
**PROSTAGLANDINS AND SKELETAL MUSCLE BLOOD FLOW/Beaty and Donald**

**Contribution of Prostaglandins to Muscle Blood Flow in Anesthetized Dogs at Rest, during Exercise, and Following Inflow Occlusion**

**ORREN BEATY III AND DAVID E. DONALD**

**SUMMARY** The role of locally formed cyclo-oxygenase products (endoperoxide intermediates, prostaglandins, or prostacyclins) in resistance to blood flow was studied in the hindlimbs of anesthetized dogs during rest, during exercise, and following release of inflow occlusion. Meclofenamic acid, indomethacin, or sodium meclofenamate reduced mean resting blood flows of 86, 153, and 118 ml/min to 54, 82, and 87 ml/min, respectively. Inhibitors of prostaglandin synthesis reduced the vasodilator response to arachidonic acid by 81%. In addition, prostaglandin synthesis inhibitors attenuated the hyperemic responses following inflow occlusions in the resting hindlimb. The attenuation was most marked following a 1-second occlusion (74%) and progressively less following a 10-second (44%) and a 300-second (24%) occlusion. However, the portion of the total postocclusive hyperemic response attributable to prostaglandins was constant and independent of occlusion duration. Inhibition of prostaglandin synthesis did not affect the hyperemia of exercise, but reduced significantly the postocclusion hyperemia that followed the release of a 1-second (83%) and a 2-second (43%) period of inflow occlusion in the exercising hindlimb; attenuation was minor following a 10-second occlusion (10%). In three of four exercising hindlimbs, the portion of the postocclusion hyperemia attributable to prostaglandins was inversely related to the duration of the occlusion. These data indicate that locally synthesized cyclo-oxygenase products, possibly prostaglandins, are important in the maintenance of blood flow in resting but not exercising muscle, contribute significantly to postocclusive hyperemia in resting and exercising hindlimbs, and mediate the hyperemia that follows occlusions of 5 seconds or less in resting and 2 seconds or less in exercising hindlimbs. *Circ Res* 44: 67-78, 1979

**PROSTAGLANDINS** have been suggested to participate in the overall control of the circulation through local regulations of blood flow to particular vascular beds (Vane and McGiff, 1975). Specifically, prostaglandin E₂, has been shown to be locally synthesized by arterial smooth muscle (Terragno et al., 1975) and to be a potent vasodilator of arterial resistance vessels (Greenberg and Sparks, 1969; Daugherty, 1971). The goal of this study was to determine whether locally synthesized products of arachidonic acid, such as prostaglandins of the E
series, might contribute to the maintenance of skeletal muscle blood flow during rest, exercise, and the hyperemia which follows the release of brief periods of arterial occlusion.

**Methods**

The experimental dogs weighed between 15 and 20 kg, and were anesthetized with sodium pentobarbital (30 mg/kg) administered intravenously. Fifty-three dogs were studied. Supplemental anesthesia was administered regularly. Prior to hindlimb perfusion the dogs were heparinized (3.0 mg/kg) and atropinized (0.2 mg/kg). Supplemental heparin (10 mg) was administered once every hour, and atropine (0.2 mg/kg) every 1.5 hours. The dogs were intubated with a cuffed endotracheal tube and ventilated with oxygen by a Harvard respirator (model 607A). The frequency of respiration was set at 10 cycles/min and the stroke volume set to produce a peak positive inspiratory pressure of 12-14/cm H2O. Arterial blood gases were monitored; P02 was always in excess of 300 mm Hg, PCO2 varied from 30 to 40 mm Hg, and the pH remained between 7.3 and 7.4.

The left hindlimb was vascularly isolated and perfused at a constant pressure of 110 mm Hg (Rowlands and Donald, 1968). Blood flow to the paw was interrupted by placing a tight ligature around the hock. The input to the pump was from the right iliac artery or the terminal aorta, and the output was to the left iliac artery. Since perfusion pressure remained constant, blood flow was inversely related to vascular resistance. Acute sympathetic denervation of the hindlimb vasculature was affected by removal of the ipsilateral lumbar sympathetic chain from L3 through S1 (Donald and Ferguson, 1970). The effect of inhibiting prostaglandin synthesis on hindlimb resistance to blood flow during rest and exercise, and on the hyperemia that followed brief periods of inflow occlusion, was examined.

