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Binding of Angiotensin by Neurohypophysis and Adenohypophysis *in Vivo* and *in Vitro*[1] (39816)

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Several laboratories have reported that angiotensin II (A II) can stimulate release of antidiuretic hormone (ADH) *in vivo* (1-4), and probably adrenocorticotrophic hormone (ACTH) as well (5). These actions of A II could be mediated at the level of the pituitary and/or higher up in the central nervous system (CNS), most likely in the hypothalamus. If either or both of these regions are CNS targets of A II, binding to receptors must occur and such binding might be detected using tracer methods. Therefore, we have studied the uptake of labeled angiotensin administered into the peripheral vascular system by CNS *in vivo*, and by isolated pituitary lobes incubated with labeled angiotensin I and II *in vitro*.

Materials and methods. In vivo studies. Pentobarbital-anesthetized rats of both sexes received a bolus of 0.125 or 0.25 ml of 0.154 *M* saline containing either 3 or 6 ng of mono-[^{125}I]iodoangiotensin I (sp act = 0.4 μCi/ng) via a carotid cannula directed cephalad. Four minutes later a sample of cerebral spinal fluid (CSF) was aspirated from the cisterna magna, and the heart and great vessels were immediately cut. After 1 min of exsanguination the rat was quenched in liquid nitrogen. Samples of CSF, pooled plasma, and tissue (still frozen) were removed, weighed, and counted in a Nuclear Chicago solid scintillation counter (efficiency 60%). The ratio T/P was calculated as:

$$\frac{T}{P} = \frac{\text{dpm/g of fresh tissue}}{\text{dpm/ml of plasma}},$$

and different tissues were compared on this basis. It is important to realize that the T/P ratio represents an instantaneous relationship which is only true at the time of sampling, since this is not a steady state. The assumption has been made that conversion of angiotensin I to II will occur *in vivo*. Furthermore, since the degradation rate of iodinated angiotensin is probably much slower than the native hormone *in vivo* (6), it has been assumed that despite several recirculations most of the hormone is still intact (see also *Discussion*). The biological activity of A II labeled with one atom of iodine is about 35-65% of the native hormone, depending on the bioassay system used (7).

In vitro studies. These studies were done to evaluate whether the observed binding of angiotensin to both pituitary lobes *in vivo* was saturable. Unanesthetized rats were decapitated, the hypophysis was removed, and the lobes were separated by needle dissection. Time required is 1 1/2 min/rat. The isolated lobes were incubated at 37° in aerated Krebs-Ringer bicarbonate buffer (KRB) + 10 m*M* glucose containing either labeled angiotensin I or II (see below). Following incubation, tissues were blotted, weighed, and prepared for counting along with a sample of medium. The ratio T/M was calculated as:

$$\frac{T}{M} = \frac{\text{dpm/ml of tissue water}}{\text{dpm/ml of incubation medium}}.$$

Included in the value of T is saturable binding, free label dissolved in tissue water, and nonspecifically bound label. Tissue water was taken as 76%. When ^{14}C was the label, tissues were solubilized in protosol and quench correction was made. All samples were counted to ±1% error. Isotope purity was confirmed by paper chromatography. Statistical significance was assessed by Stu-

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dent's *t* test. Two types of experiments were done.

^{125}I-Labeled angiotensin I (Schwartz-Mann No. 750-53; sp act = 0.4 μCi/ng). One group of pituitary lobes (neurohypophyses and adenohypophyses together) was incubated with mono-[^{125}I]iodoangiotensin I at a concentration of 1 ng/ml (0.4 μCi/ml), and another group was incubated with the same amount of labeled A I plus 75 ng/ml of cold carrier. *T/M* ratios for a given tissue were computed for both conditions and compared. Any significant decrease in *T/M* radioactivity ratio seen in the presence of excess cold angiotensin was taken to represent saturable binding. In each experiment three pairs of glands were incubated in 3 ml of medium for 10 min (beginning after 20 min of equilibration at 37° in KRB).

[^{14}C]Angiotensin II (New England Nuclear No. NEC570; sp act = 0.00025 μCi/ng). One pair of lobes was incubated in 2 ml of KRB containing 100 ng/ml of [^{14}C]angiotensin II (0.025 μCi/ml) for either 10, 20, 30, or 40 min beginning immediately after removal of the lobes. Because of the very low specific activity of this commercial preparation, and because our experiments with A I at 75 ng/ml indicated that many of the A I binding sites were already saturated at this concentration, no further addition of carrier was done. In a few experiments 3–5 mg of cerebral cortex, a neural tissue not known to be affected by A II, was incubated with the pituitaries for comparison.

