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EFFECT OF ANGIOTENSIN ON UPTAKE AND RELEASE OF NOREPINEPHRINE BY BRAIN*

DJURO PALAIĆ and PHILIP A. KHAIRALLAH

Research Division, Cleveland Clinic Foundation, Cleveland, Ohio, U.S.A.

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Abstract—Angiotensin has been shown to inhibit partially the uptake of ^{3}H-norepinephrine perfused through rat brain ventricles. This blockade most likely occurs at the cell membrane level. Angiotensin did not cause a release of norepinephrine from brain, although metanephrine, tyramine, and acetylcholine did. It is postulated that potentiation of sympathetic responses seen with angiotensin are due to inhibition of uptake or re-uptake of norepinephrine.

INTERACTION of angiotensin with the sympathetic nervous system was first described by McCubbin and Page.[1] According to them, this effect is a peripheral one which results in an increased response to procedures and agents that cause release of a sympathetic neurotransmitter.

A centrally mediated effect of angiotensin was observed by Bickerton and Buckley.[2] Angiotensin applied in brain circulation of cross-circulated dogs produced hypertension. Further studies of this phenomenon[3, 4] showed that angiotensin provoked the same effect when injected or perfused through cat brain ventricles. This effect appeared to be due to alterations in brain norepinephrine and to the activation of central sympathetic structures. Smookler *et al.*[3] suggested that norpinephrine might be a "neurotransmitter in the central nervous system regulation of sympathetic cardiovascular activity".

The aim of the present work was to analyze the possible mechanism by which angiotensin interacts with brain norepinephrine. Uptake and release of labeled norepinephrine by brain, and effects on these caused by peptides and pharmacological agents were studied.

METHODS

Rat brains were perfused through the ventricles as described by Palaic *et al.*[5] A solution of 5 μg/ml *dl*-7-^{3}H-norepinephrine HCl (New England Nuclear Corp., Boston, Mass.), containing 0·32 mc/mg was perfused at the rate of 0·04 ml/min for 1 hr. This was followed by perfusion for 1 hr with artificial cerebrospinal fluid,[6] and samples were collected every 5 min to measure washout. Cerebrospinal fluid pressure was controlled by measuring the volume of effluent, which was equal to the volume of perfusion solution entering the brain. Blood pressure was not monitored. Animals were killed by decapitation, the brains removed and homogenized in 0·1 N HCl. An

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aliquot was taken for combustion (according to the method of Kalberer and Rutschmann[7]) and counted. Other brains were homogenized in 0·32 M sucrose and centrifuged for 90 min at 100,000 *g*; both supernatant and sediment were counted. Norepinephrine was separated from its metabolites on alumina columns.[8] The amount of metabolites was determined as the difference between total radioactivity and specific norepinephrine radioactivity eluted from the column. All radioactive counts were calculated to 1 g brain tissue.

In studying effects of drugs on uptake of norepinephrine, angiotensin and related drugs were infused with norepinephrine followed by washout with cerebrospinal fluid. To determine release, norepinephrine was perfused first and drugs were added to the washout during the second hour.

Drugs used included asparagine[1]-valine[5]-angiotensin II (Hypertensin, Ciba), asparagine[1]-valine[5]-angiotensin II diamide, tyramine HCl, and acetylcholine chloride. Reserpine (Serpasil, Ciba) was injected i.p. 16 hr before perfusion of the brain at a dose of 5 mg/kg. Cocaine HCl (10 mg/kg) was given i.p. 5 min before perfusion.

RESULTS

Washout of tritiated norepinephrine

The rate of spontaneous washout of norepinephrine from brain is shown in Fig. 1. The initial slope of the curve decreased with a half-life ($T_{\frac{1}{2}}$) of about 2·0 min, while the slope of the final portion of the curve had a $T_{\frac{1}{2}}$ of around 160 min.

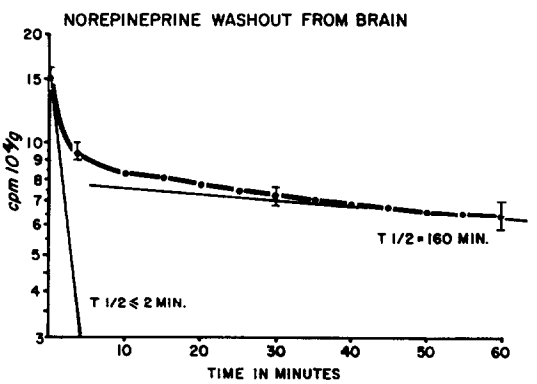

FIG. 1. Efflux of ^{3}H radioactivity from rat brain. Brains were perfused for 1 hr with ^{3}H-norepinephrine (0·2 μg/min) followed by a 1-hr perfusion with cerebrospinal fluid (perfusion rate 0·04 ml/min). Mean of 5 experiments ± S.E.M.

