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THE ROLE OF POTASSIUM IN THE CONTROL OF THE CORONARY CIRCULATION: MECHANISM OF ACTION AND TIME COURSE

by

Paul Alan Murray

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
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CHAPTER I

INTRODUCTION

The potassium ion (K^+) has been considered a possible controller of coronary vascular resistance (CVR) for almost 40 years. The hypothesis is simple and straightforward. With increased metabolic activity of the heart, there is a concomitant transmyocardial membrane flux of intracellular K^+ into the interstitial fluid that surrounds myocardial cells. This increase in interstitial fluid K^+ concentration ($IF[K^+]$) is then thought to decrease the vasomotor tone of the coronary arterioles, producing a vasodilation. The end result of this process (i.e. the vasodilation) permits the heart to match its increased metabolic demand with an increased nutrient supply. But despite a great deal of effort, and an extensive literature, the precise role of K^+ in this process is unknown. Basic questions remain unanswered or require further elucidation.

In this introduction I will review the evidence in the literature that bears on the following questions:

i. Is K^+ a vasodilator in the coronary vascular bed?

ii. Is there a correlation between increases in K^+ efflux from the heart and coronary blood flow?

iii. Are the increases in K^+ efflux fast enough to be causally related to the increases in coronary blood flow?

iv. Is the mechanism of K^+-induced vasodilation known?

Whenever relevant, information gained from the study of vascular beds from other organ systems will be cited, although extrapolations between different vascular beds should be made with caution. At the conclusion of this introduction, I will make a concise statement of the objectives of my thesis.

If K^+ is a mediator of the vascular changes that are associated with an increase in the metabolic activity of the heart, then administration of K^+ to the coronary circulation in amounts sufficient to increase IF$[K^+]$ should produce an increase in coronary blood flow.

Katz and Lindner (1938) were the first to demonstrate K^+-induced vasodilation of the coronary vascular bed. Using the isolated Langendorff dog heart preparation with fibrillating ventricles, they were able to produce an increase in coronary blood flow (52 percent) with intra-arterial injections of hypo-osmotic solutions of KCl. Even larger amounts of K^+ resulted in coronary vasoconstriction. The results of this study are difficult to interpret, since NaCl and $CaCl_2$ infusions (which are known to have virtually no effect, and a constrictor effect, respectively) also produced increases in coronary blood flow of equal or greater magnitude than the K^+ injections.

Driscol and Berne (1958), using a more physiological <u>in situ</u> beating heart preparation, found that intracoronary infusions of KCl sufficient to elevate coronary arterial plasma $[K^+]$ to levels of from 4-12 mEq/L produced increases in coronary blood flow averaging 18 percent. But they failed to find a correlation between changes in coronary blood flow and the concentration of K^+ added to the perfusate or the final plasma K^+ concentration.

Paying particular attention to the problem of changing the $[K^+]$ of coronary arterial blood without changing the total osmotic pressure of the blood, Scott et al. (1961) found that CVR decreased (15 percent) as a function of KCl infusion rate over the range of 100-800 micromoles/minute. Although these experiments were performed on in situ beating dog hearts, the hearts were performing no external work since blood was shunted around the heart and lungs.

In a very recent paper (Bünger, et al., 1976), K^+ infusions were found to produce a transient, dose-dependent increase of diastolic coronary inflow in the isolated perfused spontaneously beating guinea pig heart. Elevations in the $[K^+]$ of the perfusion medium (4-16 mEq/L) produced peak increases in diastolic coronary flow of 40 percent.

The isolated vessel technique has also been employed as a tool for investigating the effects of K^+ on coronary vascular smooth muscle. In 1936, Cohen demonstrated that increasing the extracellular $[K^+]$ resulted in a loss of resting tone in coronary arterial rings taken from cows, oxen, bulls, and calves. Konold et al. (1967) found that increasing the $[K^+]$ of the Tyrode solution bathing an isolated segment of bovine coronary artery (from 2.68-14 mmoles/liter) resulted in a 20 mm Hg decrease in intraluminal pressure, while more substantial increases in $[K^+]$ resulted in an increase in intraluminal pressure. Norton and Detar (1972) examined the effects of K^+ on the contractile tension of helical strips from rabbit free-wall left ventricular coronary arteries. They found that small changes in bath $[K^+]$ produced marked changes in the contractility of coronary vascular smooth muscle, but only if the muscle had been previously subjected to a low

K^+ environment. They suggested that the contractile activity of vascular smooth muscle may depend on the ratio of intracellular to extracellular $[K^+]$. Similarly, Gellai and Detar (1974) observed the vasoactive properties of elevated extracellular $[K^+]$ in helical strips of small coronary arteries of the rabbit. Increasing extracellular $[K^+]$ (2-6 mM) produced relaxation of resting tension in 50 percent of the strips studied, as well as a 40 percent relaxation of drug-induced contractile tension. But once again it was necessary to "condition" the strips to a low extracellular $[K^+]$.

To summarize this section, the studies cited above indicate that small increases in the extracellular $[K^+]$, both *in vivo* and *in vitro*, tend to decrease CVR or coronary vascular smooth muscle tone. Although the magnitude of the K^+-induced vasodilation appears to be only moderate and of brief duration, an important criterion for the involvement of K^+ in the control of CVR has been satisfied. But the real issue here is to ascertain the magnitude of the response of coronary vascular smooth muscle to physiologically occurring changes in $IF[K^+]$. Certainly there are problems in assessing the vascular reactivity to K^+, when K^+ is administered exogenously. Some of these difficulties will be discussed in more detail in Chapter 4.

In 1958, Berne cast a great deal of doubt about the role of K^+ in the control of CVR when he failed to demonstrate an increase in coronary sinus $[K^+]$ associated with increases in coronary blood flow produced by increasing the metabolic activity of the heart. Certainly if K^+ is to alter the caliber of the coronary arterioles, and thus act as a controller of CVR, then changes in the $IF[K^+]$ (as reflected

by changes in CS[K^+]) should be correlated with changes in coronary blood flow. As it turns out, methodological problems prevented Berne from measuring the increased efflux of K^+ from the heart. Since then many other investigators have shown that there is a clear relationship between myocardial K^+ balance, heart rate, and oxygen availability to the myocardium.

Let's first examine the relationship between myocardial K^+ balance and heart rate. In 1951, Keynes demonstrated that action potentials in nerves are associated with an increased flux of K^+ between the cell and the surrounding medium. By analogy, Wilde and O'Brien (1953) considered the possibility that a transient flux of K^+ between the myocardial cell and the interstitial fluid might occur with each cardiac cycle. This concept was supported by their effluographic findings which showed an increased efflux of K^+ from the turtle heart that was temporally related to electrical systole. It might be expected then that myocardial K^+ exchange would vary directly with heart rate. Indeed Wood and Conn (1958) found a positive linear correlation between K^+ efflux and heart rate, which was later verified by Langer et al. (1966). Sybers et al. (1965) followed changes in arterial-coronary sinus plasma [K^+] differences during the increased heart rate produced by cardiac sympathetic nerve stimulation. They found that with cardioaccelerator nerve stimulation there was an initial loss of myocardial K^+ for a brief duration, followed by an uptake of K^+ during the remainder of the stimulation period. Grupp et al. (1967) found that decreasing heart rate by vagal stimulation was associated with a decreased efflux of K^+ from the heart.

Thus, although it seemed reasonably well established that increasing the frequency of contraction of cardiac muscle is associated with a net loss of myocardial K^+, there was no real consensus concerning the mechanism involved. Hajdu (1953) first suggested that the net loss of K^+ associated with increasing heart rates was due to a decrease in the interstimulus interval, and thus less time for K^+ to be taken back up into myocardial cells during repolarization. Gilmore and Gerlings (1969) found that a number of interventions which altered the interstimulus interval (e.g. changing heart rate, paired pacing, ventricular fibrillation) also produced changes in myocardial K^+ balance, suggesting that the interstimulus interval is a determinant of myocardial K^+ balance.

However, a diminished interstimulus interval may not be the only mechanism responsible for K^+ efflux induced by increases in heart rate. Indeed it cannot account for the fact that K^+ loss is not sustained during prolonged increases in heart rate. Langer and Brady (1966) have suggested that the loss of K^+ observed by increasing heart rate is due to a lag in the Na^+-K^+ exchange pump of the myocardial cell. Although the evidence is not compelling, this hypothesis has not been disproved.

In addition to these effects of heart rate on myocardial K^+ balance, oxygen availability also appears to be a prime determinant of K^+ exchange. Flow through the coronary vascular bed is regulated to achieve a low flow and a high oxygen extraction, primarily as a result of high basal vascular tone. This forces the myocardial cells to operate at a relatively low pO_2, and it has been suggested that

this "marginally hypoxic" state provides a highly sensitive mechanism for local autoregulation of coronary blood flow. Even a small decrease in tissue pO_2 may be enough to render the cell hypoxic, and cause the release of chemical mediators. Numerous studies indicate that K^+ loss appears to be a predictable tissue response to ischemia.

Harris et al. (1954) and later Cherbakoff et al. (1957) and Cummings (1960) studied the relationship between anoxia-induced changes in myocardial K^+ balance and the development of cardiac arrhythmias. A consistent finding was that coronary artery ligation resulted in an increase in coronary sinus plasma $[K^+]$, which in turn was highly correlated with the development of cardiac arrythmias and ventricular fibrillation.

In contrast to these permanent ischemia systems, Jennings et al. (1964) examined the electrolyte shifts occurring in myocardial cells that were irreversibly damaged by transient ischemic episodes. They also found that tissue K^+ levels were decreased following a 40 minute period of ischemia.

Thus there is a considerable body of evidence which indicates that there is a loss of K^+ from irreversibly damaged myocardial cells as a result of rather gross long term coronary artery occlusion. But it was still unclear whether K^+ loss was related to the degree of oxygen deprivation or simply due to the destruction of myocardial cells.

Gerlings et al. (1969) attempted to manipulate the K^+ balance of the heart by altering the oxygen availability. They found that a net gain of myocardial K^+, induced by catecholamines, could be changed to

a net loss by limiting oxygen availability. In addition, they observed that an increase in heart rate, which at normal flows produces a transient loss of K^+, resulted in a sustained loss of K^+ during hypoxia. Parker et al. (1970) observed a greater and more sustained increase of K^+ levels in coronary sinus blood in ischemic patients than in non-ischemic patients for step increases in heart, suggesting that diminished oxygen availability as well as the elevated heart rate leads to K^+ loss. Scott et al. (1971) employing coronary occlusions of much shorter duration (30 seconds) than previously reported, observed a significant increase in K^+ efflux from the heart. Case (1971) measured K^+ efflux from the heart in response to graded, step reductions in coronary blood flow. He found that in each experiment a point was reached when a significant rise in left atrial pressure occurred, at which time there was a corresponding, abrupt rise in coronary sinus plasma $[K^+]$. Restoration of flow to levels of adequate perfusion resulted in a prompt fall in the elevated $CS[K^+]$ back to arterial levels, and an actual uptake of K^+ by the myocardium during the post-ischemic period. As stated, the onset of K^+ rise in the coronary sinus blood occurred simultaneously with a rise in left atrial pressure, as well as an increase in CS[lactate], and depression of the S-T segment of the electrocardiogram. But CVR was found to fall prior to these indicators of myocardial ischemia, and to reach a minimum value at the onset of K^+ loss. These data suggest that K^+ is not involved as a mediator of the autoregulatory response of the coronary vasculature elicited by the step reductions in coronary blood flow. But these data do not exclude the possible contribution of the elevated

K^+ levels in the maintenance of the diminished CVR during the period of apparent ischemia.

To summarize this section, these studies clearly indicate that myocardial K^+ balance can be altered either by an increase in heart rate or a decrease in oxygen availability to the myocardium. These changes in K^+ balance are likely due to a decreased interstimulus interval during increases in heart rate, or interference with the energy dependent Na^+-K^+ exchange pump of myocardial cells when oxygen supply is limiting, or perhaps a combination of these two mechanisms. Of primary importance, however, is that changes in IF[K^+] do correlate with the increases in coronary blood flow elicited by the above mentioned maneuvers. Thus it appears that a second criterion for the involvement of K^+ in the control of coronary vascular resistance has been satisfied.

It is not only necessary that there be a correlation between the changes in IF[K^+] and CVR, but these changes in [K^+] must be as fast or faster than the concomitant changes in vascular resistance. It would be clearly inappropriate to make raw comparisons between the time courses of CS[K^+] and CVR, since the time course of the changes in CS[K^+] reflect not only the time course of the changes in interstitial fluid [K^+], but also the delay and dispersion introduced by both the exchange of K^+ across the capillary endothelium and the vascular transit of blood from the capillaries to the coronary sinus sampling site. Ideally one would like to have a direct measure of the changes in myocardial interstitial fluid [K^+], but the technology to make these measurements in a beating heart still remains primarily in

the developmental stage. Thus, there is no evidence in the literature that deals with the question of whether the changes in myocardial IF[K^+] are fast enough to be causally related to the concomitant changes in CVR.

However, IF[K^+] has been measured during functional hyperemia in rabbit skeletal muscle by Gebert (1972) and Hnik et al. (1973). In each case electrical stimulation of the skeletal muscle resulted in rapid increases in IF[K^+]. In the study by Gebert, the time courses of the changes in IF[K^+] and flow appeared to be similar. But the strongest evidence regarding the dynamics of these changes comes from the study by Mohrman and Sparks (1974). They measured changes in the venous plasma [K^+] of dog skeletal muscle following one second of tetanic exercise during constant flow perfusion of the vascular bed. By utilizing a procedure that will be described in outline form in Chapter 2, they were able to determine that the time course of the estimated changes in IF[K^+] produced by the tetanic exercise was fast enough for K^+ to play a significant role in the production of the associated vascular response.

To summarize this section, the criterion of appropriate time course for K^+ has yet to be determined in the heart. One of the major objectives of my thesis is to ascertain whether the changes in myocardial IF[K^+] are fast enough to account for concomitant changes in CVR.

A number of studies have dealt with the question of the mechanism of K^+-induced changes of vascular resistance. Since K^+ is considered a possible controller of vascular resistance in a variety of

tissues, an understanding of its mechanism of action is essential. An intriguing aspect of the K^+-induced changes in vascular resistance is that they are precisely the opposite of those predicted by the Nernst and Goldman equations.

The Nernst and Goldman equations predict that increasing the $[K^+]$ of arterial blood would cause a vasoconstriction, while decreasing the $[K^+]$ would cause a vasodilation. But the observed vascular responses in both the heart and skeletal muscle are just the opposite: small increases in $[K^+]$ result in vasodilation, while decreases in $[K^+]$ produce vasoconstriction (Haddy et al., 1963). Thus the mechanism of K^+-induced alterations of vascular reactivity cannot be due solely to changes in the transmembrane concentration gradient of K^+ in vascular smooth muscle. For the purpose of discussion the most probable mechanisms of K^+-induced changes in vascular reactivity have been divided into the following categories: metabolic, neural, and direct effect on vascular smooth muscle. Since there are relatively few studies that deal explicitly with the mechanism of K^+-induced changes in coronary vascular reactivity, evidence gained from other vascular beds will be cited.

One possible mechanism of K^+-induced vasodilation is that increasing the $[K^+]$ in arterial blood results in a primary increase in tissue metabolism, producing a secondary metabolically-induced increase in blood flow. Fenn (1931) was the first to observe that the oxygen consumption of the frog sartorious muscle was increased substantially when the extracellular $[K^+]$ was raised. Van Der Kloot (1967) extended this original observation, and found that oxygen consumption begins to

rise when the membrane potential of the striated muscle falls to a critical value of about -55 mV. The fall in membrane potential results in an increase in the calcium concentration of the sarcoplasm, which in turn acts as a trigger for the increase in oxygen consumption. The threshold for this effect appears to be when the extracellular $[K^+]$ concentration is raised above 10 mM, a value similar to the observed changes of IF$[K^+]$ produced by electrical stimulation of skeletal muscle (Hnik et al., 1976). Thus some part of the dilation produced by increasing the $[K^+]$ of arterial blood could be the result of a metabolic vasodilator feedback between the striated muscle and the vasculature.

A possible metabolic component of K^+-induced vasodilation in the heart can also not be discounted. If elevated levels of coronary arterial $[K^+]$ produced increases in the primary determinants of myocardial oxygen consumption (i.e. heart rate, mean arterial pressure, or contractility), then a metabolically-induced increase in coronary blood flow could result. Driscol and Berne (1958) state that intracoronary infusions of KCl produced no significant alterations in heart rate or systemic pressure. Scott et al. (1961) also failed to observe an increase in heart rate when coronary arterial $[K^+]$ was elevated. Sarnoff et al. (1966) demonstrated that intra-coronary infusions of K^+ result in a decrease of myocardial contractility. Although these studies suggest that elevated K^+ levels do not appear to increase the metabolic activity of the heart, they provide only an indirect measure of the possible changes in myocardial oxygen consumption. In order to disprove the hypothesis that K^+-induced vasodilation

is the result of a primary increase in tissue metabolism, it is essential to directly measure the changes in tissue oxygen consumption produced by elevated K^+ levels.

K^+-induced changes in vascular reactivity may be the result of an interaction between K^+ and tissue neural elements. Several investigators have shown that the release or re-uptake of neurotransmitters from nerve endings can be influenced by the extracellular $[K^+]$. Bonaccorsi et al. (in press) have demonstrated that the increased tension development of isolated vascular smooth muscle strips of rat tail artery and dog mesenteric artery, following exposure to a K^+-free medium, is paralleled by a release of norepinephrine. This response is blocked by phentolamine, and does not occur in arteries whose nerve terminals have been destroyed by 6-hydroxydopamine. Similarly, Lindmar and Löffelhotz (1974) have observed that a diminished extracellular $[K^+]$ increases the release of norepinephrine from the perfused rabbit heart. In addition, Vanhoutte and Lorenz (1974) have reported elevated levels of extracellular $[K^+]$ inhibit both the release and re-uptake of norepinephrine in the isolated dog saphenous vein, while Bogdanski et al. (1969) found that high extracellular $[K^+]$ antagonized the uptake and storage of norepinephrine in rat heart slices.

It has been proposed that K^+-induced vasodilation is the result of an increased release of norepinephrine from local sympathetic nerve terminals, with subsequent activation of vascular beta-adrenergic receptors. A test of this hypothesis would be to observe the effects of propranolol administration on the magnitude of K^+-induced vasodilation. No data concerning the effects of propranolol on K^+-induced

vasodilation have been reported in the literature, although several investigators have stated that propranolol was without effect on K^+-induced relaxation of isolated dog cerebral artery (Toda, 1974), or K^+-induced vasodilation of the isolated perfused guinea pig heart (Bünger et al., 1976). Thus the hypothesis that K^+-induced vasodilation is the result of an increased release of norepinephrine from local sympathetic nerve terminals remains largely untested.

K^+-induced vasodilation could also be the result of an increased release of acetylcholine from local parasympathetic nerve terminals, with subsequent activation of Ach-specific vascular receptors. Beznak (1934) observed that perfusion of the frog heart with Ringer's solution containing an excess of K^+ caused the release of acetylcholine. Similar results were reported by Brown and Feldberg (1936) for nerve, and Feldberg and Guimarais (1936) for salivary gland, tongue and sweat glands. One test of this hypothesis would be to observe the effects of atropine administration on the magnitude of K^+-induced vasodilation. No data concerning the effects of atropine on the magnitude of K^+-induced vasodilation in the coronary vascular bed have been reported in the literature. Dawes (1941) states that atropine was without effect on K^+-induced vasodilation in skeletal muscle, although no data were presented to support this conclusion.

It has also been postulated that K^+-induced vasodilation might be the result of a transient withdrawal or inhibition of alpha-adrenergic mediated vascular tone. Feigl (1975) and others have demonstrated direct coronary vasoconstriction resulting from sympathetic alpha receptor stimulation. Konold et al. (1968) and Brecht

et al. (1969) have shown that small increases in the muscle bath [K^+] inhibit the vasoconstrictor action of norepinephrine in isolated bovine facial arteries. Burcher and Garlick (1975) observed that by increasing the plasma [K^+] of the venous effluent (10 mM or more) of dog gracilis muscle by intra-arterial infusions of KCl, they were able to abolish the vasoconstrictor response to sympathetic stimulation, whereas the vasoconstrictor response to exogenously administered norepinephrine was unaffected. These investigators concluded that this sympatholytic action of K^+ was the result of an inhibition of sympathetic neurotransmission due to depolarization of the adrenergic nerve terminals. However, in a recent report (Beatty et al., 1975), increases in venous plasma [K^+] (produced by intra-arterial infusions) to levels similar to those observed during the early period of exercise, significantly reduced both the vasoconstrictor response to sympathetic stimulation and norepinephrine in the dog hindlimb. These authors suggest that the sympathetic action of K^+ is due to a direct effect on alpha receptors and not to a reduction in the amount of norepinephrine released by sympathetic stimulation. At any rate, all these studies appear to support the notion that K^+-induced vasodilation could be the result of a transient withdrawal or inhibition of alpha-adrenergic mediated vascular tone. A test of this hypothesis would be to compare the magnitude of K^+-induced vasodilation before and after the administration of the alpha-adrenergic antagonist, phentolamine. Emanuel et al. (1959) observed the effect of phentolamine on K^+-induced vasodilation in the dog forelimb. These authors conclude that phentolamine was without effect on K^+-induced

vasodilation. But these results are very difficult to interpret, since the administration of phentolamine produced drastic decreases in the initial resistance of the vascular bed. In fact if one was to discount the effects of a low initial resistance on the magnitude of the vasodilator response, the data appear to indicate that phentolamine has attenuated K^+-induced vasodilation. Thus the hypothesis that K^+-induced vasodilation might be the result of a withdrawal or inhibition of alpha-adrenergic mediated vascular tone remains largely untested.

However, the interaction between K^+ and the release of neurotransmitters has been more fully investigated in circumstances where the extracellular $[K^+]$ has been lowered. Hypokalemia has been shown to produce vasoconstriction in both the heart (Brace et al., 1974) and skeletal muscle (Chen et al., 1972). Lowering the muscle bath $[K^+]$ results in an increase in the tension development of isolated vascular smooth muscle (Hendrickx and Casteels, 1974). As stated previously, the work of Bonaccorsi et al. (in press) indicates that a removal of extracellular K^+ results in an increased release of norepinephrine from isolated vascular smooth muscle, which parallels the increased tension development of the muscle strip. Moreover, this increase in tension development is blocked by the administration of phentolamine, and does not occur in arteries whose nerve terminals have been destroyed by 6-hydroxydopamine. These data suggest that at least some (hypokalemia-induced vasoconstriction) or all (K^+-free contracture) of the increases in vascular reactivity produced by a decreased extracellular $[K^+]$ are mediated through an increased release of norepinephrine.

K^+-induced changes in vascular reactivity may be the result of a direct action of K^+ on the vascular smooth muscle cell. Johansson and Bohr (1966) observed that moderate increases in the extracellular $[K^+]$ (10-30 mM) resulted in a reduction of agonist-induced rhythmic contractile activity of small, isolated subcutaneous arteries from the dog paw. Axelsson et al. (1967) found that when the extracellular $[K^+]$ was increased from a reduced to a normal level, there was an inhibition of the spontaneous activity of the isolated rat portal vein. It has been suggested that the decrease in the pacemaker activity of this type of spontaneously active vascular smooth muscle by K^+ is the result of an increase in K^+ conductance. This hypothesis has been corroborated in a forthcoming publication by Hermsmeyer et al. (in press).

But another mechanism involving the direct action of K^+ on vascular smooth muscle has been postulated to account for the K^+-induced changes in vascular reactivity of non-spontaneously active vascular smooth muscle cells. It has recently been proposed (Chen et al., 1972 and Anderson et al., 1972) that the mechanism of K^+-induced changes in vascular reactivity involves an alteration in the activity of an electrogenic Na^+-K^+ ATPase located in the membrane of the vascular smooth muscle. The hypothesis states that alterations in the interstitial fluid $[K^+]$ either slightly above or below normal levels elicit a change in the activity of the Na^+-K^+ pump. Since the pump is electrogenic, changes in pump activity result in a cellular loss or accumulation of positive charges which are inversely related to the IF$[K^+]$ and opposite to those predicted from passive ion fluxes.

The resultant change in membrane potential then produces a change in the contractile state of the vascular smooth muscle: hyperpolarization being associated with relaxation and depolarization associated with contraction.

In order to verify this hypothesis, the following criteria should be met: (1) isolation of the Na^+-K^+ ATPase (pump) from vascular smooth muscle, (2) sensitivity of the pump to physiologically occurring levels of extracellular [K^+], (3) electrogenicity of the pump, (4) alterations in pump activity resulting in changes in contractile activity of vascular smooth muscle, (5) attenuation of K^+-induced changes in vascular reactivity following inhibition of pump activity by ouabain. A growing body of evidence derived from both in vitro and in vivo studies appear to satisfy the above criteria, and thus lend support to this hypothesis.

A Mg^{++}-dependent, Na^+-K^+-stimulated ATPase has been isolated from vascular smooth muscle (dog mesenteric artery) by Limas and Cohn (1974). These investigators demonstrated that the ATPase activity was markedly stimulated by the simultaneous addition of 100 mM NaCl and 10 mM KCl, and was blocked in a dose-dependent manner by ouabain (10^{-9}-10^{-6} M).

Hendrickx and Casteels (1974) have provided evidence of the electrogenicity of the pump. Using the isolated ear artery of the rabbit, they demonstrated that exposure of this tissue to a K^+-free solution depolarized the cell, while readmission of K^+ (5.9 mM) to the bathing medium produces a transient hyperpolarization. Although a short-lasting increase in muscle tension development was observed

during exposure to the K^+-free solution (i.e. during the period of depolarization), re-admission of K^+ had no demonstrable effect on muscle tension during the period of K^+-induced hyperpolarization. The addition of ouabain (10^{-5} M) to the bathing solution elicited the predicted cell depolarization and increase in muscle tension. Moreover, the addition of ouabain abruptly terminated the hyperpolarization induced by the re-admission of K^+ to the K^+-free medium, with the membrane potential returning to the level observed during K^+-depletion. The changes in membrane potential resulting from exposure to a K^+-free medium or following the addition of ouabain do not appear to be due to changes in K^+ permeability of the smooth muscle membrane, since the small increases in the rate coefficients of K^+ efflux appeared later than the changes in cell potential or tension development.

Hypokalemia-induced constrictions of *in situ* skeletal muscle (Chen *et al.*, 1972) vascular beds are abolished or reversed by ouabain. These investigators have concluded that changes in vascular reactivity induced by hypokalemia are in large part due to depolarization of the vascular smooth muscle cells as a direct result of the inhibition of the electrogenic Na^+-K^+ exchange pump in the vascular smooth muscle. However, an alternative explanation has been offered by Bonaccorsi *et al.* (in press). Their data indicate that the K^+-free contracture of isolated vascular smooth muscle is the result of an increased release of norepinephrine following inhibition of the Na^+-K^+ ATPase in the adrenergic nerve ending.

Hyperkalemia-induced vasodilations of *in situ* skeletal muscle (Chen *et al.*, 1972) and coronary (Bünger *et al.*, 1976) vascular beds

are also attenuated or abolished by ouabain. These investigators have concluded that the change in vascular reactivity induced by hyperkalemia is due to a hyperpolarization of the vascular smooth muscle cells as a direct result of the activation of the electrogenic Na^+-K^+ exchange pump in the vascular smooth muscle. These conclusions have been largely verified by the *in vitro* study of Bonaccorsi *et al.* (in press). Using the isolated tail and femoral arteries of the rat, these investigators found that re-addition of K^+ to a K^+-free medium resulted in a dose-dependent relaxation of a K^+-free contracture. This K^+-induced relaxation was associated with a transient hyperpolarization of the vascular smooth muscle, as well as a diminished release of norepinephrine from adrenergic nerve endings. The relaxation was not dependent on the presence of nerves, and could be obtained in the presence of moderate concentrations of norepinephrine. Both the K^+-induced relaxation and the K^+-induced decrease in norepinephrine release were inhibited by ouabain. These results indicate that K^+-induced decreases in vascular reactivity could be the result of at least two mechanisms: (1) activation of the electrogenic Na^+-K^+ exchange pump in the vascular smooth muscle or, (2) decreased release of norepinephrine from adrenergic nerve endings. These investigators conclude that since the time course of the decreased release of norepinephrine is significantly slower than the time course of the K^+-induced relaxation, and since the K^+-induced relaxation can occur in the presence of moderate concentrations of norepinephrine or in the absence of nerves, activation of the Na^+-K^+ exchange pump in the vascular smooth muscle appears to be the first and most powerful mechanism of K^+-induced relaxation.

