PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **151**, 650-653 (1976)

Angiotensin Interactions with Myocardial Sympathetic Neurons: Enhanced Release of Dopamine-β-Hydroxylase during Nerve Stimulation (39278)

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Interactions between angiotensin and the sympathetic nervous system which result in facilitation of adrenergic neurotransmission have been reported by numerous investigators (1-6). This facilitatory effect of angiotensin is associated with an increase in norepinephrine in the synaptic cleft which could result from inhibition of neuronal amine uptake (2, 7, 8), stimulation of norepinephrine release (9, 10), facilitation of norepinephrine release during sympathetic nerve stimulation (11-13), or any combination of these actions. We have reported that the interaction(s) of angiotensin with myocardial adrenergic neurons can be blocked with a specific competitive antagonist of the peptide (14). Earlier studies performed in this laboratory also indicated that the neurogenic myocardial response to angiotensin only occurred during nerve stimulation (14). If angiotensin facilitates transmitter release from adrenergic neurons, then dopamine-β-hydroxylase (DβH) release should also occur. The purposes of the present investigation were (i) to determine the dependence of the induced DβH release on nerve stimulation, (ii) to determine whether the neurogenic component of angiotensin was accompanied by increased release of DβH as estimated by assay of enzyme activity, and if so (iii) to establish the susceptibility of this parameter of the angiotensin-induced neurogenic response to blockade with the reversible competitive angiotensin antagonist, saralasin.

Methods. Male New Zealand white rabbits (2-3 kg) were sacrificed by cervical dislocation, the hearts were removed within 15 sec and placed in oxygenated (95% O_2:5% CO_2) modified Krebs' solution. Isolated left atria were prepared acording to the method of Levy (15). Left atria were halved, placed in 10-ml muscle chambers containing buffer at 30 ± 1°, and were connected by silk thread to Grass force-displacement transducers (FTO3C) for recording isometric contractions with a Grass Polygraph. The atrial halves were placed between parallel coiled platinum electrodes for field stimulation (nerve and muscle) or placed in contact with two silver electrodes for point stimulation (muscle only). Stimulation parameters for field stimulation were supramaximal voltage (2× threshold), 5 msec duration, and 96 square wave pulses/min. Point-stimulated atria were run at threshold voltage (usually less than 1 V). The relationship of developed tension versus resting tension was obtained for each atrial half, and a resting tension of 50% of that tension associated with maximal contraction was applied (~1.5-2.0 g). The buffer bathing the tissues was changed every 10 min for a total equilibration time of 60 min prior to the beginning of each experiment. In some experiments, halved atria were prepared as described above but no form of electrical stimulation was used.

Activity of DβH released into the buffer bathing the halved atria was estimated using the coupled enzyme, radiochemical assay described by Molinoff *et al.* (16). For these studies, the buffer contained 0.5% bovine serum albumin (crystallized and lyophilized), and 5 ml was used in each muscle chamber. After a 20-min control period, the bath-fluid was collected, its volume measured in a syringe and placed on ice for later assay. The atria were allowed 10 min to equilibrate, the buffer was changed, and angiotensin (Ang) in final concentrations of 10^{-11} to 10^{-9} *M* was added. Twenty minutes later, the bath-fluid was collected, treated as before, and assayed for DβH activity. The assay was run at seven concentrations of $CuSO_4$ (10^{-7}-10^{-4} *M*) using phenylethylamine as substrate. Phenylethanolamine-*N*-methyl transferase was prepared from fresh bovine adrenals (16). The activity determined in boiled enzyme blanks was sub-

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tracted from experimental determinations. The data were subjected to statistical evaluation by Student's paired-*t* test, and significant differences between the means were calculated as *P* values. The materials used in the study were: angiotensin II (Hypertensin-CIBA, 83% purity) Ciba-Geigy Corp., Summit, New Jersey; catalase, sodium fumarate, S-adenosylmethionine, Sigma Chemical Co., St. Louis, Mo.; saralasin (P-113, Sar[1]-ala[8]-angiotensin II) Norwich Pharmacal, Norwich, New York; pargyline HCl (Eutonyl) Abbott Labs., North Chicago, Ill.; L-ascorbic acid, Eastman Organic Chemicals, Rochester, N.Y.; [^{3}H]S-adenosylmethionine, New England Nuclear, Boston, Mass.

Results. In general, the release of DβH from adrenergic neurons during control periods was greater in the field-stimulated than in either point-stimulated or unstimulated atria. For example, control DβH activity was detected only in baths from field-stimulated atria (Table I). We have previously reported that angiotensin facilitated sympathetic neurotransmission in the isolated left atrium only in the presence of active neuronal firing evoked by field-stimulation (14). In this regard, a concentration of angiotensin (3×10^{-10} *M*) which exerted no direct positive inotropic effect on the isolated field-stimulated atrium significantly potentiated inotropic responses to tyramine. As indicated in Table I, this concentration of angiotensin significantly increased the amount of DβH released into the bathing fluid during stimulation of the atria as estimated by enzymatic assay. In contrast, enzyme activity determined in the media from both unstimulated and point-stimulated atria was not elevated significantly over the respective controls by treatment with 1×10^{-10} to 5×10^{-7} *M* angiotensin. The DβH activities in baths from unstimulated atria were not significantly different from point-stimulated atrial preparations.

Since treatment with low concentrations of angiotensin was associated with an increase in the release of DβH from sympathetic nerve terminals during field stimulation of the rabbit split left atrium, we then determined the relationship of DβH release to various concentrations of angiotensin and attempted to antagonize this effect of the peptide with a specific angiotensin antagonist, sar[1]-ala[8]-angiotensin II (saralasin). We had reported earlier (14) that a molar ratio of 300:1 of saralasin to angiotensin was necessary to completely antagonize the neurogenic component of angiotensin. Figure 1 shows the angiotensin-induced change in DβH activity from baths of field-stimulated atria in the presence and absence of saralasin. In our experiments, saralasin (1 ×

TABLE I. EFFECT OF ANGIOTENSIN ON RELEASE OF DβH FROM ISOLATED RABBIT ATRIA.

