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Renin and Hemodynamic Changes via Central Adrenergic, Cholinergic, and Sodium Receptor Mechanisms in Conscious Rats (39152)[1]

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(Introduced by S. M. McCann)

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The phenomenon of centrally induced natriuresis was first demonstrated in the middle 1960's when Andersson's group showed that third ventricular injection or infusion of hypertonic saline into conscious goats produced an increase in sodium excretion (1, 2). Similar results were reported using anesthetized dogs, cats, and rats (3–5). In addition, a central cholinergic synapse was postulated to be involved in this type of natriuresis (6). The intraventricular injection of hypertonic saline also elicited a concomitant pressor response (3, 4, 7). In view of the important interrelationships between renin, blood pressure, and sodium excretion, this study was designed to measure serum renin activity (SRA) and blood pressure following the third ventricular injection of compounds that modify urinary sodium excretion (8).

Materials and methods. Male Holtzman rats (250–350 g) were housed individually at a constant temperature (24°) and with controlled lighting (6 AM to 6 PM). The animals were given tap water and Purina rat chow (152 mEq of Na/kg) *ad libitum*.

The two experimental protocols involved the measurement of SRA and blood pressure following various intraventricular injections into conscious rats. For experiments in which SRA was measured, chronic cannulae were placed in the third ventricle of the rat, while in the experiments in which blood pressure was measured, rats had both chronic ventricular and arterial cannulae. The surgery was performed under ether anesthesia at least 48 hr prior to the experimental procedure.

The ventricular cannulae were made from 23-gauge stainless steel tubing (20 mm in length) and were implanted according to a technique previously described (9). The injection volume was always 2 μl and this was delivered via a 10 μl Hamilton syringe. The arterial cannulae were made from polyethylene tubing (PE50). The cannula was inserted into the carotid artery, tied in place, and exteriorized posteriorly.

Blood for serum renin activity was obtained from the aorta during the first 4 sec after decapitation in siliconized glass tubes on ice as previously described (10, 11). After the blood was allowed to clot, it was centrifuged at 4° and the serum was frozen (−20°). Serum renin activity (SRA) was determined by radioimmunoassay as previously described (10) with a modification (11).

In the blood pressure experiments, the carotid cannula was flushed with saline, and the systolic and diastolic blood pressures were measured using a Statham PL3 Db strain gauge and a Beckman Dynograph recorder. The intraventricular injection was made after a control period of 10 min and the blood pressure was followed for another 90 min.

The compounds tested in this study were norepinephrine (bitartrate), carbachol (chloride), isoproterenol (hydrochloride), and a hypertonic solution of sodium chloride. Drugs were dissolved in isotonic saline which also served as the control.

The levels of significance were determined by comparison of the experimental to the control (saline) group using the Student's *t* test.

Results. Fig. 1 illustrates the changes in SRA elicited by the third ventricular injection of hypertonic saline, carbachol, and norepinephrine. SRA was unaltered by intraventricular injection of isotonic saline

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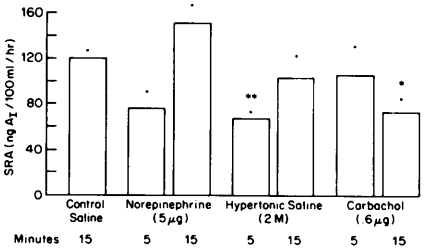

FIG. 1. The effect on renin release of intraventricular injections. The third ventricular injection of isotonic saline (2 μl of 0.9% NaCl), norepinephrine (5 μg), hypertonic saline (2 μl of 2 *M* NaCl) or carbachol (0.6 μg) was followed by decapitation and collection of the aortic blood at either 5 or 15 min after the injection. The bars represent the mean and the points designate the S.E.M. * = $P < .05$, and ** = $P < .01$ when experimental values were compared to appropriate saline controls; $n = 5$ for each group; SRA is serum renin activity.