The initial slope probably corresponds to the washout of extracellular space plus radioactivity remaining in the ventricles. The final slope probably corresponds to spontaneous efflux of radioactivity from storage sites inside the cell. This radioactivity includes both intact norepinephrine and metabolites. No separation was attempted. Extrapolation of the final slope to zero time gives a rough indication that 45 per cent of the counts in the 1-hr washout came from extracellular space, whereas 55 per cent came from intracellular sites. In subsequent figures, the designation washout indicates total counts, although in all cases there was approximately a 45:55 distribution ratio between extracellular and intracellular sources of catecholamines.

Effect of peptides on uptake of norepinephrine

Angiotensin at three levels, 8 ng, 80 ng, and 800 ng/min, bradykinin (80 ng/min), and vasopressin (80 ng/min) were perfused together with tritiated norepinephrine during the first hour equilibration phase, followed by washout for 1 hr. Total radioactivity per gram of brain and proportional amounts in the washout are shown in Fig. 2. Angiotensin and bradykinin significantly decreased radioactivity in both

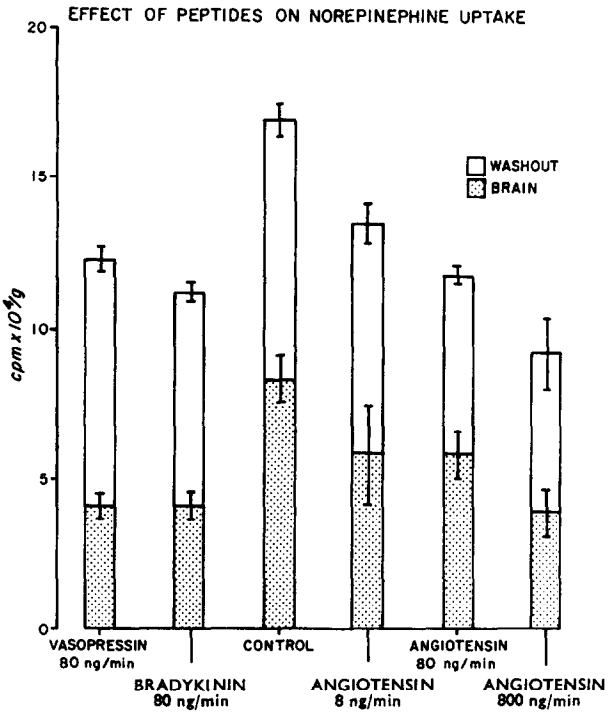

FIG. 2. Effect of peptides on norepinephrine uptake. Total radioactivity found in brain and washou fluid was calculated per gram of brain tissue. Brain was perfused for 1 hr with tritiated norepinephrine (0·2 μg/min) together with peptides, followed by a 1-hr perfusion with cerebrospinal fluid. Each column represents a mean of 5 determinations ± S.E.M.

washout and brain, whereas vasopressin decreased brain norepinephrine but did not alter amounts in the washout. Angiotensin diamide (800 ng/min), which is biologically inactive, did not produce any effect on ^{3}H-norepinephrine uptake. Decreased washout after angiotensin and bradykinin reflected the lower amount of ^{3}H-norepinephrine taken up by brain, because the rate of washout (expressed as percentage of total radioactivity found in effluent at zero time) was the same as that in control.

When brain tissue was homogenized in 0·32 M sucrose and centrifuged after angiotensin perfusion, radioactivity was mainly decreased in the sediment fraction (Fig. 3), whereas the amount in the supernatant seemed to be decreased but was not statistically significant ($P < 0{\cdot}15$).

During angiotensin perfusion of rat brain ventricles, barbiturate sleeping time was greatly shortened in most experiments. Vasopressin and bradykinin did not have this effect.

