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INCREASE IN THE ACTIVITY OF TRYPTOPHAN HYDROXYLASE FROM SLICES OF RAT BRAINSTEM INCUBATED WITH ANGIOTENSIN-11

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There is growing physiological and biochemical evidence that the octapeptide, angiotensin-11 (ANG-11) interacts with neuronal tissue (1-11). At a biochemical level ANG-11 enhances the release of norepinephrine from sympathetic nerve terminals (1,5) and acetylcholine from cortical tissue (9); it alters the content of acetylcholine in parietal cortex (10) and of 5-hydroxytryptamine (5-HT) in hypothalamus and brainstem (11) and it enhances the synthesis of catecholamines in sympathetically innervated tissues (3,7) and of 5-HT in synaptosomal preparations (11). The present communication describes a further interaction of ANC-11 with 5-HT containing neurones, namely the activation of the rate-limiting enzyme in 5-HT formation, tryptophan hydroxylase, that results when slices of rat brain stem are incubated with ANG-11. This increase in tryptophan hydroxylase activity induced by ANG-11 is blocked by addition of the ANG-11 antagonist [sar^1] - [ile^8]-ANG-11 to the brain stem incubation medium.

The procedures used in this study for the preparation and incubation of the slices of brain stem (diencephalon, midbrain and medulla pons), for the isolation of the enzyme from the slice preparation in a low speed supernatant fraction, and for the tryptophan hydroxylase assay have been described in detail elsewhere (12, 13). In the present experiments sliced rat brain stems were incubated individually with shaking at 25° in 5.0 ml of oxygenated medium (12, 13) to which different concentrations of ANG-11 or its fragments (Schwarz-Mann) were added. At the end of a ten-minute incubation period each sliced brain stem was separated from the incubation medium by centrifugation and the pelleted tissue was used as the source of the low speed supernatant preparation of tryptophan hydroxylase. Enzyme was assayed at 37° in the presence of 200 μM L-tryptophan and 50 μM (subsaturating) artificial reduced pterin cofactor, 6-methyl-5,6,7,8-tetrahydropterin (Calbiochem; $6MPH_4$), by a modification of the method of Friedman et al. (14) as described previously (12,13). The enzyme assay was checked for linearity with time and protein in each experiment. Results are expressed in pmoles 5-hydroxytryptophan (5-HTP) formed per mg protein per minute, or as percent of control enzyme activity.

Table 1 shows the effect of different concentrations of ANG-11 on the activity of tryptophan hydroxylase. An increase in enzyme activity of 27% was observed at 5×10^{-8}M ANG-11, and this reached a maximum of 65% at 5×10^{-7}M ANG-11. These concentrations of ANG-11 had no effect on control enzyme activity when added directly to the enzyme reaction mixture. Three ANG-11 peptide fragments, the hepta, hexa and pentapeptides containing, respectively, amino acids 2-8, 3-8 and 4-8 of ANG-11 were also tested on brain stem slices and on low speed supernatant preparations of control enzyme. The results are summarized

TABLE 1
Increase in the activity of tryptophan hydroxylase prepared from slices of rat brainstem incubated with various concentrations of angiotensin-II

ANG-II concentration (M)	Tryptophan Hydroxylase Activity[a] (pmoles 5-HTP/mg protein/min)	n	% Control
0	175 ± 4	14	100
10^{-9}	185 ± 14	3	106
10^{-8}	189 ± 13	3	108
5×10^{-8}	222 ± 4	3	127
10^{-7}	238 ± 12	4	136
2×10^{-7}	229 ± 15	3	131
3×10^{-7}	248 ± 11	3	142
5×10^{-7}	288 ± 8	7	165
10^{-6}	275 ± 17	3	157
10^{-5}	275 ± 8	3	157

[a]Tryptophan hydroxylase activity is expressed as the mean ± the SEM of values from 3 or more enzyme preparations, (n), each of which was made from a single brain stem. The activity of each brain stem preparation was the average of six determinations.

TABLE 2
Effect of the heptapeptide (2-8), hexapeptide (3-8) and pentapeptide (4-8) fragments of ANG-II on the activity of tryptophan hydroxylase from rat brainstem slices

Peptide Concentration (M)	Tryptophan Hydroxylase Activity[a] (% Control)		
	Heptapeptide	Hexapeptide	Pentapeptide
10^{-9}	97		
10^{-8}	108		
10^{-7}	129,129	91	108
10^{-6}	136	98	103

[a]Tryptophan hydroxylase activity is the average of six determinations on a single brain stem enzyme preparation and is expressed as % control activity. The mean activity of enzyme from the untreated (control) brain stem slice preparations was 188 ± 7 pmoles 5-HTP/mg protein/min (5 enzyme preparations).

