Effect of Angiotensin on Uptake of ^{3}H-Norepinephrine in Dog Cutaneous Arteries (36112)

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An augmentation of vascular and cardiac responses to adrenergic nerve stimulation (1–3, 6, 10–13) and a concomitant increase in release of norepinephrine (2, 10, 11, 13) has been demonstrated when angiotensin is present in the blood or medium employed to perfuse a number of different preparations. In the perfused hind paw (12), kidney (11), and cutaneous veins of the dog (3), angiotensin administration resulted in a potentiating effect on vasoconstrictor responses to nerve stimulation without any significant effect on responses to norepinephrine. Because cocaine, a blocker of catecholamine uptake, produced a similar degree of potentiation of both these responses it was apparent that angiotensin was not exerting a cocaine-like action (3, 11). In addition, Starke *et al.* (10) and Hughes and Roth (2) found that angiotensin acted to facilitate adrenergic transmitter release despite the prior blockade of catecholamine uptake by cocaine. These investigators have concluded, as have we (11, 13), that angiotensin exerts some action on the adrenergic neuron to induce release of a larger than normal quantity of norepinephrine when the nerves discharge. Other workers have favored the view that a blockade of reuptake by angiotensin may account for the increased transmitter release and end-organ response seen during nerve stimulation (5, 7). Because most studies that have suggested an inhibitory effect of angiotensin on catecholamine uptake were not carried out on vascular tissues (4, 7) or utilized high concentrations of angiotensin (5), we decided to determine the effect of angiotensin on norepinephrine uptake in blood vessels in a range of concentrations including those previously demonstrated to result in increased sympathetic responses and transmitter release (10, 12). The effect of angiotensin II amide on the uptake of ^{3}H-norepinephrine was determined and compared to that of cocaine and tyramine on vascular segments (*in vitro*) from the dog paw.

Materials and Methods. Incubation experiments. Mongrel dogs of both sexes, ranging in weight from 12 to 20 kg, were anesthetized with 30 mg/kg of sodium pentobarbital. Cranial tibial arteries were exposed in both hind paws, ligated, and removed. Control and treated segments were cut from the same portion of the vessel and matched in weight. Utilizing a procedure reported previously (8) these vascular segments, weighing 15–27 mg, were incubated with *dl*-^{3}H-norepinephrine (8.8 Ci/mmole) in a Krebs–Ringer phosphate solution at pH 7.4 and 37° in oxygen atmosphere. Labeled norepinephrine in the concentration of 5.4×10^{-8} *M* and the other agents were added simultaneously to the medium and incubated with the segment for periods of 7.5 to 60 min. Angiotensin II amide (generously supplied by Dr. A. J. Plummer of Ciba Pharmaceutical Co.), tyramine hydrochloride, and cocaine hydrochloride were the drugs used. The segment was then removed and washed with 20 drops of cold Krebs solution from a 10 ml pipette to eliminate any ^{3}H-norepinephrine adhering to the tissue. Solubilization of the segment was carried out in a counting vial with 1 ml of solubilizer (NCS, Nuclear Chicago Corporation) and 0.1 ml of distilled water. Fifteen milliliters of scintillator solution (4 g of PPO and 0.4 g of POPOP/liter of toluene) were added to vials containing the solubilized tissue and an aliquot of the medium in which the vessel was incubated. The samples were counted in a liquid scintillation spectrometer. Counting

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error was less than 2% and efficiency was determined by internal or external standardization. Data are presented as T/M ratios of tissue (dpm/g) to medium (dpm/ml).

Washout experiments. Arterial segments were incubated for 30 min with ^{3}H-norepinephrine as described above. The segment was then rinsed with Krebs solution and blotted on filter paper. It was placed in a 10 ml beaker with 2.2 ml of medium alone or with angiotensin 10^{-6} M and washed by shaking at 37° for 5, 15, 30, or 60 min. After removal, it was blotted and solubilized in a counting vial. Both this solution and an aliquot of the medium in which the segment was washed were counted as described above. Data are expressed as percentages of the total (dpm) taken up after 30 min incubation which was released into the media after the various intervals of washing.