In summary, it is known that small increases in the extracellular $[K^+]$ both *in vivo* and *in vitro* can induce decreases in the reactivity of coronary vascular smooth muscle. Very recent evidence has strongly indicated that an electrogenic Na^+-K^+ exchange pump in the vascular smooth muscle membrane may be responsible for K^+-induced vasodilation, although other possible mechanisms have not been excluded.

In addition, changes in $IF[K^+]$ do appear to be correlated with increases in heart rate or decreases in oxygen availability of the myocardium, although there is no evidence (e.g. time course comparisons) that these changes in $IF[K^+]$ are causally related to the concomitant changes in CVR.

With this background, the present study was undertaken and had several objectives. First, we have more fully assessed the vasodilator activity of K^+ in the *in situ* coronary vascular bed. Because of the transient nature of the K^+-induced vascular response, and because the magnitude of the response may depend on the rate of administration of K^+ (Duling, 1975) as well as the degree of vascular tone of the preparation, the question of the coronary vasodilator activity of K^+ has been controversial. Second, we have more fully assessed the mechanism of K^+-induced vasodilation in the coronary vascular bed. More specifically, we will determine whether K^+-induced vasodilation is the result of:

i. a primary increase in the metabolic activity of the heart, resulting in a secondary metabolically-induced increase in coronary blood flow,

ii. an increased release of norepinephrine from sympathetic nerve terminals in the heart,

iii. an increased release of acetylcholine from parasympathetic nerve terminals in the heart,

iv. a withdrawal of alpha-adrenergic mediated vascular tone,

v. an increased activity of the electrogenic Na^+-K^+ exchange pump located in the wall of coronary vascular smooth muscle.

And third, we have ascertained whether the changes in interstitial fluid $[K^+]$ are in the right direction, and have a sufficiently fast time course to be causally related to the concomitant changes in coronary vascular resistance produced by step increases in heart rate and 15 second cessations of coronary blood flow.

CHAPTER II

METHODS

1. Surgical

a. General:

Male mongrel dogs, ranging in weight from 20 to 39 kilograms, were anesthetized with morphine sulfate (4-7 mg/kg; subcutaneous injection), and sodium pentobarbital (20 mg/kg, or to effect; intravenous injection). Smaller doses of sodium pentobarbital were administered throughout the course of the experiment to maintain adequate surgical anesthesia. A cuffed endotracheal tube was positioned, and positive pressure ventilation was achieved with a Harvard large animal respirator. For the circumflex artery preparation (described below) the respiratory pump rate was set at 14 cycles per minute, while the tidal volume was set at a level approximately 15% higher than that indicated on the chart supplied by the manufacturer. For the common coronary artery preparation (described below) both respiratory pump rate and tidal volume were adjusted so that an arterial pCO_2 of 43.67 ± 1.96 mmHg (mean ± SEM) was achieved (N = 16 dogs). Body temperature was maintained at between 38-39°C throughout the course of each experiment. A femoral artery was isolated and cannulated for systemic blood pressure measurement. A femoral vein was isolated and cannulated for the return of extracorporeal blood, and the administration of a variety of pharmacologic agents (described

below). Following a priming dose of sodium heparin (500-750 units/kg), a solution containing sodium bicarbonate (15 mg/ml), sodium heparin (50 units/ml), and dextran (9 mg/ml) was infused intravenously at a constant rate (1.0-2.0 ml/min) throughout the course of the experiment to counteract anesthesia-induced acidosis, to prevent coagulation, and to correct for estimated blood loss during surgery, respectively.

b. Circumflex artery preparation:

The heart was exposed by opening the chest between the fourth and fifth ribs. The pericardium was incised and fashioned into a cradle. A short segment (1.0-2.0 cm) of the circumflex artery was isolated just distal to the bifurcation of the left common coronary artery, and catheterized according to the method of Herd and Barger (1964). An electromagnetic flow probe was positioned just proximal to this catheter for measurement of circumflex blood flow. A cotton snare was positioned between the flow probe and catheter which allowed occlusion of the artery to check for electronic baseline drift of the flow probe. A polyethylene catheter was inserted into the thoracic aorta via the femoral artery for measurement of intra-aortic blood pressure.

c. Common coronary artery preparation:

The left main coronary artery of the closed-chest dog was pump perfused with blood at a constant flow rate. Following isolation of the right common carotid artery, a balloon-tipped, metal cannula (compliments of E.O. Feigl and D.E. Mohrman, University of Washington) was passed down the carotid artery into the aorta, and guided manually

into the coronary artery. The blood perfusate was obtained from the animal's right femoral artery. The perfusion line between the femoral artery and coronary artery was composed of medical grade Silastic tubing, except for the polyethylene and metal cannulae at either end. The blood perfusate was passed through a variable speed roller pump (Cole-Parmer model 7598), and constant flow perfusion was thus obtained. The perfusion line included a blind-end side tube of Silastic tubing between the pump and the heart. When partially filled with air, this side tube acted as a windkessel and filtered out the pressure pulses generated by the pump. The dynamics of the windkessel were such (time constant$\sim$0.5 sec) that it allowed flow to vary with each cardiac cycle but did not interfere with the time course of the observed changes in coronary vascular resistance, myocardial oxygen consumption, or myocardial interstitial fluid K^+ concentration. The perfusion line also contained an access port between the pump and the heart that permitted the sampling of well-mixed arterial blood perfusing the heart. The metal cannula positioned in the coronary artery was constructed of stainless steel (3 mm ID), and contained two smaller diameter cannulae in its lumen, each with a separate exteriorized access port. One of these smaller inner cannulae was connected to the Silastic balloon around the distal end of the main cannula. Inflation of the balloon (with water) achieved a complete seal of the coronary ostium around the main stem of the perfusion cannula (criteria used to determine a complete seal described below), thus permitting constant flow perfusion of the coronary artery. The other smaller diameter inner cannula was positioned

in the main cannula so that its distal tip lay at the opening of the main cannula. The proximal end of this inner cannula was connected to a pressure transducer, via a polyethylene cannula, to measure coronary perfusion pressure.

Both jugular veins were isolated. A bipolar pacing electrode and radio-opaque catheter were passed down the jugulars and, with the aid of fluoroscopy, were positioned in the right atrium and coronary sinus, respectively.

2. Instrumentation and Measurements

Systemic arterial pressure and coronary perfusion pressure measurements were made using diaphragm strain gauge pressure transducers (Statham model P23DC).

In the open chest preparation, circumflex blood flow was measured continuously with a square wave electromagnetic flowmeter (Zepeda model SWF-3RD, or Carolina model 301). Flow probes (2.5 mm I.D.) were positioned around the circumflex artery just distal to the bifurcation of the common coronary artery. Short term (less than 10 seconds) occlusions of the circumflex artery distal to the flow probe were performed repeatedly throughout the course of the experiment to check for possible baseline electronic drift. The flow probe was calibrated in situ at the end of each experiment in the following manner. The circumflex artery was cannulated with polyethylene tubing both proximal and distal to the position of the flow probe. Blood collected from the dog was pumped through the artery via the proximal tubing (with the flow probe in position), and collected at

timed intervals from the distal tubing with a graduated cylinder and stopwatch.

The oxygen (O_2) saturation of hemoglobin in coronary sinus blood was measured continuously with a flow-through cuvette, optical densitometer (oximeter, Waters model 0-600). Blood from the coronary sinus cannula was drawn at a constant rate (16 ml/min) through the oximeter cuvette. The electrical signal from the oximeter is proportional to the oxygen content of blood, provided the hemoglobin concentration remains constant. The hemoglobin concentration in blood was measured spectrophotometrically (Hycel Cyanmethemoglobin Certified Standard no. 117 and Stable Reagent no. 116) in each experiment and was found to be constant during the 1-2 hour period of actual experimental manipulations. The electrical output of the oximeter was calibrated for total blood oxygen content by determining the oxygen content of anaerobically sampled blood with a Lex-O_2-Con-M. It should be noted that the relatively small amount of oxygen dissolved in the blood (less than 2%) is included in this oxygen content measurement. Since the contribution of dissolved oxygen to the total oxygen content is so small, we have regarded the oximeter signal as a measure of total blood oxygen content for the purposes of this thesis. Arterial blood was also drawn through the cuvette and analyzed for O_2 content at 30-90 minute intervals throughout the experiment. This allowed calculation of arterial-venous O_2 content differences across the left ventricular myocardium and, since coronary blood flow is known, calculation of myocardial oxygen consumption.

Arterial and coronary sinus blood was also sampled (anaerobically) at 30-90 minute intervals throughout the experiment for measurements of pO_2, pCO_2, and pH (Instrumentation Laboratories model 113), and hematocrit. Body temperature was measured continuously via placement of an intraesophageal thermal electrode, and maintained between 38-39°C throughout the course of the experiment.

Electrical pacing of the heart was achieved using a Grass physiological stimulator (model 54). A square wave pulse from the stimulator was sent first through a stimulus isolation unit (Grass model SIU 414) and then to a constant current unit (Grass model CCU 1A), and from there to an intraluminal bipolar electrode positioned in the right atrium. Stimulus parameters were determined for each preparation, with the pacing electrode generally delivering square waves of 0.5-1.0 mamps intensity and 5-7 msec duration. The final position of the pacing electrode was empirically determined in each experiment by slowly withdrawing the electrode from the right atrium (while applying the electrical stimulus) until a suitable pace rate was achieved. It was found on post-mortem examination that the final position of the pacing electrode (i.e. the position of the electrode which provided the best pace) was most often the junction between the superior vena cava and the right atrium. The pace rate was set by a device which acted as a voltage-to-frequency converter. The output of this device triggered the stimulator to deliver a series of pulses to the pacing electrode. The rate of these triggering signals (i.e. the pace rate) was linearly related to the input voltage to this device, and could be adjusted to a desired level with a trim pot.

Step changes in heart rate were effected by suddenly turning the stimulator on, thus increasing the heart rate up to a desired level from its inherent rate.

Arterial and coronary sinus plasma K^+ concentration was measured by collecting blood samples at timed intervals into microhematocrit capillary tubes. These tubes were centrifuged within 5 minutes, and 20 microliters of plasma were drawn from each tube into calibrated disposable glass capillary pipettes. These samples were then diluted 1:100 for determination of K^+ concentration by flame photometry. The standard deviation of duplicate determinations of the same sample was $\pm$ 0.03 mEq/L.

The outputs of the pressure transducers, gas analyzer, and oximeter were recorded on an ink-writing oscillograph (Grass model 7 B). Heart or pace rate was measured and recorded on the oscillograph with a tachograph channel (Grass model 7 P4D) using either the systemic pressure measurement signal or an ECG signal as input.

3. Drugs

a. Circumflex artery preparation:

Bolus injections (0.4 ml) of the following agents were made directly into the circumflex artery via the Herd-Barger catheter:

i. 40 micromoles of isosmotic KCl

ii. 0.9% NaCl

iii. 0.1 micrograms of isoproterenol (Isuprel; Winthrop Lab.)

iv. 0.1 micrograms of acetylcholine (Sigma Chemical)

The following agents were infused intravenously at a rate of 1.0 - 2.0 ml/minute:

i. 1.0-2.0 mg/kg propranolol hydrochloride (Inderal; Ayerst Lab.)

ii. 0.5-1.0 mg/kg atropine sulfate (Merck)

iii. 2.0-3.0 mg/kg lidocaine (Elkins-Sinn, Inc.)

iv. 50-70 μg/kg ouabain (Lilly Co.)

Phentolamine (Regitine; Ciba Pharm.) (1.0-2.0 mg/kg) was infused (0.5 ml/min) directly into the circumflex artery via the Herd-Barger catheter.

4. Protocol

a. Circumflex artery preparation:

After the circumflex artery cannulation procedure was completed, the preparation was allowed to stabilize. When circumflex artery blood flow reached a steady level, a series of infusions or bolus injections directly into the circumflex artery was begun. In the "time control" series alternate injections of KCl, NaCl, acetylcholine, and isoproterenol were made, at approximately 5 minute intervals, for a period of 3-4 hours. This series of experiments was performed to examine the effects of multiple injections on the magnitude of the vasodilation produced by these agents.

A similar protocol was used in the "blocker" experiments. Alternate injections of KCl, NaCl and either acetylcholine or isoproterenol were made at 5 minute intervals during the first hour of the experiment. This was followed by an infusion of one of the various blocking agents (atropine, propranolol, phentolamine, lidocaine, ouabain), which in turn was followed by a 2 hour period of injections of the

vasodilator agents listed. These experiments were designed to examine the effect of the various blocking agents on the magnitude of the K^+-induced vasodilation.

At the termination of each experiment the animal was sacrificed by the rapid intravenous injection of a saturated potassium chloride solution. The flow probe was calibrated in situ by making timed collections of blood into a graduated cylinder. The heart was then excised and weighed.

b. Common coronary artery preparation:

Following the initial placement of the coronary cannula, the preparation was allowed to stabilize. During this period, the coronary flow was set by adjusting the pump rate so that coronary perfusion pressure approximately equalled systemic arterial blood pressure. An acceptable positioning of the coronary cannula and seal of the coronary ostium was achieved when the following criteria were met:

i. a smooth, rapid (less than 10 second) fall in coronary perfusion pressure to 15 mm Hg or less during a 15 second cessation of coronary blood flow,

ii. the return of coronary sinus O_2 content to approximately pre-occlusion levels with the re-institution of flow following a 15 second cessation of coronary blood flow,

iii. a smooth fall in coronary perfusion pressure and coronary sinus oxygen content to new steady state levels, and their return to pre-stimulation levels, in response to step changes in heart rate (in animals where it was possible to achieve a step change in heart rate).

When perfusion pressure had reached an acceptable and steady level, the following maneuvers were performed:

i. Step increase in heart rate: Heart rate was increased in a stepwise fashion an average of 75 ± 6.56 (mean ± S.E.M) beats per minute (range = 50-110) above the inherent rate. The elevated rate was maintained for a period of 2.5-3.0 minutes. This maneuver was performed 1-3 times in 9 dogs.

ii. 15 second cessation of coronary blood flow: The pump perfusing the left common coronary artery was turned off for 15 seconds. At the end of the 15 second period flow was restored in a stepwise fashion to pre-cessation levels. Throughout this entire sequence heart rate either remained unchanged or was maintained at the pre-cessation-of-flow level by pacing the heart at a rate slightly above the inherent rate. This maneuver was performed 2-3 times in 9 dogs.

Timed arterial and coronary sinus plasma or whole blood K^+ concentration measurements were made just before and during the periods of decreased coronary vascular resistance that were elicited by either a step increase in heart rate or a 15 second cessation of coronary blood flow. Blood samples were collected at 5 second intervals, starting 30 seconds prior to these experimental maneuvers, and continuing for 2.5-3.0 minutes.

The distribution of intravascular transit times was obtained in each preparation by injecting an intravascular dye (indigo carmine) as a bolus into the coronary artery through the coronary perfusion pressure cannula, and then monitoring the relative concentration of

the dye in the coronary sinus blood with the oximeter. From 3-5 dye curves were obtained for each preparation. The coronary blood flow was always the same as that at which the heart rate steps or cessations of flow were performed.

At the termination of each experiment a solution containing crystal violet stain was injected into the perfusion line. Five to ten seconds after injection of the stain the animal was sacrificed by the rapid intravenous injection of a saturated potassium chloride solution and the cessation of the coronary perfusion. The chest was opened and the heart exposed. The positions of the common coronary cannula, the coronary sinus cannula, and the pacing electrode were confirmed by visual inspection. The heart was then excised and examined. As a further check on the proper placement of the common coronary artery cannula, and the complete seal of the coronary ostium, the following criteria had to be met for an acceptable experimental preparation:

i. no staining of the walls of the aorta with crystal violet in the area of the coronary ostium,

ii. no staining of the right ventricular myocardium,

iii. staining of both the circumflex artery and left anterior descending artery vascular beds,

iv. staining of the septal artery vascular bed.

5. Data Analysis and Presentation

a. Circumflex artery preparation:

The calculated changes in resistance (i.e. mean aortic

pressure/circumflex blood flow) produced by KCl, isoproterenol, and acetylcholine injections represent the difference between the peak change in resistance and the average resistance over the 30 second period that immediately preceded administration of the vasoactive agent. All responses have been corrected for the small, non-specific vascular effect produced by the intra-circumflex artery bolus injection, as determined by isotonic NaCl injections of equal volume (0.4 ml).

The data have been divided into three separate categories for grouping and statistical analyses.

i. Effect of initial resistance on the magnitude of K^+-induced vasodilation: In this series, 177 vascular responses to K^+ over a wide range of spontaneously varying initial resistances were collected from 18 animals during the first hour of each experiment. The individual responses were then grouped within different ranges of initial resistance (intervals of 0.5 PRU starting with initial resistance of 1.0 PRU) and expressed as the mean ± one standard error of the mean for that range of initial resistance.

ii. Effect of K^+ on HR and MAP was examined simultaneously in 7 animals (119 observations). HR during the minute immediately preceding the administration of K^+ was compared to the HR during the minute immediately following K^+ administration. At the same time the MAP associated with the peak increase in circumflex blood flow produced by K^+ was compared to the average MAP during the 30 second period immediately preceding K^+ administration. These data were

statistically analyzed using Student's t test modified for paired replicates (Dixon and Massey, 1969).

iii. Effect of time (N = 7 dogs), propranolol (N = 10 dogs), atropine (N = 8 dogs), phentolamine (N = 5 dogs), lidocaine (N = 4 dogs), and ouabain (N = 8 dogs) on the magnitude of K^+-induced vasodilation: In these series, the effect of time and the effect of the various blocking agents on the magnitude of K^+-induced vasodilation was tested. In all cases a number of control vascular responses to K^+ were obtained during the first hour of each experiment, and were then compared to those vascular responses obtained during the succeeding 2-3 hours of each experiment, either in the absence of any experimental intervention (time controls), or following administration of a pharmacologic blocking agent. The data were grouped and analyzed in the following manner:

(a) Each series of experiments was treated separately.

(b) "Control" responses (i.e. vascular responses obtained during the first hour of the experiment) and "experimental" responses (i.e. vascular responses obtained during the succeeding 2-3 hours of the experiment) were treated separately.

(c) A given set of responses for each dog was separated into several intervals of initial resistance (intervals of 1.0 PRU, starting with initial resistance of 1.0 PRU). There were two exceptions to this convention: (1) the effects of ouabain-lidocaine on acetylcholine-induced vasodilation, where the responses were grouped in initial

resistance intervals of 1.0-2.0, 2.0-2.5, and 2.5-3.5 PRU. (2) the effects of atropine on acetylcholine-induced vasodilation, where the experimental responses were grouped in initial resistance intervals of 1.0-1.5, 1.5-2.0, 2.0-4.0 PRU.

(d) Responses within each interval of initial resistance for each dog were averaged and the mean value used in all subsequent analyses. In this way the response of each dog was weighted equally.

(e) The average responses within each interval of initial resistance from all dogs within a series were averaged, and the variance among dogs determined.

(f) Within each interval of initial resistance a statistical analysis of the difference in the mean values between "control" and "experimental" vascular responses was performed using Student's t test (where sample size (N) equals the number of dogs in the "control" and "experimental" groups for that particular interval of initial resistance).

This method of grouping permitted a direct comparison of the effects of time alone or the various blocking agents on the magnitude of K^+-induced vasodilation that is independent of the effects of initial resistance.

b. Common coronary artery preparation:

i. Digitization

The curvilinear oscillograph records of coronary perfusion

pressure and coronary sinus O_2 content were digitized using a clear plastic, overlaying grid. Values for the two variables were determined at 0.6 second intervals starting at 6.0 seconds prior to and ending 150-200 seconds after the step increase in heart rate or the 15 second cessation of coronary blood flow.

ii. Normalization

Each individual digitized response of coronary perfusion pressure and coronary sinus O_2 content, as well as the measured changes in coronary sinus plasma K^+ concentration, in response to either an increase in heart rate or a 15 second cessation of coronary blood flow was normalized in the following manner prior to any averaging:

(a) Step increase in heart rate: The pre-step baseline value of the variable (defined as the average of the values of the variable determined at the points from -6 seconds [-30 seconds for K^+ only] up to and including time zero) was set equal to zero. The post-step value of the variable which corresponded to the peak change of the variable was set equal to 100. The variable values at all points of that particular response were then adjusted to conform to this new 0 to 100 scale.

(b) Fifteen second cessation of flow: The release point (corresponding to the point at which flow was re-instituted after a 15 second cessation of flow) was defined as time zero.

For coronary sinus O_2 content and coronary sinus plasma $[K^+]$ the post-step value of the variable which corresponded to the peak change of the variable was set equal to 0. The steady state level to which

these variables returned after the 15 second cessation of flow was set equal to 100. This value was obtained by averaging the values of the variables over a 10 second period (O_2) or a 30 second period (K^+) immediately following the return of these variables to a steady state level. The values at all the points of that particular response were then adjusted to conform to this new 0 to 100 scale.

The initial rapid rise in coronary perfusion pressure immediately following the re-institution of flow (as seen in figure 19) is due to the passive refilling of the coronary arterial vascular bed, and will not be considered for the purpose of this thesis. Of primary interest is the comparison of time courses for K^+ and the active vascular response elicited by the cessation of flow, as reflected by the gradual return of coronary perfusion pressure to a steady state level. The following convention was thus employed. The rate of change of coronary perfusion pressure following the re-institution of flow was computed point by point at 0.6 second intervals by the following equation:

$$\text{rate of change in pressure} = \frac{\text{pressure}_{(N+1)} - \text{pressure}_{(N)}}{\text{time}_{(N+1)} - \text{time}_{(N)}}$$

where N refers to the number of the digitized unit. When the rate of change in coronary perfusion pressure decreased below 5.0 mm Hg/ 0.6 sec, N was set equal to 0. This point occurred 1.61 ± 0.16 sec (mean ± S.E.M.; N = 16 individual cessations of flow; range = 0.6 to 3.0 sec) following the re-institution of flow. The steady state level to which perfusion pressure returned after the 15 second cessation of flow was set equal to 100. This value was obtained by

averaging the values of the variable over a 10 second period immediately following the return of perfusion pressure to a steady level. The values at all the digitized points of that particular response were then adjusted to conform to this new 0 to 100 scale.

The units of this scale (i.e. 0 to 100) can be thought of as being: (1) the percent of the peak change of the value of the variable for heart rate steps, and (2) the percent of return of the variables from their peak values to the post-step steady state values as a result of the re-institution of flow following a 15 second cessation of flow. This normalization of the magnitude of the responses allows us to compare their time courses.

iii. Grouping

Responses of coronary perfusion pressure, coronary sinus O_2 content and coronary sinus plasma K^+ concentration to either step increases in heart rate or 15 second cessations of coronary blood flow were grouped for averaging, convolution (see below), and statistical analyses as follows:

(a) Responses to increases in heart rate and responses to 15 second cessations of coronary blood flow were treated separately.

(b) For each preparation, the average response of the individually normalized responses (as described above) for each variable in response to either increases in HR or 15 second cessations of flow was obtained by averaging, point-by-point, the corresponding values from each of the individually normalized responses. This produced one data set per preparation, with all the responses in that data set obtained from the same preparation, for the same

maneuver. There were a total of 9 data sets of step increases in heart rate (involving 21 individual steps) from 9 dogs. There were a total of 6 data sets of 15 second cessations of flow (involving 16 individual steps) from 6 dogs. These data sets were used as the basic units for statistical analysis. The time course parameters (described below) were also obtained from these data sets. And, finally, the perfusion pressure components of the data sets were the functions used in the convolution procedure (described below).

(c) The overall average responses of coronary perfusion, coronary sinus O_2 content, and coronary sinus plasma K^+ concentration to either step increases in heart rate or 15 second cessations of flow were obtained by point-by-point averaging of the data sets, so that the responses from individual dogs were weighted equally.

iv. Time course parameters

Since neither perfusion pressure, O_2 content, nor K^+ time courses were well described by monoexponential curves, it was deemed inappropriate to quantify these time courses with the use of time constants (reciprocal of monoexponential rate constants). Instead, the following parameters were used to describe the time courses of the responses of the measured variables to either step increases in heart rate, or 15 second cessations of coronary blood flow.

The t_{10}, t_{50}, and t_{90} are the times after time zero at which the average set response of the variable in question has changed by 10, 50, or 90 percent of the final peak change for step increases in heart rate, and returned to the steady state level from the peak

change in response to a 15 second cessation of coronary blood flow.

The values for t_{10}, t_{50}, t_{90} were obtained by linear interpolation between adjacent points from the average set responses of coronary perfusion pressure, coronary sinus O_2 content, coronary sinus plasma K^+ concentration, and the convoluted functions described below.

v. Comparison of time courses

It is inappropriate to draw conclusions concerning the relative time courses of changes in myocardial interstitial fluid K^+ concentrations and coronary vascular resistance from a comparison of the time courses of coronary sinus plasma K^+ concentration and coronary perfusion pressure. This is because the coronary sinus plasma K^+ concentration reflects not only transient changes in myocardial interstitial fluid K^+ concentration, but also: (1) the delay introduced by the transport of K^+ across the capillary endothelium from interstitial fluid to blood, and (2) the delay and dispersion introduced by vascular transit of blood from the capillary to the site where coronary sinus blood samples were collected and later analyzed for plasma K^+ concentration. The effect of the capillary endothelium on interstitial fluid-blood K^+ exchange will not be dealt with on a quantitative basis in this thesis, though the possible implications of this effect will be discussed. But the problem of delay and dispersion introduced by vascular transit has been approached, utilizing techniques reported previously from this laboratory (Mohrman and Sparks, 1974; Belloni and Sparks, in press). The reader is directed to these studies for a detailed analysis, but the general approach

can be outlined as follows:

(a) The effects of transcoronary vascular transit were measured for each preparation by measuring the distribution of transcoronary transit times of a bolus intravascular tracer (indigo carmine dye) injected close arterially and detected in coronary sinus blood by the cuvette densitometer.

(b) The effects of vascular transit between the coronary capillaries and the coronary sinus sampling site were calculated by taking a constant percentage (91.1%) of the total transcoronary transit time. This percentage is based on a separate series of experiments performed by Belloni and Sparks (in press) involving a comparison of the total transcoronary vascular transit (as estimated from dye transits) and the pre-capillary transit (as estimated by the arrival of microspheres in tissue following their intra-arterial injection).

(c) We imposed the effects of vascular transit between the coronary capillaries and coronary sinus sampling site on the time course of the coronary vascular response to either a step increase in heart rate or a 15 second cessation of coronary blood flow by use of the convolution integral:

$$CVR^*(t) = \int_0^t CVR(T)\ h(t-T)\ dt$$

where CVR(t) = coronary vascular resistance (proportional to coronary perfusion pressure with constant flow), h(t) = the distribution of vascular transit times between coronary capillaries and the coronary sinus measuring site, and T = the dummy variable of integration.

Thus, CVR*(t) includes the time course of vascular resistance plus the estimated effects of vascular transit.

(d) CVR*(t) was compared to the time course of coronary sinus plasma K^+ concentration which reflects the time course of the changes in myocardial interstitial fluid K^+ concentration plus the effects of vascular transit.

If, then, the effects of vascular transit are equal in CVR*(t) and in the time course of coronary sinus plasma K^+ concentration, differences in their time courses reflect differences in the time courses of coronary vascular resistance and myocardial interstitial fluid K^+ concentration.

This method involves a number of assumptions. The dye curves represent an attempt to measure the frequency distribution of transit times through the coronary vascular bed. We have assumed that the distribution of transit times is both unaffected by the number of dye particles introduced (assumption of linearity), and is time-invariant (assumption of stationarity). This second assumption is the basis for the constant flow used in this study, since the rate of blood flow through the vascular bed greatly influences the transit time distribution. In addition, we have assumed that the dye particles have transit times which are representative of the transit times of blood through the coronary vascular bed. Since the dye particles are believed to attach to plasma proteins they are probably confined to the intravascular space and, so, are good tracers for blood flow. Finally, we have assumed that there is no recirculation of dye particles. Although this is not strictly true, the dilution

of the particles in the total blood volume is sufficient to practically eliminate any problem in this regard.

