19. Guisado R, Arieff AI, Massry SG: Effects of glycerol infusions on brain water and electrolytes. Am J Physiol **227:** 865-872, 1974
20. Buchborn E, Edel H: Akutes Nierenversagen. *In* Handbuch der Inneren Medizin VIII/2, edited by H Schwiegk. New York, Springer-Verlag, 1968, pp 942-1083
21. Dunn FL, Brennan TJ, Nelson AF, Robertson GL: The role of blood osmolality and volume in regulating vasopressin secretion in the rat. J Clin Invest **52:** 3212-3219, 1973
22. Paton AM, Lever AF, Oliver NWJ, Medina A, Briggs JD, Morton JJ, Brown JJ, Robertson JIS, Fraser R, Tree M, Gavras H: Plasma angiotensin II, renin, renin-substrate and aldosterone concentrations in acute renal failure in man. Clin Nephrol **3:** 18-23, 1975
23. Konrads A, Hofbauer KG, Bauereiss K, Hackenthal E, Möhring B, Möhring J, Gross F: Systemic vasoconstriction in glycerol-induced acute renal failure of rats. Pfluegers Arch **365** (suppl): R-13, 1976
24. Nakano J: Cardiovascular actions of vasopressin. Jap Circ J **37:** 363-381, 1973
25. Owen K, Desautels R, Walter CW: Experimental renal tubular necrosis—the effect of pitressin. Surg Forum **4:** 459-463, 1953
26. Oken DE, Arce ML, Wilson DR: Glycerol-induced hemoglobinuric acute renal failure in the rat. I. Micropuncture study of the development of oliguria. J Clin Invest **45:** 724-735, 1966
27. Wilson DR, Thiel G, Arce ML, Oken DE: The role of the concentration mechanism in the development of acute renal failure: micropuncture studies using diabetes insipidus rats. Nephron **6:** 128-139, 1969
28. Bauereiss K, Hofbauer KG, Konrads A, Gross F: Effects of serum and saralasin in glycerol-induced acute renal failure of rats. Naunyn Schmiedebergs Arch Pharmacol **293** (suppl): R-38, 1976
29. Hofbauer KG, Bauereiss K, Zschiedrich H, Gross F: Effects of saralasin on renal function in the rat. Prog Biochem Pharmacol **12:** 63-83, 1976
30. Hofbauer KG, Bauereiss K, Hackenthal E, Konrads A, Möhring B, Möhring J: Vasopressin and renin in glycerol-induced acute renal failure of rats. Eur J Clin Invest **6:** 322, 1976
31. Hofbauer KG, Bauereiss K, Konrads A, Hackenthal E, Möhring B, Möhring J, Gross F: Release of vasopressin and renin in glycerol-induced acute renal failure of rats. Pfluegers Arch, **362** (suppl): R-12, 1976

5-Hydroxytryptamine and Neurotransmitter Release in Canine Blood Vessels

Inhibition by Low and Augmentation by High Concentrations

MICHAEL A. MCGRATH

SUMMARY **Experiments were designed to determine the effect of 5-hydroxytryptamine on adrenergic neurotransmission in blood vessels. Strips from canine saphenous veins and tibial arteries were incubated in norepinephrine[7-^{3}H] and mounted for superfusion and isometric tension recording. The superfusate was collected for estimation of total radioactivity and for column chromatographic separation of norepinephrine[7-^{3}H] and its metabolites. 5-Hydroxytryptamine (5-HT), 10^{-8} M and 10^{-7} M, inhibited the increase in smooth muscle tension and the release of norepinephrine[7-^{3}H] caused by transmural electric stimulation of the sympathetic nerve endings. By contrast, the increase in tension caused by direct stimulation of the smooth muscle with norepinephrine was either unchanged or augmented. In addition, 5-HT augmented the increase in tension with tyramine but did not affect the release of radiolabeled compounds by this substance. In unstimulated preparations low concentrations of 5-HT (10^{-7} M) caused contraction but did not affect the release of norepinephrine[7-^{3}H]. With a higher concentration (10^{-5} M) the release of neurotransmitter was markedly increased. This response was inhibited by cocaine. Whereas 5-HT-induced contractions were inhibited by phentolamine and methysergide, these antagonists had no effect on its inhibitory action at the sympathetic nerve ending. Hence, low concentrations of 5-HT depress sympathetic tone by inhibiting the release of transmitter during nerve depolarization. At higher concentrations 5-HT has a direct excitatory effect on vascular smooth muscle together with an indirect effect which involves the uptake of 5-HT into the sympathetic nerve ending via the cocaine-sensitive mechanism and the release of norepinephrine.**

IN HUMANS the intra-arterial infusion of 5-hydroxytryptamine (5-HT) consistently causes a decrease in skin blood flow.[1-3] However, its effect on total limb and skeletal muscle blood flow is more complex. For example, in small doses it causes an increase in forearm and calf blood flows but when large doses are infused the increase in flow usually is followed by a dose-dependent decrease.[1, 3-5] Studies on isolated blood vessels support the conclusion that the vasoconstriction is predominantly a direct effect of 5-HT on the vascular smooth muscle.[6-9] In several tissues, for example, rabbit heart,[10] guinea pig vas deferens,[11] and cat spleen capsule,[12] 5-HT causes the release of norepinephrine from the sympathetic nerve endings; however, it is not known whether an indirect sympathomimetic action contributes to the vasoconstriction induced by this substance.

Although the mechanism of the increase in limb blood flow with 5-HT is unknown, there are several observations which suggest that this may result, at least in part, from the inhibition of neurogenic vasoconstriction. For example, it has been demonstrated in animal experiments that the

From the Department of Physiology and Biophysics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.

Michael A. McGrath was supported by a Bushell Travelling Fellowship in Medicine or the Allied Sciences of the Royal Australasian College of Physicians and by the Mayo Foundation. This investigation was supported in part by Research Grant HL 5883 from the National Institutes of Health.

Address for reprints: Dr. Michael A. McGrath, c/o Section of Publications, Mayo Clinic, 200 First Street S.W., Rochester, Minnesota 55901.