**Experimental Manipulations**

Rhythmic contraction of the hindlimb was accomplished by monophasic electrical impulses delivered by a Grass stimulator (model SD5) to four needle electrodes (two positive and two negative) at 0.8 msec, 12 V, and frequencies of 1, 2, and 4 Hz. One pair of the needle electrodes was placed in the anterior thigh muscles on either side of the femoral neurovascular bundle. The other pair was placed in the posterior thigh muscles on either side of the sciatic nerve.

Reactive hyperemia was produced by mechanically occluding inflow to the resting hindlimb for 1, 5, 10, 20, 30, 60, 120, and 300 seconds, and for 1, 2, 5, and 10 seconds in the exercising hindlimb contracting once a second. The magnitude of the response was measured as the difference between control and peak postocclusion blood flows.

Prostaglandin synthesis was inhibited by treating the dog with 9 mg/kg doses of either meclofenamate,* or indomethacin† (Romero et al., 1976). The meclofenamic acid and indomethacin were dissolved in 12 ml of ethyl alcohol and diluted with 60 ml of a phosphate buffer at pH 7.4-8.0. Sodium meclofenamate was dissolved in 72 ml of normal saline. The inhibitors were infused at a rate of 1.23 ml/min into the inflow side of the perfusion pump.

To evaluate completeness of prostaglandin synthesis inhibition, the hindlimb hyperemic responses to injections of arachidonic acid, 5 µg/ml, (NuChek Prep, Inc.) were compared before and after infusion of the inhibitor drug. Prostaglandin E1 (PGE1), 9 ng/ml, was injected before and after inhibition of prostaglandin synthesis to demonstrate that exposure to the inhibitor had not affected the ability of the hindlimb resistance vessels to dilate when exposed to prostaglandins. The amount of arachidonic acid and of PGE1 injected was adjusted in accordance with the reduced blood flow so that each drug was given in the same concentration before and after the inhibitors of prostaglandin synthesis.

The arachidonic acid was prepared in 100 mM Na2CO3, stirred continuously, and stored in a nitrogen atmosphere. Prior to injection, the arachidonic acid was diluted in saline. The amount injected was 0.7 mg in a volume of 1 ml. The vehicle, 0.1 ml of sodium carbonate in 0.9 ml normal saline, had no effect on vascular resistance when injected.

In a separate series of hindlimb perfusions, inflow occlusions were carried out before and after control blood flow to the resting hindlimb had been reduced by a close intra-arterial infusion of norepinephrine to approximately 65% of that recorded prior to administration of the drug. Occlusions of 1, 5, 10, 60, and 120 seconds were made. The infusion of norepinephrine was stopped and started coincident with occlusion and release of blood flow. In those dogs in which each hindlimb was perfused in succession, the input to the pump was taken from the terminal aorta.

Prostaglandin synthesis is augmented by bradykinin (Lonigro et al., 1971; McGiff et al., 1972; Messina et al., 1975; Needleman et al., 1975; Terragno et al., 1975). This substance was dissolved in normal saline and infused into the resting hindlimb at a rate of 0.49 ng/min. The resulting blood concentration of bradykinin did not alter resting blood flow. The hyperemic response following the release of inflow occlusion was recorded before and during the continuous close-arterial infusion of bradykinin. The infusion of bradykinin was stopped coincident with inflow occlusion.

The data were analyzed by Student's t-test for paired observations and by a one-sample t-test (Dixon and Massey, 1969). The level of significance

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* Courtesy of Parke-Davis.
† Courtesy of Merck, Sharp & Dohme.
was taken as 0.05%. Grouped data are presented as means and standard errors unless stated otherwise.

Results

Presence of Prostaglandin Synthesis in the Skeletal Muscle Vascular Bed

As shown in Figure 1, the injection of arachidonic acid, the substrate for prostaglandin synthesis, resulted in a mean increase in hindlimb blood flow of 254 ± 13 ml/min (n = 4). After inhibition of prostaglandin synthesis with sodium meclofenamate, the injection of arachidonic acid resulted in a mean increase in hindlimb blood flow of 54 ± 16 ml/min (n = 4), or 19% of the previous response. Figure 1 also shows that the ability of the hindlimb resistance vessels to respond to exogenous prostaglandins was not altered by inhibition of prostaglandin synthesis. The dose of PGE\( _2 \) used was less than that required for maximal vasodilation of the hindlimb resistance vessels.