Since a certain fraction of this measured total transcoronary transit time is then used to correct for the effects of delay and dispersion of the end-capillary plasma $[K^+]$, an implicit assumption in this entire analysis is that the effects of vascular transit are identical for both the dye particles and K^+. Thus we have assumed that the plasma $[K^+]$ changes linearly when blood samples of unequal concentration are mixed during vascular transit. In addition we have assumed that the plasma $[K^+]$ changes occurred simultaneously in all capillaries and that the mass of plasma K^+ is conserved during vascular transit. Moreover, we have assumed that neither the experimental manipulations themselves (i.e. increased heart rate or 15 second cessation of flow) nor the changes in plasma $[K^+]$ elicited by those maneuvers has had any significant effect on the distribution of intravascular times. Belloni (1975) has shown that the distribution of intravascular transit times for a given coronary blood flow were not significantly different when heart rate was increased from 146 to 164 beats per minute. These assumptions and their possible implications on the interpretation of experimental results will be discussed more fully in chapter 4.

vi. Statistical analyses

All statistical tests performed on the time course parameters defined above used Student's t distribution for paired samples. Statistical analyses were performed using an Amdahl 470V/6 computer and the Michigan Interactive Data Analysis System.

CHAPTER III

RESULTS

Part 1: Characterization of the Vasodilator Activity of K^+ in the Coronary Vascular Bed

Figure 1 shows the results of our initial series of experiments in which isosmotic KCl, in amounts ranging from 12-140 micromoles per minute was infused into the circumflex artery. In each of the 4 dogs studied, K^+ administration resulted in a dose-dependent decrease in coronary vascular resistance, averaging from 22.75 ± 7.47 (mean ± S.E.M) to 39.92 ± 6.12 percent of control values. Despite the fairly large degree of variability in the magnitude of the vascular response for any particular amount of K^+ infused, these preliminary data satisfied an important, although not sufficient, criterion for the involvement of K^+ in the control of coronary vascular resistance: i.e. that K^+ is a vasodilator in the coronary vascular bed. It thus seemed reasonable for us to extend our investigation concerning the role of K^+ in the control of coronary vascular resistance.

The resistance changes associated with intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl observed in 18 dogs are shown in figure 2. Myers and Honig (1968) have demonstrated that the magnitude of vasodilation in response to certain vasomotor stimuli is a linear function of the initial resistance prior to stimulation. As shown in figure 2 the magnitude of K^+-induced vasodilation

Figure 1

Effects of intra-circumflex artery infusions of isosmotic KCl on coronary vascular resistance. Data from each dog are presented separately. N = 4 dogs.

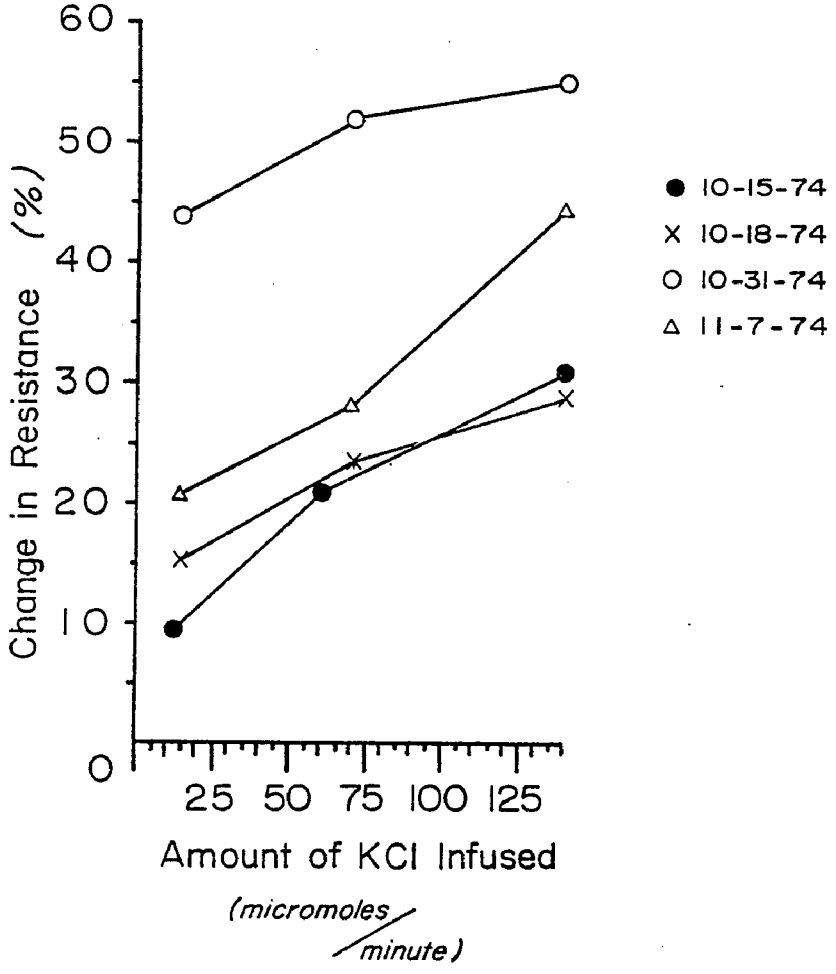
60
50
40
30
20
10
0
Change in Resistance (%)
25 50 75 100 125
Amount of KCl Infused
(micromoles / minute)
10-15-74
10-18-74
10-31-74
11-7-74

Figure 1

Figure 2

Effects of initial resistance on the magnitude of K^+-induced vasodilation produced by intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl. The best fit regression line is shown. Both the slope and the y-intercept are significantly different from zero ($p < .0000$). Numbers in parentheses refer to the individual responses within the 0.5 PRU interval of initial resistance. 177 responses were observed in 18 dogs. Vertical and horizontal bars indicate one S.E.M.

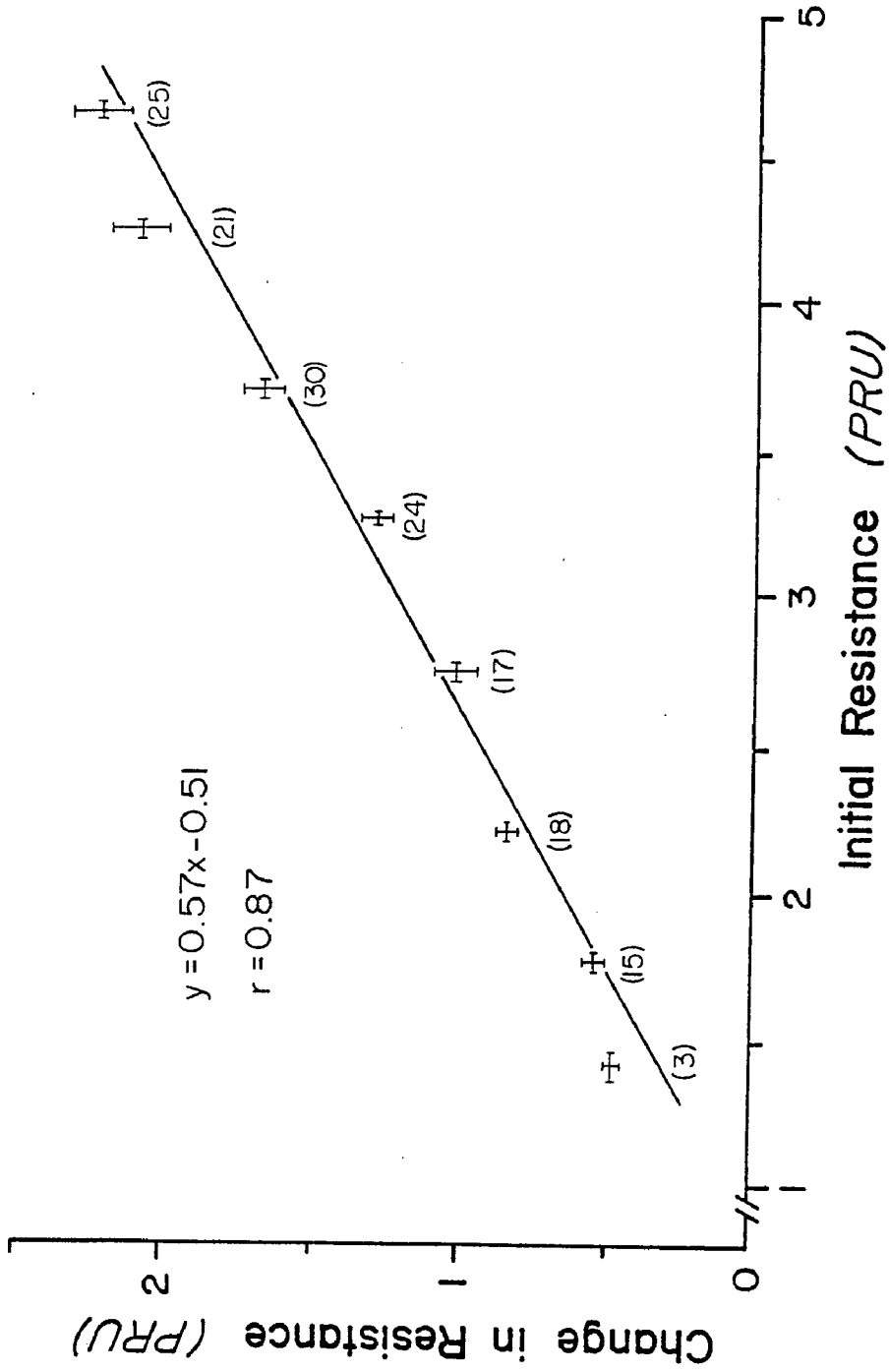
Change in Resistance (PRU)
Initial Resistance (PRU)
y = 0.57x - 0.51
r = 0.87
(3)
(15)
(18)
(17)
(24)
(30)
(21)
(25)
0
1
2
3
4
5

Figure 2

is linearly related to the initial resistance of the vascular bed ($y = 0.57 x - 0.51$), with a correlation coefficient of 0.87. This amount of K^+ produces a decrease in coronary vascular resistance of 34 to 48 percent, depending on the initial resistance.

To summarize this section, these data indicate that K^+ is a significant vasodilator in the coronary vascular bed.

Part 2: The Mechanism of K^+-induced Vasodilation in the Coronary Vascular Bed

1. Metabolic:

We tested the hypothesis that K^+-induced vasodilation was the result of a primary increase in the metabolic activity of the heart, resulting in a secondary, metabolically-induced increase in coronary blood flow.

The first two panels of figure 3 depict the changes in mean arterial pressure (MAP) and heart rate (HR) produced by 40 micromole bolus injections of isosmotic KCl into the circumflex artery of 7 dogs at free flow. Data from individual dogs are summarized in table 1. Paired-t analyses indicate that K^+ administration produces a small (4 mmHg), though statistically significant, decrease in MAP, and no change in HR.

Since it would not be possible to measure a transient in myocardial O_2 consumption directly with the free flow circumflex artery preparation, we examined the effects of K^+ administration on myocardial O_2 consumption using the constant flow common coronary artery preparation. The third panel in figure 3 depicts the changes in coronary sinus O_2 content (which reflect changes in myocardial O_2 consumption

Figure 3

Effects of intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl on mean arterial pressure and heart rate (panels 1 and 2). 119 responses observed in 7 dogs. Panel 3 shows effects of K^+ injections on coronary sinus O_2 content in constant flow common coronary artery preparation. 15 responses observed in 5 dogs. Vertical bars indicate one S.E.M. Asterisk (*) indicates $p < .05$ with paired-t analyses.

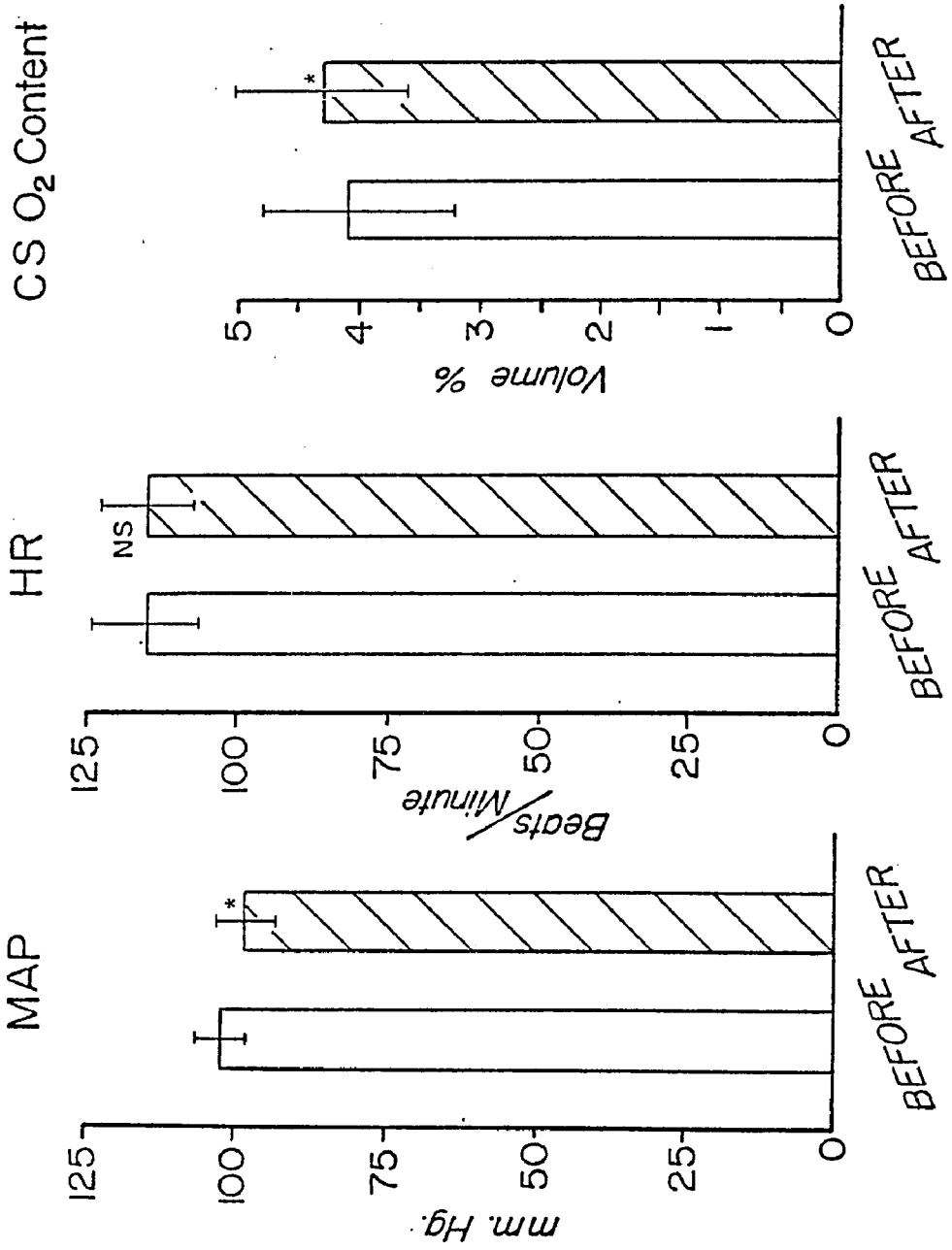
MAP
mm. Hg.
125
100
75
50
25
0
*
BEFORE
AFTER
HR
Beats/Minute
125
100
75
50
25
0
NS
BEFORE
AFTER
C S O_2 Content
Volume %
5
4
3
2
1
0
*
BEFORE
AFTER

Figure 3

TABLE 1 EFFECTS OF K^+ ON MAP, HR, AND CSO_2 CONTENT

Dog	# Obs.	MAP (mm Hg) Before	MAP (mm Hg) After	HR (beats/min) Before	HR (beats/min) After	Dog	# Obs.	CSO_2 Content (vol %) Before	CSO_2 Content (vol %) After
12-5-74	11	106.67	103.13	110	110	2-3-76	3	5.66	5.91
12-6-74	7	109.10	104.29	119	116	2-5-76	3	3.54	3.83
12-13-74	22	85.83	79.78	101	99	5-26-76	3	3.69	3.90
12-14-74	23	106.98	102.30	105	105	5-26-76	3	5.69	5.85
12-16-74	22	98.77	95.75	85	93				
1-3-75	23	87.49	83.36	129	130				
1-9-75	11	116.64	113.64	155	155				
MEAN		101.64	97.46	114.86	115.43			4.10	4.30
S.E.M.		4.34	4.57	8.50	8.00			0.72	0.73
p		<.005		>.50				<.01	

at constant flow) produced by 40 micromole bolus injections of isotonic KCl into the left common coronary artery of 4 dogs. Data from individual dogs are summarized in table 1. Paired-t analysis indicates that K^+ administration produces a small (0.2 vol%), though statistically significant, increase in coronary sinus O_2 content, reflecting a small decrease in myocardial O_2 consumption.

To summarize this section, these results indicate that K^+-induced vasodilation is not the result of a primary increase in the metabolic activity of the heart.

2. Neural:

It has been postulated that the mechanism of K^+-induced vasodilation may be the result of an interaction between K^+ and tissue neural elements. Figure 4 shows the effects of time alone on the magnitude of K^+-induced vasodilation. The vascular responses obtained during the first hour of each experiment (corresponding to the "control" period in the blocker experiments that follow) were compared to the vascular responses obtained during the succeeding 2-3 hours of each experiment (corresponding to the responses obtained in the presence of blocker in the series that follow) in 7 dogs. Statistical analyses indicate that the magnitude of K^+-induced vasodilation was somewhat attenuated (21 percent at the highest initial resistance) at the higher levels of initial resistance.

We tested the hypothesis that K^+-induced vasodilation was the result of an increased release of norepinephrine from sympathetic nerve terminals in the heart, with subsequent activation of coronary

Figure 4

Effects of time on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 192 individual responses were observed in 7 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

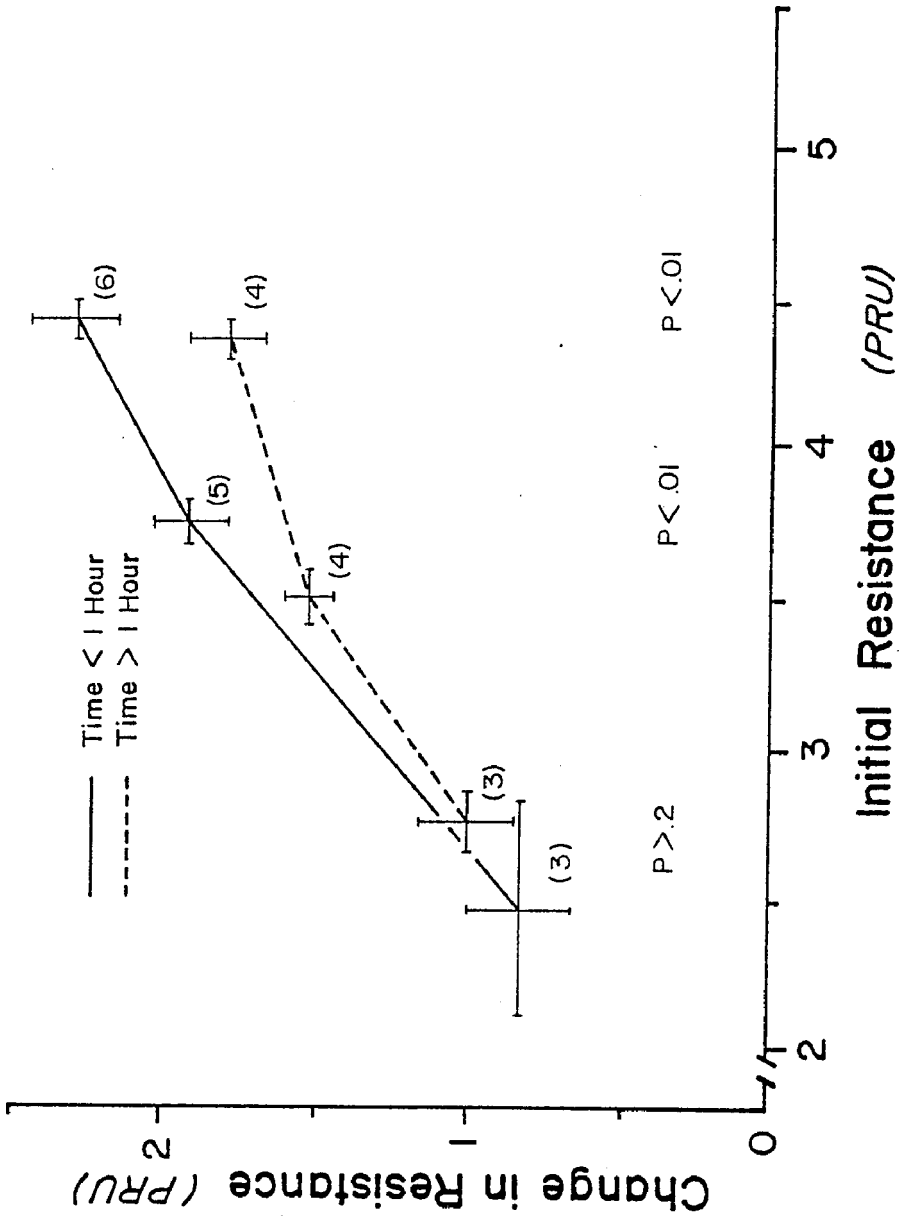
Change in Resistance (PRU)
Initial Resistance (PRU)
Time < 1 Hour
Time > 1 Hour
(6)
(5)
(4)
(4)
(3)
(3)
P>.2
P<.01
P<.01
0
1
2
3
4
5

Figure 4

vascular beta receptors. Figure 5 depicts the effects of propranolol on the magnitude of K^+-induced vasodilation in 10 dogs. Statistical analyses indicate that the magnitude of K^+-induced vasodilation was not diminished following the administration of propranolol, although this dose of propranolol was found to almost totally abolish isoproterenol-induced vasodilation (figure 6).

We next attempted to ascertain whether K^+-induced vasodilation was the result of an increased release of acetylcholine from parasympathetic nerve terminals in the heart, with subsequent activation of Ach-specific receptors on coronary vascular smooth muscle. Figure 7 depicts the effects of atropine on the magnitude of K^+-induced vasodilation in 8 dogs. Statistical analyses indicate that K^+-induced vasodilation was not diminished following the administration of atropine, although this dose of atropine was found to almost completely block acetylcholine-induced vasodilation (figure 8).

We next tested the hypothesis that K^+-induced vasodilation was the result of a transient withdrawal of alpha-adrenergic mediated coronary vascular smooth muscle tone. We compared the magnitude of K^+-induced vasodilation before and after the administration of phentolamine in 6 dogs. The results are shown in figure 9. Statistical analyses indicate that K^+-induced vasodilation was not significantly diminished after the administration of phentolamine.

To summarize this section, these results indicate that K^+-induced vasodilation is not the result of either an increased local release of norepinephrine or acetylcholine, or a withdrawal of alpha-adrenergic mediated vascular tone.

Figure 5

Effects of propranolol on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 145 individual responses were observed in 10 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

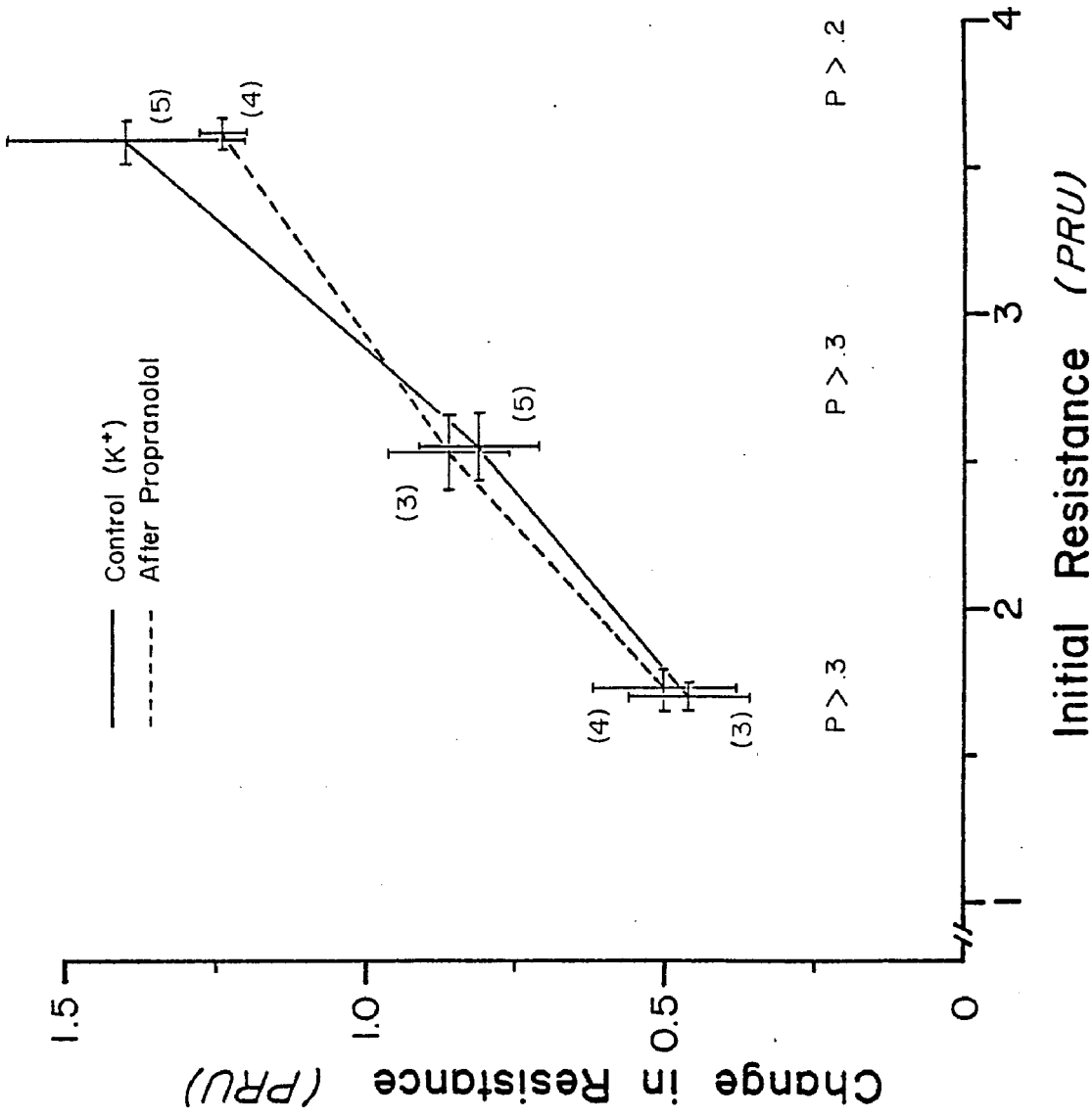
Change in Resistance (PRU)
1.5
1.0
0.5
0
1
2
3
4
Initial Resistance (PRU)
Control (K+)
After Propranolol
(5)
(4)
(3)
(5)
(4)
(3)
P > .3
P > .3
P > .2

Figure 5

Figure 6

Effects of propranolol on the magnitude of isoproterenol-induced vasodilation (0.1 micrograms). Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 55 individual responses were obtained from 10 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

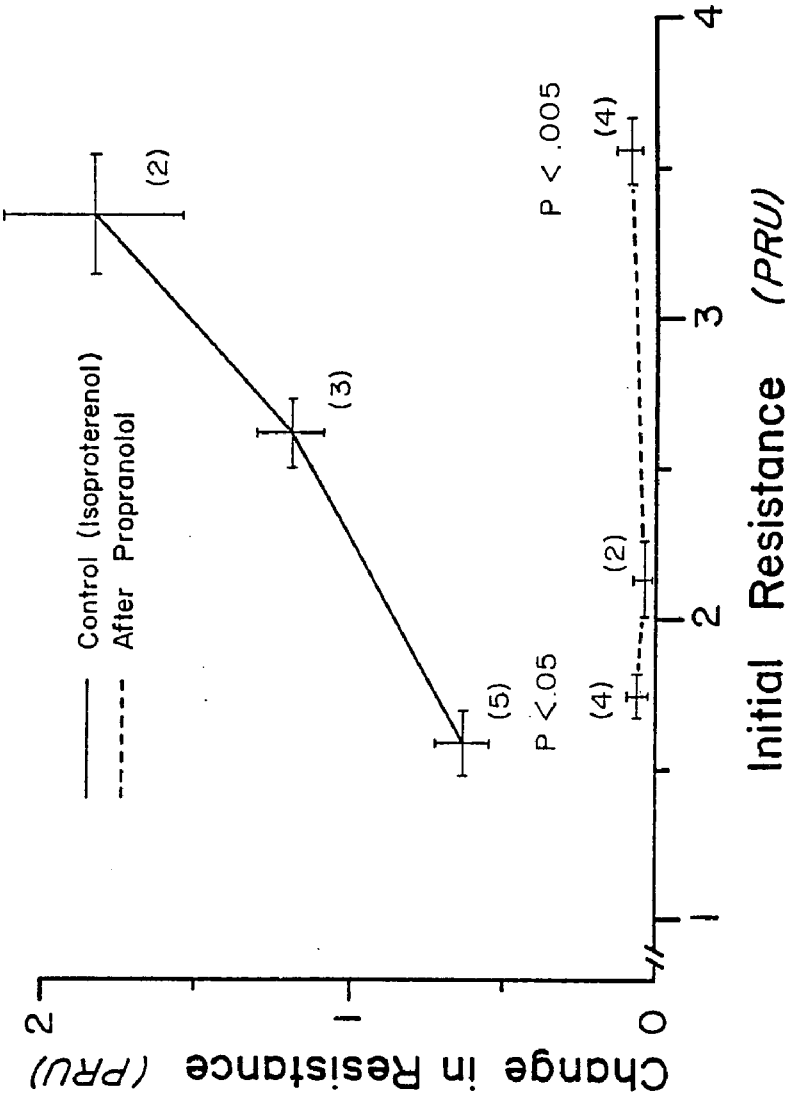
Change in Resistance (PRU)
Initial Resistance (PRU)
Control (Isoproterenol)
After Propranolol
P < .05
P < .005
(2)
(3)
(5)
(4)
(2)
(4)
0
1
2
3
4