Results and discussion. In vivo. Figure 1 shows the relative *T/P* ratios 4 min after intravascular injection of labeled A I. The greatest accumulation of label was in two known target tissues of A II, namely adrenal and kidney (8). Intestine and heart, which are affected to a lesser degree (8), had lower *T/P* ratios. Skeletal muscle and fat, which are not thought to be targets (8), had low values. Thus, there appeared to be increased uptake of label in known target tissues. It was therefore of interest that both pituitary lobes accumulated label.

T/P ratios for neurohypophysis varied from 0.61 to 2.4, and from 0.7 to 1.5 for adenohypophysis. Without exception, other regions of brain sampled, including hypothalamus, had low activity. Failure to find increased uptake of label by CNS except in pituitary could mean that angiotensin II does not have its effect on ADH release in the hypothalamus. However, it is important to realize that if there were small regions of increased uptake in a large tissue sample with an overall low *T/P,* such binding would likely be missed. There was also no detectable label in CSF, but we did not sample from the third ventricle where A II infusion has been shown to stimulate ADH release (3).

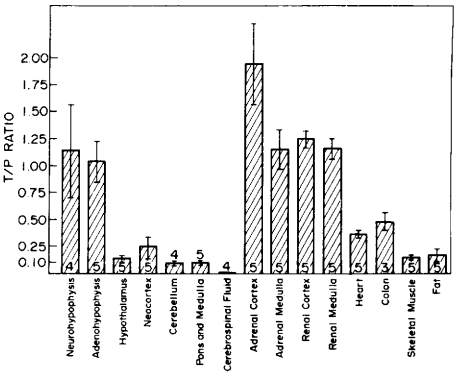

FIG. 1. Tissue to plasma radioactivity ratios for various tissues 4 min after carotid injection of 3 or 6 ng of mono-[^{125}I]iodoangiotensin I (1.25 or 2.5 μCi.) MEANS ± SEM. Numbers at the bars indicate number of experiments.

It is not known whether the label in both pituitary lobes represents A I, A II, A III (2–8 heptapeptide), or smaller degradation products. However, the half-time of ^{131}I-labeled angiotensin II has been reported to be hours (6), rather than minutes. Thus, iodination appears to decrease markedly the rate of metabolic degradation of A II. Therefore, it is very likely that most of the label represents either A I (if conversion is slow or did not occur) or A II. Furthermore, metabolic degradation products of [^{3}H]A II accumulate in brain (9), but we found very low activity in all regions of brain tested except pituitary.

The uptake of label by both pituitary lobes was consistent with the idea that they might be target tissues. Therefore, an attempt was made to determine whether the uptake of label represented saturable or nonspecific binding.

In vitro. ^{125}I-labeled angiotensin I. Table I summarizes the results of 10-min incuba-

TABLE I. EFFECT OF INCUBATING ANTERIOR AND POSTERIOR PITUITARY IN 1 ng/ml (0.4 μCi/ml) OF ^{125}I-LABELED ANGIOTENSIN I IN THE PRESENCE (HOT AND COLD) AND ABSENCE (HOT) OF A 75-FOLD EXCESS OF UNLABELED ANGIOTENSIN I.[a]

Tissue	*T/M* Hot	*T/M* Hot and cold	Mean decrease in binding (%)	*P*
Posterior pituitary n = 6 (3)[b]	2.59 ± 0.20	1.74 ± 0.06	33	0.003
Anterior pituitary n = 6 (3)	1.55 ± 0.06	1.30 ± 0.04	16	0.003

[a] Means ± SEM.
[b] Number of pairs of glands per experiment is given in parentheses.

tions of anterior and posterior pituitary with 1 ng/ml of labeled A I in the presence and absence of 75 ng/ml of cold A I. Both lobes showed a significant reduction in binding of label in the presence of excess cold carrier, indicating that some of the binding of A I by both pituitary lobes is saturable. The percentage decrement for neurohypophysis was nearly twice that for anterior lobe. The absolute amount of label bound per gram of tissue was also significantly greater for neurohypophysis ($P < 0.001$).

In these studies we neither blocked conversion of angiotensin I to II, nor measured the rate of conversion. However, since the volume of the medium is very large compared to the total volume of tissue (~15 mg of tissue/3 ml of medium), it is unlikely that such a small amount of tissue could convert to A II and/or metabolize a significant fraction of the total mass of A I present (225 ng) in 10 min. Therefore, labeled A II or labeled metabolites released into the medium from the tissue probably constitute a negligible proportion of the total counts present in the medium. On the other hand, we did not measure the relative proportions of angiotensin I, II, and metabolite(s) in the tissue, and significant binding or trapping of these substances in the tissue would raise the *T/P* ratio. However, unless angiotensin II or its metabolites compete for the same sites as angiotensin I, the decrement in binding of label seen when carrier is added should primarily reflect competition between carrier and isotope for angiotensin I binding sites.