Role of Prostaglandins in the Maintenance of Resting Skeletal Muscle Blood Flow

Figure 2 displays resting hindlimb blood flow data obtained before and after inhibition of prostaglandin synthesis with either meclofenamic acid, indomethacin, or sodium meclofenamate, and similar data obtained before and after the administration of the inhibitor drug vehicle. Blood flow data were obtained in 17 dogs both before and from 70 to 90 minutes after infusion of the inhibitor was begun. Prostaglandin synthesis inhibition reduced hindlimb blood flow in each dog, the average reduction being 39% of the mean control blood flow of 114 ± 13 ml/min (n = 17). The vehicle did not affect hindlimb blood flow significantly (n = 5).

Contribution of Prostaglandins to Exercise Hyperemia

In four dogs, blood flow was measured in the hindlimb perfused at constant pressure and contracted rhythmically at 1, 2, and 4 Hz. The measurements were repeated after treatment with either indomethacin or sodium meclofenamate. As shown in Figure 3, inhibition of prostaglandin synthesis reduced the blood flow in the hindlimb at rest but not during exercise. These data indicate that, over the workloads studied, inhibition of prostaglandin synthesis does not reduce the vasodilatory response to exercise. In three dogs, arachidonic acid was injected into the hindlimb contracting at 1 Hz. Blood flow was increased significantly from a mean control of 172 ± 17 ml/min to a mean peak of 289 ± 16 ml/min.

Prostaglandins and Post-Occlusive Hyperemia

Inhibition of Prostaglandin Synthesis

Reactive hyperemia was produced in the resting dog hindlimb by occluding arterial inflow for 1, 5, 10, 20, 30, 60, 120, and 300 seconds. Figure 4 shows that the hyperemic responses that followed these periods of inflow occlusion were significantly reduced by inhibiting prostaglandin synthesis. The mean percent reduction in the postocclusion hyperemia was 74% following a 1-second occlusion and...
became progressively less as the duration of inflow occlusion was prolonged. Mean reduction was 44% after 10 seconds of occlusion and 24% after 300 seconds. Infusion of the vehicle alone did not influence the hyperemic response that followed the release of inflow occlusions (Fig. 4, lower panel).

To estimate the portion of the hyperemic response attributable to prostaglandins, the postocclusion flow increase in milliliters per minute following treatment with the inhibitor was subtracted from the postocclusion flow increase prior to treatment. These blood flow differences were regressed linearly on time, and the slope and intercept computed for each dog. A one-sample t-test showed that the mean intercept (67 ± 21.2 ml/min, n = 5) was significantly greater than zero. Thus, the effect of the prostaglandin synthesis inhibitor was significant. The mean slope of the line of regression (—0.03 ± 0.18 ml/min per second of occlusion, n = 5) was not significantly different from zero. This indicated that the hyperemic contribution of endogenous prostaglandins following the release of inflow occlusion lasted 1–300 seconds was independent of occlusion duration.

**Reduction in Control Blood Flow**

Mean data from eight hindlimb perfusions are shown in Figure 5. Mean control blood flow was 148 ± 10 ml/min; blood flow during norepinephrine infusion was 96 ± 7 ml/min, an average reduction of 35% of control blood flow. Analysis by the one-sample t-test of the 40 pairs of postocclusion increases in blood flow in the control state and during norepinephrine infusion showed that the catecholamine-induced reduction in baseline blood flow had no significant effect on the absolute value of the hyperemic response. The increases in blood flow that followed occlusions of 5, 10, 60, and 120 seconds in the control state and during the infusion of norepinephrine were not significantly different. The

**Figure 3** Failure of inhibition of prostaglandin synthesis to depress vasodilation during exercise in four dogs. Measurement of blood flow in dog hindlimb perfused at constant pressure at rest and during muscle contractions, at 1, 2, and 4 per second before (○) and after (●, □) treatment with indomethacin or with sodium meclofenamate. Blood flows measured 75 minutes after treatment with indomethacin (○) were not different from those measured after 165 minutes (■).