Figure 6

Figure 7

Effects of atropine on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 170 individual responses were observed in 8 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

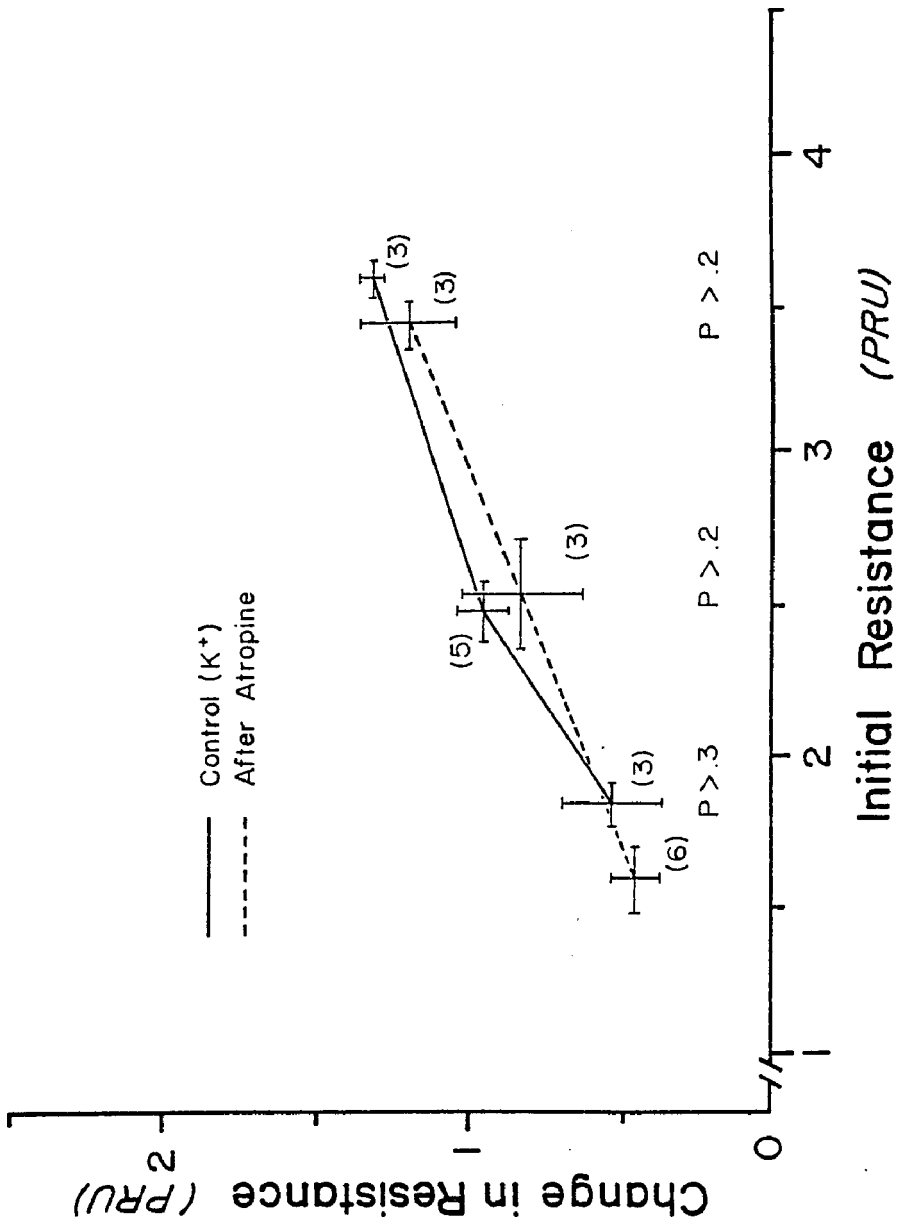
Change in Resistance (PRU)
Initial Resistance (PRU)
Control (K+)
After Atropine
(3)
(3)
(5)
(3)
(3)
(6)
P > .3
P > .2
P > .2
0
1
2
3
4

Figure 7

Figure 8

Effects of atropine on the magnitude of acetylcholine-induced vasodilation (0.1 micrograms). Numbers in parentheses refer to the number of dogs from which responses within the 0.5 or 1.0 PRU intervals of initial resistance were collected. In all, 46 individual responses were observed in 8 dogs. P values obtained by Student's t test analyses of differences in means of corresponding control and experimental responses for each interval of initial resistance indicated. Vertical and horizontal bars indicate one S.E.M.

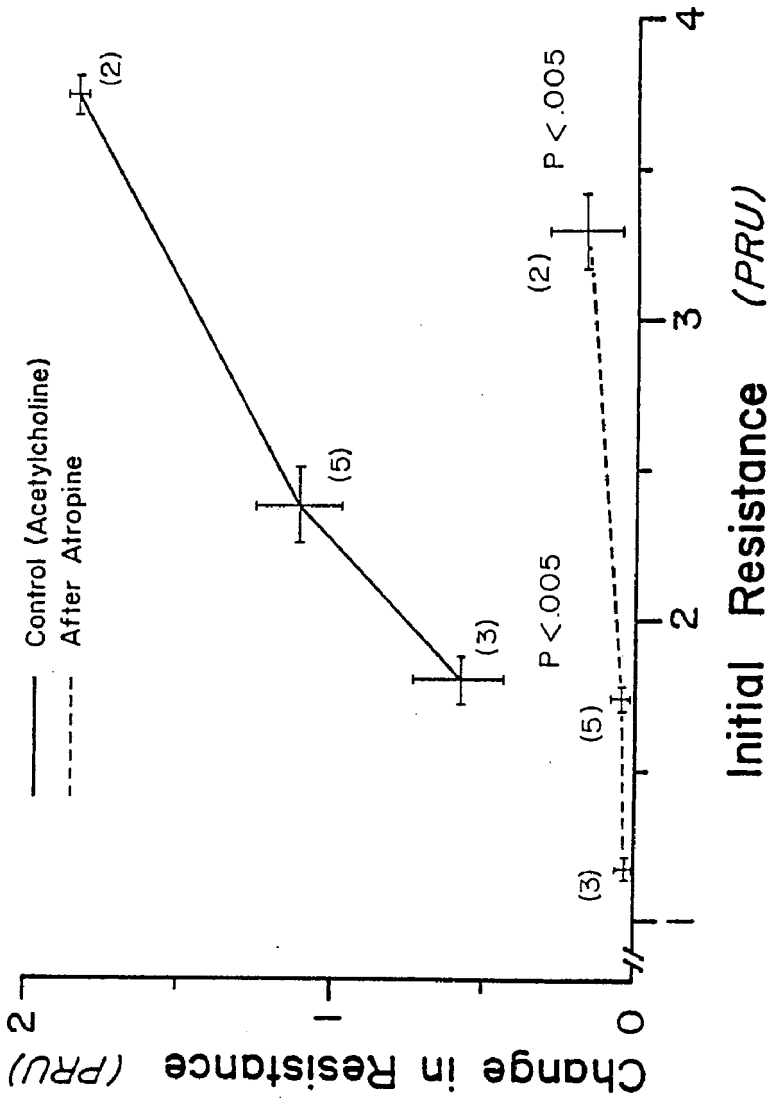
Change in Resistance (PRU)
2
1
0
Control (Acetylcholine)
After Atropine
(2)
(5)
(3)
P<.005
(3)
(5)
(2)
P<.005
1
2
3
4
Initial Resistance (PRU)

Figure 8

Figure 9

Effects of phentolamine on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 145 individual responses were observed in 5 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

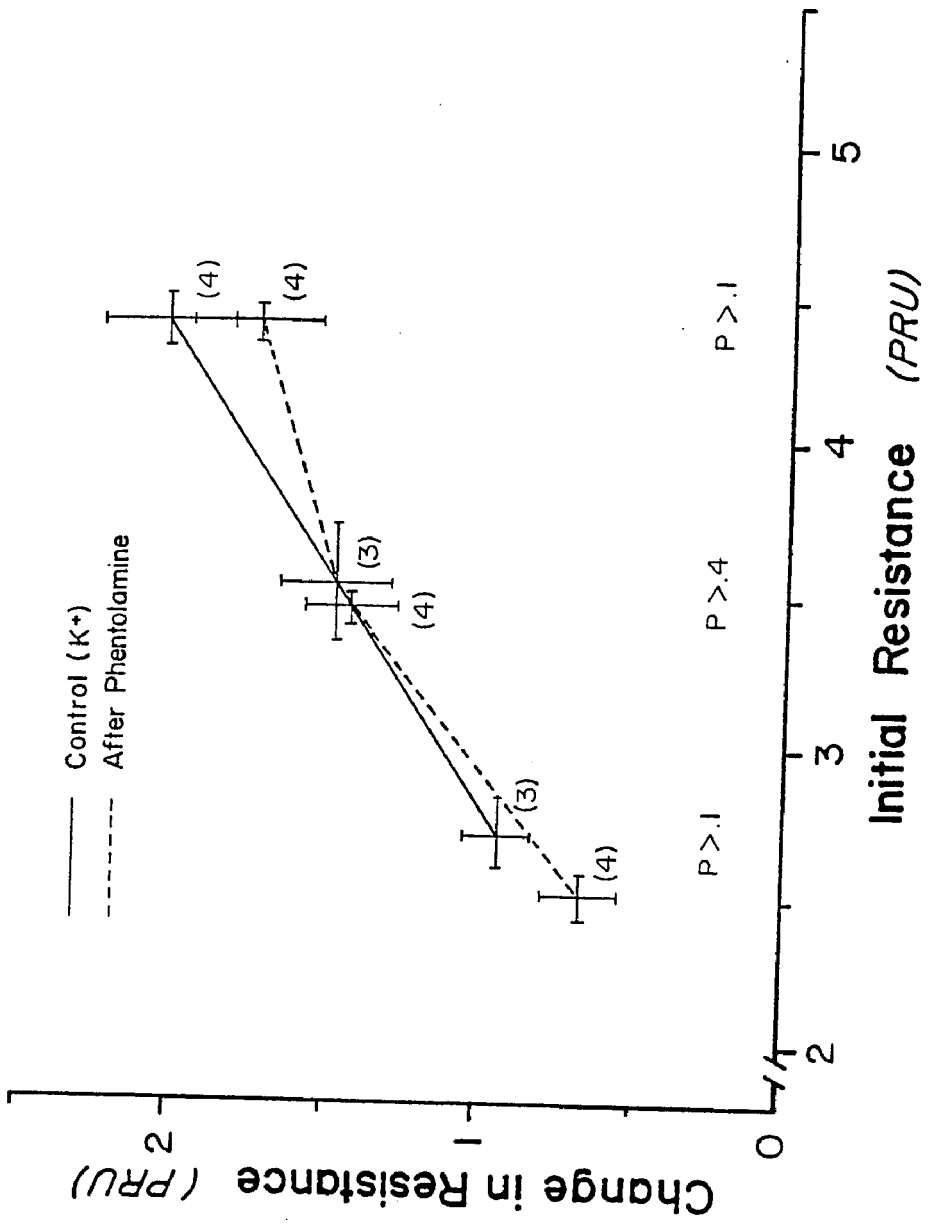
Change in Resistance (PRU)
Initial Resistance (PRU)
Control (K+)
After Phentolamine
(4)
(4)
(3)
(4)
(3)
(4)
P>.1
P>.4
P>.1
0
1
2
3
4
5

Figure 9

3. Direct effect on vascular smooth muscle:

Having previously excluded a metabolic mechanism, and having eliminated the classic neural mechanisms, we turned our attention to a possible direct effect of K^+ on coronary vascular smooth muscle. In a series of 8 dogs we tested the hypothesis that K^+-induced vasodilation was the result of an activation of the electrogenic Na^+-K^+ exchange pump located in the walls of coronary vascular smooth muscle. We compared the magnitude of K^+-induced vasodilation before and after the administration of a solution containing ouabain and lidocaine. The results are shown in figure 10. Statistical analyses indicate that K^+-induced vasodilation has been attenuated over the entire range of initial resistances observed (54 percent at the highest initial resistance).

To determine whether this inhibition was due primarily to the effects of ouabain, we compared the magnitude of K^+-induced vasodilation before and after the administration of lidocaine alone in 4 dogs. As shown in figure 11, lidocaine alone did have a tendency to slightly diminish the magnitude of K^+-induced vasodilation, although this reduction only approached statistical significance at the highest initial resistance. Since statistical analyses indicated that the control vascular responses for the ouabain-lidocaine, and lidocaine alone series were not significantly different (figure 12), it was possible to make a direct comparison of the magnitude of K^+-induced vasodilation after ouabain-lidocaine, and after lidocaine alone. These results are shown in figure 13. Statistical analyses indicate that K^+-induced vasodilation after ouabain-lidocaine is

Figure 10

Effects of ouabain-lidocaine on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 188 individual responses were observed in 8 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

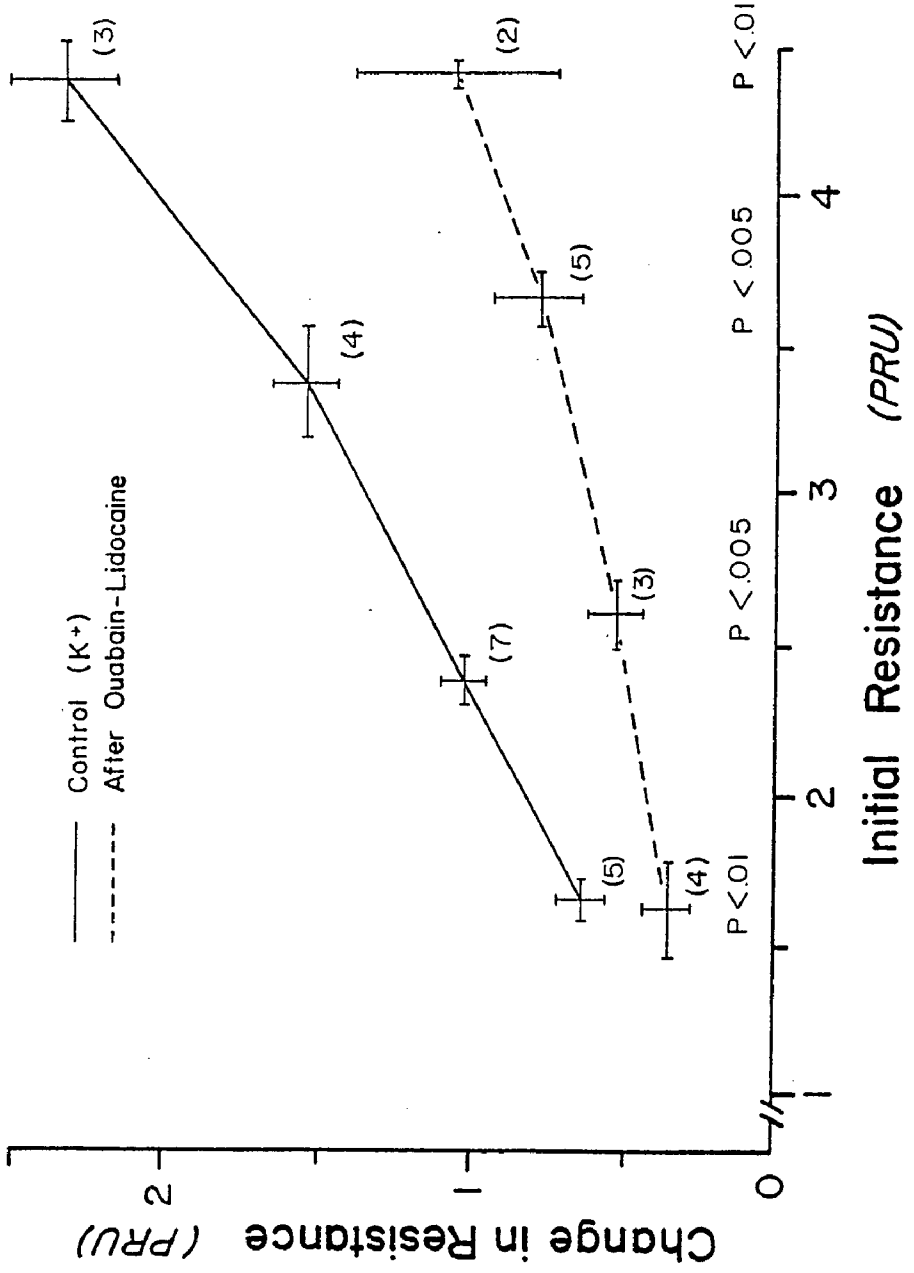
Change in Resistance (PRU)
Initial Resistance (PRU)
Control (K+)
After Ouabain-Lidocaine
(3)
(4)
(7)
(5)
(2)
(5)
(3)
(4)
P<.01
P<.005
P<.005
P<.01
0
1
2
3
4

Figure 10

Figure 11

Effect of lidocaine alone on the magnitude of K^+-induced vasodilation. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 127 individual responses were observed in 4 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

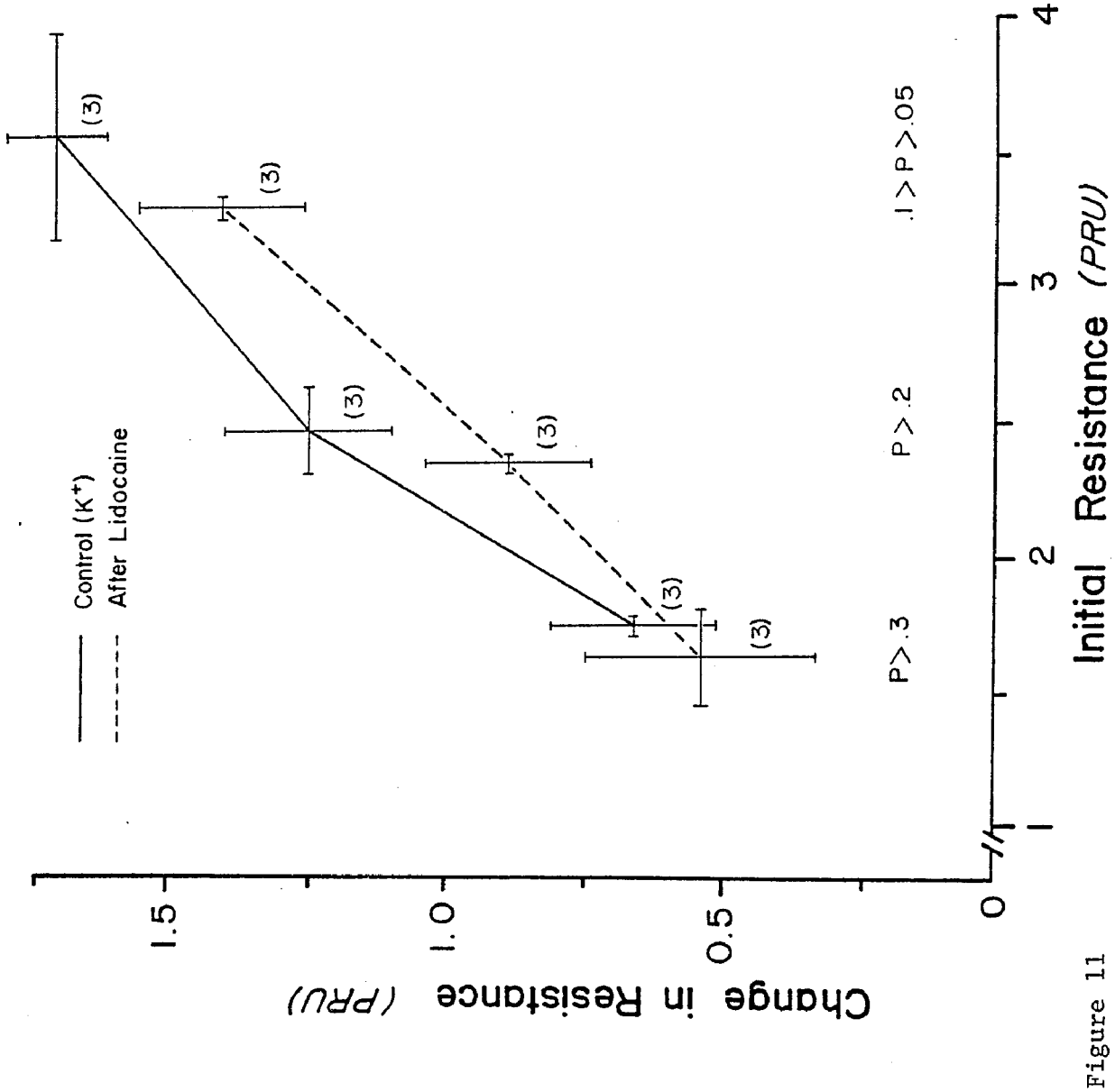
Change in Resistance (PRU)
1.5
1.0
0.5
0
1
2
3
4
Initial Resistance (PRU)
Control (K+)
After Lidocaine
(3)
(3)
(3)
(3)
(3)
(3)
P>.3
P>.2
.1>P>.05

Figure 11

Figure 12

Comparison of control (pre-block) vascular responses to K^+ for lidocaine alone, and ouabain-lidocaine series. Numbers in parentheses refer to the number of dogs from which responses within the 1.0 PRU intervals of initial resistance were collected. In all, 198 individual responses were observed in 12 dogs. P values obtained by Student's t test analyses of differences in means of control responses for each series for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

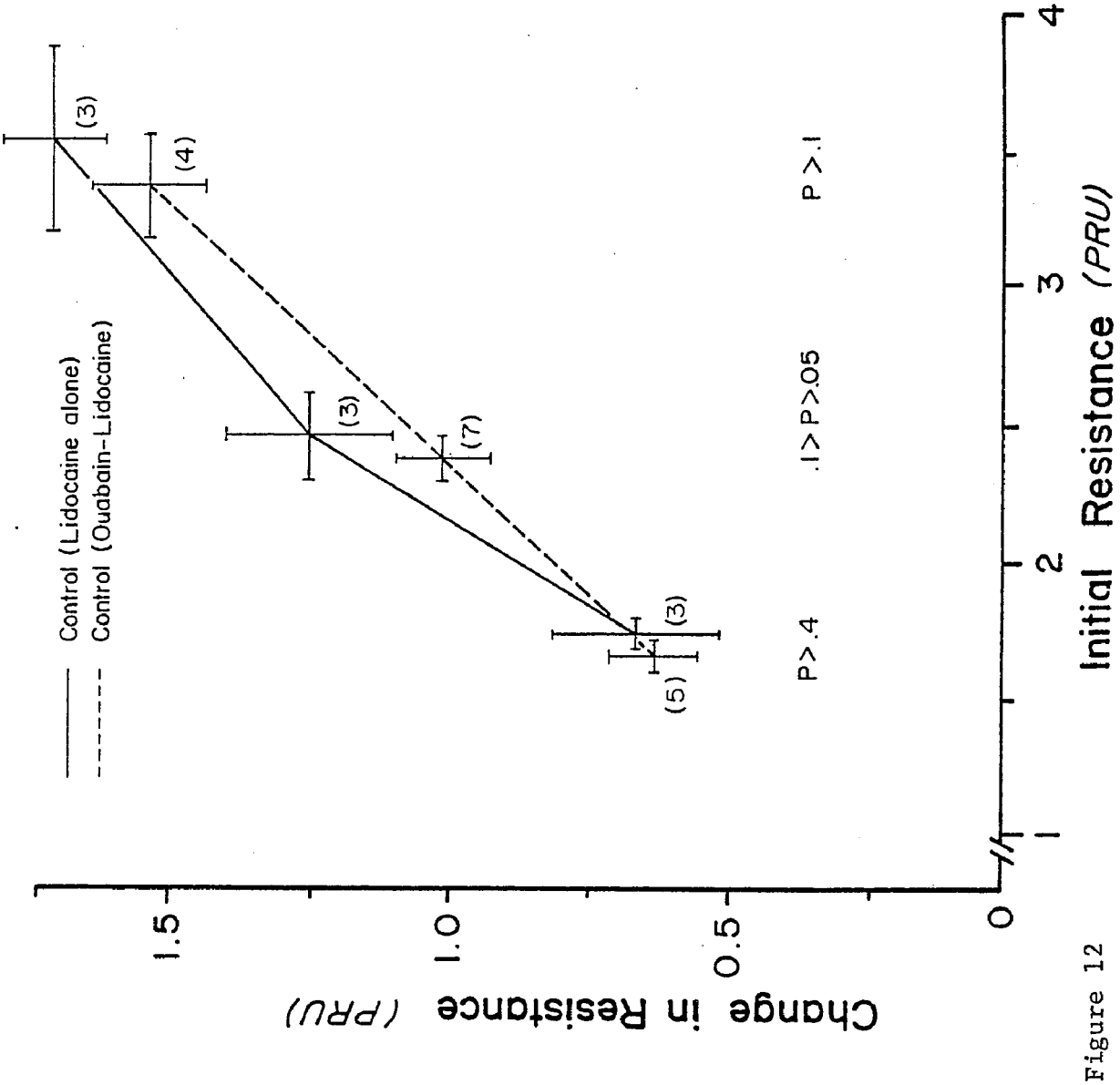
Control (Lidocaine alone)
Control (Ouabain-Lidocaine)
(3)
(4)
(3)
(7)
(3)
(5)
P>.4
.1>P>.05
P>.1
Change in Resistance (PRU)
Initial Resistance (PRU)
0
0.5
1.0
1.5
1
2
3
4

Figure 12

Figure 13

Comparison of K^+-induced vasodilation after lidocaine alone, and after ouabain-lidocaine. Numbers in parentheses refer to the number of dogs from which the responses within the 1.0 PRU intervals of initial resistance were collected. In all, 117 individual responses were observed in 12 dogs. P values obtained by Student's t test analyses of differences in means of each group for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

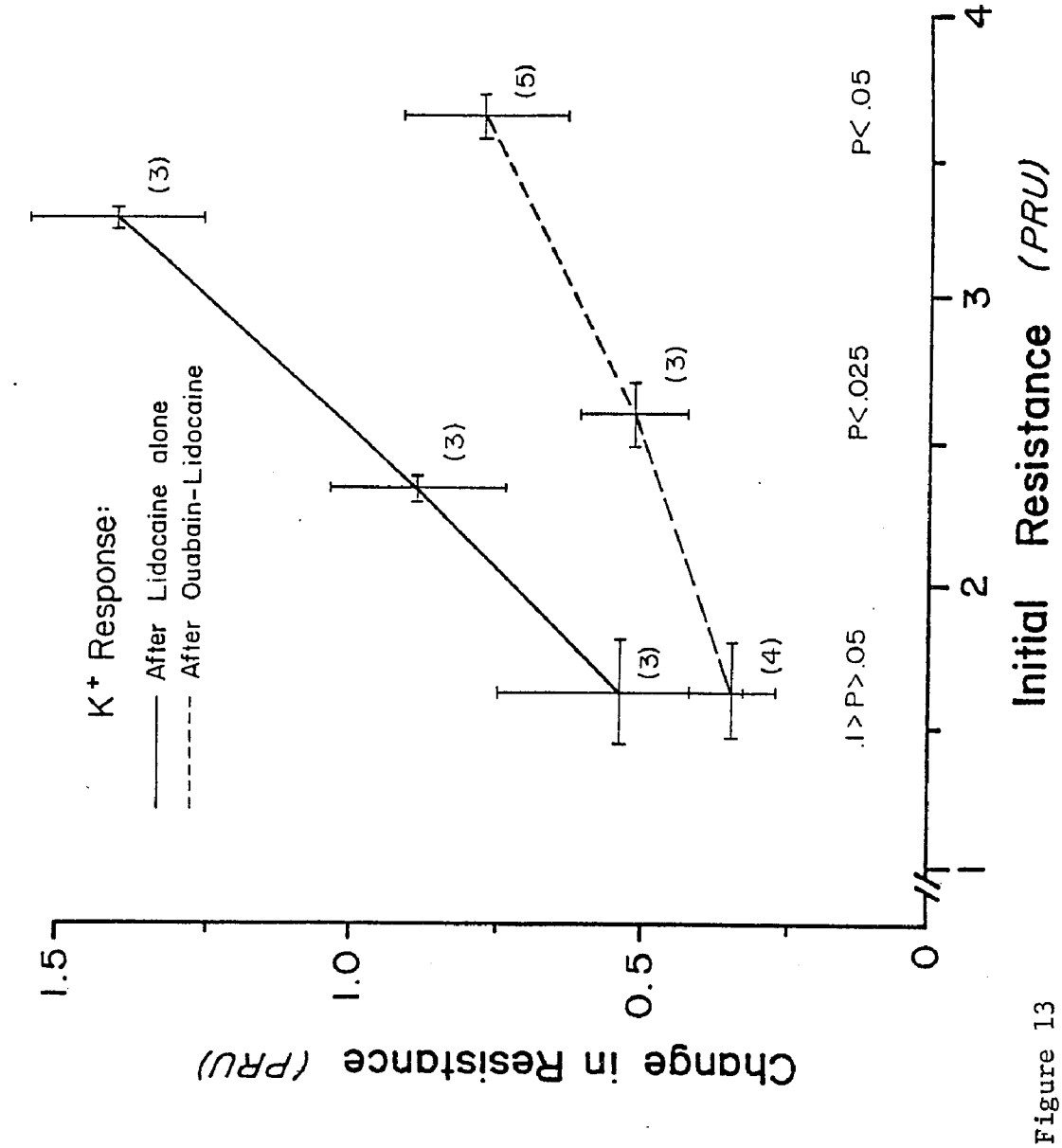
Change in Resistance (PRU)
1.5
1.0
0.5
0
1
2
3
4
Initial Resistance (PRU)
K+ Response:
After Lidocaine alone
After Ouabain-Lidocaine
(3)
(3)
(3)
(4)
(5)
(3)
.1 > P > .05
P < .025
P < .05

Figure 13

significantly attenuated for both the middle and high initial resistance values, and approaches statistical significance at the lowest initial resistance.