[^{14}C]Angiotensin II. These studies were done to determine to what extent both pituitary lobes could accumulate label over time. An effect of A II on ADH release can be seen in less than 10 min (2), so if the binding is related to this process, uptake should be rapid. The time course of the postulated effect of A II on ACTH release has not been studied, but an effect on cortisol can be seen in 30 min (5).

For both lobes of pituitary, *T/M* increased to a maximum of approximately 5 (Fig. 2) at 30 min and then, for neurohypophysis, began to decline ($P < 0.05$). The apparent decrease for adenohypophysis is not significant. The amount of activity was the same for either lobe except at 20 min. At 10 min there was no difference between either lobe of pituitary and cerebral cortex, but by 30 min a clear difference was present ($P < 0.01$). The plateau in binding at 30 min suggests that saturation of binding sites may have occurred. In fact, a peak in binding at 30 min with a subsequent decline has been reported for other target tissues of angiotensin II (10, 11). However, this is a difficult finding to interpret physiologically since no known effects of angiotensin require nearly so long to be manifested.

It is also possible that the concentration of

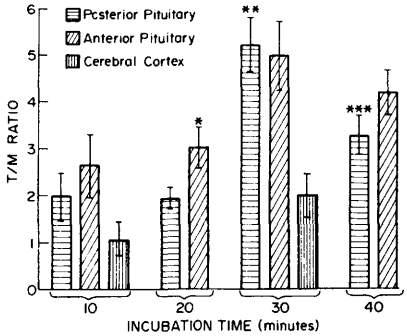

FIG. 2. Effect of incubating different tissues with 100 ng/ml of [^{14}C]angiotensin II (0.025 μCi/ml) for varying lengths of time. Means ± SEM. Number of experiments for each incubation time: 10 min, two; 20 min, five; 30 min, three; 40 min, five. *, $P = 0.05$ adenohypophysis (AP) > neurohyophysis (PP); **, $P = 0.01$ AP and PP > cerebral cortex; ***, $P = 0.03$ PP (30 min) > PP (40 min).

label by both lobes of pituitary at longer incubation times represents accumulation of metabolic products of A II. The long incubation time would permit increased degradation of the hormone by tissue, even though there is a large mass of A II available (200-ng total) and a very small volume of tissue. It has been shown that homogenates of brain can metabolize [^{3}H]A II (12). In addition, Khairallah *et al.* (9) have reported a prominent accumulation of labeled degradation products of [^{3}H]A II in brain 30 min after intravascular administration. Only adrenal, kidney, and urine had higher activity.

Thus, athough binding of labeled angiotensin *in vivo* and *in vitro* is consistent with the hypothesis that both pituitary lobes may be targets of angiotensin, more data are required before this conclusion may be drawn. Binding of a hormone, even saturable binding, is not sufficient evidence. For example, binding of angiotensin I could represent binding to the converting enzyme. Both pituitary lobes contain considerable amounts of the enzyme, the posterior pituitary more than the anterior (13). This is entirely consistent with our data that both pituitary lobes specifically bind angiotensin I and that per gram of weight, neurohypophysis binds more than adenohypophysis.

Binding may also represent such processes as sequestration, transport, and metabolic degradation. Very many tissues contain angiotensinases which rapidly degrade angiotensin II. These enzymes would also show saturable binding of A II. Therefore, in order to assert that angiotensin II binding is related to release of ADH and/or ACTH, additional data are required. Specifically, it must be shown that binding is accompanied by hormone release. One laboratory has reported that incubation of isolated neural lobes with angiotensin II is accompanied by ADH release (14), but this finding has been disputed by two laboratories (15, 16). Thus, the question of the physiological correlate(s) of angiotensin binding by pituitary remains to be answered.

Summary. Following intra-arterial injection of ^{125}I-labeled angiotensin I *in vivo* (which is presumed to be converted to angiotensin II), there was similar and significant accumulation of label by both lobes of pituitary. Other regions of brain including hypothalamus showed very little uptake of label. *In vitro* incubations of both pituitary lobes with angiotensin I showed that neurohypophysis bound more hormone per unit weight than adenohypophysis, and that a portion of the binding in both lobes was saturable. Studies with angiotensin II *in vitro* showed that both lobes could concentrate label equally to a level five times that of the medium. A plateau in binding occurred at 30 min for both lobes, which is consistent with the interpretation that a portion of this binding is also saturable. However, most actions of angiotensin II occur within 5 min, so the peak in binding at 30 min may not be related to a physiological action of angiotensin II on neurohypophysis or adenohypophysis. Thus, the physiological correlates of angiotensin I and II binding by both pituitary lobes *in vivo* and *in vitro* remain to be established.

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