Received September 7, 1976; accepted for publication February 23, 1977.

response of skeletal muscle and limb resistance vessels depends on their basal resistance. 5-HT increased vascular resistance in the presence of an initial low resistance, but this increase could be reversed by increasing baseline resistance via sympathetic nerve stimulation.[13-15] By contrast, 5-HT does not oppose the vasoconstriction by norepinephrine or tyramine.[15] In other experiments it was shown that, after the intravenous infusion of this substance, blood pressure falls when neurogenic tone is high and rises when it is low.[16] In addition, direct microscopic studies have demonstrated that 5-HT causes a relaxation of mesenteric vessels constricted by sympathetic nerve stimulation[15] and an active dilation of human skin vessels that appears to be related to the initial vasoconstrictor tone of these vessels.[17]

These observations prompted experiments designed to determine whether 5-HT effects adrenergic neurotransmission in blood vessels and to analyze the relationship between actions on the sympathetic nerve ending and its direct effect on the vascular smooth muscle cells.

Methods

The experiments were performed on helical strips (20–25 mm long, 2–3 mm wide, 55–75 mg) of lateral saphenous veins and anterior tibial arteries taken from dogs (15–30 kg) anesthetized with sodium pentobarbital (30 mg/kg, iv). Each strip was placed in a chamber filled with Krebs-Ringer solution (NaCl, 118.2 mM; KCl, 4.7 mM; $MgSO_4$, 1.2 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.5 mM; $NaHCO_3$, 25.0 mM; calcium disodium ethylenediaminetetraacetate, 0.026 mM; and glucose, 5.5 mM) maintained at 37°C and aerated with a 95% O_2-5% CO_2 mixture. The strips were connected to a force transducer (Grass FT .03C) for isometric tension recording. The transducer was mounted on a movable support that allowed fine adjustment of the strip length. The strip tension was recorded on a direct pen-writing recorder (Gould Brush 220). Two rectangular platinum electrodes (30 mm by 4 mm; 0.5 mm thick) were positioned parallel to the vessel strips. The electrical impulses consisted of square waves (9 V, 2 msec) from a direct current power supply with a switching transistor (RCA 1-2N-3034) triggered by a stimulator (Grass SD5).

After an equilibration period (30–60 minutes), the length of each strip was increased progressively by increments of 2 mm until the contraction caused by a standard electrical stimulus reached a maximum. The length at which this occurred was maintained throughout the experiment.

In other experiments the saphenous vein and tibial artery strips were incubated for 2 hours in Krebs-Ringer solution containing norepinephrine[7-^{3}H] at 7.8×10^{-8} M (specific activity = 8.634 Ci/mmol, New England Nuclear) and ascorbic acid (1.1×10^{-6} M). Then the strips were transferred to a fresh labeled solution for 2 more hours. At the end of incubation the strips were rinsed in fresh Krebs-Ringer solution and mounted for superfusion. Samples (1 ml) of the superfusate were added to 10 ml of Insta-Gel emulsifier (Packard), and radioactivity was measured in a liquid scintillation counter. Corrections for quenching were made with an external standard. The counting efficiency was 36%. Unless otherwise indicated, data are expressed as disintegrations per minute (dpm) per minute of superfusion. Selected samples of the superfusate were taken for column chromatographic separation of norepinephrine and its metabolites as previously described.[18] Catechol compounds (norepinephrine and deaminated metabolites) were separated from non-catechol compounds (deaminated, *O*-methylated metabolites and normetanephrine) and from tritiated water by adsorption on alumina at pH 8.4 and subsequent elution with acid. Norepinephrine was separated from deaminated metabolites and normetanephrine was separated from deaminated, *O*-methylated metabolites by adsorption on Dowex 50W-X4 resin. Radioactivity was measured on 1-ml duplicate samples of each effluent and eluate. A standard sample of norepinephrine[7-^{3}H] was run through the chromatographic procedure with each experiment. The overall recovery of this sample was 79 ± 1%.

The following pharmacological agents were used: *l*-norepinephrine bitartrate (Levophed, Winthrop); tyramine hydrochloride (Abbott); 5-hydroxytryptamine creatinine sulfate complex (Sigma); methysergide maleate (Sandoz); metiamide (Smith, Kline and French); sodium meclofenamate (Parke, Davis); atropine sulfate (Lilly); phentolamine mesylate (Regitine, CIBA); morphine sulphate; and propranolol hydrochloride (Ayerst). The doses of each drug are expressed as final bath concentrations of the salts. Dose-response studies were carried out in a cumulative manner. The drugs were removed from the bath by overflowing the preparations with aerated Krebs-Ringer solution at 37°C. In experiments using potassium chloride the concentration of this salt was increased in equimolar replacement for sodium chloride in the Krebs-Ringer solution.

In each group of experiments, only one vessel strip was used from any one dog. The data are expressed as means ± SE. Statistical analyses were performed using Student's *t*-test. A P value of less than 0.05 was considered significant.

Results

RESTING STRIPS

5-HT caused saphenous vein strips to contract. In six strips the effects of increasing concentrations of this substance were determined and compared with the response to norepinephrine in the same preparations. The maximum contraction with 5-HT was about half the maximum response to norepinephrine whereas the median effective dose (ED_{50}) for norepinephrine was almost 10 times greater than the ED_{50} for 5-HT (Fig. 1). Tachyphylaxis to 5-HT was not observed.

The log dose-response curve to 5-HT was shifted to the right in a parallel manner by phentolamine (3.6×10^{-6} M; dose ratio = 5; $n = 6$) and methysergide (10^{-7} M; dose ratio = 6; $n = 6$). At these concentrations the antagonists had no direct contractile effects.

In six strips, cocaine (3.3×10^{-5} M) caused an increase in the maximum contraction by 5-HT and the ED_{50} was reduced from 1.1×10^{-7} to 7×10^{-8} M (Fig. 2). By contrast, in six other strips, cocaine (3.3×10^{-5} M) caused a parallel shift of the norepinephrine log dose-response

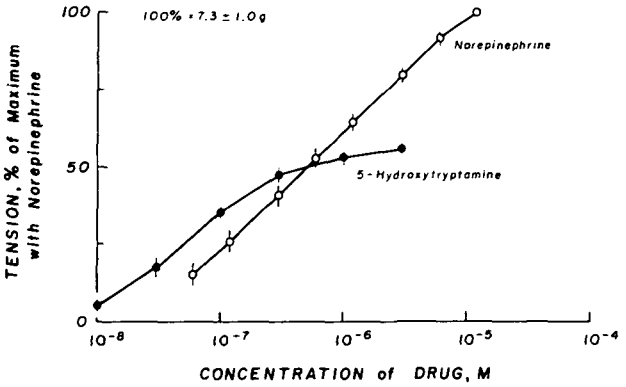

FIGURE 1 *Cumulative dose-response curves of canine saphenous vein strips with 5-hydroxytryptamine and norepinephrine (mean ± SE; six strips).*

curve to the left. The ED_{50} was reduced from 9×10^{-7} to 1.7×10^{-7} M.