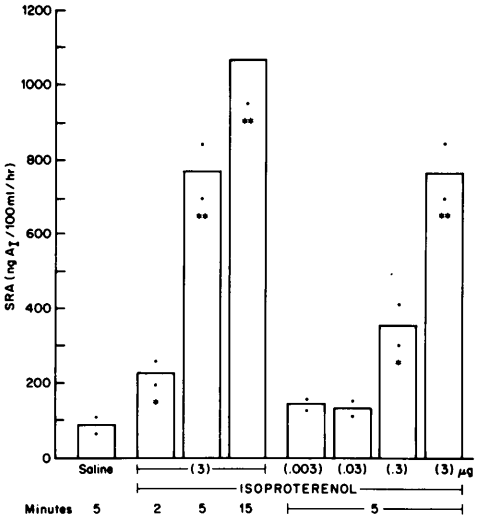

FIG. 2. Chronology and dose-response of isoproterenol-induced renin release. The third ventricular injection of either isoproterenol at 0.003, 0.03, 0.3, 3.0 μg or isotonic saline (2 μl of 0.9% NaCl) was followed 5 min later by decapitation and collection of the aortic blood for SRA. For the chronology, 3 μg of isoproterenol was injected into the third ventricle followed at 2, 5, and 15 min by decapitation. The bars represent the mean and the points designate the S.E.M. * = $p < .05$ and ** = $p < .01$ when experimental values were compared to saline controls; $n = 4$ for each group; SRA is serum renin activity.

(121 ± 16) when compared with noninfected (117 ± 28) conscious rats. The apparent lowering of SRA by NE at 5 min after intraventricular injection was not statistically significant ($P > .05$). At 15 min, SRA was at or above control levels. An increase in the sodium concentration of the cerebrospinal fluid (CSF) produced by the injection of 2 μl of a 2 *M* solution of sodium chloride resulted in a significant decrease in SRA at the 5-min interval with return toward control levels at 15 min. Cholinergic stimulation with carbachol (0.6 μg) produced a similar, but delayed response with a significant decrease evident after 15 min.

Isoproterenol, a beta-adrenergic agonist, elicited a dose related increase in SRA (Fig. 2) in contrast to the decrements produced by hypertonic saline and carbachol. The lowest effective dose tried was 0.3 μg. The effect of isoproterenol on SRA was evident as early as 2 min and lasted for at least 15 min (Fig. 2).

Blood pressure changes induced by the various intraventricular injections are summarized in Table I. Both hypertonic saline and carbachol caused an increase ($P < .05$) in systolic pressure of rapid onset. The pressor response produced by hypertonic saline lasted only 10 min, while that of carbachol was evident for 20 min. Norepinephrine and isoproterenol both induced blood pressure lowering ($P < .05$) after intraventricular injection. The changes in blood pressure appeared to be specific responses and not the result of the injection itself, since there was no change following the isotonic injection.

Discussion. The results of the present investigation indicate that the intraventricular administration of compounds that affect sodium excretion (8) are also effective in modifying serum renin activity and systemic blood pressure. It was previously reported that a decrease in CSF sodium concentration in dogs (12) or sheep (13) produced an increase in plasma renin levels. These changes were not accompanied by any hemodynamic response, nor were they prevented by renal denervation (14). Thus, an increase in CSF sodium concentration might be expected to produce a decrease in renin levels as was the case in our experiments.

Although the ventriculocisternal infusion of a low sodium solution produced no change in blood pressure (12, 13), the injec-

TABLE I. CHANGE IN BLOOD PRESSURE FOLLOWING INTRAVENTRICULAR INJECTIONS OF HYPERTONIC SALINE, CARBACHOL, NOREPINEPHRINE, OR ISOPROTERENOL.[a]

Maximal mean change in blood pressure	0.9% NaCl (7)[b]	Hypertonic saline (9)	Carbachol (7)	Norepinephrine (6)	Isoproterenol (8)
Δ systolic	0	+35** ± 6.3(1)	+26 ** ± 5.5(2)[b]	−9* ± 2.0(7)[b]	−13* ± 3.0(3)[b]
Δ diastolic	0	+28** ± 4.6(1)	+10[d] ± 3.0(1)[b]	−11* ± 2.0(9)[b]	−13* ± 3.0(2)[b]

[a] Values are mean ± SE in mm Hg.
[b] Number of animals per group.
[c] Time (minutes) to maximal change.
[d] Not significant.
* $P < .05$ vs preinjection pressure.
** $P < .01$ vs preinjection pressure.

tion of hypertonic saline was reported to elevate blood pressure in anesthetized dogs (3, 7) and cats (4). The pressor response observed following intraventricular hypertonic saline in the rat is in agreement with these earlier reports.