	DβH activity (nmole prdt[a]/hr/bath)	
	Control	Angiotensin treated (3×10^{-10} *M*)
Field-stimulated	1.0	28.0[b]
	2.5	22.3[b]
	5.2	23.8[b]
	9.1	31.8[b]
Unstimulated	<1.0	<1.0[c]
Point-stimulated	<1.0	<1.0[c]

[a] [^{3}H]*N*-methylphenylethanolamine.
[b] Indicates significantly different from controls $P < 0.05$.
[c] Represents three to four experimental observations.

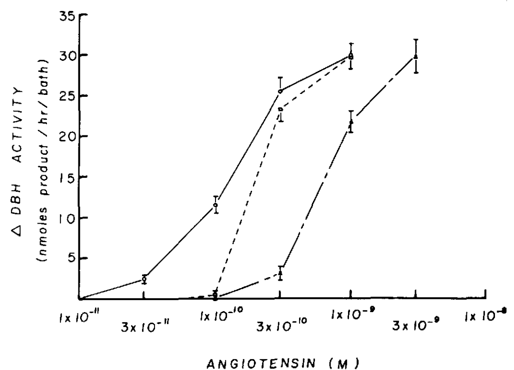

FIG. 1. Effect of saralasin (P-113) on the change in DβH activity produced by angiotensin in field-stimulated rabbit split left atria. O——O represents control responses to angiotensin; □- - -□ represents responses to the same doses of angiotensin in the presence of saralasin (1×10^{-8} *M*); Δ- - -Δ represents responses to angiotensin in the presence of saralasin (3×10^{-8} *M*). Saralasin was added to the atrial baths 5 min prior to beginning the angiotensin dose-response curve. The product measured was [^{3}H]*N*-methylphenylethanolamine. Each point represents the mean of five experimental observations.

10^{-9} to 5×10^{-7} M) failed to alter the basal release of DβH induced by field stimulation. Other investigators have reported that α-adrenergic blocking agents can increase the overflow of DβH during nerve stimulation (17, 18). In the present investigation, saralasin did not attenuate the overflow of DβH induced by phenoxybenzamine. A concentration of 1×10^{-8} M saralasin was required to significantly antagonize the increase in DβH release evoked by angiotensin (1×10^{-10} M). When the data were graphed, it was observed that treatment with saralasin (3×10^{-8} M) shifted the low end of the DβH dose-response curve about one log unit of dose to the right. This figure clearly shows the reversible competitive nature of the inhibition achieved with saralasin in that the dose-response curves to angiotensin are shifted in a parallel fashion to the right, and the same maximal response can be obtained in the presence of the antagonist. The nature of this antagonism permits us to calculate the K_B value (19) which is a quantitative indication of the dissociation constant of the receptor–antagonist complex. The negative logarithm of K_B has been termed the pA_x value by Schild (20). The pA_2 is indicative of the molar concentration of antagonist which reduces the effect of a double concentration of agonist to that of a single concentration. A pA_2 value of 8.0 was determined for saralasin with regard to its effect on atrial adrenergic neurons.

Discussion. We have previously reported that the neurogenic receptors for angiotensin in rabbit atria, which apparently mediate the facilitatory effect of the peptide on adrenergic neurotransmission, differ from atrial myogenic receptors in their sensitivity to angiotensin antagonists (14). The present study determined that activation of the neuronal angiotensin receptor was associated with increased release of DβH from myocardial sympathetic neurons. This release of DβH from field-stimulated atria was dose-dependent and could be antagonized only by high concentrations of saralasin. As reported by Blumberg *et al.* (14), an antagonist:agonist ratio of 300:1 was required to abolish the effect of angiotensin on sympathetic neurons, whereas a threefold excess of saralasin blocked the direct inotropic effect of angiotensin. The present study confirms these data in that a ratio of 200:1 (antagonist:agonist) was required to attenuate the increase in DβH evoked by angiotensin.

The data also indicate that active neuronal firing was necessary for angiotensin to facilitate neurotransmission in the rabbit atrium. If angiotensin acted to directly stimulate secretion-coupling in peripheral adrenergic neurons, an increase in DβH activity in baths of unstimulated and point-stimulated atria should have been observed. The results of the present study do not disprove an inhibitory action of angiotensin on catecholamine uptake which might well operate in concert with a facilitatory action of angiotensin on neurotransmitter release to ultimately enhance sympathetic neurotransmission.

The present study is in accord with the reports of Starke (12) and Starke *et al.* (21) who reported an increase in [^{14}C]- norepinephrine overflow from isolated stimulated rabbit hearts treated with low doses of angiotensin. The effect these investigators observed was not mediated by inhibition of amine uptake. Our data amplify these studies in that we observed an increase in DβH activity in baths from angiotensin-treated atria.

In summary, the present study is further evidence for an interaction of angiotensin with adrenergic neurons in the myocardium. Concentrations of the peptides which do not display inotropic activity in point-stimulated atria result in marked facilitation of the release of neurotransmitter and DβH from adrenergic neurons in field-stimulated atrial preparations. The neuronal receptor for angiotensin is relatively resistant to blockade with an angiotensin antagonist, saralasin.

We acknowledge the generous gift of saralasin (P-113) from Dr. Alan Castellion of Norwich Pharmacal, Norwich, New York, and the technical assistance of Ms. Kellee Chiknas.

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Received December 8, 1975. P.S.E.B.M. 1976, Vol. 151.