STIMULATED STRIPS

Figure 3 shows the record from an experiment in which 5-HT (10^{-8} M and 10^{-7} M) was given during contraction by electrical stimulation (1 Hz), tyramine (5.8×10^{-6} M), and norepinephrine (3×10^{-7} M). This frequency of stimulation and the doses of the agonists are all approximately ED_{50} for this preparation. 5-HT caused a relaxation of the electrically induced contractions; at the higher concentration the response to 5-HT was characterized by a secondary increase in tension. There was no evidence of a relaxation during contractions caused by tyramine and norepinephrine. Thus, 5-HT caused a decrease in tension when the neurogenic tone was high and an increase in tension when it was low. The results of similar experiments on paired strips from 10 dogs are summarized in Figure 4. In these experiments the tension was measured at 1 minute after the addition of 5-HT to the bath.

Experiments were carried out to determine whether the smooth muscle relaxation caused by 5-HT was mediated either through β-adrenergic, H_2-histamine, or muscarinic acetylcholine receptors or by the release of prostaglandins. Each strip was made to contract by electrical stimulation (9 V, 1 Hz), and 5-HT (10^{-8} M) was added to cause a relaxation. The experiment then was repeated after incubation of the strip for 10 minutes in Krebs-Ringer solution containing either propranolol (3.9×10^{-6} M; four strips), atropine (1.5×10^{-7} M; four strips), metiamide (10^{-5} M; four strips), or sodium meclofenamate (3×10^{-5} M; four strips). The doses of these antagonists are sufficient to significantly inhibit the effect of the specific agonists or prostaglandin metabolic pathway in this preparation;[18] however, none of them attenuated the 5-HT-induced relaxation.

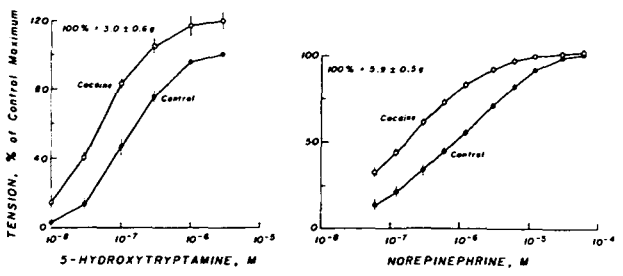

FIGURE 2 *Comparison of the effects of cocaine (3.3×10^{-5} M) on cumulative dose-response curves of canine saphenous vein strips with 5-hydroxytryptamine and norepinephrine (mean ± SE; six strips).*

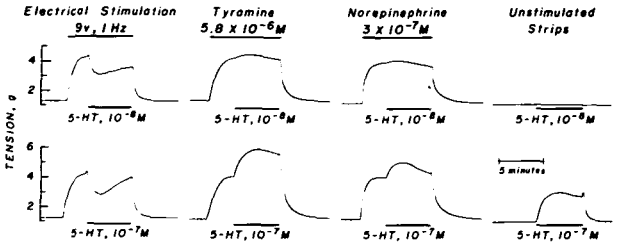

FIGURE 3 *Effects of two concentrations of 5-hydroxytryptamine on contractions of canine saphenous vein strips induced by electrical stimulation, tyramine and norepinephrine. The direct effect of these concentrations of 5-hydroxytryptamine is shown.*

RELEASE OF RADIOLABELED NOREPINEPHRINE AND METABOLITES BY 5-HT

In four saphenous vein strips the effects of two concentrations of 5-HT on tension and the release of radioactive norepinephrine and its metabolites were determined. The contractions that occurred with 10^{-7} M were not associated with any significant change in the release of norepinephrine[7-^{3}H] (Fig. 5 and Table 1). The higher concentration of 5-HT (10^{-5} M) caused a marked increase in the release of norepinephrine[7-^{3}H] and of the deaminated and *O*-methylated metabolite fractions.

In four additional strips, cocaine (3.3×10^{-5} M) inhibited the release of radioactive compounds caused by 5-HT (10^{-5} M) but did not reduce the contractions (Fig. 5).

RELEASE OF RADIOLABELED COMPOUNDS BY ELECTRICAL STIMULATION

In six saphenous vein strips electrical stimulation (1 Hz) caused a sustained contraction and an augmentation of the release of radioactive compounds into the superfusate. 5-

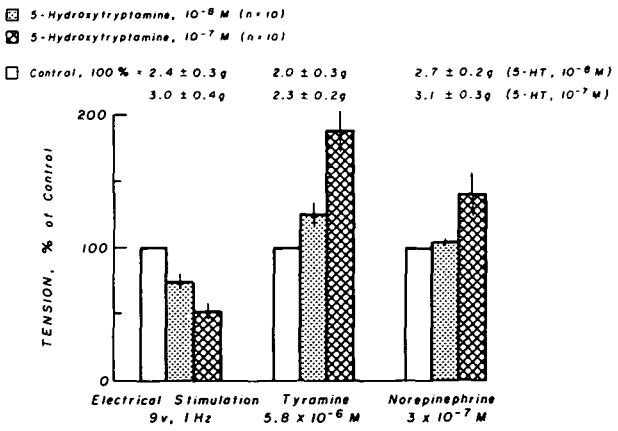

FIGURE 4 *Effects of two concentrations of 5-hydroxytryptamine on contractions of canine saphenous vein strips by electrical stimulation, tyramine and norepinephrine. The direct effect of the low and high concentrations of 5-hydroxytryptamine was <0.1 g and 2.7 ± 0.2 g, respectively (mean ± SE). n = number of strips.*