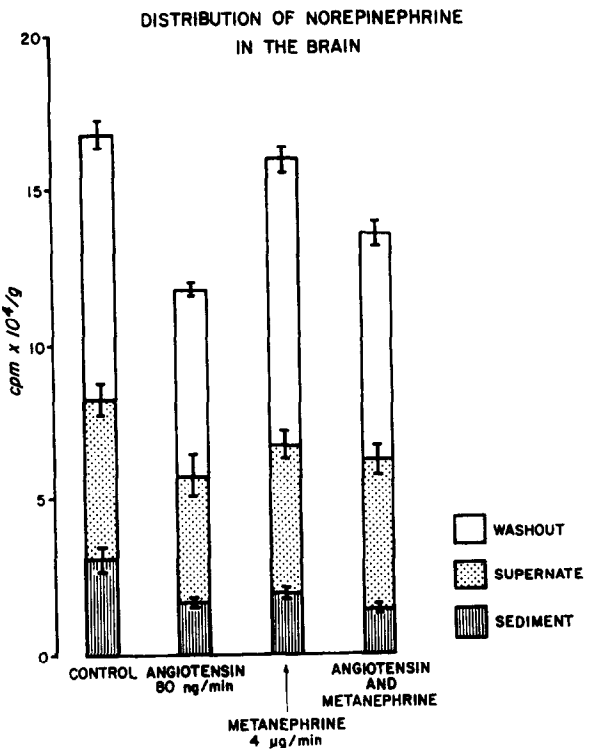

FIG. 3. Distribution of norepinephrine in brain. Total radioactivity in sucrose homogenates of brain after ultracentrifugation (sediment and supernatant). Brain was perfused for 1 hr with 0·2 μg tritiated norepinephrine/min with angiotensin or metanephrine, followed by a 1-hr perfusion with cerebrospinal fluid. Columns represent a mean of 5 determinations $\pm$ S.E.M.

Effect of other drugs with angiotensin on uptake of norepinephrine

Metanephrine perfused at 4 μg/min inhibited uptake of norepinephrine as measured in the sediment fraction of brain homogenate, but to a lesser degree than did angiotensin at 80 ng/min. There was no change in radioactivity of the supernatant or washout fractions. Angiotensin and metanephrine perfused together decreased the radioactivity found in the sediment fraction, with no change in the supernatant; radioactivity in the washout fraction was intermediate between angiotensin and metanephrine separately (Fig. 3).

Tyramine, 4·0 μg/min, interfered with norepinephrine uptake, as demonstrated by the significant decrease of radioactivity in brain, but did not change the amount in the washout. Together, tyramine and angiotensin decreased amounts both in brain and in washout (Table 1). Acetylcholine (400 ng/min) had no effect on uptake of norepinephrine, but with angiotensin it decreased amounts in washout but not in brain (Table 1).

Effect of angiotensin and other drugs on metabolism of norepinephrine

Brain tissue was homogenized in 0·1 N HCl and the supernatant was passed through alumina columns to separate norepinephrine from metabolites. Although the total amount of radioactivity was less in brain after angiotensin (80 ng/min), the

TABLE 1. EFFECT OF ANGIOTENSIN, TYRAMINE, AND ACETYLCHOLINE ON NOREPINETHRINE UPTAKE*

Drug	Total radioactivity			Ratio washout/brain
	Washout	Brain	Total	
Control	8·675 ± 0·495	8·305 ± 0·865	16·880 ± 1·285	1·04
Angiotensin	5·978 ± 0·268 <0·0025	5·846 ± 0·844 <0·05	11·825 ± 0·754 <0·005	1·02
Tyramine	7·461 ± 0·983 N.S.	4·958 ± 0·601 <0·01	12·419 ± 0·967 <0·025	1·50
Tyramine + angiotensin	5·623 ± 1·539 <0·05	5·686 ± 0·067 <0·0125	11·310 ± 1·481 <0·025	0·98
Acetylcholine (ACh)	9·064 ± 0·732 N.S.	7·611 ± 0·836 N.S.	16·523 ± 1·554 N.S.	1·19
Acetylcholine + angiotensin	7·211 ± 0·486 <0·05	8·102 ± 0·678 N.S.	15·314 ± 0·325 N.S.	0·89

* Brains were perfused for 1 hr with tritiated norepinephrine (0·2 μg/min) together with angiotensin (80 ng/min), tyramine (4 μg/min), and acetylcholine (0·4 μg/min). Results are expressed as cpm × 10^4/g tissue, an average of 5 determinations ± S.E.M. P values express level of significance between the control and treated group; N.S. = not significant.

ratio of metabolites to norepinephrine did not differ from control. Similar results were obtained with tyramine (4 μg/min). Perfused metanephrine (4 μg/min), acetylcholine (400 ng/min), and i.p. reserpine (5 mg/kg) given to rats 16 hr prior to brain perfusion significantly increased amounts of metabolites. When cocaine (10 mg/kg) was given i.p. 5 min before perfusion no metabolites were detected (Fig. 4).