And finally we examined the possibility that the blocking action of ouabain was the result of a non-specific inhibition of coronary vascular smooth muscle activity. Figure 14 shows the effects of ouabain-lidocaine on acetylcholine-induced vasodilation in 6 dogs. Statistical analyses indicate that the magnitude of acetylcholine-induced vasodilation is not significantly diminished by ouabain-lidocaine.

To summarize this section, these data indicate that K^+-induced vasodilation is significantly blocked by the administration of ouabain-lidocaine. This inhibitory action is independent of the effects of lidocaine, and is not the result of a non-specific depression of coronary vascular smooth muscle vasodilator activity. These data are consistent with the hypothesis that the mechanism of K^+-induced vasodilation is the result of an activation of the electrogenic Na^+-K^+ exchange pump located in the wall of coronary vascular smooth muscle.

Part 3: Comparison of the Dynamics of Interstitial Fluid K^+ Concentration and Coronary Vascular Resistance

1. Step increases in heart rate:

The pre-stimulation and peak change data from all the sets of heart rate steps are presented in table 2. The values shown for coronary vascular resistance, coronary sinus O_2 content, myocardial O_2 consumption, and coronary sinus plasma K^+ concentration are the average of the values immediately preceding each individual heart rate step

Figure 14

Effects of ouabain-lidocaine on the magnitude of acetylcholine induced vasodilation. Numbers in parentheses refer to the number of dogs from which the responses within the 0.5 or 1.0 PRU intervals of initial resistance were collected. In all, 84 individual responses were observed in 6 dogs. P values obtained by Student's t test analyses of differences in means of control and experimental responses for each interval of initial resistance. Vertical and horizontal bars indicate one S.E.M.

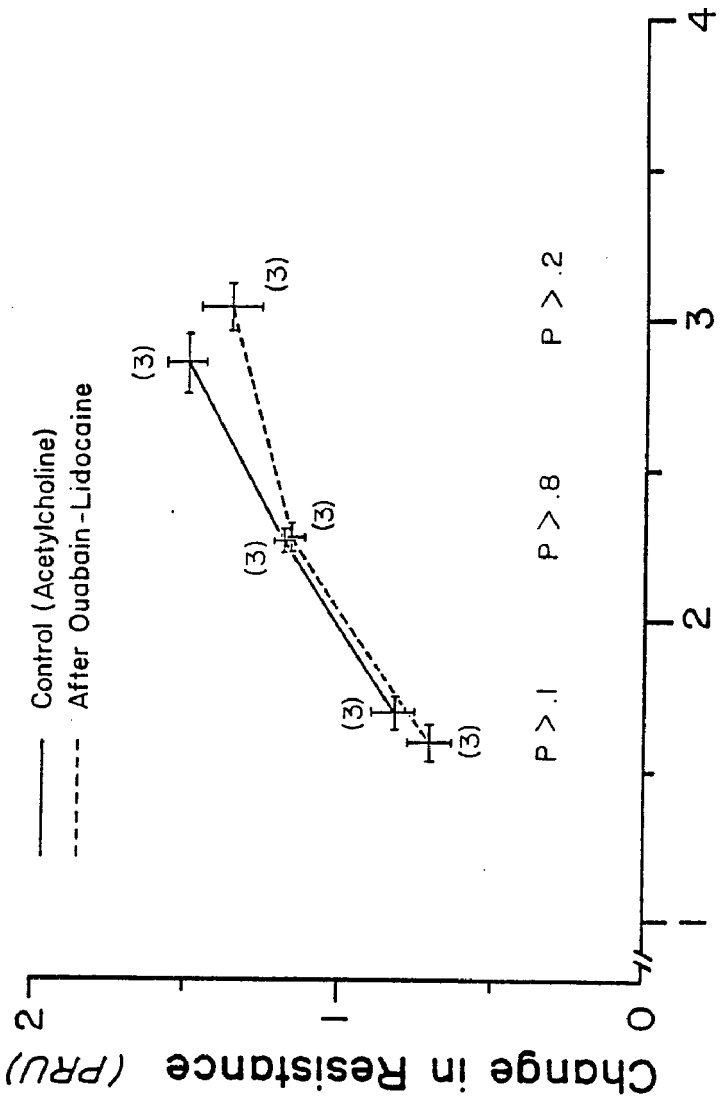
Change in Resistance (PRU)
2
1
0
Control (Acetylcholine)
After Ouabain-Lidocaine
(3)
(3)
(3)
(3)
(3)
P > .1
P > .8
P > .2
1
2
3
4
Initial Resistance (PRU)

Figure 14

TABLE 2 STEADY STATE AND TRANSIENT DATA: HEART RATE INCREASE

Dog	Data Set	Heart Rate (beats/min)	Coronary Blood Flow (ml/min/100 g)	Coronary Vascular Resistance (PRU_{100})	Coronary Sinus O_2 Content (vol %)	Myocardial O_2 Consumption (ml/min/100 g)	Coronary Sinus Plasma K^+ Concentration (mEq/L)
Baseline Values Before Step Increases in Heart Rate							
2-3-76	1	90	77.24	1.51	8.87	3.96	4.65
2-5-76	2	100	80.50	1.75	6.41	9.05	4.00
2-24-76	3	110	88.24	1.11	6.63	8.62	3.99
3-5-76	4	160	84.35	1.32	3.84	10.02	4.98
3-9-76	5	80	74.19	1.73	3.07	8.11	3.90
3-10-76	6	160	89.51	1.40	4.01	11.58	3.50
3-12-76	7	160	74.37	1.33	3.67	8.93	4.36
3-30-76	8	105	100.65	1.32	5.42	7.03	3.14
4-1-76	9	160	105.95	1.00	8.43	12.25	4.28
Mean		125	86.11	1.40	5.54	8.84	4.09
± S.E.M.		11.43	3.75	0.08	0.73	0.82	0.19
Peak Changes After Step Increases in Heart Rate							
2-3-76	1	200	77.24	1.24	3.96	7.75	5.12
2-5-76	2	190	80.50	0.74	1.73	12.82	4.79
2-24-76	3	200	88.24	0.17	4.61	10.40	4.47
3-5-76	4	220	84.35	0.73	1.72	11.81	5.30
3-9-76	5	150	74.19	0.61	0.50	10.02	4.82
3-10-76	6	210	89.51	1.13	3.32	12.21	3.90
3-12-76	7	220	74.37	0.93	1.95	8.93	4.83
3-20-76	8	190	100.65	0.71	2.63	9.83	3.72
4-1-76	9	220	105.95	0.90	6.59	14.20	4.64
Mean		200	86.11	0.89	3.00	11.03	4.62
± S.E.M.		7.45	3.75	0.07	0.62	0.64	0.17

in that data set, as well as the peak changes in those values produced by the step increase in heart rate.

Some typical responses of coronary sinus O_2 content, coronary vascular resistance (CVR), and coronary sinus plasma K^+ concentration ($CS[K^+]$) to a step increase in heart rate are shown in figure 15. In general, when heart rate was increased, CVR gradually fell to a new steady state level. Coronary sinus O_2 content remained unchanged for several seconds after the heart rate increase and then gradually fell to a new steady state value. $CS[K^+]$ also remained unchanged for several seconds following the heart rate increase, and then gradually increased to a peak value before returning to control level during the period of increased heart rate. When data from the 9 sets of step increases in heart rate were grouped, the average increase in heart rate was 75.0 ± 6.56 (mean ± S.E.M.) beats per minute. This resulted in a 0.51 ± 0.12 PRU_{100} decrease in CVR, a 2.19 ± 0.36 ml O_2/min/100g increase in myocardial O_2 consumption, and a 0.53 ± 0.07 mEq/L increase in $CS[K^+]$. Coronary blood flow averaged 86.11 ± 3.75 ml/min/100g.

Figure 16 depicts the average changes in $CS[K^+]$ produced by step increases in heart rate. The ordinate of this graph has the units: percentage of peak change. Following a step increase in heart rate, $CS[K^+]$ increased in all 9 dogs studied. But in only 3 dogs was this increase sustained throughout the period of increased heart rate. In the remaining 6 dogs $CS[K^+]$ returned to pre-stimulation levels even while the increase in heart rate was maintained. Although these responses are clearly separable in terms of the steady state changes

Figure 15

Some typical responses of coronary sinus O_2 content, coronary vascular resistance, and coronary sinus plasma K^+ concentration to a step increase in heart rate. Coronary blood flow was held constant at 89.51 ml/min/100g. The heart rate step size was 60 beats/min.

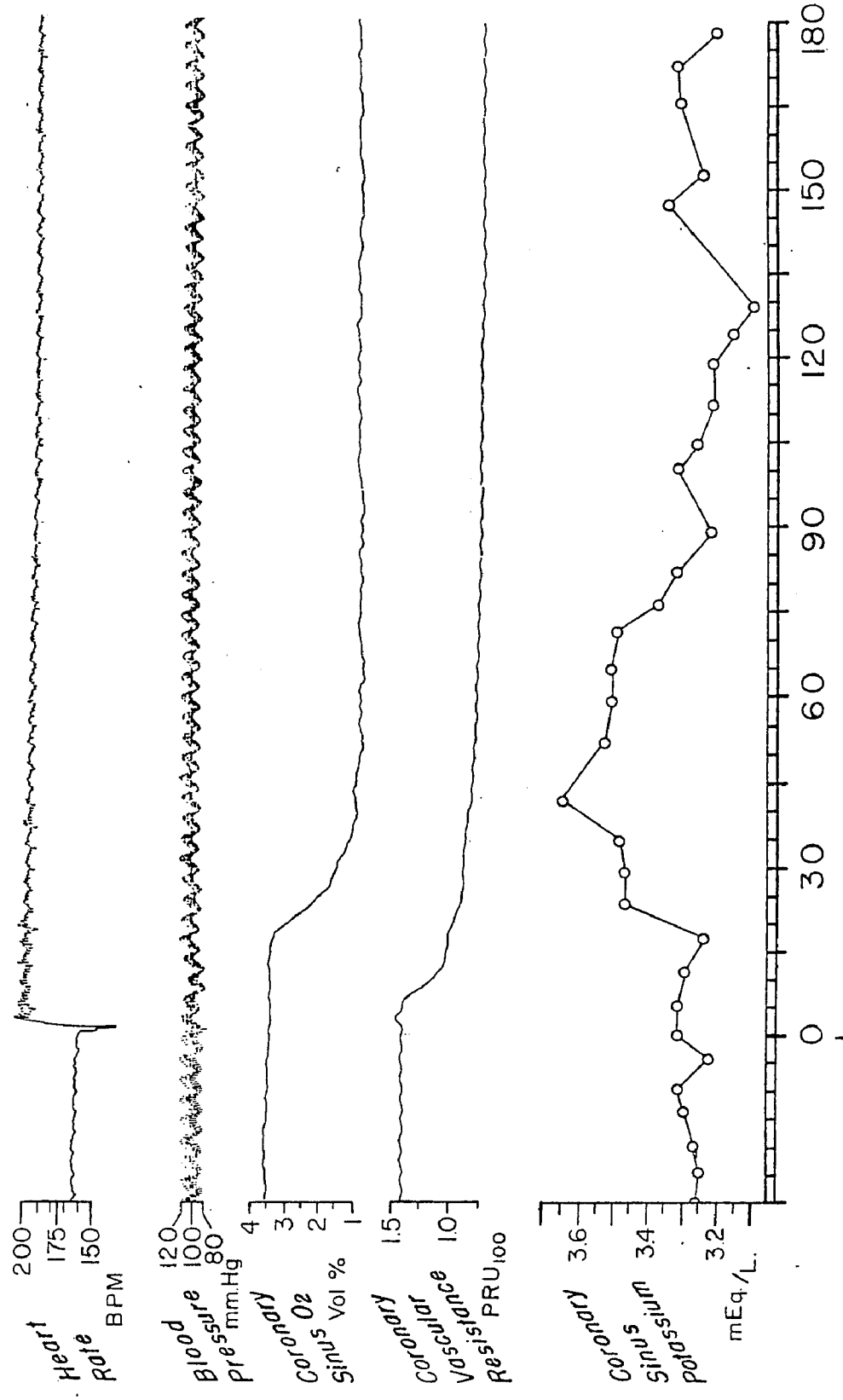
Heart Rate BPM
200
175
150
Blood Pressure mm.Hg
120
100
80
Coronary Sinus Oz Vol %
4
3
2
1
Coronary Vascular Resistance PRU100
1.5
1.0
Coronary Sinus Potassium mEq/L.
3.6
3.4
3.2
0
30
60
90
120
150
180
TIME (Seconds)
INCREASE HEART RATE

Figure 15

Figure 16

Average response of coronary sinus plasma K^+ concentration to step increases in heart rate. Heart rate increases were induced at time zero. Normalization procedure is explained in chapter II. Responses divided into 2 groups as described in text. N = 9 data sets of heart rate steps from 9 dogs. Vertical bars indicate one S.E.M.

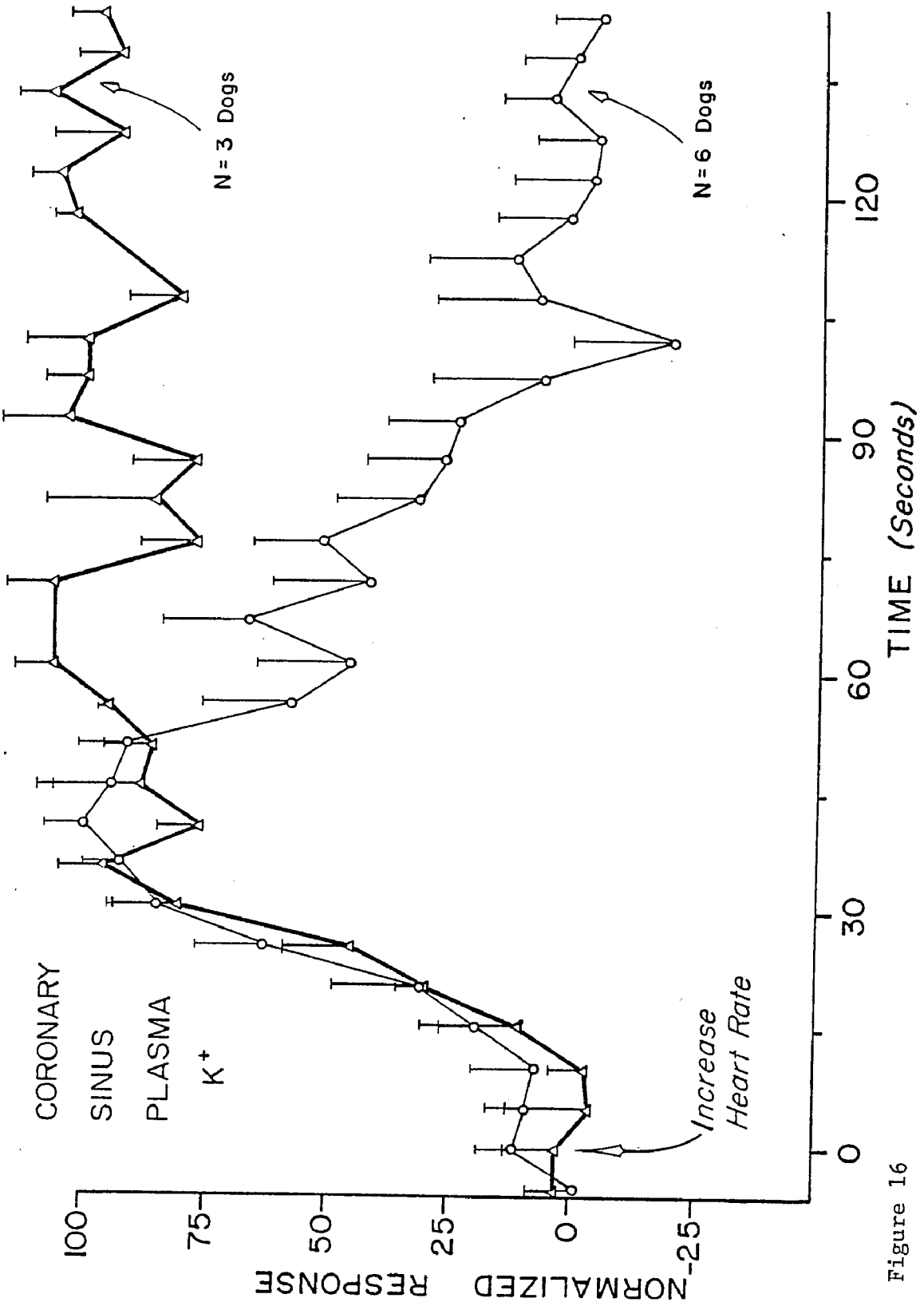
CORONARY
SINUS
PLASMA
K+
N=3 Dogs
N=6 Dogs
Increase
Heart Rate
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
30
60
90
120
TIME (Seconds)

Figure 16

in $CS[K^+]$ produced by step increases in heart rate, the time required to reach a peak change in $CS[K^+]$ is identical for both groups. Thus, for the purpose of determining the dynamics of the changes in $CS[K^+]$ for step increases in heart rate, the time course parameters (i.e. the time required to reach 10, 50 and 90 percent of the peak change: see Methods) from both groups of dogs have been combined.

Figure 17 shows the average response of $CS[K^+]$ and CVR to step increases in heart rate. It should be noted once again that the ordinate of this plot has the units: percentage of peak change (i.e. peak increase for $CS[K^+]$, or peak decrease for CVR). It is clear from this figure that CVR changes more rapidly than $CS[K^+]$ following step increases in heart rate. Table 3 summarizes the time course parameters which characterize the time courses of these responses. Paired-t analyses (the CVR and $CS[K^+]$ responses from one data set equal one pair) show that the t_{50} for CVR is significantly lower (i.e. faster) than the corresponding parameters which characterize the average $CS[K^+]$ time course. The t_{90} for CVR is also lower than the t_{90} for $CS[K^+]$, with the p value approaching the conventional 0.05 significance level ($p = 0.081$).

A simple comparison of CVR and $CS[K^+]$ time courses is inappropriate if the primary purpose is to ascertain whether the changes in interstitial K^+ concentration are fast enough to be causally related to the concomitant changes in CVR produced by step increases in heart rate. On the one hand, changes in CVR are reflected almost instantaneously by the measured changes in coronary perfusion pressure. But the changes in $CS[K^+]$ reflect not only the time course of the

Figure 17

Average response of coronary sinus plasma K^+ concentration and coronary vascular resistance to step increases in heart rate. Heart rate increases were induced at time zero. These data represent responses which have been normalized but otherwise untreated (i.e. no correction for vascular transit). Normalization procedure explained in chapter II. N = 9 data sets of heart rate steps from 9 dogs. Vertical bars indicate one S.E.M.

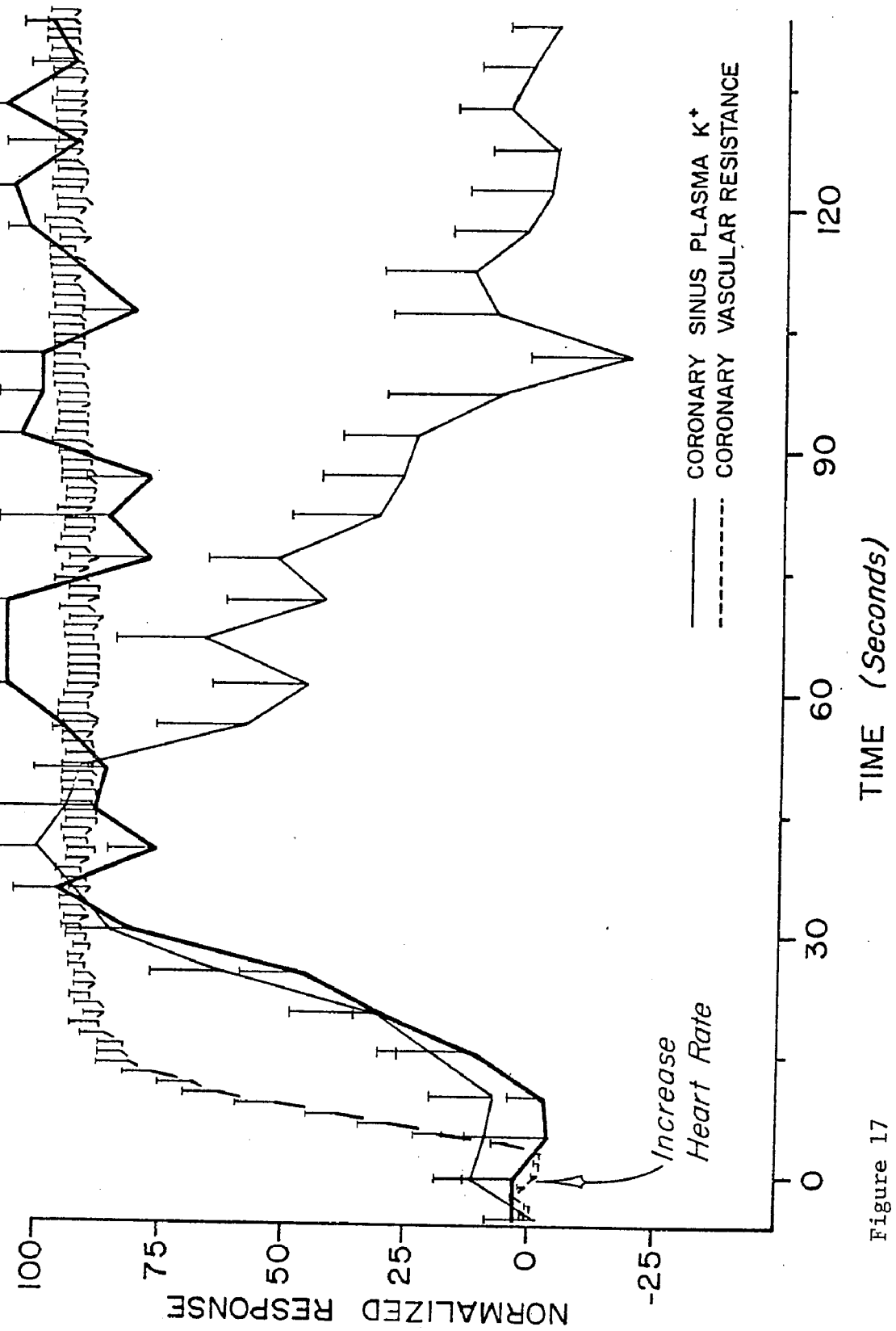
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
30
60
90
120
TIME (Seconds)
CORONARY SINUS PLASMA K+
CORONARY VASCULAR RESISTANCE
Increase
Heart Rate

Figure 17

TABLE 3 COMPARISON OF RAW TIME COURSES: HEART RATE INCREASE

	(seconds)		
	t_{10}	t_{50}	t_{90}
n = 9 data sets			
Coronary Vascular Resistance	5.36 ± 0.64	9.36 ± 0.78	32.57 ± 6.69
Coronary Sinus Plasma K^+ Concentration	6.58 ± 2.50	26.56 ± 1.23	43.79 ± 4.59
p	.333	<.005	.081

changes in interstitial fluid K^+ concentration but also the delay introduced in the exchange of K^+ from the interstitium to the blood, as well as the delay and dispersion introduced by the vascular transit of blood from the capillaries to the coronary sinus sampling site.

We have not attempted to account for the delay introduced by the exchange of K^+ across the capillary endothelium. Our methods for estimating the delay and dispersion introduced by vascular transit have been discussed in the Methods section. The basic approach has been to account for the effects of vascular transit by adjusting the time course of CVR (CVR*) by use of the convolution integral. Thus, since the effects of vascular transit are represented in CVR* and in the time course of $CS[K^+]$, differences in their time courses reflect differences in the time courses of CVR and myocardial interstitial fluid K^+ concentration.

Figure 18 shows the average responses of $CS[K^+]$ and CVR* to step increases in heart rate. Table 4 summarizes the time course parameters which characterize the time courses of these responses. Paired-t analyses (the CVR* and $CS[K^+]$ responses from one data set equal one pair) show that the t_{10} and t_{50} for $CS[K^+]$ are significantly lower (i.e. faster) than the corresponding parameters which characterize the CVR* time course. The t_{90} for $CS[K^+]$ is also less than the t_{90} for CVR* with the p value approaching the conventional 0.05 significance level ($p = 0.087$).

To summarize the results of this section, the data indicate that a step increase in heart rate elicits either a transient or sustained

Figure 18

Average response of coronary sinus plasma K^+ concentration and coronary vascular resistance * to step increases in heart rate. Heart rate increases were induced at time zero. These data represent responses which have been normalized. Normalization procedure explained in chapter II. Coronary vascular resistance * includes the coronary vascular resistance time course as well as the correction for vascular transit. Convolution is indicated by *. N = 9 data sets of heart rate steps from 9 dogs. Vertical bars indicate one S.E.M.