Results. Influence of angiotensin, tyramine, and cocaine on uptake of ^{3}H-norepinephrine. We assumed that total ^{3}H represents principally ^{3}H-norepinephrine since in a previous study involving these vessels (8) the radioactive material bound in the segments consisted almost entirely of unchanged amine. Figure 1 depicts the T/M ratios in arteries obtained after incubation with ^{3}H-norepinephrine for 7.5, 15, 30, and 60 min in presence and absence of angiotensin (1×10^{-6} M). The mean T/M ratios found in the angiotensin-treated vessels were larger than those of the control arteries after 30 and 60 min of incubation ($p<.05$). These increments in T/M expressed as percentages were 39 and 29%, respectively. After incubation for 30 min with lower concentrations of angiotensin (10^{-8} and 10^{-7} M), similar increases in the T/M ratios were obtained (Fig. 2), which did not differ significantly from that obtained using the highest concentration.

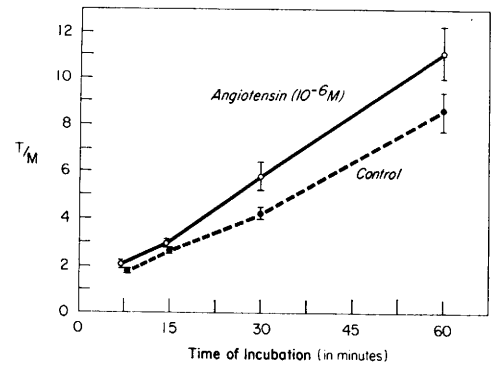

FIG. 1. T/M ratios of dpm of total radioactivity in arteries incubated for 7.5, 15, 30, and 60 min with ^{3}H-norepinephrine: (- - - -) T/M ratios of arteries incubated without angiotensin; and (—) T/M ratios of arteries incubated with angiotensin, 10^{-6} M. Values are mean ± SEM obtained in 8 experiments.

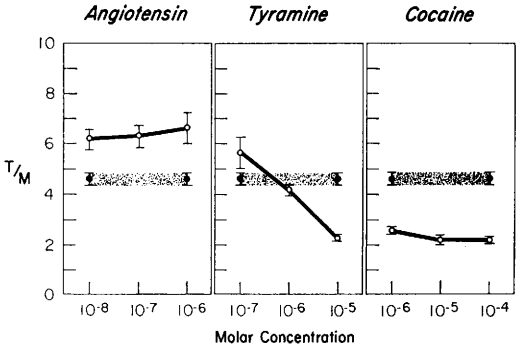

FIG. 2. T/M ratios of dpm of total radioactivity of arteries incubated with ^{3}H-norepinephrine for 30 min in presence of angiotensin, tyramine, and cocaine in various concentrations: T/M ratios obtained in absence of drugs are within stippling. Values are mean ± SEM obtained in 9 experiments.

Since in the presence of angiotensin the accumulation of ^{3}H-norepinephrine was augmented and not decreased, it was of interest to compare its effect with that of other agents which are known to influence the binding of catecholamines in sympathetic nerve endings. Tyramine, which releases norepinephrine from adrenergic nerve endings, and cocaine, which blocks neuronal uptake of catecholamines, were employed in concentrations of 10^{-7} to 10^{-5} M and 10^{-6} to 10^{-4} M, respectively. Tyramine in the lower concentrations did not exert any significant effect on the uptake of ^{3}H-norepinephrine, but in the concentration of 10^{-5} M an approximately 50% inhibition of uptake was achieved (Fig. 2). Cocaine in the concentrations employed reduced uptake of ^{3}H-norepinephrine to a similar degree in these vessels as that reported previously (8).

In another group of animals, paired experiments were performed to determine the effect

of angiotensin, cocaine, and angiotensin in presence of cocaine on the uptake of ^{3}H-norepinephrine (Fig. 3). As before, the *T/M*

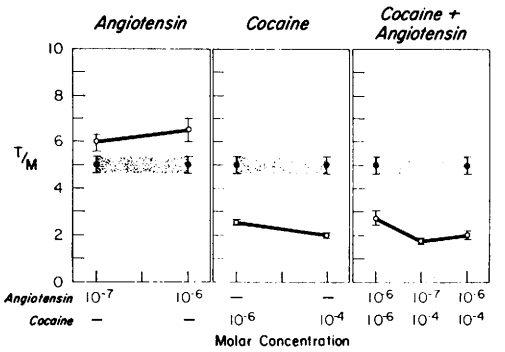

FIG. 3. *T/M* ratios of total radioactivity in arteries incubated with ^{3}H-norepinephrine for 30 min in presence of angiotensin, cocaine, and angiotensin + cocaine: concentrations of angiotensin and cocaine employed (abscissa). *T/M* ratios obtained in absence of drugs are within stippling. Values are mean ± SEM of 8 experiments with angiotensin alone and 5 experiments with cocaine and angiotensin + cocaine.

ratios were increased by angiotensin in the two concentrations employed ($p<.02$ and $p<.01$, respectively). Cocaine also blocked the uptake of ^{3}H-norepinephrine to a similar degree as in the previous experiments. In those arteries treated with the combination of cocaine and angiotensin, *T/M* ratios did not differ significantly from the ratios obtained in the arteries subjected to cocaine alone.