**Figure 4** Mean (± SE) resting and peak blood flows in dog hindlimb perfused at constant pressure following arterial occlusions of 1–300 seconds before (○, ◦) and after (●, □) inhibition of prostaglandin synthesis by meclofenamic acid (top panel). The hyperemic response following each occlusion was attenuated significantly following inhibition of prostaglandin synthesis. As the period of occlusion was increased, the percent attenuation of the hyperemic response was progressively smaller, but the absolute reduction in terms of milliliters per minute remained constant. Infusion of the vehicle alone was without significant effect on either resting or peak hyperemic blood flows (bottom panel).
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600
- 400
O
O
O
- 200
Q
200
- 100
O
O
O
- 0
Q
O
O
O
- 400
P<br>Baseline OCCLUSION

FIGURE 5
Mean (± SE) resting and peak blood flows in dog hindlimbs perfused at constant pressure following occlusions of 1-120 seconds before (●, △) and during (○, ▲) close intra-arterial infusion of norepinephrine. Baseline blood flow was reduced by an average of 35% during catecholamine infusion. Hyperemic responses during infusion of norepinephrine were not significantly less than in control situation.

Increase in blood flow that followed a 1-second occlusion was greater during norepinephrine infusion in seven of eight perfusions. The mean ratio of the postocclusion increase in blood flow in the control state to that during infusion of norepinephrine was 0.87 ± 0.06 (n = 8). The ratio of the hyperemic responses before and after inhibition of prostaglandin synthesis was 1.86 ± 0.07 (n = 5). These ratios are significantly different.

Augmentation of Prostaglandin Synthesis

To evaluate further the role of prostaglandins in postocclusion hyperemia, bradykinin was infused with the object of increasing the local synthesis and release of prostaglandins. Figure 6 shows mean data from four dogs. The infusion of bradykinin at 49 ng/min did not alter significantly the resting hindlimb blood flow (72 ± 12 ml/min before, and 78 ± 13 ml/min during bradykinin infusion). However, in each dog, treatment with bradykinin augmented significantly the hyperemic responses following the release of the 5- and 10-second periods of arterial occlusion. The mean increase in blood flow of 208 ± 21 ml/min following release of a 5-second arterial occlusion during the infusion of bradykinin was significantly greater than the mean control increase of 145 ± 24 ml/min. Similarly, during bradykinin infusion, the mean increase in blood flow of 209 ± 40 ml/min after the release of a 10-second occlusion was significantly greater than the mean control increase of 152 ± 24 ml/min.

Reactive Hyperemia during Exercise

Reactive hyperemia also was produced before and after indomethacin or sodium meclofenamate in the rhythmically contracting dog hindlimb (1 contraction/sec) by occlusion of inflow for 1, 2, 5, and 10 seconds. Neither of the drugs significantly affected the control exercise blood flows. Figure 7 shows that inhibition of prostaglandin synthesis significantly reduced the ability of the resistance vessels of exercising skeletal muscle to dilate following inflow occlusions of 1 second (63% reduction) and 2 seconds (43% reduction). At 5 and 10 seconds of occlusion, the hyperemic response was attenuated by 16% and 10%, respectively. As in the resting hindlimb, the hyperemic response increased progressively with the duration of the occlusion. The portion of the postocclusion hyperemia attributable to the vasodilator effect of endogenous prostaglandins was depressed on the time of occlusion as described above for the quiescent hindlimb. These data are presented in Figure 8. The mean intercept for the four lines of regression (60.0 ± 12.4) was significantly greater than zero, indicating
Blood Flow PG Inhibition Exercise Post-Occlusion
Before After

**FIGURE 7** Control and peak blood flows following arterial occlusions of 1–10 seconds before and after inhibition of prostaglandin synthesis in four dog hindlimbs perfused at constant pressure and contracted once per second. The hyperemic response following 1 second of occlusion was reduced by 63% following inhibition of prostaglandin synthesis. The flow response following 10 seconds of occlusion was reduced by an average of 10%.