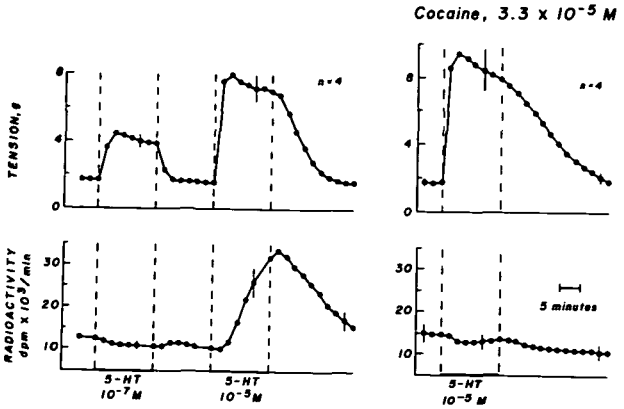

FIGURE 5 *Comparison of the effects of two concentrations of 5-hydroxytryptamine on tension (top) and efflux of radiolabeled compounds (bottom) from canine saphenous vein strips. The influence of cocaine on the responses to the high concentration of 5-hydroxytryptamine is demonstrated (mean ± SE). n = number of strips.*

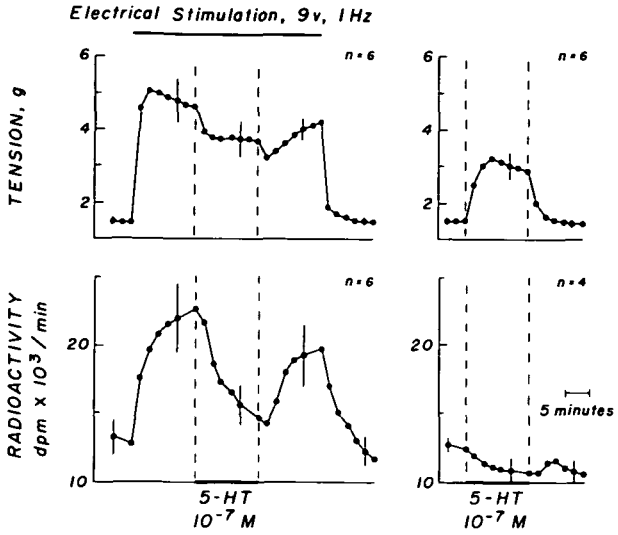

FIGURE 6 *Effects of 5-hydroxytryptamine on tension (top) and efflux of radiolabeled compounds (bottom) from canine saphenous vein strips during electrical stimulation and at rest (mean ± SE). n = number of strips.*

HT (10^{-7} M) given during the contraction caused an immediate relaxation that was paralleled by a marked decrease in the radioactivity of the superfusate. These effects were reversible. In the absence of electrical stimulation, 5-HT (10^{-7} M) applied to these strips caused a contraction (Fig. 6).

Samples of the superfusate were collected during four 2-minute periods for analysis by column chromatography. These periods were (1) immediately prior to electrical stimulation (basal), (2) after 10 minutes of electrical stimulation, (3) during electrical stimulation in the presence of 5-HT, and (4) 10 minutes after stopping the infusion of 5-HT. Electrical stimulation increased the release of norepinephrine[7-^{3}H] and, to a lesser degree, its metabolites. 5-HT caused a significant and reversible decrease in the release of all fractions; the norepinephrine[7-^{3}H] was decreased more than its metabolites (Fig. 7). Cocaine (3.3 × 10^{-5} M) did not inhibit this action of 5-HT (four strips).

Experiments were carried out to determine whether the inhibition of adrenergic neurotransmission was mediated by prejunctional α-adrenergic receptors. Control studies (three saphenous vein strips) demonstrated that norepinephrine (6 × 10^{-7} M) markedly inhibited the release of radiolabeled norepinephrine during electrical stimulation (1 Hz) of saphenous vein strips superfused with Krebs-Ringer solution containing cocaine (3.3 × 10^{-5} M). This action of norepinephrine was inhibited by phentolamine (3.6 × 10^{-6} M) (Fig. 8). However, phentolamine (3.6 × 10^{-6} M) had no effect on the depressor action of 5-HT (10^{-7} M) either in the presence (three strips) or absence (two strips) of cocaine (3.3 × 10^{-5} M).

Additional experiments were carried out with methysergide (10^{-7} M; four strips) and morphine (6.5 × 10^{-6} M; four strips). Neither substance inhibited the depressor action of 5-HT during electrical stimulation. At higher concentrations each of these substances had a direct depressor effect on adrenergic neurotransmission which prevented their use in further attempts to define the nature of the prejunctional 5-HT receptor.

In four tibial artery strips, electrical stimulation (2 Hz) caused a sustained contraction (1.0 ± 0.2 g) together with an increase in the release of radiolabeled compounds from 6.1 ± 0.3 dpm × 10^3 (basal) to 11.7 ± 1.2 dpm × 10^3. 5-

TABLE 1 *Effect of 5-Hydroxytryptamine (5-HT) on Overflow of Norepinephrine[7-^{3}H] and Metabolites From Canine Saphenous Vein Strips*

Experimental condition	Radioactivity (dpm)*			
		Metabolites		
	Norepinephrine	Deaminated	Deaminated, *O*-methylated	*O*-methylated
Basal	1.0 ± 0.1	10.3 ± 1.7	7.4 ± 0.7	0.7 ± 0.1
5-HT (10^{-7} M)	0.9 ± 0.1	9.6 ± 1.9	6.4 ± 0.5†	0.6 ± 0.1
% basal	85 ± 5	92 ± 4	86 ± 2	89 ± 4
Basal	0.9 ± 0.1	9.6 ± 1.9	6.8 ± 0.7	0.6 ± 0.1
5-HT (10^{-5} M)	11.2 ± 1.9†	27.1 ± 4.3†	7.5 ± 0.6	4.0 ± 0.5†
% basal	1266 ± 146	286 ± 10	114 ± 14	733 ± 70

* Radioactivity is expressed as disintegrations per minute × 10^3 per collection time (2 minutes); results are given as mean ± SE for four strips.
† $P < 0.05$ compared to basal.

