Effects of intracerebroventricular injections of angiotensin II on prolactin plasma levels in the Rhesus monkey

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Summary. Intracerebroventricular injections of angiotensin II (A II) induced a sharp and dose-dependent increase in plasma prolactin (PRL) levels in the awake Rhesus monkey. Our results suggest that in primates, A II may be involved in the mechanisms controlling PRL secretion.

Prolactin release is tonically inhibited by the hypothalamus, mainly via neurotransmitters such as dopamine (DA)[2] and γ-amino-butyric acid (GABA)[3,4]. An increasing number of substances have been shown recently to stimulate plasma prolactin (PRL) release; among these are various neuropeptides such as β-endorphin[5], substance P (SP)[6] and vasoactive intestinal peptide (VIP)[7]. Angiotensin II (A II) is an octapeptide known to induce drinking behavior[8] and vasopressin (AVP) release[9] when administered into the brain. Recently, A II immunoreactivity was identified in the hypothalamus and median eminence of rat[10], monkey and human[11] brains. These observations suggest that, like other neuropeptides, A II could be involved in the control of pituitary function.

This work reports the effect of intracerebroventricular (i.c.v.) administration of various doses of A II on PRL levels in the conscious Rhesus monkey. Because of the central hypertensive effect of A II[12], the result of such injections on arterial blood pressure was also evaluated.

Methods. Experiments were performed on 3 ovariectomized adult Rhesus monkeys (*Macaca mulatta*) (b.wt: 4.9–5.6 kg) which had been trained to chair restraining. The animals were fitted with a cannula chronically implanted in the III[rd] ventricle according to a method already described[9]. Water supply to the animals was suppressed 1 h before the beginning of each experiment. Angiotensin II (synthetic, BACHEM) was administered over 30 sec in 5 µl of synthetic cerebrospinal fluid (CSF) in doses of 0, 1, 10 and 50 µg. Blood samples were collected via a chronic cardiac catheter at 30 and 5 min before the microinjection, and then at 5, 10, 20, 30, 45, 60, 90 and 120 min after the end of the microinjection. Prolactin was measured by radioimmunoassay using materials and methods described for human PRL by Reuter et al.[13]. The standard was a monkey PRL preparation, WP-XI-49-29[14], which completely cross-reacts with human PRL.

In separate experiments, arterial blood pressure was measured in 2 animals. The monkeys were tranquillized by Ketamine HCl, and their right femoral artery was joined to a miniature pressure transducer (NARCO-System) connected to a polygraph. They were then returned to the chair and 3 h later received, at hourly intervals, i.c.v.-injections of CSF containing successively 1, 10 and 50 µg A II.

Results and discussion. The effect of i.c.v.-injections of 50, 10 and 1 µg A II on mean PRL plasma levels is illustrated in figure 1. A dose-dependent effect of A II on PRL concentrations was observed. When the 50 µg dose was used (n = 7) a significant rise in plasma hormone levels was seen over 60 min. Prolactin levels rose by 5 min and peaked at 10 min. The peak concentration of PRL was approximately 10 times higher than basal levels. 10 µg A II (n = 5) caused a PRL release of a lesser magnitude and hormone values were only significantly increased from 5 to 20 min post-injection. No significant changes in PRL levels were