There was a negative correlation between the blood flow response due to the presence of endogenous prostaglandins and the duration of occlusion in three of four rhythmically contracting hindlimbs (−0.953, −0.961, −0.939 ml/min per second of occlusion).

**Discussion**

This study indicates that local synthesis and release of prostaglandins contribute significantly to the maintenance of blood flow in the resting dog hindlimb, and principally to the increase in blood flow that follows release of short-lasting inflow occlusions in resting and exercising hindlimbs. Inhibition of prostaglandin synthesis increased resting hindlimb vascular resistance but did not alter the vascular resistance of the rhythmically contracting hindlimb.

The possibility that the reduction in blood flow to the resting hindlimb and in the postocclusion hyperemia seen after inhibition of prostaglandin synthesis was due to a nonspecific vasoconstrictive action of the inhibitory agent must be considered. The following observations would argue against this being the case. In the present study, three different inhibitors of prostaglandin synthesis were used, two of which were completely different in molecular structure (Flower, 1974). Blood flow to the resting hindlimb was reduced to a similar degree following treatment with each drug (average reduction, 39%). After inhibition of prostaglandin synthesis, the absolute value of the hyperemic response was on the average 65% of that recorded prior to treatment with the inhibitor drug. After reduction of blood flow to the resting limb to a degree similar to that caused by inhibition of prostaglandin synthesis (average reduction induced by norepinephrine was 35%), the absolute value of the hyperemic response was not significantly different from that observed before the catecholamine-induced vasoconstriction. Repetitive electrically induced contractions of the hindlimb muscles at 1 Hz increased blood flow by an average of 100 ml/min. Inhibition of prostaglandin synthesis did not reduce this exercise-induced hyperemia, but did result in reductions of 63% and 43% in the increases in blood flow that followed occlusions of 1 and 2 seconds, respectively. Similarly, intravenous infusion of indomethacin (10 mg/kg) into conscious dogs has been reported not to alter renal blood flow or resistance, but to reduce significantly the increase in blood flow that followed a 15-second occlusion of the renal artery (Swain et al., 1975). Thus it is more likely that the reductions

**FIGURE 8** Relation of the difference in the postocclusion blood flow responses before and after inhibition of prostaglandin synthesis to the duration of occlusion in four exercising dog hindlimbs. The calculated regression lines and actual data points are shown for each dog. In three of the dogs there is a significant negative correlation between the blood flow response attributable to prostaglandins and the duration of the inflow occlusion.
in blood flow to the resting hindlimb and in the hyperemia that followed brief occlusions of inflow to resting and exercising muscle were due to suppression of prostaglandin synthesis than to a non-specific vasoconstrictive action of the drug.

In the kidney, blockade of prostaglandin synthesis reduced renal blood flow and the renal venous concentration of prostaglandin-like substances, and abolished the release of prostaglandins induced by the infusion of angiotensin II into the renal artery (McGiff et al., 1970; Aiken and Vane, 1973; Lonigro et al., 1973). There is thus ample evidence for a continuous synthesis and release of prostaglandin from the renal vasculature. There are fewer studies on the hindlimb vessels, and the data are conflicting. Lonigro et al. (1973) reported a 13% increase in hindlimb vascular resistance 15 minutes after treatment with indomethacin. Aiken and Vane (1973) found no statistically significant change in hindlimb blood flow or resistance following treatment with this agent. They also reported that an intra-arterial infusion of angiotensin II into the hindlimb was not accompanied by the appearance of prostaglandin-like substances in the venous outflow. In another study, prostaglandin-like substances were found in the venous effluent from the dog hindlimb during simulated exercise or inflow occlusion (Herbaczynska-Cedro et al., 1974).