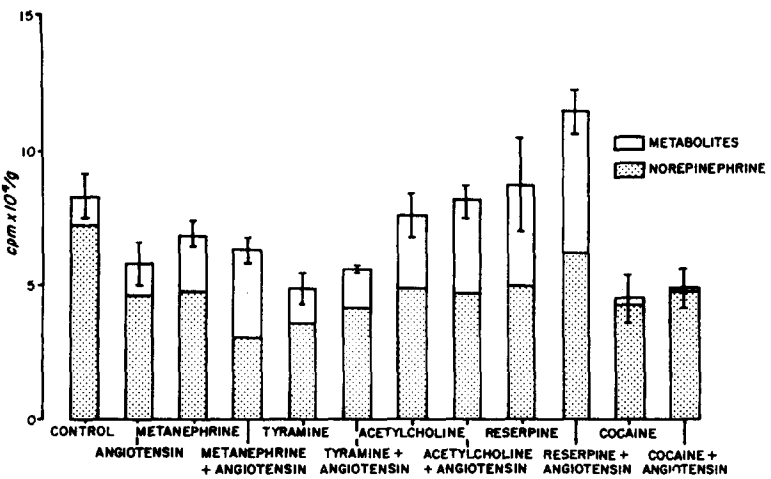

FIG. 4. Norepinephrine and metabolites in brain homogenates. Brains were perfused for 1 hr with tritiated norepinephrine (0·2 μg/min) and angiotensin (80 ng/min), metanephrine (4 μg/min), tyramine (4 μg/min), and acetylcholine (0·4 μg/min) followed by a 1-hr perfusion with cerebrospinal fluid. Reserpine (5 mg/kg) was given i.p. 16 hr before and cocaine (10 mg/kg) 5 min before brain perfusion. Each column represents the means of 5 or 6 determinations ± S.E.M.

Angiotensin did not change the amount of metabolites when given with tyramine, acetylcholine (ACh), reserpine or cocaine, but significantly increased the amount of metabolites when given together with metanephrine (Fig. 4).

Effect of angiotensin and other drugs on release of norepinephrine

In the previous experiments, angiotensin, other peptides and drugs were given during the first hour perfusion together with norepinephrine, and measurements of influx of radioactivity were monitored. During the second hour, the brain was perfused with artificial cerebrospinal fluid containing no drugs. In the following experiments norepinephrine alone was given during the first hour, and the drugs and peptide were added to the cerebrospinal fluid during the second hour, the washout phase. Results are presented in Fig. 5.

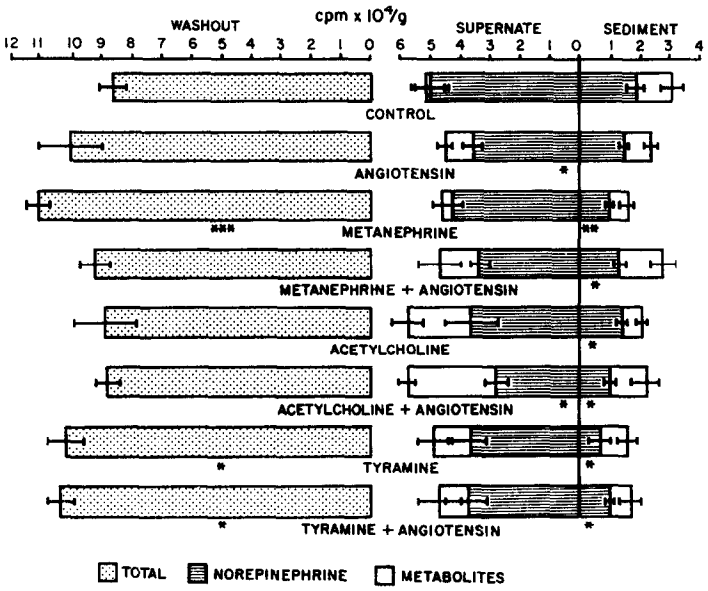

FIG. 5. Effect of drugs on release of norepinephrine and metabolites from brain. Brains were perfused with tritiated norepinephrine (0·2 μg/min) for 1 hr followed by a 1-hr perfusion with cerebrospinal fluid containing angiotensin (80 ng/min), metanephrine (4 μg/min), tyramine (4 μg/min), and acetylcholine (0·4 μg/min). Brains were homogenized in sucrose, centrifuged, and metabolites separated from norepinephrine. Each column represents the means of 5 or 6 experiments ± S.E.M. * Indicates $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ when compared to control.

Angiotensin seemed to increase the radioactivity in the washout fraction, but this was only significant at the 5 per cent level. There was no change in norepinephrine or metabolites in the sediment fraction, but a significant decrease in norepinephrine was observed in the supernatant. Metanephrine significantly decreased norepinephrine in the sediment fraction and increased radioactivity in the washout fraction. Acetylcholine and tyramine also decreased the norepinephrine content of the sediment fraction and tyramine increased the radioactivity in the washout fraction. Angiotensin added along with metanephrine or tyramine did not cause any extra change. All drugs tested increased the metabolite content in the supernatant fractions, and angiotensin increased this further when given with metanephrine and ACh, but not with tyramine.