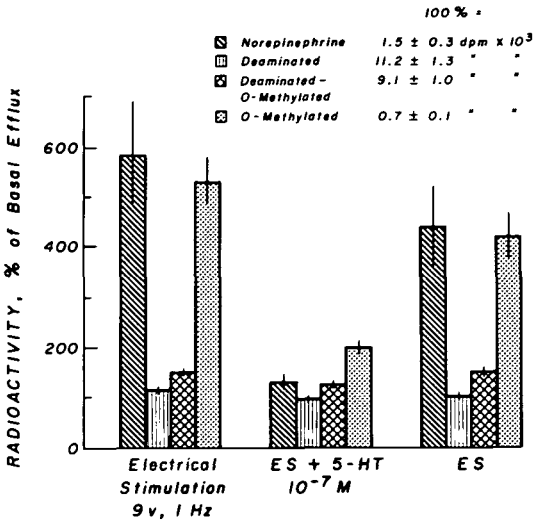

FIGURE 7 *Effect of 5-hydroxytryptamine on efflux of radiolabeled norepinephrine and metabolites during electrical stimulation (ES) of canine saphenous vein strips (n = 6) after incubation in norepinephrine[7-³H]. Radioactivity is given as disintegrations per minute (dpm) × 10³ per collection time (2 minutes) (mean ± SE).*

HT (10^{-7} M) caused a significant decrease in the latter to 8.5 ± 1.0 dpm × 10^3. There was no significant effect on tension presumably because the depressor effect of the decrease in neurotransmitter release is opposed by the direct smooth muscle excitatory action (0.3 ± 0.1 g) of 5-HT.

This concentration of 5-HT had no effect on the spontaneous release of radiolabeled compounds in this tissue.

RELEASE OF RADIOLABELED COMPOUNDS BY TYRAMINE

In four saphenous vein strips tyramine, at a concentration of 2.3 × 10^{-5} M (ED_{50} approximately under the conditions of superfusion), caused a sustained contraction and an augmentation of the release of radiolabeled compounds. 5-HT (10^{-7} M) given during the contraction caused an increase in the tension of the strips whereas the radioactivity of the superfusate was unaltered; control studies with tyramine in the absence of 5-HT showed a progressive decrease in the efflux of radioactivity which was not significantly different from that observed during the infusion of 5-HT. In the absence of tyramine, 5-HT (10^{-7} M) applied to these strips caused a contraction (Fig. 9).

RELEASE OF RADIOLABELED COMPOUNDS BY POTASSIUM

In four saphenous vein strips, potassium (40 mM) caused a sustained contraction (4.5 ± 0.3 g) together with an increase in the release of radiolabeled compounds from 9.8 ± 0.4 dpm × 10^3 (basal) to 19.6 ± 1.7 dpm × 10^3. 5-HT (10^{-7} M) caused a significant decrease in the latter to 15.4 ± 1.4 dpm × 10^3. There was no significant effect on the tension presumably because the depressor effect of the decrease in neurotransmitter release is opposed by the direct smooth muscle excitatory action of 5-HT.

Discussion

The lateral saphenous vein of the dog was chosen for the majority of these experiments because it contains a relatively large amount of smooth muscle and has a dense sympathetic innervation.[19] Strips taken from this vein react strongly both to activation of the sympathetic nerves by transmural electrical stimulation[20] and to direct stimulation of the smooth muscle cells. By analyzing the action of a vasoactive substance on the smooth muscle tension and on the release of neurotransmitter under basal conditions and during different modes of stimulation it is possible to determine its separate effects on the sympathetic nerve endings and smooth muscle cells of blood vessels.

The observation that contractions caused by electrical

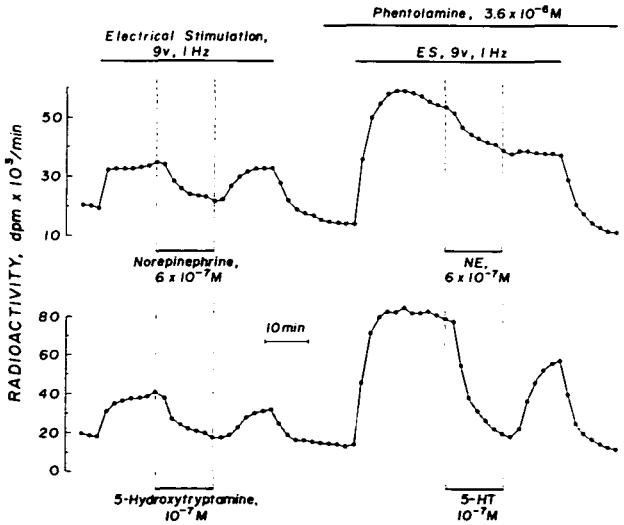

FIGURE 8 *Equivalent inhibition of adrenergic neurotransmission in cocaine-treated canine saphenous vein strips by norepinephrine (top) and 5-hydroxytryptamine (bottom). Comparison is shown of the effects of α-adrenergic blockade with phentolamine on the inhibition.*

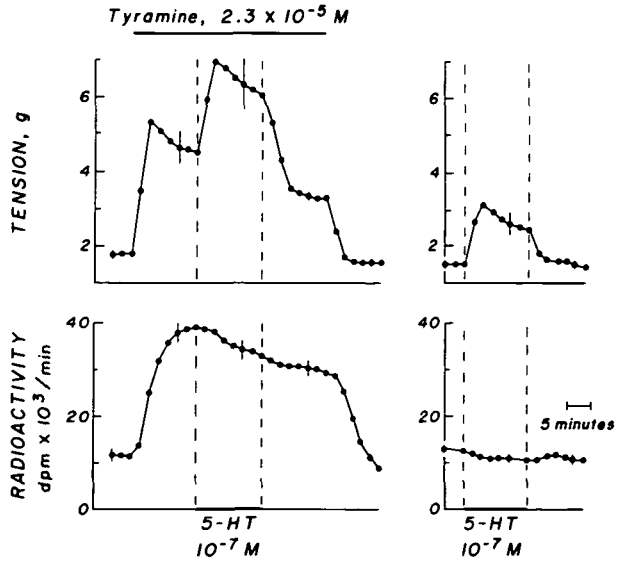

FIGURE 9 *Effect of 5-hydroxytryptamine on tension (top) and efflux of radiolabeled compounds (bottom) from canine saphenous vein strips during contractions caused by tyramine and at rest (mean ± SE; four strips).*

stimulation are depressed by low concentrations of 5-HT whereas contractions caused by norepinephrine are either unchanged or augmented demonstrates that 5-HT can cause smooth muscle relaxation when the neurogenic tone is high and an increase in tension when it is low. The conclusion that the relaxation was due to an inhibitory action on the sympathetic nerve endings was confirmed by the demonstration that 5-HT decreased the release of radioactive norepinephrine from the saphenous vein and tibial artery strips during contractions caused by depolarization of the sympathetic nerve endings. The additional finding that cocaine failed to attenuate the smooth muscle relaxation or the decreased release of radioactive compounds indicates that 5-HT acts by inhibiting the release of neurotransmitter through a mechanism which is independent of the amine uptake process.