in Table 2. There was a 36% increase in enzyme activity after treatment of the brain stem slice preparation with 10^{-6}M ANG-II heptapeptide. None of these peptides had any effect on enzyme activity when added directly to the enzyme preparation. Finally the ANG-II antagonist [sar^1] - [ile^8]-ANG-II (15) (Beckman) was tested to see if it would block the increase in tryptophan hydroxylase activity observed after incubation of the brain stem slice preparation with 5×10^{-7} M ANG-II. The antagonist had no effect on tryptophan hydroxylase activity when incubated with the brain stem slices in the absence of ANG-II

TABLE 3

Antagonism of the ANG-II-induced increase in tryptophan hydroxylase activity by addition of [sar^1] - [ile^8]- ANG-II to the brainstem slice incubation medium

[Sar^1] - [ile^8]-ANG-II concentration (M)	Tryptophan Hydroxylase Activity[a] Control	n	ANG-II[b]	n
0	100	3	155 ± 5	6
5×10^{-7}	100 ± 2	3	123 ± 1	3
7.5×10^{-7}	101	1	120 ± 6	3
10^{-6}	104 ± 4	3	105 ± 7	3
5×10^{-6}	100 ± 3	3	99 ± 2	3

[a]Tryptophan hydroxylase activity is expressed as a percentage of untreated control enzyme (100 per cent) ± the S.E.M. The number of individual brain stem enzyme preparations assayed is given under n. The average control enzyme activity was 175 ± 6 pmoles 5-HTP/mg protein/min (n = 8).

[b]The concentration of ANG-II in the brain slice incubation medium was 5×10^{-7}M.

TABLE 4

Effect of ANG-II on the kinetic properties of tryptophan hydroxylase from brainstem slices[a]

	I Tryptophan		II $6MPH_4$	
Incubation medium	K_m (μM)	V_{max}	K_m (μM)	V_{max}
Control	81 ± 5	196 ± 6	173 ± 10	214 ± 5
ANG-II	63 ± 4	267 ± 7	104 ± 5	284 ± 4
	P < 0.05	P < 0.001	P < 0.005	P < 0.001

[1]Tryptophan concentration was varied in the presence of 300 μM $6MPH_4$.

[II]$6MPH_4$ concentration was varied in the presence of 500 μM L-tryptophan.

[a]The concentration of ANG-II in the brain slice incubation medium was 5×10^{-7}M. Each value of K_m and V_{max} is the mean ± the S.E.M. of the reciprocals of the intercepts from three ($6MPH_4$ concentration varied) or six (tryptophan concentration varied) Lineweaver-Burk plots determined by linear regression. The statistical significance of differences between values of K_m and V_{max} for control and ANG-II-treated enzymes was determined by the student t-test.

(Table 3). However, the ANG-II-induced increase in enzyme activity was partially blocked in the presence of 5×10^{-7} M [sar^1] - [ile^8]-ANG-II and was completely blocked when the antagonist concentration was increased to 5×10^{-6}M (Table 3).

The effects of ANG-II on the kinetic properties of tryptophan hydroxylase are summarized in Table 4. There was a small but significant decrease in the K_m of the enzyme for both substrate and artificial cofactor and a modest increase in V_{max}.

The concentration of ANG-II required to produce this activation of tryptophan hydroxylase is very high, a fact which raises questions regarding the physiological relevance of these observations. On the other hand the peptide must diffuse into the 250 μ slices of brain stem and much of it is presumably degraded in the process. Experiments are in progress to determine whether lower concentrations of ANG-II are effective in the presence of protease inhibitors.

The increase in enzyme activity reported here is seen only when ANG-II is incubated with the brain slice preparation and never when it is added directly to the enzyme supernatant. In contrast Nahmod et al. (11) obtained an incease in tryptophan hydroxylase activity when ANG-II was added directly to their enzyme preparation. One explanation for the discrepancy between the data presented here and those of Nahmod et al. may lie in the nonlinearity of the assay used by Nahmod et al.

Many of the events triggered by ANG-II such as smooth muscle contraction, an increase in the contractile force of cardiac muscle and the release of catecholamines from the adrenal medulla are calcium dependent. In addition, recent evidence indicates that ANG-II enhances the permeability of cardiac muscle to calcium (16). These observations suggest that ANG-II may alter calcium availability within excitable tissues and they raise the possibility that the ANG-II-induced activation of tryptophan hydroxylase reported here may also be calcium mediated. The fact that tryptophan hydroxylase is activated when prepared from brain stem slices which have been incubated with drugs or under conditions that promote calcium uptake or intraneuronal release (12,13) lends support to this hypothesis. Preliminary data indicate that the ANG-II-induced increase in tryptophan hydroxylase activity is reduced, though not abolished, when the brain stem slices are incubated in a calcium free medium. The possibility that calcium ions may mediate the effects of ANG-II on tryptophan hydroxylase is now under investigation.

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