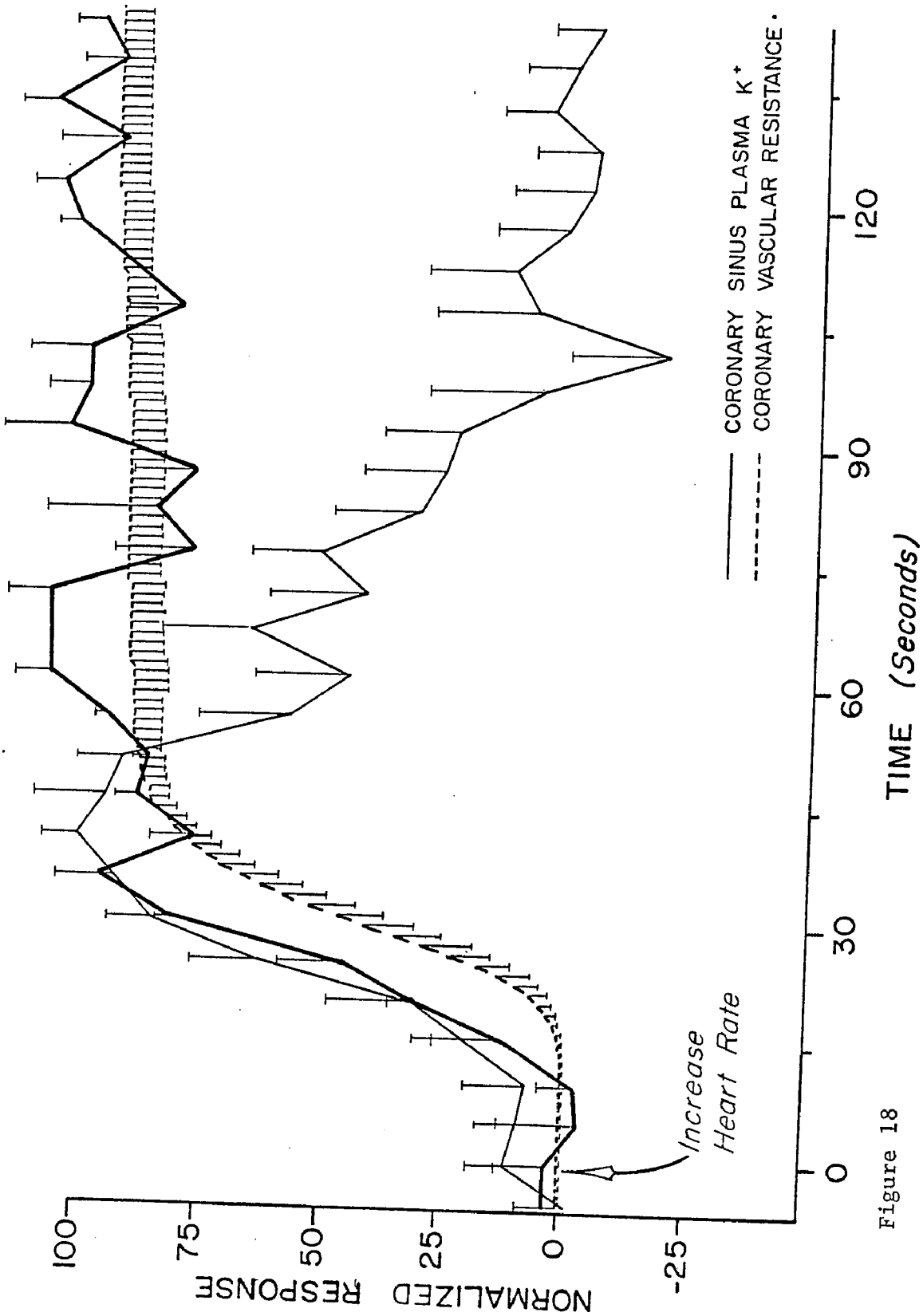
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
30
60
90
120
TIME (Seconds)
CORONARY SINUS PLASMA K+
CORONARY VASCULAR RESISTANCE
Increase Heart Rate

Figure 18

TABLE 4 COMPARISON OF CORRECTED TIME COURSES: HEART RATE INCREASE

	(seconds) t_{10}	t_{50}	t_{90}
n = 9 data sets			
Coronary Vascular Resistance *	24.46 ± 1.39	32.58 ± 1.96	53.32 ± 5.35
Coronary Sinus Plasma K^+ Concentration	6.58 ± 2.50	26.56 ± 1.23	43.79 ± 4.59
p	<.005	.011	.087

increase in myocardial interstitial fluid K^+ concentration with a time course that is fast enough to be causally related to the concomitant changes in CVR.

2. Fifteen second cessation of coronary blood flow:

The steady state and transient data from all the sets of fifteen second cessations of flow are presented in table 5. The values shown for CVR and $CS[K^+]$ are the average baseline values of these variables immediately preceding the cessation of flow in that data set, as well as the peak changes of CVR and $CS[K^+]$ produced by the cessation of flow.

Some typical responses of coronary sinus O_2 content, CVR, and $CS[K^+]$ to a 15 second cessation of flow are shown in figure 19. At minus 15 seconds the pump delivering blood to the left common coronary artery was stopped. CVR (as reflected by coronary perfusion pressure) fell immediately and rapidly (less than 10 seconds) to a very low value and remained at this level throughout this stop flow period. At time zero the pump was turned on, and flow was re-instituted. CVR rose quickly to about one third of its pre-cessation-of-flow level, and then gradually returned to control levels. It is this gradual return of CVR to control levels following the re-institution of flow that is of primary interest for the purposes of this thesis. Coronary sinus O_2 content remained unchanged for several seconds following the 15 second cessation of flow, and then gradually decreased to a peak value before returning to control levels. This decrease in coronary sinus O_2 content reflects a transient imbalance

TABLE 5 STEADY STATE AND TRANSIENT DATA: FIFTEEN SECOND CESSATION OF FLOW

Dog	Data Set	Coronary Blood Flow (ml/min/100 g)	Coronary Vascular Resistance (PRU_{100})	Coronary Sinus Plasma K^+ Concentration (mEq/L)
Baseline Values Before Cessation of Flow				
2-3-76	1	77.24	1.54	4.59
2-4-76	2	116.67	0.96	4.51
2-5-76	3	81.01	1.28	7.22
2-11-76	4	55.09	2.27	5.26
2-13-76	5	51.98	2.04	4.82
2-24-76	6	88.24	1.04	4.46
Mean		78.37	1.52	5.14
± S.E.M.		9.68	0.22	0.43
Peak Changes After Cessation of Flow				
2-3-76	1	77.24	0.31	5.08
2-4-76	2	116.67	0.39	5.24
2-5-76	3	81.01	0.31	8.13
2-11-76	4	55.09	0.29	5.61
2-13-76	5	51.98	0.29	5.17
2-24-76	6	88.24	0.08	4.94
Mean		78.37	0.28	5.90
± S.E.M.		9.68	0.04	0.50

Figure 19

Some typical responses of coronary sinus O_2 content, coronary vascular resistance, and coronary sinus plasma K^+ concentration to a 15 second cessation of coronary blood flow. The pump used to perfuse the left common coronary artery with blood at a constant rate was turned off at minus 15 seconds, and turned back on at time zero.

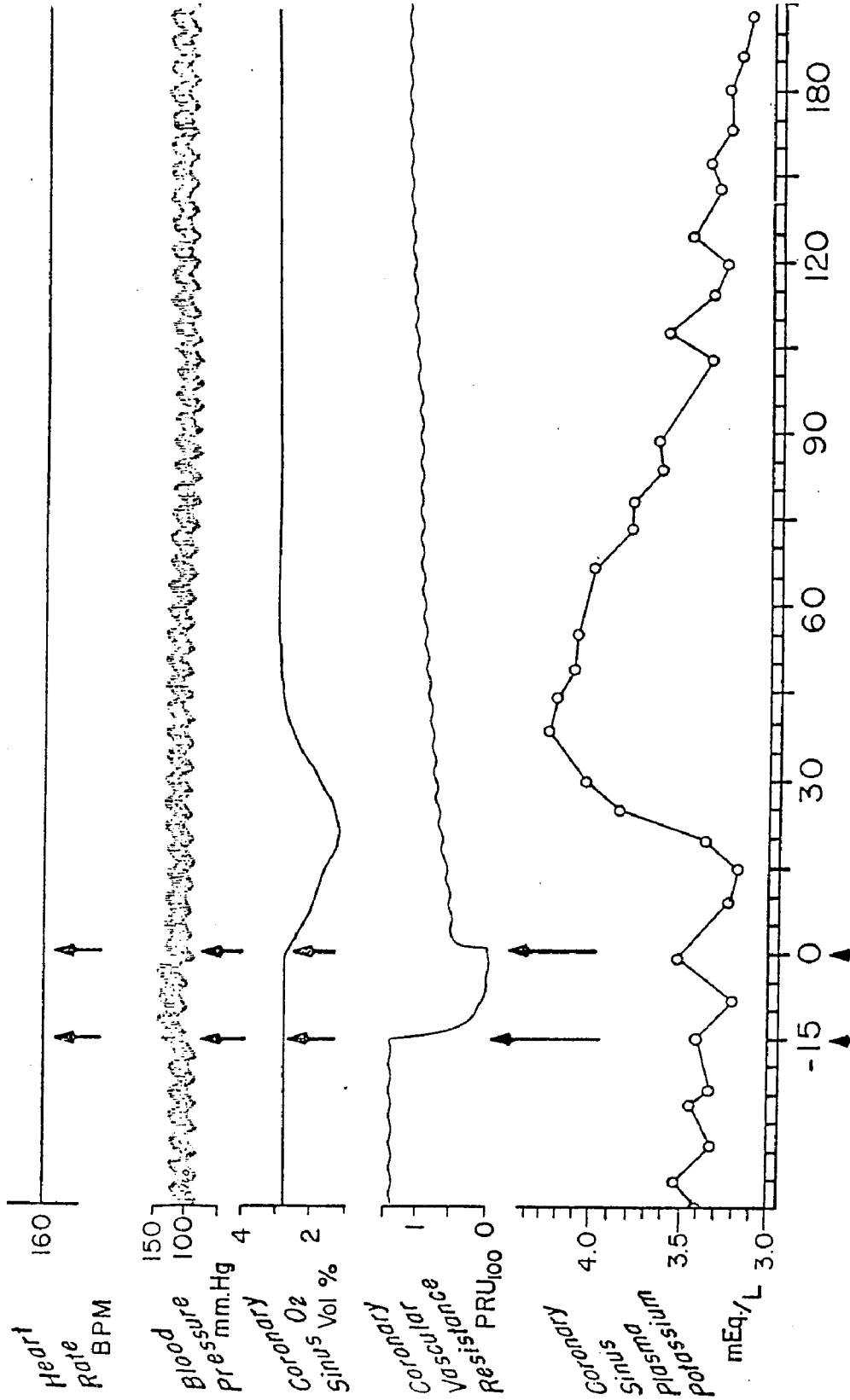
Heart Rate BPM
160
Blood Pressure mm.Hg
150
100
Coronary Sinus O2 Vol %
4
2
Coronary Vascular Resistance PRU100
1
0
Coronary Sinus Plasma Potassium
mEq./L
4.0
3.5
3.0
STOP FLOW
-15
START FLOW
0
30
60
90
120
180
TIME (Seconds)

Figure 19

between O_2 supply and O_2 demand of the myocardium. $CS[K^+]$ also remained unchanged for several seconds following the 15 second cessation of flow, and then gradually increased to a peak value before returning to control levels. Heart rate either did not change, or was held constant by external pacing throughout this entire period. When data from the 6 sets of cessation of flow were grouped, the average peak decrease in CVR was 1.25 ± 0.22 PRU_{100}, while the average peak increase in $CS[K^+]$ was 0.55 ± 0.09 mEq/L. Coronary blood flow averaged 78.37 ± 9.68 ml/min/100g.

Figure 20 depicts the average changes in $CS[K^+]$ produced by 15 second cessations of coronary blood flow. Following the 15 second cessation of flow, $CS[K^+]$ increased in all 6 dogs studied, reaching a peak value approximately 30 seconds after the re-institution of flow.

In order to exclude the possibility that this increase in $CS[K^+]$ was the result of an efflux of K^+ from red blood cells as they traversed the coronary vascular bed, we measured the coronary sinus whole blood K^+ concentration following 15 second cessations of flow. The results are shown in figure 21. Coronary sinus whole blood K^+ concentration was found to increase in all 3 dogs studied, with peak increases averaging 0.52 ± 0.08 mEq/L.

Figure 22 shows the average response of $CS[K^+]$ and CVR to 15 second cessations of coronary blood flow. The ordinate of this plot has the units: percentage of control values (where control values are the values to which both $CS[K^+]$ and CVR return from their peak changes produced by the 15 second cessation of flow). It is clear from this

Figure 20

Average response of coronary sinus plasma K^+ concentration to 15 second cessations of coronary blood flow. Flow was stopped at minus 15 seconds, and started at time zero. Heart rate constant. Normalization procedure described in chapter II. N = 6 data sets of cessation of flow from 6 dogs. Vertical bars indicate one S.E.M.

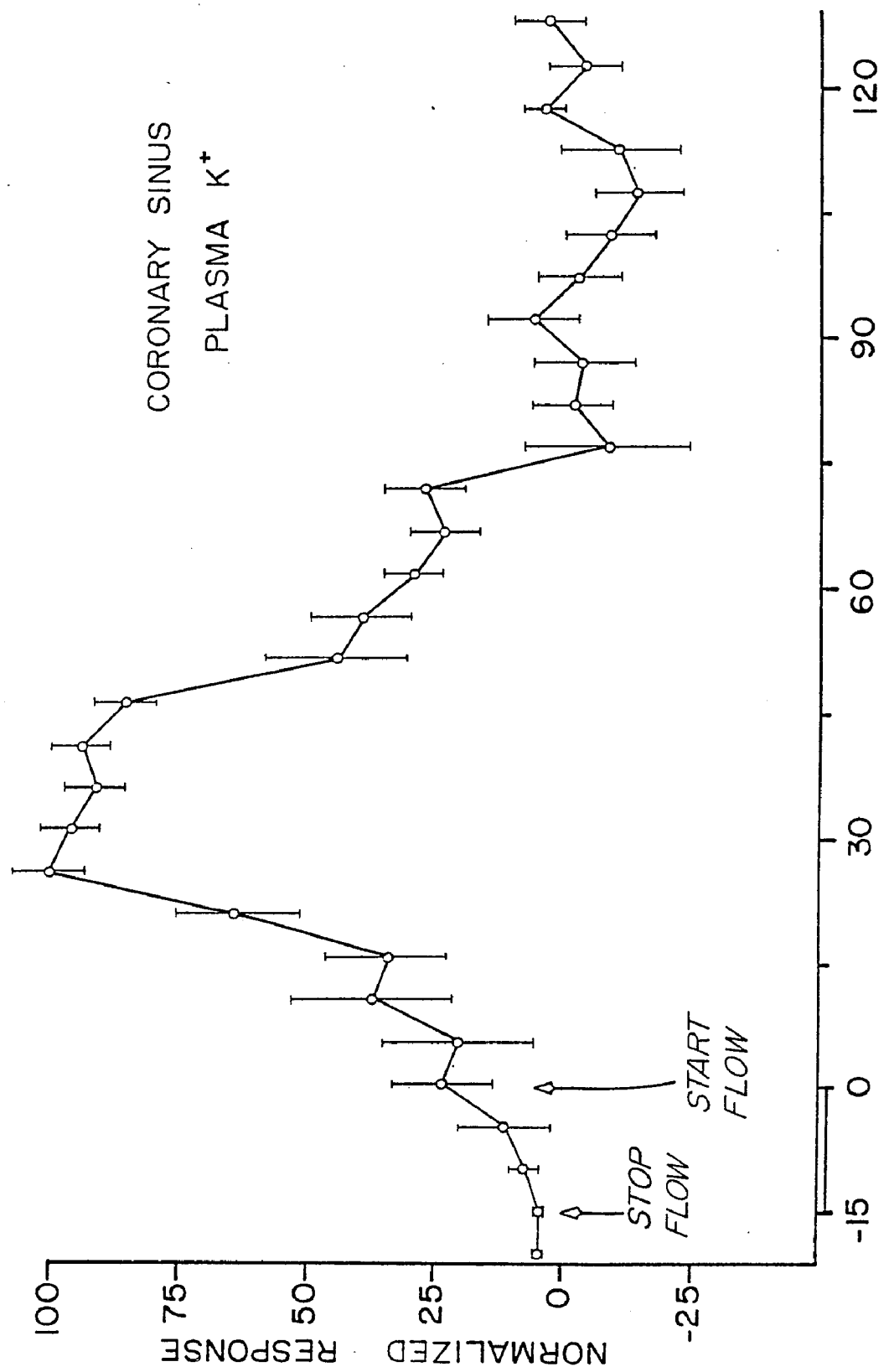
CORONARY SINUS
PLASMA K+
NORMALIZED RESPONSE
100
75
50
25
0
-25
-15
0
30
60
90
120
TIME (SECONDS)
START FLOW
STOP FLOW

Figure 20

Figure 21

Average response of coronary sinus whole blood K^+ concentration to 15 second cessations of coronary blood flow. Flow was stopped at minus 15 seconds, and started at time zero. Heart rate constant. Normalization procedure explained in chapter II. N = 3 data sets of cessation of flow from 3 dogs. Vertical bars indicate one S.E.M.

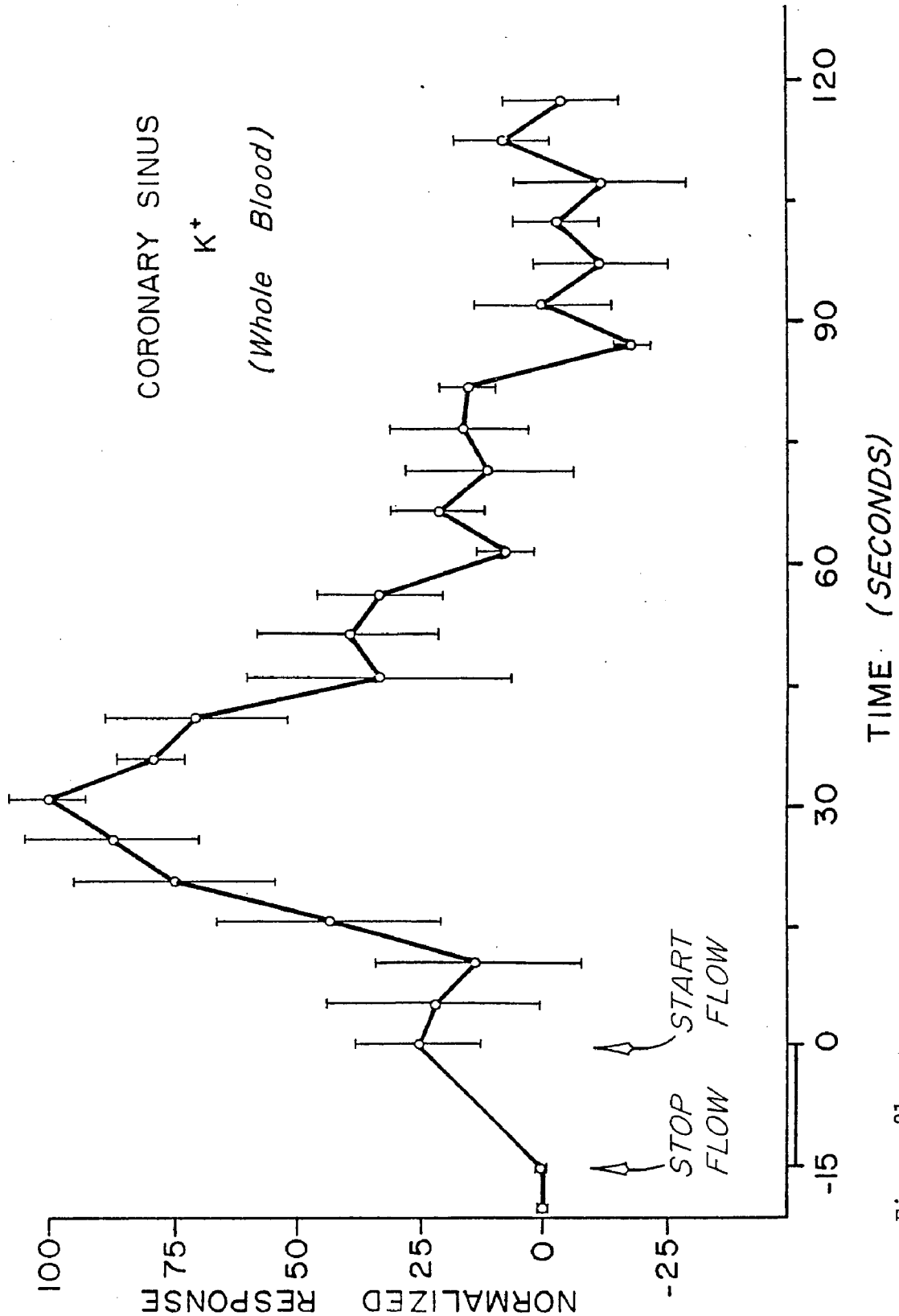
CORONARY SINUS
K+
(Whole Blood)
NORMALIZED RESPONSE
100
75
50
25
0
-25
-15
0
30
60
90
120
TIME (SECONDS)
STOP FLOW
START FLOW

Figure 21

Figure 22

Average response of coronary sinus plasma K^+ concentration and coronary vascular resistance to 15 second cessations of coronary blood flow. Flow was re-instituted at time zero. These data represent responses which have been normalized but otherwise untreated (i.e. no correction for vascular transit). Normalization procedure explained in chapter II. N = 6 data sets of cessation of flow from 6 dogs. Vertical bars indicate one S.E.M.

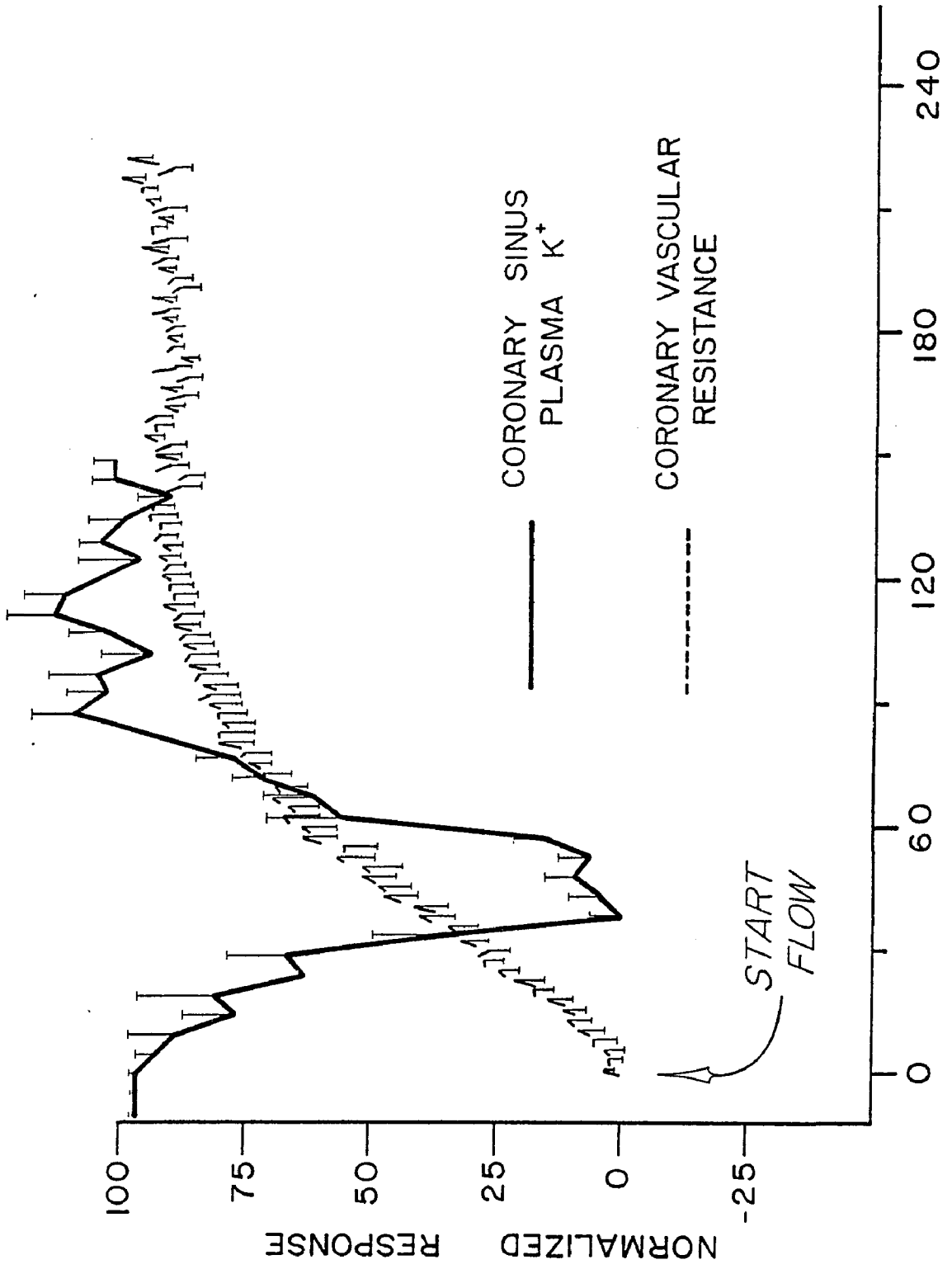
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
60
120
180
240
TIME (Seconds)
CORONARY SINUS PLASMA K+
CORONARY VASCULAR RESISTANCE
START FLOW

Figure 22

figure that over a large fraction of the total time course CVR returns to control levels from its peak change more rapidly than $CS[K^+]$. Table 6 summarizes the time course parameters which characterize the time courses of these responses. Paired-t analyses (the CVR and $CS[K^+]$ responses from one data set equal one pair) show that the t_{10} and t_{50} for CVR are significantly lower (i.e. faster) than the corresponding parameters which characterize the average $CS[K^+]$ time course. The t_{90} for $CS[K^+]$ is smaller than the corresponding parameter for CVR ($p = .058$).

But as stated previously, a simple comparison of CVR and $CS[K^+]$ is inappropriate if the primary purpose is to ascertain whether the changes in interstitial fluid K^+ concentration are fast enough to be causally related to the concomitant changes in CVR produced by the 15 second cessation of flow. Thus, we have once again adjusted CVR (CVR*) to account for the effects of vascular transit. Since the effects of vascular transit are now represented in CVR* and in the time course of $CS[K^+]$, differences in their time courses reflect differences in the time course of CVR and myocardial interstitial fluid K^+ concentration. Figure 23 shows the average responses of $CS[K^+]$ and CVR* to 15 second cessations of coronary blood flow. Once again the ordinate has the units: percentage of control values. These data indicate that the $CS[K^+]$ time course appears to lag behind the CVR* time course over approximately the initial 30 percent of the CVR* time course, while over the later 70 percent of the CVR* time course the $CS[K^+]$ time course is as fast or faster than the CVR* time course. Table 7 summarizes the time course parameters which

TABLE 6 COMPARISON OF RAW TIME COURSES: CESSATION OF FLOW

	(seconds)		
	t_{10}	t_{50}	t_{90}
n = 6 data sets			
Coronary Vascular Resistance	11.93 ± 3.45	47.73 ± 6.07	106.28 ± 18.99
Coronary Sinus Plasma K^+ Concentration	43.65 ± 3.03	60.98 ± 1.91	78.18 ± 4.73
p	< .005	.008	.058

Figure 23

Average response of coronary sinus plasma K^+ concentration and coronary vascular resistance * to 15 second cessations of coronary blood flow. Flow was re-instituted at time zero. These data represent responses which have been normalized. Normalization procedure explained in chapter II. Coronary vascular resistance * includes the coronary vascular resistance time course as well as the correction for vascular transit. Convolution is indicated by *. N = 6 data sets of cessation of flow from 6 dogs. Vertical bars indicate one S.E.M.