The central natriuretic action of carbachol was previously demonstrated (6); however, there have been no reports concerning the drug's effect on SRA. In view of the similarity of the urinary response to intraventricular hypertonic saline and carbachol, it is not surprising that this cholinergic agonist also produced a decrease in SRA and an increase in blood pressure. Perhaps the delay noted in its effect on SRA and blood pressure, when compared to hypertonic saline, might be due to the fact that it is not stimulating the primary sodium sensitive receptor, but a synapse in this pathway.

Pressor responses have been shown to occur following the lateral ventricular injection of acetylcholine and carbachol in dogs (15, 16). As might be expected, the natriuretic and pressor responses to hypertonic saline and carbachol were inversely related to SRA. Curiously, norepinephrine was also effective in eliciting a natriuretic response (8), although it did not alter SRA. In addition, a decrement in blood pressure was observed after the norepinephrine injection.

Other investigators have raised the possibility of a beta-adrenergic component of renin release. The systemic injection of isoproterenol was shown to produce an increase in SRA (17). Further support for the control was demonstrated by the effectiveness of beta-adrenergic blockade with propranolol in preventing the renin release elicited by anesthesia (17, 18), stress (17), and drug-induced vasodilation (10). In the present experiments, isoproterenol produced a marked increase in SRA with a dose as low as 0.3 μg. The central administration seems to be more efficacious than the systemic, since the minimum effective intraperitoneal dose (17) was 30 times greater than the minimum intraventricular dose in the pres ent study. This suggests the possibility of a central nervous system role in the renin release by beta-adrenergic drugs. Since the intraventricular injection of isoproterenol produced a decrease in sodium excretion (8) as well as blood pressure, an inverse relationship between these parameters and SRA was again observed.

Changes in serum renin levels can be induced by the injection of beta-adrenergic and cholinergic drugs and hypertonic saline into the third ventricle of unanesthetized rats. It is well established that changes in mean blood pressure or in renal sodium load can elicit changes in SRA (19); therefore, the blood pressure or sodium excretion changes might be instrumental in producing the observed changes in SRA. It is notable that in each case (hypertonic, saline, carbachol, isoproterenol) in which SRA is modified, blood pressure and/or urinary sodium output are also changed in the reciprocal directions. Norepinephrine, however, does not alter SRA, which might be due to a balance between its actions to decrease blood pressure and increase so-

role of the beta-adrenergic system in renin dium excretion (8). At present, there is no direct evidence that sodium excretion and/or blood pressure are the causative mechanism (or mechanisms) in this renin response. Possibly, central injections activate other pathways (i.e., neural or hormonal) which directly or indirectly modify SRA. Even though the mechanism of action of these compounds remains unclear, these results demonstrate that beta-adrenergic and cholinergic stimulation of the central nervous system, as well as specific osmoreceptor activation by hypertonic saline via the third ventricular route, can induce definitive changes in renin release.

Summary. The effects of centrally administered autonomic drugs and hypertonic saline on renin release were studied in the conscious rat. A 0.3 μg intraventricular dose of isoproterenol, which is one-thirtieth of the intraperitoneal dose required to stimulate renin release, induced the release of renin into the systemic circulation. Norepinephrine had no effect on renin release in the same dose range. Hypertonic saline and carbachol suppressed renin release. Alterations in renin release were preceded by a reciprocal change in blood pressure. These results suggest a central nervous system site for sodium, beta-adrenergic, and cholinergic receptors in altering renin release and blood pressure.

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