In the present study, indirect evidence indicated that the vasculature of the dog hindlimb contained the necessary components for the synthesis of prostaglandins. Arachidonic acid, the substrate for the production of prostaglandin compounds, elicited an increase in blood flow when injected into the hindlimb circulation. Specific inhibition of prostaglandin synthesis reduced this hyperemic response to injected arachidonic acid by 81% but did not alter the hyperemic response to injected PGE1. The residual response to arachidonic acid was attributed to a direct effect of this fatty acid on the resistance vessels and to incomplete inhibition of prostaglandin synthesis. Similar vasodilator effects of injected arachidonic acid have been described previously in the dog (Rose et al., 1974; Ryan and Zimmerman, 1974) and in the spontaneously hypertensive rat (Cohen et al., 1973). Thus it is reasonable to consider that the dog hindlimb vasculature is capable of synthesizing prostaglandins. However, Fitzpatrick et al. (1977) have reported the opposite finding that intra-arterial infusion of arachidonic acid resulted in vasoconstriction of the perfused hindlimb. There is no immediate explanation for this difference. In the preparation used by Fitzpatrick et al. (1977), the hind paw circulation was intact. This area is predominantly a cutaneous vascular bed, and frequently exhibits a different behavior from that of the vasculature of the hindlimb muscles (Hanley et al., 1971). In addition, according to Fitzpatrick et al. (1977), only that portion of the hindlimb distal to the right femoral artery was perfused. The paw would in this case account for a considerably greater portion of the circulation. Recently, Greenberg et al. (1977) have shown that prostaglandin B2, a potent constrictor of both pulmonary and cutaneous vasculatures, causes vasoconstriction of the canine hind paw.

The ability of hindlimb muscle resistance vessels to dilate during exercise was not affected by inhibition of prostaglandin synthesis. In the working heart, inhibition of prostaglandin synthesis has been reported either not to alter coronary blood flow (Afonso et al., 1974; Alexander et al., 1975; Block et al., 1975; Owen et al., 1975) or to cause a slight decrease which was statistically significant in one study (−14%) (Hintz and Kaley, 1977) but not in the other (−13%) (Alexander et al., 1975). Thus prostaglandins do not appear to make a major contribution to the lowered resistance to blood flow which accompanies rhythmic contraction of cardiac and skeletal muscle. However, prostaglandins have been detected in the venous outflow from contracting skeletal muscle of the dog (Herbaczynska-Cedro et al., 1974) and from the working heart of the dog and the rabbit (Alexander et al., 1975; Block et al., 1975). After treatment with indomethacin, prostaglandins either were not detected (Herbaczynska-Cedro et al., 1974; Block et al., 1975) or were produced in reduced amounts (Alexander et al., 1975).

In the present study, the infusion of arachidonic acid into the exercising hindlimb resulted in a further increase in blood flow. Similarly, in the intact heart of the dog, an intracoronary injection of arachidonic acid caused an increase in coronary blood flow; this increase was abolished following intravenous administration of indomethacin (Hintz and Kaley, 1977). Thus there is the paradox that the prostaglandin enzyme system appears to be intact and functioning in contracting skeletal and cardiac muscle, yet blockade of synthesis does not result in an increase in vascular resistance such as was observed in the resting limb.

In contrast to the hyperemia of exercise, the local release of prostaglandins contributed significantly to postocclusion hyperemia in the hindlimb. As has been reported previously for the kidney (Aiken and Vane, 1973; Lonigro et al., 1973; Swain et al., 1975; Herbaczynska-Cedro and Vane, 1974), inhibition of prostaglandin synthesis in the present studies reduced both the basal level of blood flow in the resting hindlimb and the reactive hyperemia that followed release of inflow occlusion. Infusion of bradykinin, a substance previously reported to increase local synthesis and release of prostaglandins (Lonigro et al., 1971; McGiff et al., 1972; Messina et al., 1973; Needleman et al., 1975; Terragno et al., 1975), significantly increased the hyperemic response that followed inflow occlusions of 5 and 10 seconds in the resting hindlimb. Endogenous prostaglandins thus appeared to be principally responsible for the increase in blood flow which followed release of a 1- to 5-second occlusion in resting muscle, and a 1- to 2-second occlusion in exercising
muscle. As occlusion was prolonged beyond these times, accumulated vasodilator metabolites became the principal determinants of the hyperemic response in both resting and exercising muscle. The observations that reactive hyperemia in the contracting heart is not influenced by blockade of prostaglandin synthesis (Owen et al., 1975; Hintz and Kaley, 1977) are not at variance with the present observations in contracting skeletal muscle. In the former studies the shortest period of occlusion was 5 seconds; in contracting skeletal muscle there was no significant difference in the hyperemic response to a 5-second occlusion before and after blockade of prostaglandin synthesis. However, Alexander et al. (1975) have reported that, in the beating dog heart, blockade of prostaglandin synthesis results in a significant reduction in reactive hyperemia and in release of prostaglandin-like substances following occlusions of 10-20 seconds.