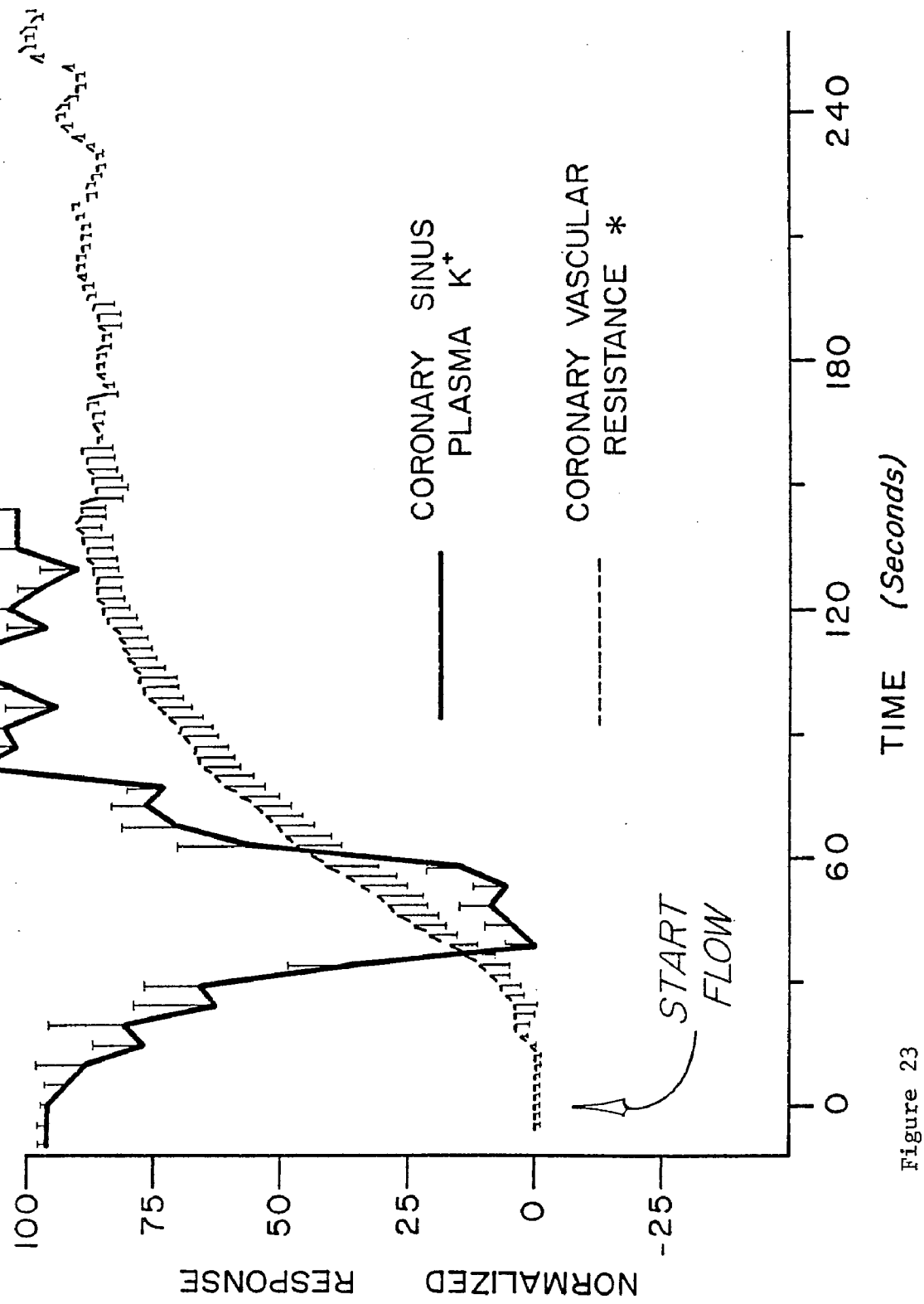
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
60
120
180
240
TIME (Seconds)
CORONARY SINUS PLASMA K+
CORONARY VASCULAR RESISTANCE *
START FLOW

Figure 23

TABLE 7 COMPARISON OF CORRECTED TIME COURSES: CESSATION OF FLOW

	(seconds)		
	t_{10}	t_{50}	t_{90}
n = 6 data sets			
Coronary Vascular Resistance *	33.80 ± 3.75	69.23 ± 7.84	131.70 ± 22.63
Coronary Sinus Plasma K^+ Concentration	43.65 ± 3.03	60.98 ± 1.91	78.18 ± 4.73
P	.041	.143	.019

characterize the time courses of these responses. Paired-t analyses (the CVR* and $CS[K^+]$ responses from one data set equal one pair) show that the t_{10}, t_{50}, and t_{90} for $CS[K^+]$ is slower, not different, and significantly faster, respectively, than the corresponding parameters which characterize the average CVR* time course.

To summarize the results of this section, the data indicate that a 15 second cessation of coronary blood flow elicits a transient increase in myocardial interstitial fluid K^+ concentration that is independent of changes in heart rate, and with a time course that is fast enough to be causally related to at least the last 70 percent of the concomitant changes in CVR.

CHAPTER IV

DISCUSSION

The theory that K^+ is involved in the control of CVR holds that, in association with an increase in myocardial metabolic activity, there is an increase in the $[K^+]$ of the interstitial space, which in turn causes relaxation of the coronary vascular smooth muscle in a dose-related fashion. We have presented data designed to test this hypothesis, and to more fully discern the mechanism of K^+-induced vasodilation in the coronary vascular bed.

The determinants of coronary blood flow, and thus the possible mechanism of K^+-induced vasodilation, can be conveniently divided into five categories: (1) perfusion pressure, (2) myocardial systolic compression of coronary vessels, (3) myocardial metabolism, (4) neural control of the coronary vessels, (5) direct action on coronary vascular smooth muscle tone.

Perfusion Pressure: Coronary blood flow is directly related to coronary perfusion pressure, and so increases in coronary blood flow produced by K^+ administration might result from an increase in aortic blood pressure. In this study a 34 to 48 percent decrease in coronary vascular resistance (figure 2) was associated with a 4 mm Hg fall in aortic blood pressure (figure 3). This result is clear evidence of coronary vasodilation rather than a passive increase in flow secondary to an increase in pressure.

Myocardial Systolic Compression: Contraction of the heart during systole impedes coronary blood flow by compressing the coronary vascular bed. K^+-induced vasodilation could, therefore, be the result of a decrease in the myocardial systolic compressive effect on coronary blood flow. We made no direct measurement of myocardial tension development. We did, however, observe a very slight decrease (4 mm Hg) in mean aortic pressure with the administration of K^+. Even if this small decrease in aortic pressure reflects a slight decrease in systolic compression, it is exceedingly unlikely that this effect could account for a significant portion of the K^+-induced increases in blood flow. Sabiston and Gregg (1957) studied the effect of systolic compression on coronary blood flow and observed only a 26 percent increase in coronary inflow following fibrillation of normally beating dog hearts. Moreover, the possible effects of systolic compression can be avoided by observing K^+-induced changes in flow during diastole. Indeed Bünger et al. (1976) observed peak increases of 40 percent in diastolic coronary blood flow following K^+ administration in isolated perfused guinea pig hearts. Thus, it appears that K^+-induced vasodilation is not primarily the result of a decrease in myocardial systolic compression.

Myocardial Metabolism: Increases in myocardial metabolism are known to be associated with increases in coronary blood flow. K^+-induced vasodilation could, then, be the result of a primary increase in the metabolic activity of the heart followed by a secondary metabolically-induced increase in coronary blood flow. In these studies we observed that K^+ administration had no significant effect on heart rate, and

produced a small decrease in aortic blood pressure. Furthermore, in the constant flow preparation we found that K^+ administration produced a small (0.2 vol %), but significant, increase in coronary sinus oxygen content (figure 3), reflecting an actual decrease in myocardial oxygen consumption. These data indicate that K^+-induced vasodilation is not the result of an increased myocardial metabolic activity.

Neural Control of Coronary Vessels: Sympathetic alpha receptor coronary vasoconstriction (Feigl, 1967) and parasympathetic cholinergic coronary vasodilation (Feigl, 1969) have been demonstrated. Furthermore, changes in extracellular $[K^+]$ alter the release and reuptake of neurotransmitters into nerve endings (Bonaccorsi et al., in press). Thus, it seemed possible that the mechanism of K^+-induced vasodilation might involve an interaction between K^+ and tissue neural elements. The strategy employed in these studies was straightforward: if K^+-induced vasodilation was the result of an increased release of vasodilator neurotransmitter or a decreased release of vasoconstrictor neurotransmitter, then K^+-induced vasodilation should be attenuated or abolished following the administration of pharmacologic antagonists specific to the agonists in question.

We first tested the hypothesis that K^+-induced vasodilation was the result of an increased release of norepinephrine from sympathetic nerve terminals in the heart, with subsequent activation of coronary vascular beta receptors. This hypothesis was disproved by demonstrating that a dose of propranolol sufficient to abolish

isoproterenol-induced vasodilation had no effect on K^+-induced vasodilation (figures 5 and 6).

K^+-induced vasodilation also does not appear to be the result of an increased release of acetylcholine from parasympathetic nerve terminals in the heart, with subsequent activation of Ach-specific receptors on coronary vascular smooth muscle. A dose of atropine sufficient to completely block acetylcholine-induced vasodilation had no effect on K^+-induced vasodilation (figures 7 and 8).

Bonaccorsi and Bohr et al. (in press) have demonstrated that re-admission of K^+ to a K^+-free medium resulted in a dose-dependent relaxation of isolated vascular smooth muscle. This K^+-induced relaxation was associated with a diminished release of norepinephrine from adrenergic nerve endings. It seemed possible, therefore, that K^+-induced vasodilation might be the result of a withdrawal of alpha adrenergic mediated coronary vascular tone. But in our study the administration of phentolamine failed to have a significant effect on K^+-induced vasodilation (figure 9).

Thus, the failure of propranolol, atropine, or phentolamine to have a significant effect on K^+-induced vasodilation strongly indicates that the mechanism of K^+-induced vasodilation does not necessarily involve an interaction between K^+ and the tissue neural elements discussed.

Direct Effect on Vascular Smooth Muscle Tone: Having essentially excluded the above mentioned mechanisms as being responsible for K^+-induced vasodilation, we examined the possibility that K^+-induced

vasodilation was the result of a direct action on the coronary vascular smooth muscle. Specifically, we tested the hypothesis that K^+-induced vasodilation is the result of an activation of the electrogenic Na^+-K^+ exchange pump located in the wall of the coronary vascular smooth muscle. If K^+ exerts its effect by increasing pump activity, then K^+-induced vasodilation should be attenuated following inhibition of the pump with ouabain. The data clearly indicate that K^+-induced vasodilation is partially blocked by ouabain-lidocaine (figure 10). This attenuation of K^+-induced vasodilation appears to be primarily due to the effects of ouabain, since lidocaine alone has little, if any, effect on K^+-induced vasodilation (figure 11). Moreover, the effect of ouabain is not due to a non-specific depression of vascular smooth muscle activity, since it is without effect on acetylcholine-induced vasodilation (figure 14).

Since the K^+-induced vasodilation was not completely abolished by ouabain, the possibility that K^+ may exert its vasodilator action through some other mechanism cannot be discounted. But it is also possible that the response could be more fully abolished by administering larger doses of ouabain. This is difficult to demonstrate in a practical sense, since larger doses of ouabain would further inhibit the Na^+-K^+ ATPase of myocardial cells, and thus increase the incidence of ouabain-induced arrhythmias. Indeed it was the administration of lidocaine, which delays the onset of ouabain-induced arrhythmias, (Kniffen et al., 1974) that allowed us to deliver the dose of ouabain necessary to achieve the attenuation of K^+-induced vasodilation.

A more complete blockade of K^+-induced vasodilation in the coronary vascular bed by ouabain has been very recently reported by Bünger et al. (1976). But these results must be accepted with some degree of reservation for the following reasons: (1) no time controls were performed to ascertain whether the vasodilator response to K^+ decreased as a result of successive administrations of K^+, (2) no data were presented to show that for this preparation (isolated Krebs-perfused spontaneously beating guinea pig heart) the effect of ouabain was not the result of a non-specific depression of vascular smooth muscle reactivity, and (3) the administration of ouabain produced an increase in resting flow levels, a result which is both inconsistent with the proposed mechanism of K^+-induced vasodilation and with our own findings and those of other investigators (Bloor et al., 1972).

Thus our data lend further support to the mounting evidence that suggests that at least one mechanism of K^+-induced vasodilation is the result of an activation of the electrogenic Na^+-K^+ exchange pump located in the wall of vascular smooth muscle. Moreover, because our experiments were performed in situ, it was possible for us to examine other potential mechanisms of K^+-induced vasodilation. We have presented evidence which suggests that K^+-induced vasodilation is not the result of: (1) a passive increase in flow secondary to an increase in coronary perfusion pressure, (2) an increase in the metabolic activity of the heart, and (3) an interaction between K^+ and the classic neurotransmitters, norepinephrine and acetylcholine. In addition we have argued that based on the findings of

other investigators, and our own evidence that K^+ administration was associated with only a small decrease (4 mm Hg) in aortic pressure, that it is very unlikely that any significant portion of K^+-induced vasodilation is the result of a decrease in myocardial systolic compression.

If K^+ is a mediator of the decreases in CVR associated with increases in the metabolic activity of the heart, then the following conditions must be met: (1) the $[K^+]$ in the environment of the vascular smooth muscle must increase at least as fast as the decrease in CVR, and (2) these changes in $[K^+]$ of the interstitial space must be of sufficient magnitude to account for at least some portion of the associated vascular response. We have presented evidence that bears on both of these points.

K^+ has been shown to be a potent vasodilator in the coronary vascular bed. Intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl produced decreases in CVR of 34 to 48 percent (figure 2) depending on the initial resistance of the bed. This apparent dependence of the magnitude of K^+-induced vasodilation on the initial resistance of the bed may simply reflect the "dilution" of K^+ by the flow rate at the time of administration (i.e. a dose-effect relationship between $[K^+]$ and CVR). The data presented in figure 2 have been re-plotted and are shown in figure 24. In this figure the change in resistance is plotted as a function of the amount of K^+ injected (40 micromoles) divided by the flow rate at the time of injection. The abscissa of this graph can be viewed as the concentration of K^+ added to the coronary arterial blood. The data

Figure 24

Changes in coronary vascular resistance produced by intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl. These data are a re-plot of the data shown in figure 2. The abscissa represents the amount of K^+ administered divided by the flow rate in the circumflex artery immediately prior to the injection. The best fit regression line is shown. Numbers in parentheses refer to the individual responses observed. 177 responses were observed in 18 dogs. Vertical and horizontal bars indicate one S.E.M.

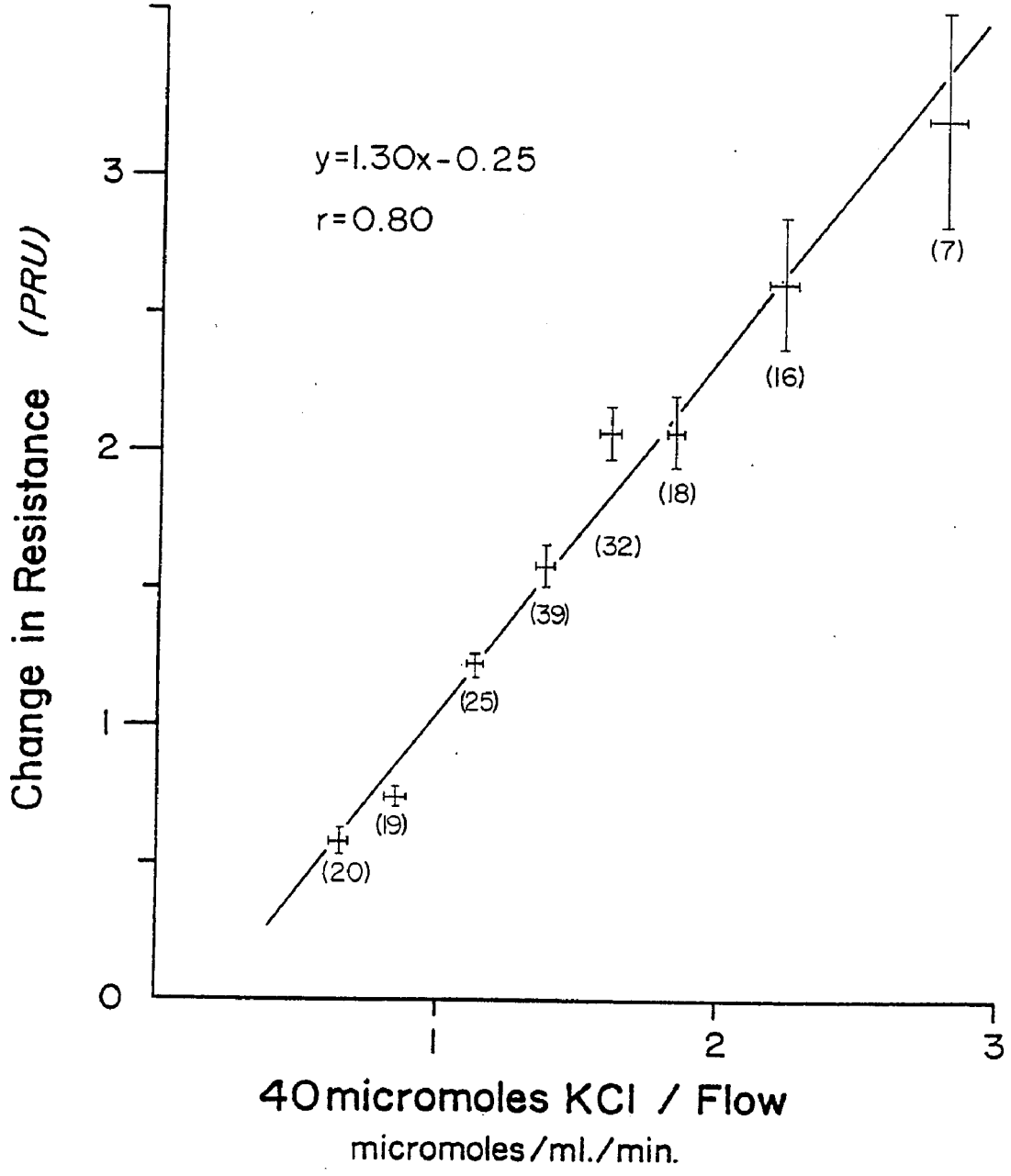
y=1.30x-0.25
r=0.80
Change in Resistance (PRU)
0
1
2
3
(20)
(19)
(25)
(39)
(32)
(18)
(16)
(7)
1
2
3
40 micromoles KCl / Flow
micromoles/ml./min.

Figure 24

suggest a positive, linear relationship between the $[K^+]$ added to the coronary arterial blood and the magnitude of the vascular response evoked.

But the real issue here is to ascertain the magnitude of the response of coronary vascular smooth muscle to physiologically occurring changes in interstitial fluid $[K^+]$. To achieve this end we must: (1) estimate the changes in interstitial $[K^+]$ elicited by our experimental maneuvers (i.e. heart rate steps and 15 second cessations of flow), (2) estimate the changes in interstitial fluid $[K^+]$ produced as a result of increasing the coronary arterial $[K^+]$, and (3) for an equivalent estimated change in interstitial fluid $[K^+]$ compare the magnitude of the vascular response elicited either by an experimental maneuver or by the administration of K^+.

Direct measurements of myocardial interstitial fluid $[K^+]$ have not been made. Thus, we must attempt to estimate the changes in interstitial fluid $[K^+]$ produced by either step increases in heart rate or 15 second cessations of coronary blood flow. These maneuvers produced peak increases in coronary sinus plasma $[K^+]$ of approximately 0.5 mEq/L. But the magnitude of these changes in coronary sinus plasma $[K^+]$ almost certainly underestimate the magnitude of the changes in interstitial fluid $[K^+]$, depending on the value of the product of capillary permeability and surface area (Renkin's PS; Renkin, 1959). Interstitial fluid $[K^+]$ can be estimated if one knows arterial and venous $[K^+]$, flow, and the capillary PS for K^+ These data and the calculated changes in interstitial $[K^+]$ for step increases in heart rate are shown in table 8. There is some

TABLE 8 ESTIMATED CHANGES IN INTERSTITIAL $[K^+]$: HEART RATE INCREASE

Dog	Heart Rate Increase (beats/min)	Plasma Flow (ml/min/100 g)	Plasma $[K^+]$ (mEq/L)		Estimated Interstitial $[K^+]$ (mEq/L)*		Peak Decrease CVR (percent)
			Arterial	Coronary Sinus	PS = 5	PS = 10	
3-9-76	70	34.13	3.90	4.82	10.47	7.58	65
3-12-76	60	38.67	4.36	4.83	8.28	6.40	30
3-20-76	85	46.30	3.14	3.72	8.94	6.19	46

*Interstitial $[K^+]$ was calculated according to the equation: $(C_A - C_V)/(C_A - C_I) = 1 - \exp(-PS/Q)$, where C_A, C_V, and C_I are K^+ concentrations in arterial plasma, coronary sinus plasma, and interstitial space, respectively, PS is the permeability-surface area product for the capillaries, and Q is the plasma flow.

question as to the appropriate value for PS because it increases with increased metabolism (Sheehan and Renkin, 1972). The values presented in table 8 are based on the findings of Tancredi et al. (1975) and represent reasonable values of PS for control (PS = 5) and experimental (PS = 10) conditions. One assumption of this model is that the measured changes in plasma $[K^+]$ are equivalent to end-capillary plasma $[K^+]$. This may not be true for the transient changes in coronary sinus plasma $[K^+]$ observed for some heart rate steps and 15 second cessations of flow. But it probably does apply for the step increases in heart rate where $CS[K^+]$ remained elevated at the peak level throughout the stimulation period. For this reason the estimated changes in interstitial $[K^+]$ produced by step increases in heart rate have only been calculated for these data. But since the peak changes in $CS[K^+]$ were roughly equivalent for 15 second cessations of flow and heart rate steps regardless of whether $CS[K^+]$ returned to control or remained elevated, these calculated values of interstitial $[K^+]$ may well represent reasonably good estimates of the changes in interstitial $[K^+]$ produced by each of our experimental maneuvers.

It is apparent from table 8 that the interstitial $[K^+]$ levels are significantly higher than the coronary sinus levels for these step increases in heart rate. The estimated peak increases in interstitial $[K^+]$ range from 6.19 to 10.47, with the lower values associated with the higher PS. These estimated increases in interstitial $[K^+]$ are associated with peak decreases in CVR of from 30 to 65%.

Having estimated the changes in interstitial [K^+] produced by step increases in heart rate, we must next estimate the changes in interstitial [K^+] produced by 40 micromole bolus injections of K^+ into the circumflex artery. This is a complex estimation and requires a number of assumptions. First, we assume that the mass of injected K^+ is conserved during arterial to capillary transit. This assumption is based on the study by Frazier et al. (1954) who report that the half-time for plasma-red cell K^+ exchange is 36 hours for dog blood at 37°C. Second, we assume that 50 percent of the mass of K^+ arriving in the capillary is extracted by the heart and is distributed uniformly throughout the interstitial space. This assumption is based on the study by Ziegler and Goresky (1971) who found that 50 percent of radio-labelled rubidium was extracted by the heart in one pass through the coronary vascular bed. Third, to simplify our calculations we ignore the active uptake of interstitial K^+ into the myocardial cells. By ignoring this active cellular re-uptake mechanism for K^+ we almost certainly over-estimate the final peak concentration of interstitial fluid K^+ produced by our bolus injection. Fourth, we assume that K^+ only exerts its effect on vascular smooth muscle from the interstitial space.

Given these assumptions the estimated changes in interstitial fluid [K^+] and the associated changes in CVR produced by a 40 micromole bolus injection of K^+ into the circumflex artery are presented in table 9. We estimate that in this hypothetical heart, where the circumflex artery supplies 60 g of left ventricle containing 9 ml of interstitial fluid, a 40 micromole bolus injection of K^+

TABLE 9: ESTIMATED CHANGES IN INTERSTITIAL $[K^+]$: BOLUS INJECTIONS OF K^+

Left Ventricle Weight (g)	Ventricular Weight Supplied by Circumflex Artery (g)	Interstitial Space (ml)	Amount of K^+ injected (micromoles)	Amount of K^+ extracted (micromoles)	Estimated Interstitial $[K^+]$ (mEq/L) Before Injection	Estimated Interstitial $[K^+]$ (mEq/L) After Injection	Peak Decrease in Resistance (percent)
100	60	9.0	40	20	4.0	6.22	34-48

delivered into the circumflex artery increases interstitial [K^+] from a baseline of 4.0 to a peak of 6.22 mEq/L. This increase in interstitial [K^+] is associated with a 34 to 48 percent decrease in CVR. These changes compare favorably with the estimated changes in interstitial [K^+] and the observed changes in CVR produced by step increases in heart rate (as shown in table 8).

These calculations are admittedly rough and are only as good as the assumptions made. Nevertheless, they suggest that changes in interstitial [K^+] are of sufficient magnitude to play a significant role in the production of the vascular response to either a step increase in heart rate or a 15 second cessation of flow.

A second condition which must be satisfied in order to demonstrate that K^+ is a mediator of the decreases in CVR associated with increases in myocardial metabolism is the criterion of time course: the [K^+] in the environment of the vascular smooth muscle must increase at least as fast as the decrease in CVR.

Our data show that for step increases in heart rate, the increases in CS[K^+] precede the decreases in CVR after correcting for the effects of vascular transit. In addition, the time course of the return of interstitial [K^+] to control levels is as fast or faster than a large fraction of the associated changes in CVR produced by 15 second cessations of coronary blood flow. Thus the data appear to satisfy the time course criterion. But there are several potential errors in logic and methodology which should be discussed.

First, it should be noted that the correction for vascular transit permits a comparison of capillary [K^+] and CVR time courses. However our primary interest is to compare the interstitial [K^+] and CVR time courses. Thus, if we had taken into account the delay introduced by the capillary endothelium in the exchange of K^+ between the interstitial space and the blood, the time course of the changes in CS[K^+] would be even faster relative to the CVR time course (corrected for vascular transit). This may partially account for the apparently slower K^+ time course relative to the CVR time course during the initial phase of the response to a 15 second cessation of flow.

Second, the comparison between K^+ and CVR time courses relies heavily on our ability to accurately measure the distribution of vascular transit times and dispersion between the end of the capillaries and our sampling site. This method was outlined in Chapter 2. In our adjustment of the CVR time course to include the effects of vascular transit and dispersion, we assume that neither the experimental manipulations (i.e. increased heart rate or 15 second cessation of flow), nor the changes in plasma [K^+] elicited by these maneuvers, have any significant effect on the distribution of intravascular transit times. But in the case of the 15 second cessations of flow this may not be true. If the 15 second period of zero flow resulted in a decrease in the blood volume of the coronary vascular bed, then following the re-institution of flow some period of time would elapse before the blood volume of the coronary bed would return to the pre-cessation-of-flow level. The net effect would almost

certainly be an increased delay (and perhaps dispersion) in the arrival of the elevated plasma K^+ levels at our sampling site. This effect could be appreciable. The coronary blood volume has been estimated to be approximately 7.5% of the heart weight (Howe and Winbury, 1969). The mean weight of the perfused myocardium in this series of experiments was 150 g. If the coronary blood volume had decreased by one half during the 15 second cessation of flow, it would require 4-5 seconds to restore the coronary blood volume at the perfusion rates employed (78 ml/min/100 g). No adjustment in the CVR time course has been made for this possible delay, which would mean that interstitial $[K^+]$ could be changing more rapidly relative to CVR than is estimated by our analysis. Thus, this factor may also partially account for the apparently slower K^+ time course relative to the CVR time course during the initial phase of the response to a 15 second cessation of flow.

To summarize this section, our data show that the dynamics for the changes in interstitial $[K^+]$ are fast enough to account for the vascular responses associated with step increases in heart rate or 15 second cessations of flow. This is despite the fact that we have not accounted for several factors which, if considered, would further strengthen this conclusion. But we must emphasize that by satisfying the criterion of time course we have not proved that the changes in interstitial $[K^+]$ cause the associated changes in CVR. Rather, these results fail to reject the hypothesis that K^+ is involved in the control of CVR. But this result of a sufficiently fast time course, and the fact that we have demonstrated that K^+ is a vasodilator

in the coronary vascular bed, lends considerable support to the hypothesis that K^+ is involved in the control of CVR.

However, our data indicate that K^+ cannot be responsible for the steady state changes in CVR produced by step increases in heart rate in 6 of the 9 dogs studied. In these animals the plasma $[K^+]$ of the coronary sinus blood increased to a peak value and then returned to the control level even while the increase in heart rate was maintained. It has been suggested that there may be two separable components of the vascular response associated with changes in the metabolic activity of a muscle, an early transient phase, and a later sustained phase (Mohrman et al, 1973). Our data support the concept that K^+ could be a mediator of the vascular response at the onset of exercise, but in two-thirds of the cases could not be involved in the steady state vascular response.

We also measured the changes in myocardial oxygen consumption associated with these same step increases in heart rate. As shown in figure 25 these changes in myocardial oxygen consumption precede the associated changes in CVR, and are sustained throughout the period of elevated heart rate. Table 10 summarizes the time course parameters which characterize the time course of these responses. Paired-t analyses indicate that the t_{10}, t_{50}, and t_{90} for CS O_2 content are significantly lower (i.e. faster) than the corresponding parameters which characterize the CVR* time course. Thus, changes in the concentration of a vasodilator linked to myocardial oxidative metabolism could also be responsible for the initial decrease in CVR, but in addition may account for the more sustained decreases in CVR

Figure 25

Average response of coronary sinus oxygen content and coronary vascular resistance * to step increases in heart rate. Heart rate increases were induced at time zero. These data represent responses which have been normalized. Normalization procedure explained in chapter II. Coronary vascular resistance * includes the coronary vascular resistance time course as well as the correction for vascular transit. Convolution is indicated by *. N = 9 data sets of heart rate steps from 9 dogs. Vertical bars indicate one S.E.M.