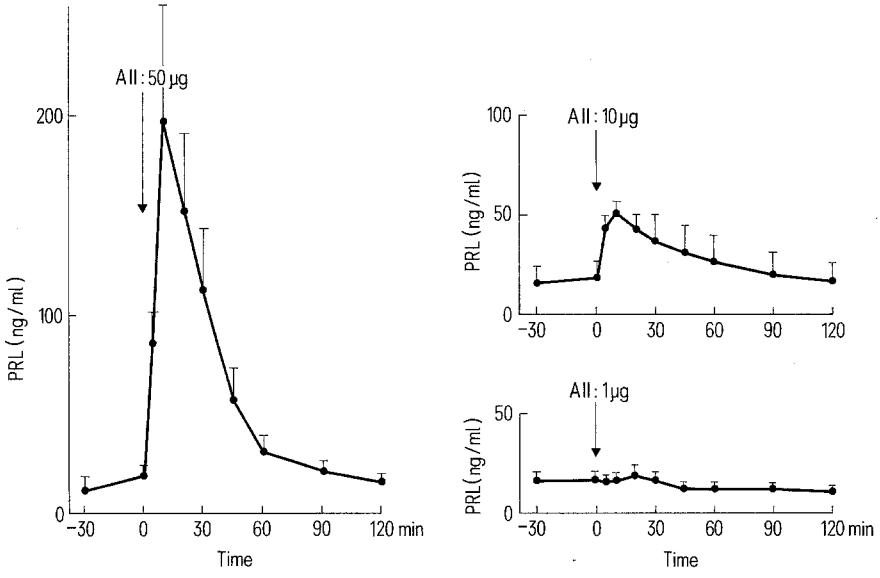

Figure 1. Effect of 50, 10 and 1 µg A II on prolactin plasma levels in ovariectomized Rhesus monkeys. Each point represents mean ± SEM.

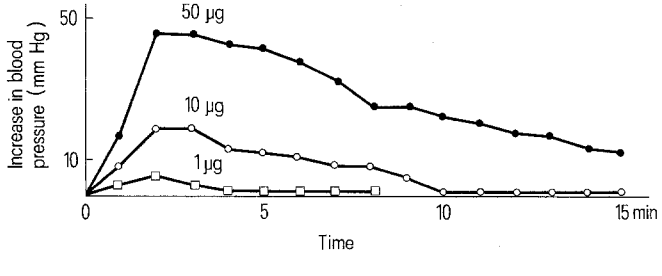

Figure 2. Modifications of arterial blood pressure following 3rd ventricle injections of different doses of A II in monkey 112. Injections were started at time zero.

observed when either 1 µg A II (n = 5) or the solvent alone (n = 3; not shown) were used. I.c.v. injections of A II also increased blood pressure in a dose-dependent fashion. Results were similar in the 2 animals studied and are presented in figure 2 for monkey 112, in which blood pressure increments of 6, 19 and 46 mm Hg were observed after administration of 1, 10 and 50 µg A II respectively.

The effect of A II on PRL release in the monkey differs from that described in the rat. In the latter, Steele et al.[15] have found that i.c.v.-injections of A II lowered PRL plasma levels. Such a discrepancy may be attributed to species differences in the distribution of A II binding sites[16]. The present study indicates that A II may be involved in the control of PRL secretion in primates. The observation that i.c.v.-injections of A II modify in a parallel way both blood pressure and PRL plasma levels suggests the possibility of a link between the 2 phenomena. However, our results do not allow us to decide whether A II increases PRL secretion by acting directly on the nervous mechanisms involved in hormone release or indirectly, via its central effect on arterial blood pressure.

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2 J. Meites and C.S. Nicoll, Rev. Physiol. *28*, 57 (1966).
3 A.V. Schally, T.W. Redding, R.A. Arimura, A. Dupont and G.L. Linthicum, Endocrinology *100*, 681 (1977).
4 G. Racagni, J.A. Apud, V. Locatelli, D. Cocchi, G. Nistico, R.M. Di Giorgio and E.E. Muller, Nature *281*, 575 (1979).
5 A. Dupont, L. Cusan, F. Labrie, D.H. Cox and C.J. Li, Biochem. biophys. Res. Commun. *75*, 76 (1977).
6 C. Rivier, M. Brown and W. Vale, Endocrinology *100*, 751 (1977).
7 M. Ruberg, W.H. Rotsztejn, S. Arancibia, J. Besson and A. Enjalbert, Eur. J. Pharmac. *51*, 319 (1978).
8 J.T. Fitzsimons, Physiol. Rev. *52*, 468 (1972).
9 G. Simonnet, F. Rodriguez, F. Fumoux, P. Czernichow and J.D. Vincent, Am. J. Physiol. *237*, R 20 (1979).
10 D.G. Changaris, L.C. Keil and W.B. Severs, Neuroendocrinology *25*, 257 (1978).
11 J.T. Quinlan and I. Philips, Brain Res. *205*, 212 (1981).
12 W.B. Severs, J. Summy-Long, J.S. Taylor and J.D. Connor, J. Pharmac. exp. Ther. *174*, 27 (1970).
13 A.M. Reuter, F. Kennes, Y. Gevaert and P. Franchimont, Int. J. nucl. Med. Biol. *3*, 21 (1976).
14 WP-XI-49-29 (biological activity 40 IU/mg) was a gift of Dr W.D. Peckham (Pittsburgh, USA).
15 M.K. Steele, A. Negro-Vilar and S.M. McCann, Endocrinology *109*, 893 (1981).
16 J.W. Hardling, L.P. Stone and J.W. Wright, Brain Res. *205*, 265 (1981).