The present experiments do not provide an explanation for the finding that, in the resting hindlimb, the vasodilator contribution of endogenous prostaglandins to reactive hyperemia was constant and independent of duration of occlusion, whereas in the contracting hindlimb the vasodilator contribution progressively diminished as the duration of occlusion was prolonged. It could be argued that the reduction in vascular tension which followed occlusion made a constant amount of the substrate, arachidonic acid, available for the synthesis of vasoactive products. As has been previously reported, this reaction requires oxygen (Van Dorp et al., 1964; Samuelsson, 1965; Nugteren et al., 1967; Samuelsson et al., 1967). In the resting muscle there apparently was sufficient O2 for the reaction to be completed, and thus, up to 300 seconds of occlusion, the vasodilator products of prostaglandin synthesis made a constant contribution to the postocclusion hyperemia. However, in the exercising muscle, the tissue PO2 would decline rapidly following occlusion. This would progressively limit the synthetase activity such that at 5 seconds of occlusion an insignificant amount of vasodilator product would be generated from the substrate made available by reduction in vascular tension. This explanation remains speculative, since the mechanism for the increased production of prostaglandins remains to be determined (Piper and Vane, 1971; Afonso et al., 1974). Also remaining to be determined is whether this increase is due to a greater availability of substrate, accelerated synthetase activity, or both.

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The Effect of Brief Vagal Stimulation on the Isolated Rabbit Sinus Node

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SUMMARY We developed an isolated rabbit atrial preparation which responds consistently and reproducibly to brief, submaximal stimulation of the autonomic nerves contained in it. In 8 of 11 preparations in the presence of propranolol (1 mg/liter), the time course of changes in the atrial rate following 120 msec vagal stimulation was bimodal. The maximal slowing occurred at 0.64 ± 0.16 second, and the peak secondary slowing occurred at 2.3 ± 1.0 seconds. An acceleratory component occurred between the first and second peaks between 0.8 and 1.8 seconds. The total time course of vagal effect lasted for 5.0 ± 2.0 seconds. These changes in rate could not be explained by shifts in the location of the primary pacemaker. The acceleratory component was due to a 4.7 ± 2.0 (SD) mV depolarization of the maximum diastolic membrane potential of the primary pacemaker of the sinus node which lasted for 1.8 ± 0.3 seconds. Following vagal stimulation, there was an increase of 0.2 mM in the activity of potassium in the extracellular space recorded with a potassium-sensitive electrode; this peaked between 1.4 and 2.5 seconds and cleared with an exponential time course. The half-times for recovery ranged between 2.8 and 4.6 seconds. The initial peak slowing of the bimodal time course and the acceleratory component therefore appear to be direct effects of acetylcholine. The secondary slowing occurs after acetylcholine presumably has been inactivated and occurs coincidently with the accumulation of potassium in the extracellular space. Circ Res 44: 75-88, 1979

IN 1845, Weber and Weber were first to demonstrate that vagal stimulation slows the heart. In 1934, Brown and Eccles described for the cat sinus node the time course of the changes in spontaneous rhythm following a single vagal volley. Characteristically, the time course of rhythm change exhibits two inhibitory peaks separated by a transient period of acceleration in which spontaneous rate may exceed control. Similar time courses have been described for vagal stimulation in both dogs and rabbits (Stade and Weiss, 1956; Levy et al. 1970; Spear and Moore, 1973). In addition, it has been shown that there is a double-peaked inhibitory time course following vagal stimulation for pacemakers located within the atrioventricular junction (Spear and Moore, 1973).

Experiments from several laboratories have provided information about the mechanism of this double-peaked response to vagal stimulation. Since it is not abolished by β-adrenergic blockade (Brown and Eccles, 1964; Levy et al., 1970; Spear and Moore, 1973), the acceleratory component of the time course of vagal effect cannot be due to a contribution from sympathetic stimulation. Also, the response of the sinus node to sympathetic stim-
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