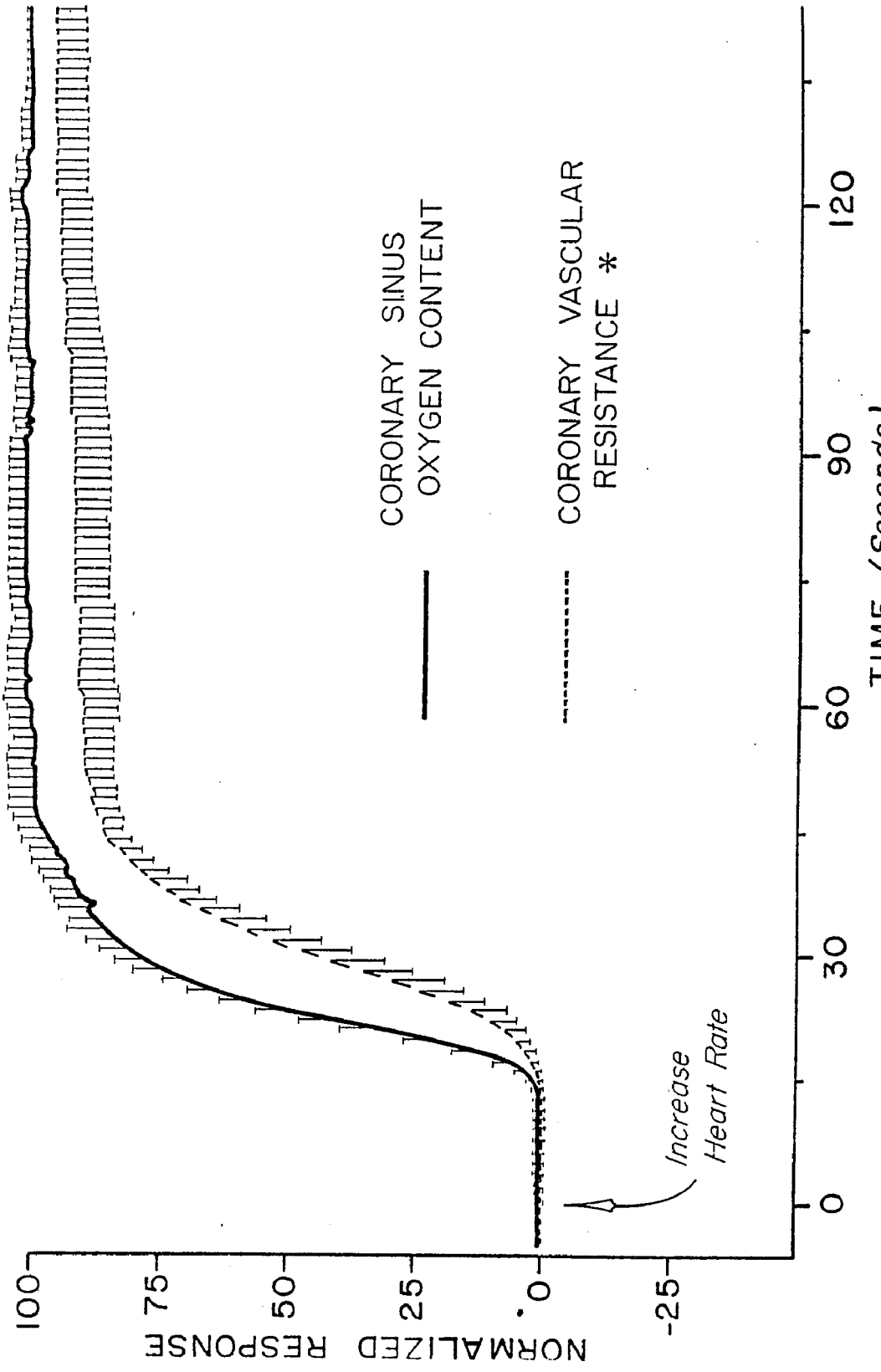
NORMALIZED RESPONSE
100
75
50
25
0
-25
0
30
60
90
120
TIME (Seconds)
CORONARY SINUS
OXYGEN CONTENT
CORONARY VASCULAR
RESISTANCE *
Increase
Heart Rate

Figure 25

TABLE 10 COMPARISON OF CORRECTED TIME COURSES: HEART RATE INCREASE

	(seconds)		
	t_{10}	t_{50}	t_{90}
n = 9 data sets			
Coronary Vascular Resistance *	24.46 ± 1.39	32.58 ± 1.96	53.32 ± 5.35
Coronary Sinus Oxygen Content	18.44 ± 0.53	23.98 ± 0.58	40.69 ± 4.58
p	<.005	<.005	.016

produced by step increases in heart rate. These findings lend further support to the hypothesis that work-induced changes in vascular resistance are due to multiple factors.

The fact that K^+ remained elevated throughout the period of increased heart rate in 3 dogs is worthy of note for several reasons. First, this steady state correlation between the increase in interstitial [K^+] and the decrease in CVR prevents us from excluding K^+ as a possible controller of CVR under these circumstances. Second, this observation may provide some insight into the possible importance of K^+ in the control of CVR in circumstances where the myocardium is relatively hypoxic. In those animals where CS[K^+] returned to control level during the period of elevated heart rate the CS O_2 content was significantly higher (3.66 ± 0.76 vol % vs. 1.69 ± 0.63 vol %) than in those animals where CS[K^+] remained elevated ($p < .05$). These data support the contention that in the well-oxygenated heart the active myocardial cellular re-uptake process for K^+ maintains the myocardial K^+ balance and prevents a potentially deleterious build-up of interstitial [K^+]. In contrast, a condition of relative hypoxia could result in a depletion of the available energy pool (i.e. ATP) that is necessary to drive the electrogenic Na^+-K^+ exchange pump of the myocardial cells. The effect would be a build-up of interstitial [K^+] and a subsequent loss of myocardial K^+. Interestingly, this effect could be offset by the vasodilator action of the elevated interstitial [K^+] on the coronary vascular smooth muscle, in that the resultant increase in flow would increase the supply of oxygen to the hypoxic area, which in turn could be used to generate the ATP necessary to run the cellular re-uptake mechanism for K^+.

Under these circumstances K^+ may assume an extremely important role in the control of CVR.

Further support for this hypothesis is provided by the transient release of K^+ elicited by the 15 second cessation of flow. This increase in K^+ efflux from the heart could not be due to a decrease in interstimulus interval, since heart rate was held at a constant level. We feel it is more likely due to a transient imbalance between nutrient supply and demand, resulting in a decrease in the available energy supply necessary to maintain the full activity of the myocardial Na^+-K^+ exchange pump.

The transient nature of the K^+-induced changes in vascular reactivity produced by either elevating the extracellular $[K^+]$ in the isolated vessel preparation (Gellai and Detar, 1974) or increasing the $[K^+]$ of arterial blood perfusing a resting muscle (Brace, 1974) apparently argues against the participation of K^+ in the sustained phase of work-induced decreases in vascular resistance. It is postulated that the decay of vasodilator activity may be due to a decrease in the intracellular $[Na^+]$ of vascular smooth muscle as a result of the K^+-stimulated increase in the activity of the electrogenic Na^+-K^+ exchange pump (where Na^+ is pumped out of the cell at a faster rate than K^+ is pumped in). This fall in intracellular $[Na^+]$ results in a gradual slowing of the electrogenic pump, a waning of the hyperpolarization, and thus a waning of the vascular response. Although these observations suggest that K^+-induced changes in vascular reactivity via alterations in the activity of the electrogenic Na^+-K^+ exchange pump may be of a transient nature, they do not

exclude the possibility that, during periods of increased metabolic activity, K^+ may interact with other agents (e.g. neurotransmitters; vasodilator metabolites) which in turn may have vasoactive properties. Thus, any effort to discount the possible involvement of K^+ in the sustained phase of functional hyperemia on the basis that K^+-induced vasodilation wanes with time must be made with some caution.

In addition to the proposed involvement of K^+ in functional and reactive hyperemia, it has been postulated that K^+ may be involved in a number of pathological processes.

Overbeck et al. (1976) have initiated a series of studies to determine whether a defect in the ouabain-sensitive Na^+-K^+ pump of vascular smooth muscle is responsible for some part of the vasoconstriction of hypertension. These investigators have found a significant attenuation of K^+-induced vasodilation in dogs with chronic one-kidney perinephritic hypertension (1972), and in a proportion of men with essential hypertension (1974). Moreover, hypertensive dogs were found to have a decreased activity of the electrogenic Na^+-K^+ exchange pump (as measured by radioactive rubidium uptake) in both mesenteric arteries and splanchnic veins. These investigators argue that a depression of the electrogenic Na^+-K^+ pump could result in a partial depolarization of the vascular smooth muscle, which in turn could account for the elevated vascular resistance.

Rhabdomyolysis associated with intense physical conditioning in a hot climate may be a consequence of acute K^+ depeletion (Knochel et al., 1972). These investigators have suggested that this acute

K^+ depletion may result in a diminished release of K^+ from the exercising skeletal muscle, which in turn would diminish the active hyperemic response that is normally associated with exercise. The lack of a sufficient nutrient supply to the exercising skeletal muscle could then result in ischemia-induced damage to the skeletal muscle cells. They tested this hypothesis by monitoring the changes in blood flow and K^+ efflux produced by electrical stimulation of the isolated gracilis muscle of the dog following chronic dietary K^+ depletion (1973). The results of this study appear to confirm their hypothesis, since following K^+ depletion both the K^+ efflux and active hyperemia associated with muscular exercise were absent. The results of this extremely important study have not been duplicated. If verified, however, these results would offer definitive proof for the participation of K^+ in functional hyperemia.

Proof of the involvement of K^+ in these and other pathologic processes requires further investigation. But it is apparent that the potential importance of the interaction of K^+ and vascular smooth muscle cannot be underestimated.

To summarize this section, our data indicate that two necessary, but not sufficient, criteria for the involvement of any putative mediator of coronary vascular resistance have been satisfied by K^+: changes in interstitial fluid $[K^+]$ (1) have a sufficiently fast time course, and (2) are of appropriate magnitude, to be causally related to the concomitant changes in CVR produced by either step increases in heart rate or 15 second cessations of coronary blood flow. Further critical tests of this hypothesis must follow to prove beyond question

that K^+ is a controller of CVR. For example, if either (1) an attenuation of K^+-induced vasodilation, or (2) an alteration in the efflux of K^+ from myocardial cells to the interstitial space, could be shown to alter either the time course or the steady state changes in CVR produced by, for example, a step increase in heart rate, then this would offer definitive evidence for the participation of K^+ in the control of CVR. The converse result would not disprove the role of K^+ in the control of CVR, since redundant control mechanisms could mask the K^+-dependent component of the vascular response. These experiments should be done.

CHAPTER V

SUMMARY

This thesis has been primarily concerned with three separate questions:

1. Is K^+ a vasodilator in the coronary vascular bed?
2. What is the mechanism of K^+-induced vasodilation?
3. Are the changes in interstitial [K^+] produced by step increases in heart rate or 15 second cessations of coronary blood flow fast enough and of sufficient magnitude to be causally related to the associated changes in coronary vascular resistance?

Our findings can be summarized as follows:

1. Intra-circumflex artery bolus injections of 40 micromoles isosmotic KCl produced decreases in coronary vascular resistance of from 34 to 48 percent depending on the initial resistance of the bed. It was estimated that this dose of K^+ administered intra-arterially produced changes in myocardial interstitial fluid [K^+] well within the range of those occurring physiologically.

2. The mechanism of K^+-induced vasodilation in the coronary vascular bed was found not to be the result of:

   (a) a passive increase in flow secondary to an increase in coronary perfusion pressure,

   (b) a decrease in myocardial systolic compression,

(c) a primary increase in the metabolic activity of the heart, resulting in a secondary metabolically-induced increase in coronary blood flow,

(d) an increased release of norepinephrine from sympathetic nerve terminals in the heart,

(e) an increased release of acetylcholine from parasympathetic nerve terminals in the heart,

(f) a withdrawal of alpha-adrenergic mediated vascular tone.

3. K^+-induced vasodilation in the coronary vascular bed does appear to be at least partly the result of an activation of the electrogenic Na^+-K^+ exchange pump located in the wall of coronary vascular smooth muscle.

4. Step increases in heart rate were associated with increases in coronary sinus plasma $[K^+]$ (peak = 0.53 ± 0.07 mEq/L) in 9 of 9 dogs studied. Fifteen second cessations of coronary blood flow were associated with increases in coronary sinus plasma $[K^+]$ (peak = 0.55 ± 0.09 mEq/L) in 6 of 6 dogs studied.

5. When the effects of venous vascular transit are accounted for, the changes in coronary sinus plasma $[K^+]$ precede the associated changes in coronary vascular resistance over the entire time course for step increases in heart rate and over a large fraction of the time course for 15 second cessations of flow. These data indicate that the changes in interstitial $[K^+]$ elicited by these experimental maneuvers are fast enough to be causally related to the associated changes in coronary vascular resistance.

6. In 6 of 9 experiments the increases in coronary sinus $[K^+]$ produced by step increases in heart rate were not sustained throughout the period of elevated heart rate, and thus could not be responsible for the steady state changes in coronary vascular resistance. On the other hand, the changes in myocardial oxygen consumption produced by step increases in heart rate were sufficiently fast and were sustained, so that some vasodilator agent linked to these changes could be responsible for the associated changes in coronary vascular resistance.

7. In 3 of 9 experiments the increases in coronary sinus $[K^+]$ produced by step increases in heart rate were sustained throughout the period of elevated heart rate. It would appear that in circumstances of limited oxygen availability K^+ may well participate in the steady state control of the associated changes in coronary vascular resistance.

REFERENCES

1. Anderson, D.K., Roth, S.A., Brace, R.A., Radawski, D., and Haddy, F.J.: Effect of hypokalemia and hypomagnesemia produced by hemodialysis on skeletal muscle vascular resistance; role of potassium in active hyperemia. Circ. Res. 31:165-173, 1972.

2. Axelsson, J., Wahlstrom, B., Johansson, B., and Johsson, O.: Influence of the ionic environment on spontaneous electrical and mechanical activity of the rat portal vein. Circ. Res. 21:609-618, 1967.

3. Beaty, O., Tyce, G.M., and Donald, D.E.: Mechanism of "sympatholysis" in exercising skeletal muscle. Physiol. 18:134, 1975.

4. Belloni, F.L.: Dynamics of myocardial oxygen comsumption and coronary vascular resistance. Univ. of Michigan dissertation, 1975.

5. Belloni, F.L., and Sparks, H.V.: Dynamics of myocardial oxygen consumption and coronary vascular resistance. Amer. J. Physiol. (in press).

6. Beznak, A.B.L.: On the mechanism of the autocoid function of parasympathetic nerves. J. Physiol. 82:129-153, 1934.

7. Bloor, C.M., Walker, D.E., and Pensinger, R.R.: Ouabain induced primary coronary vasoconstriction. Proc. Soc. Exp. Biol. Med. 140:1409-1413, 1972.

8. Bogdanski, D.F., and Brodie, B.B.: The effects of inorganic ions on the storage and uptake of H^3-norepinephrine by rat heart slices. J. Pharm. Exp. Ther. 165:181-189, 1969.

9. Bonaccorsi, A., Hermsmeyer, K., Aprigliano, O., Smith, C.B., and Bohr, D.F.: Mechanism of potassium relaxation of arterial vascular smooth muscle. Amer. J. Physiol. (in press).

10. Bonaccorsi, A., Hermsmeyer, K., Smith, C.B., and Bohr, D.F.: Norepinephrine release in isolated arteries induced by K^+-free solution. Amer. J. Physiol. (in press).

11. Brace, R.A.: Time course and mechanisms of the acute effects of hypokalemia and hyperkalemia on vascular resistance. Proc. Soc. Exp. Biol. Med. 145:1389-1394, 1974.

12. Brace, R.A., Anderson, D.K., Chen, W.T., Scott, J.B., and Haddy, F.J.: Local effects of hypokalemia on coronary resistance and myocardial contractile force. Amer. J. Physiol. 227:590-597, 1974.

13. Brecht, K., Konold, P., and Gebert, G.: The effect of potassium, catecholamines and other vasoactive agents on isolated arterial segments of the muscular type. Physiol. Bohem. 18:15-22, 1969.

14. Brown, G.L., and Feldberg, W.: The action of potassium on the superior cervical ganglion of the cat. J. Physiol. 86:290-305, 1936.

15. Bünger, R., Haddy, F.J., Querengasser, A., and Gerlach, E.: Studies on potassium induced coronary dilation in the isolated guinea pig heart. Pflug. Arch. 363:27-31, 1976.

16. Burcher, E., and Garlick, D.: Effects of exercise metabolites on adrenergic vasoconstriction in the gracilis muscle of the dog. J. Pharm. Exp. Ther. 192:149-156, 1975.

17. Case, R.B.: Ion alterations during myocardial ischemia. Cardiology 56:245-262, 1971.

18. Chen, W.T., Brace, R.A., Scott, J.B., Anderson, D.K., and Haddy, F.J.: The mechanism of the vasodilator action of potassium. Proc. Soc. Exp. Biol. Med. 140:820-824, 1972.

19. Cherbakoff, A., Toyama, S., and Hamilton, W.F.: Relation between coronary sinus plasma potassium and cardiac arrhythmia. Circ. Res. 5:517-521, 1957.

20. Cohen, S.E.: The influence of the Ca and K ions on tonus and adrenaline response of the coronary arteries. Arch. Int. Pharmacodyn. 54:1-16, 1936.

21. Cummings, J.R.: Electrolyte changes in heart tissue and coronary arterial and venous plasma following coronary occlusion. Circ. Res. 8:865-869, 1960.

22. Dawes, G.S.: Vasodilator action of potassium. J. Physiol. 99: 224-238, 1941.

23. Dixon, W.J., and Massey, F.J.: Introduction to statistical analysis, McGraw-Hill Book Co., New York, 1969.

24. Driscol, T.E., and Berne, R.M.: Role of potassium in regulation of coronary blood flow. Proc. Soc. Exp. Biol. Med. 96:505-508, 1958.

25. Duling, B.R.: Effects of potassium ion on the microcirculation of the hamster. Circ. Res. 37:325-332, 1975.

26. Emanuel, D.A., Scott, J.B., and Haddy, F.J.: Effect of potassium on small and large blood vessels of the dog forelimb. Amer. J. Physiol. 197:637-642, 1959.

27. Feigl, E.O.: Sympathetic control of coronary circulation. Circ. Res. 20:262-271, 1967.

28. Feigl, E.O.: Parasympathetic control of coronary blood flow in dogs. Circ. Res. 25:509-519, 1969.

29. Feigl, E.O.: Control of myocardial oxygen tension by sympathetic coronary vasoconstriction in the dog. Circ. Res. 37:88-95, 1975.

30. Feldberg, W., and Guimarais, J.A.: The liberation of acetylcholine by potassium. J. Physiol. 86:306-314, 1936.

31. Fenn, W.O.: The oxygen consumption of muscles made non-irritable by sugar solutions. Amer. J. Physiol. 97:635-647, 1931.

32. Frazier, H.S., Sicular, A., and Solomon, A.K.: Potassium uptake by dog erythrocyte. J. Gen. Physiol. 37:631-641, 1954.

33. Gebert, G.: Messung der K^+- und Na^+- activitat mit mikroglaselektroden im extracellularraum des Kaninchenskelet muskels bei muskelarbeit. Pflug. Arch. 331:204-214, 1972.

34. Gellai, M., and Detar, R.: Evidence in support of hypoxia but against high K^+ and hyperosmolality as possible mediators of sustained vasodilation in rabbit cardiac and skeletal muscle. Circ. Res. 35:681-691, 1974.

35. Gerlings, E.D., Miller, D.T., and Gilmore, J.P.: Oxygen availability: a determinant of myocardial potassium balance. Amer. J. Physiol. 216:559-562, 1969.

36. Gilmore, J.P., and Gerlings, E.D.: Influence of interstimulus interval on myocardial potassium balance. Amer. J. Physiol. 217:136-141, 1969.

37. Grupp, G., Acheson, G.H., and Charles, A.: Heart rate and paired ventricular stimulation: effects on contractile force and K^+ exchange. Amer. J. Physiol. 212:607-611, 1967.

38. Haddy, F.J., Scott, J.B., Florio, M.A., Daugherty, R.M., and Huizenga, J.N.: Local vascular effects of hypokalemia, alkalosis, hypercalcemia, and hypomagnesemia. Amer. J. Physiol. 204:202-212, 1963.

39. Hajdu, S.: Mechanism of staircase and contracture in ventricular muscle. Am. J. Physiol. 174:371-380, 1953.

40. Harris, A.S., Toth, L.A., and Hoey, T.E. Arrhythmic and antiarrhythmic effects of sodium, potassium, and calcium salts and of glucose injected into coronary arteries of infarcted and normal hearts. Circ. Res. 6:570-579, 1958.

41. Hendrickx, H., and Casteels, R.: Electrogenic sodium pump in arterial smooth muscle cells. Pflug. Arch. 346:299-306, 1974.

42. Herd, J.A., and Barger, A.C.: A simplified technique for chronic catheterization of blood vessels. J. Appl. Physiol. 19:791-792, 1964.

43. Hnik, P., Holas, M., Krekule, I., Mejsnar, J., Smiesko, V., Ujec, E., and Vyskocil, F.: Work-induced potassium changes in skeletal muscle and effluent venous blood assessed by liquid ion-exchanger microelectrodes. Pflug. Arch. 362:85-94, 1976.

44. Hnik, P., Vyskocil, F., Kriz, N., and Holas, M.: Work-induced increase of extracellular K^+ concentration in muscle measured by ion-specific electrodes. Brain Res. 40:559-562, 1972.

45. Howe, B.B., and Winbury, M.M.: Effect of acute coronary occlusion on myocardial nutritional circulation in swine. Amer. J. Physiol. 216:1069-1074, 1969.

46. Jennings, R.B., Sommers, H.M., Kaltenbach, J.P., and West, J.J.: Electrolyte alterations in acute myocardial ischemic injury. Circ. Res. 14:260-269, 1964.

47. Johansson, B., and Bohr, D.F.: Rhythmic activity in smooth muscle from small subcutaneous arteries. Amer. J. Physiol. 210:801-806, 1966.

48. Katz, L.N., and Lindner, E.: The action of excess Na^+, Ca^{++}, and K^+ on the coronary vessels. Amer. J. Physiol. 124:155-160, 1938.

49. Keynes, R.D.: The leakage of radioactive potassium from stimulated nerve. J. Physiol. 113:99-114, 1951.

50. Kniffen, F.J., Lomas, T.E., Nobel-Allen, N.L., and Lucchesi, B.R.: The comparative antiarrhythmic actions of lidocaine and its quarternary derivative, methyl lidocaine. Circulation 49: 264-271, 1974.

51. Knochel, J.P., Dotin, L.N., and Hamburger, R.J.: Pathophysiology of intense physical conditioning in a hot climate. I. Mechanisms of potassium depletion. J. Clin. Inv. 51:242-255, 1972.

52. Knochel, J.P. and Schlein, E.M.: Absence of active hyperemia in potassium depleted skeletal muscle. Clin. Res. 19:87, 1971.

53. Knochel, J.P., and Schlein, E.M.: On the mechanism of rhabdomyolysis in potassium depletion. J. Clin. Inv. 51:1750-1758, 1972.

54. Konold, P., Gebert, G., Brecht, K.: The antagonism of potassium and catecholamines on the vascular tone of isolated arterial segments. Experentia 24:692, 1968.

55. Konold, P., Gebert, G., and Brecht, K.: The mechanical response of isolated arteries to potassium. Experentia 24:247-248, 1967.

56. Kramer, K., Elam, J.O., Saxton, G.A., and Elam, W.M., Jr.: Influence of O_2 saturation, erythrocyte concentration and optical depth upon the red and near infrared light transmittance of whole blood. Amer. J. Physiol. 165:229-246, 1951.

57. Langer, G.A., and Brady, A.J.: Potassium in dog ventricular muscle: Kinetic studies of distribution and effects of varying frequency of contraction and potassium concentration of perfusate. Circ. Res. 18:164-177, 1966.

58. Limas, C.J., and Cohn, J.N.: Stimulation by vasodilators of the (Na^+-K^+)-ATPase of vascular smooth muscle. Circ. Res. 35:601-607, 1974.

59. Lindmar, R., and Löffelholz, K.: The neuronal efflux of noradrenaline: Dependency on sodium and facilitation by ouabain. Naunyn-Schmiedebergs Arch. Pharmacol. 284:93-100, 1974.

60. Mohrman, D.E., Cant, J.R., and Sparks, H.V.: Time course of vascular resistance and venous oxygen changes following brief tetanus of dog skeletal muscle. Circ. Res. 33:323-336, 1973.

61. Mohrman, D.E., and Sparks, H.V.: Resistance and venous oxygen dynamics during sinusoidal exercise of dog skeletal muscle. Circ. Res. 33:337-345, 1973.

62. Mohrman, D.E., and Sparks, H.V.: Role of potassium ions in the vascular response to a brief tetanus. Circ. Res. 35:384-390, 1974.

63. Myers, H.A., and Honig, C.R.: Influence of initial resistance on magnitude of response to vasomotor stimuli. Amer. J. Physiol. 216:1429-1436, 1969.

64. Norton, J.M., and Detar, R.: Potassium and isolated coronary vascular smooth muscle. Amer. J. Physiol. 222:474-479, 1972.

65. Overbeck, H.W.: Vascular responses to cations, osmolality, and angiotensin in renal hypertensive dogs. Amer. J. Physiol. 223:1358-1364, 1972.

66. Overbeck, H.W., Derifield, R.S., Pamnani, M.B., and Sozen, T.: Attenuated vasodilator responses to K^+ in essential hypertensive men. J. Clin. Inv. 53:678-686, 1974.

67. Overbeck, H.W., Pamnani, M.B., Akera, T., Brody, T.M., and Haddy, F.J.: Depressed function of a ouabain-sensitive sodium-potassium pump in blood vessels from renal hypertensive dogs. Circ. Res. 38:II-48 - II-52.

68. Parker, J.O., Chiong, M.A., West, R.O., and Case, R.B.: The effect of ischemia and alterations of heart rate on myocardial potassium balance in man. Circ. 42:205-217, 1970.

69. Renkin, E.M.: Transport of potassium-42 from blood to tissue in isolated mammalian skeletal muscles. Amer. J. Physiol. 197: 1205-1210, 1959.

70. Sabiston, D.C., and Gregg, D.E.: Effect of cardiac contraction on coronary blood flow. Circulation 15:14-20, 1957.

71. Sarnoff, S.J., Gilmore, J.P., McDonald, R.H., Daggett, W.M., Weisfeldt, M.L., and Mansfield, P.B.: Relationship between myocardial K^+ balance, O_2 consumption, and contractility. Amer. J. Physiol. 211:361-375, 1966.

72. Scott, J.B., Frohlich, E.D., Hardin, R.A., and Haddy, F.J.: Na^+, K^+, Ca^{++}, and Mg^{++} action on coronary vascular resistance in the dog heart. Amer. J. Physiol. 201:1095-1100, 1961.

73. Scott, J.B., and Radawski, D.: Role of hyperosmolarity in the genesis of active and reactive hyperemia. Circ. Res. 28:I-26 - I-32, Suppl. 1, 1971.

74. Scott, J.B., Rudko, M., Radawski, D., and Haddy, F.J.: Role of osmolarity, K^+, H^+, Mg^{++}, and O_2 in local blood flow regulation. Amer. J. Physiol. 218:338-345, 1970.

75. Sheehan, R.M., and Renkin, E.M.: Capillary, interstitial, and cell membrane barriers to blood-tissue transport of potassium and rubidium in mammalian skeletal muscle. Circ. Res. 30:588-607, 1972.

76. Sybers, R.G., Sybers, H.D., Helmer, P.R., and Murphy, Q.R.: Myocardial potassium balance during cardioaccelerator nerve and atrial stimulation. Amer. J. Physiol. 209:699-701, 1965.

77. Tancredi, R.G., Yipintsoi, T., and Bassingthwaighte, J.B.: Capillary and cell wall permeability to potassium in isolated dog hearts. Amer. J. Physiol. 229:537-544, 1975.

78. Toda, N.: Responsiveness to potassium and calcium ions of isolated cerebral arteries. Amer. J. Physiol. 227:1206-1211, 1974.

79. Van Der Kloot, W.G.: Potassium-stimulated respiration and intracellular calcium release in frog skeletal muscle. J. Physiol. 191:141-165, 1967.

80. Vanhoutte, P.M., and Lorenz, R.R.: Inhibition of norepinephrine uptake by potassium ions in vascular smooth muscle. Arch. Int. Pharm. Ther. 208:377-382, 1974.

81. Wilde, W.S.: Pulsatile nature of the release of potassium from heart muscle during systole. Ann. NY Acad. Sci. 65:693-699, 1957.

82. Wood, J.C., and Conn, H.L.: Potassium transfer kinetics in the isolated dog heart. Influence of contraction rate, ventricular fibrillation, high serum potassium and acetylcholine. Amer. J. Physiol. 195:451-458, 1958.

83. Ziegler, W.H., and Goresky, C.A.: Kinetics of rubidium uptake in the working dog heart. Circ. Res. 29:208-220, 1971.