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The Effect of Angiotensin II Infusion on Plasma Catecholamines in the Conscious Rabbit (41242)

BRIAN P. ROWE[1] AND ALBERTO NASJLETTI

Department of Pharmacology, University of Tennessee, Memphis, Tennessee 38163

Abstract. Plasma levels of norepinephrine, epinephrine, and dopamine were measured in conscious, male rabbits. Twenty-minute infusions of 5, 50, and 500 ng/kg/min angiotensin II elevated mean arterial blood pressure by 8 ($P < 0.05$), 23 ($P < 0.01$), and 33 mm Hg ($P < 0.01$), respectively. Plasma catecholamines were unchanged at all levels of angiotensin II infusion except epinephrine which rose from 15 ± 3 to 52 ± 17 pg/ml at 500 ng/kg/min. These data suggest an action of the peptide at the adrenal medulla but offer no evidence of a generalized stimulation of sympathetic discharge by antiotensin II.

Blockade of α-adrenergic receptors attenuates the rise in blood pressure induced by angiotensin II in the rabbit (1) and rat (2) suggesting involvement of adrenergic mechanisms in the pressor action of the peptide. This is consistent with evidence implicating angiotensin II in the promotion of sympathetic nervous activity at several levels. For example, angiotensin II stimulates the central nervous system and sympathetic ganglia, releases catecholamines from the adrenal medulla, induces synthesis of the adrenergic transmitter, facilitates transmitter release from nerve fibers, and inhibits neuronal reuptake of norepinephrine (3). However, much of this information is derived from studies *in vitro*, and in anesthetized animals subject to surgical stress, and, consequently, may not be applicable to more physiological settings. The present study was designed to investigate the effects of angiotensin II on plasma catecholamine levels in the conscious rabbit.

Methods. Seven male New Zealand White rabbits (1.9–2.7 kg) were fed Purina Rabbit Chow (Na content: 100 meq/kg; K content: 570 meq/kg) and water *ad libitum*. Conscious animals were placed in a restraining box and a polyethylene cannula (PE50, Clay Adams) was inserted into a central ear artery under local anesthesia (Xylocaine, Astra). Blood pressure was monitored with a Statham pressure transducer (P 23Db) connected to a Grass polygraph (Model 5C). Drugs were infused into a marginal ear vein via a 23-gauge needle. The animals remained undisturbed for at least 30 min before infusions were started.

Two different protocols, separated by 4 to 8 day intervals were followed in each animal. Each protocol consisted of six 20-min infusion periods (0.18 ml/min). In one protocol, Ile^5-antiotensin II (Sigma) was infused in periods 2, 4, and 6 at 5, 50, and 500 ng/kg/min, respectively, whereas the vehicle (5% dextrose) was given in periods 1, 3, and 5. In the second protocol, 5% dextrose was given in all six periods.

One-milliliter blood samples were withdrawn from the central ear artery at the end of each 20-min infusion period and collected in chilled glass test tubes containing 20 μl of a solution containing ethylene glycol bis (β-aminoethyl ether) N,N'-tetraacetic acid (EGTA, 90 mg/ml), and glutathione (60 mg/ml). Samples were centrifuged and the plasma was stored at −20°C prior to assay. Radioenzymatic assay for catecholamines was performed with a commercially available kit (Cat-a-kit) supplied by Upjohn diagnostics. This procedure is a modification of that described by Passon and Peuler (4).

To confirm that we could detect changes in plasma catecholamines using our methodology, we determined plasma norepinephrine and epinephrine levels in blood samples (2 ml) withdrawn from a central ear

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[1] Reprint requests should be addressed to: Brian P. Rowe, Ph.D., Department of Physiology, Quillen–Dishner College of Medicine, Box 19780A, East Tennessee State University, Johnson City, Tenn. 37614.

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TABLE I. EFFECTS OF ANGIOTENSIN II ON PLASMA EPINEPHRINE, NOREPINEPHRINE, DOPAMINE, AND MEAN ARTERIAL BLOOD PRESSURE ($n = 7$)