In a recent study of the rabbit basilar artery it was noted that 5-HT (8.4×10^{-8} M) decreased the contractions caused by nerve stimulation, but not those by exogenous norepinephrine.[21] The reason for this difference was not examined but, on the basis of the evidence provided by the present study, it likely involves the depression of adrenergic neurotransmission in the rabbit basilar artery by 5-HT.

The ability of dichloroisoproterenol, a β-adrenergic blocking drug, to antagonize the vasodilator action of 5-HT in the presence of neurogenic vasoconstriction in the dog hindlimb and kidney led to the suggestion that 5-HT causes selective sensitization of vascular receptors to neurally released norepinephrine.[2, 15] This mechanism was excluded in the present study by the failure of propranolol to block the vasodepression, which is consistent with the observation that propranolol does not inhibit the vasodilator effect of 5-HT in the human forearm.[5] Similarly, the studies with phentolamine provide evidence against the possibility that 5-HT activates or sensitizes the prejunctional α-adrenergic receptors which can mediate a feedback inhibition of norepinephrine release from the sympathetic nerve endings.[22]

5-HT can cause the release of histamine and acetylcholine from different tissues.[7, 23, 24] The possibility that the inhibitory action of 5-HT is mediated indirectly by these substances was tested because both of them can inhibit the release of the neurotransmitter from postganglionic sympathetic adrenergic nerve endings by the activation of prejunctional histamine-H_2 and muscarinic receptors, respectively.[18, 20] However, the involvement of histamine and acetylcholine appears unlikely since the specific receptor antagonists failed to influence the response, and, in addition, 5-HT appears to be many times more potent than either of these substances in this tissue. Similarly it could be argued that 5-HT may stimulate prostaglandin synthesis. Since the presumed inhibition of prostaglandin synthesis by meclofenamate failed to attenuate the relaxation it is unlikely that prostaglandins mediate the response.

Hence the possibility remains that 5-HT activates specific receptors on the sympathetic nerve ending. The studies with phentolamine and methysergide suggest that the nature of the prejunctional 5-HT receptor is different from the receptor on the smooth muscle cell membrane, since a concentration of each of these antagonists which significantly inhibited the smooth muscle contraction failed to affect the inhibition of adrenergic neurotransmission by 5-HT. In further attempts to define the nature of this receptor morphine was used because this substance inhibits the nervous effects of 5-HT in several other tissues;[25] however, it was without effect in this preparation.

The failure of 5-HT to inhibit the release of radioactive compounds caused by tyramine, in contrast to its effect on norepinephrine release by nerve depolarization, suggests several possible mechanisms of action. Whereas the latter is calcium-dependent, the displacement of norepinephrine by tyramine is not.[26] The depressor effect of 5-HT on the sympathetic nerve terminal could therefore be mediated directly by inhibition of the calcium influx associated with nerve depolarization or indirectly by a change in the resting membrane potential.

High concentrations of 5-HT were shown to have an indirect contractile effect on the saphenous vein which appears to involve the uptake of this substance into the sympathetic nerve ending and the release of norepinephrine. This conclusion is supported by three sets of observations: (1) the high concentration of 5-HT caused an increase in the release of norepinephrine and its metabolites; (2) the pattern of radioactive compounds released is similar to that released by tyramine[27] but differs from the release by nerve depolarization in that there was a greater proportion of deaminated metabolites; and (3) the increased release of norepinephrine was inhibited by cocaine. This indirect sympathomimetic action of 5-HT has been observed previously in other tissues including rabbit heart,[10] guinea pig vas deferens,[11] and cat spleen capsule.[12] However, the fact that the contractions caused by 5-HT were not depressed by cocaine at a concentration that inhibits the amine uptake process suggests that the vasoconstrictor action of 5-HT predominantly involves the direct excitation of the vascular smooth muscle cells. By contrast, the action of tyramine is almost totally limited to its indirect sympathomimetic effect.[18,28] In addition, cocaine caused an increase in the maximum contractile response to 5-HT. This is in contrast to the effect of cocaine on norepinephrine-induced contractions in this tissue and suggests a selective postjunctional action. Whether this response is mediated by an increase in available receptors or an increase in the efficacy of the 5-HT-receptor complex cannot be determined. Previous investigations have concluded that cocaine has a direct action on blood vessels, sensitizing them to amines by a mechanism that is unrelated to amine inactivation.[29,30]

The conclusion that the contractions of the vessel strips caused by 5-HT were principally due to a direct action on the smooth muscle cells is supported by the observation that contractions occurred in the absence of a change in the release of radiolabeled norepinephrine (Fig. 5 and Table 1). As has been observed for other smooth muscle tissues[7, 8, 25] the contractions were antagonized by methysergide and by the α-adrenergic blocking drug phentolamine. Whether 5-HT activates α-adrenergic receptors cannot be determined on the available data. However, the maximum contractions obtained with 5-HT were less than those caused by norepinephrine. This pattern has been reported for other blood vessels[7,31,32] and the inequality

has been correlated with differences in the smooth muscle electrophysiological responses to these substances.[33] By contrast, pulmonary, cerebral, and umbilical vessels are more potently constricted by 5-HT.[6-8,32,34] The relative differences in sensitivity to 5-HT and norepinephrine among different blood vessels suggest the existence of specific receptors for each agonist.

It has been reported that 5-HT potentiates responses of human limb and rabbit ear arteries to norepinephrine.[31,35] This was not observed in the present study although, in some vessels, there appeared to be a synergistic interaction between 5-HT and tyramine. This might have occurred at the sympathetic nerve ending, since both these substances have an indirect sympathomimetic action. The fact that 5-HT failed to affect the release of radioactive compounds by tyramine suggests that the potentiation is caused by the preferential neuronal uptake of tyramine resulting in a reduced uptake of 5-HT, hence, a greater concentration of the latter available to activate the smooth muscle cell directly.