DISCUSSION

Norepinephrine injected into the lateral ventricles of rats is rapidly taken up by neurones, and appears in the nerve ending fraction obtained after density gradient centrifugation of homogenized brain tissue.[9] Two mechanisms have been postulated for the uptake of norepinephrine.[10] One involves a 'membrane pump' at the cell membrane level, and the other the storage granule. If the latter uptake mechanism is inhibited, by reserpine for example, less norepinephrine will be stored, but since it is in cytoplasm by the action of the membrane pump, it may be metabolized. If the cell membrane uptake is inhibited, by cocaine or desipramine for example, less norepinephrine will enter the cell and the ratio of intracellular norepinephrine to metabolites should be equal to control levels. Another hypothesis has been presented[11] indicating that reserpine acts on the cell membrane level and desipramine on the granule level. However, at present, there is not enough experimental evidence to prove this hypothesis.

We have shown that angiotensin partially inhibited norepinephrine uptake, and thus possibly re-uptake also. The effect of angiotensin was reflected mostly in a decrease of norepinephrine in the high-speed sediment fraction (Fig. 3), and there was no change in norepinephrine metabolites (Fig. 4). This possibly indicates that angiotensin inhibits the membrane uptake mechanism of norepinephrine. The basis of angiotensin effect on norepinephrine uptake is not completely explained. Our preliminary experiments suggest that it is probably mediated by ion movement across the cell membrane. Metanephrine also decreased norepinephrine uptake in brain (Fig. 3), similar to results obtained by Iverson[12] in rat hearts. Metanephrine, however, increased the amount of norepinephrine metabolites, which suggests that it interferes with norepinephrine storage causing more to accumulate in cytoplasm and to be metabolized. When angiotensin was added along with metanephrine, interference in storage occurred, and thus the metabolic fraction increased to almost 50 per cent of the total radioactivity.

Vasopressin and bradykinin also partially inhibited norepinephrine uptake, but their site of action has not been identified. Angiotensin diamide, which is biologically inactive and which was used as control for angiotensin, did not show any effect on norepinephrine uptake. Therefore, the effect of angiotensin is not due to the 'mass action effect'.

The results obtained with reserpine and cocaine (Fig. 4) also can be explained by Carlsson's theory.[10] Cocaine blocked the catecholamine membrane uptake causing a decrease of total radioactivity in brain. However, the mechanism by which cocaine did interfere with norepinephrine metabolism remains unexplained. On the other hand, reserpine blocked the storage granule concentrating mechanism, but had no effect on the membrane pump. Reserpine did not change the total amount of radioactivity in the brain (Fig. 4), but greatly increased the proportion of metabolites. A similar effect was produced by ACh. This indicates that ACh probably also interferes with storage of norepinephrine. The possible blockade of storage causing norepinephrine to diffuse extraneuronally may explain the role of ACh in norepinephrine release.[13]

The effect of angiotensin and other drugs on efflux of norepinephrine from pre-equilibrated brains is shown in Fig. 5. Assuming that the radioactivity in the sediment fraction represents catecholamines in storage granules and that the supernatant

fraction represents cell cytoplasm, it does not appear that angiotensin released norepinephrine from storage granules. The decreased norepinephrine in the supernatant can probably be explained by partial inhibition of re-uptake. Metanephrine and tyramine, on the other hand, released norepinephrine from storage granules as shown by a decreased radioactivity in the sediment fraction with no change in the supernatant fraction and an increase in the washout fraction. Angiotensin combined with these indirectly acting sympathetic amines did not change the pattern greatly.

Our data suggest that angiotensin partially blocks uptake of norepinephrine in brain, acting on the membrane pump, and does not cause a release of norepinephrine. Since termination of transmitter action is mainly by re-uptake of released norepinephrine, angiotensin, by blocking this, increases the amount of neurotransmitter at the receptor sites, thus probably inducing central sympathetic excitation. Our results obtained with rat spleen slices and aortic strips showed that inhibition of norepinephrine uptake by angiotensin occurred also in peripheral sympathetic nerves,[14] suggesting that it is a generalized phenomenon. Knowing this, one possibly may explain the findings of McCubbin and Page,[1] and of Khairallah *et al.*,[15] that angiotensin potentiates the pressor and myotropic effects of tyramine and metanephrine by a mechanism of supersensitivity similar to that seen with cocaine and imipramine.[16]

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