Hypothyroidism lowers blood pressure, adenylate cyclase and Na^+,K^+- and K^+,Ca^{++}-ATPase activities in normotensive and spontaneously hypertensive rats[1]

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Summary. Myocardial isoproterenol-stimulated adenylate cyclase, Na^+,K^+-ATPase and K^+,Ca^{++}-ATPase activities are elevated in the spontaneously hypertensive rat and can be lowered by methimazole-induced hypothyroidism which also prevents the development of hypertension. Although thyroid hormone levels are similar between untreated SHRs and WKY rats, the thyroid is apparently necessary for the expression of spontaneous hypertension.

The spontaneously hypertensive rat (SHR) displays a transitory increase in cardiac index during the development of hypertension[2] similar to that observed in some forms of human hypertension[3]. The altered reactivity of the SHR myocardium may be due to several factors including thyroid status and the activities of adenylate cyclase or the electrolyte transporting enzymes Na^+,K^+-ATPase and K^+,Ca^{++}-ATPase.

Since hypothyroidism attenuates hypertension in the SHR[4] and lowers adenylate cyclase activity[5], an alteration in this enzyme may be a factor in the development or maintenance of hypertension. Hypothyroidism has been shown to alter myocardial Na^+,K^+-ATPase as well as K^+,Ca^{++}-ATPase activity[6] and alterations in these ion transporting enzymes could be one possible explanation for alterations in the contractile properties and reactivity of the SHR myocardium.

The present study was undertaken to determine if, during the development of hypertension, changes occur in adenylate cyclase, ATPase enzymes, or thyroid hormone levels which could account for the altered cardiac index observed in the prehypertensive SHR. Since these enzyme activities of the myocardium appear to be altered by thyroid hormones they were also assessed in hypothyroid animals.

Methods. Hypothyroidism was induced in neonate SHR and WKY rats by the continuous administration of 0.01% methimazole via the drinking water to lactating dames and later to the weaned rats. Blood pressure was measured with a Friedman: Freed Microphonic manometer and tail cuff on conscious, restrained rats after gentle prewarming. Rats were sacrificed at 0, 5, 10, 20, 30 and 100 days of age by decapitation. The blood serum of each group was collected, pooled and stored below -25 °C. The ventricles were rapidly removed and placed in ice-cold homogenization buffer (250 mM sucrose/50 mM Tris HCl, pH 7.5). The pooled ventricles of each group were homogenized in ice-cold homogenization buffer (10 ml/g) by 3 30-sec pulses of a Brinkmann Polytron PT-10 tissue homogenizer ($^1/_2$ maximal speed). The homogenate was centrifuged at 4 °C, 1000 × g for 10 min. The supernatant was centrifuged at 4 °C at 40000 × g for 30 min and the resulting pellet was resuspended in homogenization buffer (approximately 8 mg protein/ml). Protein was determined by the method of Lowry et al.[7]. Adenylate cyclase activity was assessed by measuring the conversion of [$\alpha - ^{32}$P] ATP to cyclic [^{32}P] AMP[8]. ATPase activities were measured at 37 °C by monitoring the release of inorganic phosphorus from 3 mM Tris ATP[9]. Total Na^+,K^+-ATPase activity[10] and K^+,Ca^{++}-ATPase activity[11] were measured by previously determined protocols. Serum T_3 and T_4 levels were measured by competitive binding RIA using procedures outlined by Clinical Assay Division of Travenol Laboratories. Statistical significance was by Student's t-test and analysis of covariance with age as the covariant.