The influence of sympathetic tone on the hemodynamic response to an infusion of 5-HT can therefore be explained by the direct effects of this substance on adrenergic neurotransmission. For example, the decrease in total limb and skeletal muscle vascular resistance by 5-HT in the presence of a high sympathetic tone[13-15] can be attributed to a potent inhibition of neurotransmitter release. At high concentrations this decrease in resistance is opposed by two additional actions of 5-HT: (1) a direct excitatory action on the vascular smooth muscle cells, and (2) an indirect sympathomimetic effect mediated by the neuronal amine uptake process. The identification of dose-dependent prejunctional actions of this substance helps to explain previous observations in man that small doses cause an increase in limb blood flow, but when large doses are infused, the increase in blood flow is usually followed by a dose-dependent decrease.[1,3-5,16]

The vasomotor response of a given vessel to this substance will depend on the summation of its separate effects on the sympathetic nerve endings and on the smooth muscle cells and therefore will be influenced by other factors, including the density and distribution of the sympathetic nerves within the vessel wall. For example, it would be expected that the effect on the sympathetic nerve endings would be most apparent in those vessels in which there is dense innervation of the media and this factor might explain regional differences in the response to this substance.

5-HT normally circulates in association with the blood platelets and is released during their aggregation. Recent studies have also identified this substance in vascular tissue[36] and suggest that it may have a local role in the maintenance of vascular tone. It is also found in the small intestine and central nervous system and although its functional relationship with the sympathetic nerves in these regions remains speculative the present study would support an inhibitory interaction.

Acknowledgments

It is a pleasure to acknowledge the advice and suggestions by Dr. John T. Shepherd and the excellent technical assistance by Robert R. Lorenz. The methysergide was kindly supplied by Sandoz and the metiamide by Smith, Kline and French.

References

1. Bock KD, Dengler H, Kuhn HM, Matthes K: Die wirkung von 5-Hydroxyptryptamin auf Blutdruck, Haut- und Muskeldurchblutung des Menschen. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol **230:** 257-273, 1957
2. Page IH: Serotonin. Chicago, Year Book Medical Publishers, 1968, p 44
3. Whelan RF: Serotonin. *In* Control of the Peripheral Circulation in Man. Springfield, Ill., Charles C Thomas, 1967, p 27
4. Glover WE, Marshall RJ, Whelan RF: The antagonism of the vascular effects of 5-hydroxytryptamine by BOL 148 and sodium salicylate in the human subject. Br J Pharmacol **12:** 498-503, 1957
5. Walsh JA: Antagonism by methysergide of vascular effects of 5-hydroxytryptamine in man. Br J Pharmacol Chemother **30:** 518-530, 1967
6. Altura BM, Malaviya D, Reich CF, Orkin LR: Effects of vasoactive agents on isolated human umbilical arteries and veins. Am J Physiol **222:** 345-355, 1972
7. Erspamer V: Peripheral physiological and pharmacological actions of indolealkylamines. *In* Handbook of Experimental Pharmacology, vol 18, edited by O Eichler, A Farah. New York, Springer-Verlag, 1966, p 245-359
8. Somlyo AP, Somlyo AV: Vascular smooth muscle. II. Pharmacology of normal and hypertensive vessels. Pharmacol Rev **22:**249-353, 1970
9. Daugherty RM, Scott JB, Emerson TE, Haddy FJ: Comparison of iv and ia infusion of vasoactive agents on dog forelimb blood flow. Am J Physiol **214:** 611-619, 1968
10. Fozard JR, Mwaluko GMP: Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. Br J Pharmacol **57:** 115-125, 1976
11. Ozawa H, Katsuragi T: Ouabain-induced potentiation on the contractions of the guinea pig vas deferens. Eur J Pharmacol **25:** 147-154, 1974
12. Pluchino S: Direct and indirect effects of 5-hydroxytryptamine and tyramine on cat smooth muscle. Naunyn Schmiedebergs Arch Pharmacol **272:** 189-224, 1972
13. Emerson TE Jr, Meier PD, Daugherty RM Jr: Dependence of skeletal muscle vascular response to serotonin upon the level of vascular resistance. Proc Soc Exp Biol Med **142:** 1185-1188, 1973
14. Haddy FJ, Gordon P, Emanuel D: The influence of tone upon responses of small and large vessels to serotonin. Circ Res **7:**123-130, 1959
15. McCubbin JW, Kaneko Y, Page IH: Inhibition of neurogenic vasoconstriction by serotonin; vasodilator action of serotonin. Circ Res **11:** 74-83, 1962
16. Page IH, McCubbin JW: The variable arterial pressure response to serotonin in laboratory animals and man. Circ Res **1:** 354-362, 1953
17. Maricq HR: Local effect of serotonin on blood vessels of human skin. Microvasc Res **4:** 258-263, 1972
18. McGrath MA, Shepherd JT: Inhibition of adrenergic neurotransmission in canine vascular smooth muscle by histamine; mediation by H_2-receptors. Circ Res **39:** 566-573, 1976
19. Coimbra A, Ribeiro-Silva A, Oswald W: Fine structural and autoradiographic study of the adrenergic innervation of the dog lateral saphenous vein. Blood Vessels **11:** 128-144, 1974
20. Vanhoutte P: Inhibition by acetylcholine of adrenergic neurotransmission in vascular smooth muscle. Circ Res **34:** 317-326, 1974
21. Bevan JA, Duckles SP, Lee TJF: Histamine potentiation of nerve and drug-induced responses of a rabbit cerebral artery. Circ Res **36:** 647-653, 1975
22. Langer SZ: Presynaptic regulation of catecholamine release. Biochem Pharmacol **23:** 1793-1800, 1974
23. Brownlee G, Johnson ES: The release of acetylcholine from the isolated ileum of the guinea pig induced by 5-hydroxytryptamine and dimethylphenylpiperazinium. Br J Pharmacol **24:** 689-700, 1965
24. Feldberg W, Smith AN: Release of histamine by tryptamine and 5-hydroxytryptamine. Br J Pharmacol Chemother **8:** 406-411, 1953
25. Gyermek L: Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines. *In* Handbook of Experimental Pharmacology, vol 18, edited by O Eichler, A Farah. New York, Springer-Verlag, 1966, pp 471-528
26. Thoa NB, Wooten F, Axelrod J, Kopin IJ: On the mechanism of release of norepinephrine from sympathetic nerves induced by depolarizing agents and sympathomimetic drugs. Mol Pharmacol **11:** 10-18, 1975
27. Lorenz RR, Vanhoutte PM: Inhibition of adrenergic neurotransmission in isolated veins of the dog by potassium ions. J Physiol (Lond) **246:** 479-500, 1975
28. Trendelenburg U: Classification of sympathomimetic amines. *In*