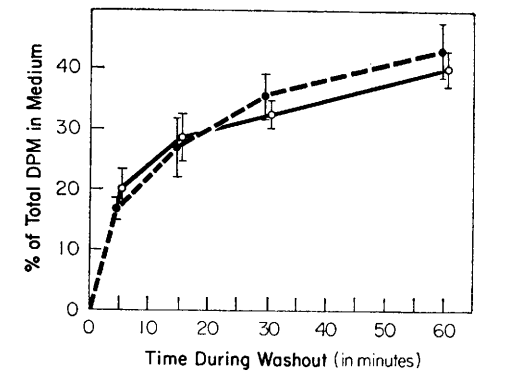

FIG. 4. Rate of efflux of ^{3}H-norepinephrine from arterial segments in presence (—); or absence (- -) of angiotensin (10^{-6} *M*) expressed as percentage of dpm washed into the media from the tissue. Intervals of washing were 5, 15, 30, and 60 min. Values are mean ± SEM of 4 experiments.

Rate of washout of ^{3}H-norepinephrine in arteries in presence of angiotensin. To test the possibility that the increase in uptake of ^{3}H-norepinephrine produced by angiotensin might have been due to release of endogenous norepinephrine and exchange of labeled norepinephrine for endogenous amine, washout experiments were performed. In Fig. 4 are the washout curves obtained in presence and absence of angiotensin. It can readily be seen that the quantity of labeled amine washed into the medium (expressed as the % of the total amount which had been taken up into the tissue) did not differ between the control and angiotensin-treated vessels.

Discussion. We have presented evidence which indicates that angiotensin does not decrease but increases, to a small degree, uptake of norepinephrine in canine cutaneous arteries. Inasmuch as angiotensin did not result in release of bound catecholamine as determined by the washout experiments, the increased uptake was not due to greater exchange of labeled for endogenous amine. Furthermore, tyramine, an agent known to release catecholamine from adrenergic nerve endings, did not result in a significantly greater accumulation of labeled norepinephrine. There have been conflicting reports on the influence of angiotensin on the uptake of norepinephrine. Several workers contend that angiotensin inhibits catecholamine uptake based on results obtained employing various tissues of the rat (5), and rabbit (7), and in the cat mesenteric vascular bed (6). However, Hughes and Roth (2) found an inconsistent effect of angiotensin on uptake of labeled norepinephrine in the rabbit portal vein and coeliac artery and rat heart. Schumann *et al.* (9) found no inhibitory effect of angiotensin on uptake in the rabbit heart unless a very large concentration (13 μg/ml) of the polypeptide was employed. Since no evidence for blockade of catecholamine uptake by angiotensin was obtained in the present investigation, this mechanism does not appear to play a role in the potentiation of responses to sympathetic nerve stimulation in the dog paw (12, 13).

The slightly augmented uptake of norepi-

nephrine found in presence of angiotensin appears to involve an effect of the polypeptide on the adrenergic nerve ending. The evidence for a neuronal action of angiotensin is based on the finding that cocaine blocked the increased uptake normally obtained with angiotensin. Thus, when no norepinpehrine can cross the neuronal membrane, the effect of angiotensin is absent. The *T/M* ratio of 2 obtained in presence of cocaine alone, probably represents extraneuronal binding of the catecholamine and it is significant that angiotensin exerted no effect on this process.

Summary. In presence of angiotensin, uptake of ^{3}H-norepinephrine into canine cutaneous arteries (*in vitro*) was slightly increased rather than decreased compared to control. Tyramine and cocaine both inhibited the uptake process in these vessels, and cocaine interfered with the effect of angiotensin. Angiotensin did not increase the rate of norepinephrine washout from the arteries, therefore the increased uptake produced by angiotensin was not attributable to increased exchange of the amine with its endogenous store.

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