		Vehicle		Angiotensin II (5 ng/kg/min) or vehicle		Vehicle		Angiotensin II (50 ng/kg/min) or vehicle		Vehicle		Angiotensin II (500 ng/kg/min) or Vehicle
Mean arterial blood pressure (mm Hg)	Experimental	75 ± 3	*	83 ± 3	*	75 ± 3	**	98 ± 3	**	77 ± 3	**	110 ± 4
	Control	76 ± 4		76 ± 4		75 ± 2		73 ± 2		71 ± 2		72 ± 2
Plasma norepinephrine (pg/ml)	Experimental	224 ± 145		128 ± 54		172 ± 79		83 ± 34		134 ± 46		142 ± 31
	Control	185 ± 55		170 ± 56		155 ± 57		250 ± 96		141 ± 46		133 ± 53
Plasma epinephrine (pg/ml)	Experimental	24 ± 9		21 ± 10		16 ± 8		13 ± 4		15 ± 3		52 ± 17*
	Control	42 ± 23		21 ± 8		16 ± 5		26 ± 6		17 ± 3		18 ± 4
Plasma dopamine (pg/ml)	Experimental	95 ± 18		74 ± 14		71 ± 17		55 ± 8		61± 7		92 ± 20
	Control	87 ± 14		66 ± 10		60 ± 13		87 ± 21		55 ± 10		56 ± 4

Note. Each figure represents a mean ± SEM. Asterisks between two means denote a significant difference between those two means. A superscript asterisk indicates that the figure is significantly different from the remaining five treatment groups. The vehicle for angiotensin II was 5% dextrose.

* $p < 0.05$.

** $p < 0.01$.

artery before and after 5-min infusion of tyramine (270 μg/kg/min iv) in five conscious rabbits. Tyramine elevated mean arterial blood pressure from 92 ± 3 to 108 ± 7 mm Hg ($P < 0.05$) and plasma norepinephrine from 248 ± 25 to 955 ± 59 pg/ml ($P < 0.001$). Epinephrine levels were not significantly altered (25 ± 9 vs 37 ± 6 pg/ml).

Data were treated by analysis of variance and a Newman–Keuls test was used to contrast individual means. A P value less than 0.05 was considered significant.

Results. Table I shows the blood pressure and catecholamine data during the infusion of angiotensin II or vehicle. Angiotensin II infused at 5, 50, and 500 ng/kg/min elevated mean arterial blood pressure by 8, 23, and 33 mm Hg, respectively. Plasma epinephrine was significantly elevated (52 ± 17 versus 15 ± 3 pg/ml) during infusion of angiotensin II at 500 ng/kg/min, but it was not affected by infusion of the peptide at 5 and 50 ng/kg/min. Plasma norepinephrine and dopamine levels did not change with infusion of angiotensin II at any dose. In control studies, the intravenous infusion of 5% dextrose, the vehicle for angiotensin, had no effect on either blood pressure or plasma catecholamines.

Discussion. In the present study, angiotensin II infusion at extremely high rates did not affect plasma norepinephrine and dopamine levels. However, studies *in vitro* and in anesthetized animals indicate that angiotensin II facilitates norepinephrine release from sympathetic nerves and inhibits its neuronal reuptake (3), both of which actions are likely to result in elevation of plasma norepinephrine. With regard to this inconsistency it is possible that angiotensin II is not an efficacious promotor of sympathetic activity in the conscious rabbit at rest or, if it is, the effects may be transient. Alternatively, promotion of sympathetic mechanisms by angiotensin II might be offset by pressure-induced baroreflex inhibition of sympathetic activity in the conscious animal.

Infusion of angiotensin II at 500 ng/kg/min in the present study increased plasma epinephrine levels. As circulating epinephrine arises primarily, if not exclusively, from the adrenal medulla, it is reasonable to infer that the rise in plasma epinephrine caused by angiotensin II results from stimulation of the adrenal medulla. This finding extends to the conscious rabbit, earlier observations indicating that angiotensin II promotes release of catecholamines from the adrenal gland in anesthetized animals subject to varying degrees of surgical stress, and from the adrenal gland perfused *ex vivo* (3). However, there are reports that angiotensin II does not affect the plasma concentration of catecholamines in man (5) or the urinary excretion of catecholamines in the conscious rabbit (6) and man (7). This conflict between results may arise from differences in experimental conditions, species, and dosage of angiotensin II. The importance of the latter is evidenced in our study, as at lower dosages (5–50 ng/kg/min) the infusion of angiotensin II did not increase plasma epinephrine. Therefore, it would appear that angiotensin II increases plasma epinephrine only when substantial amounts of the peptide are administered, establishing circulating levels that are probably pathological. Yet, recent observations indicate that the medullary catecholamine secretory response elicited by hemorrhage (8) or hypoglycemia (9) is inhibited by maneuvers that suppress the renin–angiotensin system, e.g., nephrectomy or angiotensin receptor blockade. Moreover, administration of angiotensin II in amounts that do not cause direct stimulation of epinephrine release from the adrenal medulla restores the adrenal secretory response to hemorrhage in the nephrectomized animal. This suggests that endogenous angiotensin II facilitates reflex epinephrine release. A direct effect on release appears to be evident only with higher circulating levels of the peptide.

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