Handbook of Experimental Pharmacology, vol 33, edited by H Blaschko, E Muscholl. New York, Springer-Verlag, 1972, pp 336-362
29. Kalsner S, Nickerson M: Mechanism of cocaine potentiation of responses to amines. Br J Pharmacol **35:** 428-439, 1969
30. Reiffenstein RJ, Triggle CR: Cocaine-induced supersensitivity in the human umbilical artery. Can J Physiol Pharmacol **52:** 687-698, 1974
31. de Lande IS, Cannell VA, Waterson JG: The interaction of serotonin and noradrenaline on the perfused artery. Br J Pharmacol Chemother **28:** 255-272, 1966
32. Toda N, Fujita Y: Responsiveness of isolated cerebral and peripheral arteries to serotonin, norepinephrine and transmural electrical stimulation. Circ Res **33:** 98-104, 1973.
33. Somlyo AV, Vinall P, Somlyo AP: Excitation-contraction coupling and electrical events in two types of vascular smooth muscle. Microvasc Res **1:** 354-373, 1969
34. Yashon D, Blocker D, Brown RJ, Hunt WE: Quantitative in vitro vasoactivity of 5-hydroxytryptamine on the human basilar artery. Surg Neurol **3:** 295-300, 1975
35. Scroop GC, Walsh JA: Interactions between angiotensin, noradrenaline and serotonin on the peripheral blood vessels in man. Aust J Exp Biol Med Sci **46:** 573-580, 1968
36. Jarrott B, McQueen A, Graf L, Louis WJ: Serotonin levels in vascular tissue and the effects of a serotonin synthesis inhibitor on blood pressure in hypertensive rats. Clin Exp Pharmacol Physiol (Suppl) **2:** 201-205, 1975

Triggered and Automatic Activity in the Canine Coronary Sinus

ANDREW L. WIT AND PAUL F. CRANEFIELD

SUMMARY **The effects of norepinephrine on the canine coronary sinus and atrial muscle outside of the sinus ostium were studied. In the absence of electrical stimulation, norepinephrine caused spontaneous activity which initially arose in the atrial muscle outside of the ostium, but the pacemaker soon shifted into the coronary sinus. When the atrium was separated from the coronary sinus and norepinephrine was added, spontaneous activity occurred in the atrium but not in the sinus. Stimulation of the coronary sinus, at this time, triggered sustained rhythmic activity. These observations suggested that the focus of sustained rhythmic activity in atrial tissue outside of the ostium of the coronary sinus had properties different from foci found along the length of the coronary sinus. Atrial fibers outside the ostium had high resting potentials (−82 ± 4 mV) and action potentials with relatively rapid upstrokes ($\dot{V}_{ax}$ = 80 ± 12 V/sec). They developed spontaneous diastolic depolarization when exposed to norepinephrine. Coronary sinus fibers also had high resting potentials (−75 to −85 mV) and action potentials with relatively rapid upstrokes ($\dot{V}_{ax}$ = 129 ± 9 V/sec). Some coronary sinus cells lost resting potential and became inexcitable when not stimulated. Norepinephrine increased resting potential in these cells, restored excitability, and caused the appearance of a delayed afterhyperpolarization and afterdepolarization. Afterhyperpolarizations and afterdepolarizations also occurred in some cells which remained excitable after a quiescent period. The amplitude of the afterdepolarizations increased when stimulus rate increased or after a premature stimulus and nondriven action potentials often arose from the peak of the afterdepolarization. Triggered sustained rhythmic activity followed. Afterdepolarizations and triggering were sometimes facilitated by a prior period of quiescence. They were inhibited by verapamil and acetylcholine.**

IN 1907 Erlanger and Blackman[1] reported that the area around the orifice of the coronary sinus in rabbits possessed a "high degree of rhythmicity." In 1912 Zahn[2] produced tachycardia by warming the coronary sinus of the canine heart. Many later studies on animals and humans have suggested that impulses arising in the region of the coronary sinus may cause atrial extrasystoles, ectopic rhythms, and tachycardias,[3-5] but that concept has not been universally accepted.

We know of no electrophysiological studies designed to determine whether there are cells in the coronary sinus capable of initiating impulses, exactly where such cells might be, or the way in which they might become rhythmically active. Possible causes of rhythmic activity include reentry and diastolic depolarization; diastolic depolarization, in turn, may be spontaneous or may result from afterdepolarizations that reach threshold.[6] In this article, we report our investigation of the electrical activity of atrial fibers in the canine coronary sinus and around its orifice. We have found two regions in or near the coronary sinus that can act as pacemakers; one region shows spontaneous phase 4 depolarization whereas the other region shows "triggered" activity[6] arising from delayed afterdepolarizations.

Methods

Preparations were isolated from 50 canine hearts. Mongrel dogs weighing 10-15 kg were anesthetized with sodium pentobarbital (30/mg per kg, iv). The hearts were removed rapidly through a thoracotomy and dissected at room temperature in a modified Tyrode's solution with the following millimolar composition: NaCl, 137; $NaHCO_3$, 13; dextrose, 5.5; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; $CaCl_2$, 2.8; and KCl, 4.0. The Tyrode's solution was equilibrated with a gas mixture of 95% O_2-5% CO_2.

From The Rockefeller University, New York, New York, and The Department of Pharmacology, Columbia University College of Physicians and Surgeons, New York, New York.
Dr. Wit is a Career Scientist of the Irma T. Hirschl Trust.
Supported by U.S. Public Health Service Grants HL-14899 and HL-12738 from the National Heart and Lung Institute.
Address for reprints: Dr. Paul F. Cranefield, the Rockefeller University, 1230 York Avenue, New York, New York 10021.
Received February 22, 1977; accepted for publication May 17, 1977.