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Central and Hormonal Regulation of Renin Release by Baboon Kidneys (40950)

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Abstract. Renin release from renal cortical slices of baboon kidneys was measured. It was found that the basal release rate was directly proportional to the renin content. Release was stimulated in a dose-dependent fashion by isoproterenol and was inhibited both by angiotensin II and vasopressin. Maximum inhibition was approximately 30%. Experiments also were done in which angiotensin II and converting enzyme inhibitor were infused into the lateral ventrical of anesthetized baboons. Angiotensin caused a reduction in plasma renin activity (PRA), and converting enzyme inhibitor an increase in PRA, independent of changes in blood pressure.

The central role of the renin–angiotensin system in the control of salt and water balance has been known for some time. Angiotensin II, the end product of renin secretion, acts both centrally and peripherally to alter parameters which affect the metabolic balance of both sodium chloride and water (1). For example, in dogs the intraventricular infusion of angiotensin II leads to a decrease in aldosterone levels (2). On the other hand, at the renal level, intravenous or intra-arterial infusion of angiotensin II and antidiuretic hormone (ADH) cause the kidney to reduce the output of renin (3, 4). Much of what is known about the renin–angiotensin system has been learned from studies using dogs and rats; few data have been accumulated in primates. The experiments reported here were designed to test the hypothesis that the renin secretory rate in a primate species is affected by the same stimuli as in nonprimates and also that the central action of angiotensin in primates, as in nonprimates, is to reduce the circulating levels of renin.

Methods. Experiments were performed on one female and four male baboons (*Papio cynocephalus*). They weighed 11 to 18 kg, were in an excellent state of health and nutrition, and were estimated from dentition and body conformation to be 3 to 5 years old. Three days prior to experimentation the standard complete diet of monkey chow (Ralston Purina Company) was changed to a low-sodium diet consisting of oranges, apples, and bananas.

Anesthesia was induced with ketamine hydrochloride (Vetalar, Warner Lambert–Parke-Davis; 20 mg/kg administered intramuscularly). Surgical anesthesia was maintained throughout the experimental period, lasting up to 8 hr, by administering pentobarbital sodium (5 mg/kg) every 20 or 30 min, or as needed, via an indwelling femoral vein cannula. The adequacy of anesthesia was evaluated continuously by direct clinical observation of the baboons, by frequent testing of palpebral and pedal reflexes, and by periodic measurement of femoral arterial pressure. Rectal temperature was monitored periodically. Surgical drapes and a warming lamp were used occasionally as aids in maintaining normal body temperature.

Following cannulation of a femoral artery and vein, the baboons were placed in a stereotaxic holder, and a 25-gauge needle was advanced slowly into the right lateral ventricle. The needle was attached to polythylene tubing which in turn was connected via a T-connector to a constant infusion pump and a pressure transducer. Artificial CSF was infused at 10 μl/min and the pressure in the tubing was monitored. As the needle was slowly lowered through the brain, pressure rose steadily. A sudden drop in pressure signalled needle entrance into the ventricle. At this time the infusion

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rate was increased to 30 μl/min. At the end of the experiment, needle placement was verified by perfusion of blue dye. Only if the entire ventricular system was found stained upon necropsy was needle placement considered successful. Four of five experiments met this criterion.

Following needle placement a 1-hr equilibration period was allowed, at the end of which 5 ml of blood was drawn for determination of plasma renin activity (PRA). The infusion was then changed to one containing one of the following: angiotensin II (200 ng/ml), converting enzyme inhibitor, SQ20881 (17 μg/ml), or the competitive inhibitor of angiotensin, saralasin or P-113 (200 ng/ml). A second blood sample was taken at the end of 2 hr and the infusion was changed back to CSF without drug. Two hours later a recovery sample was drawn for PRA and the last experimental period began. During the second experimental period one of the other two drugs was added to the CSF. In this way using four baboons we were able to obtain four periods of SQ20881 infusion, three of AII infusion, and one of P-113 infusion, each with its own control period. The sequence of drug infusion was randomized. At the end of the last experimental period a final sample for PRA was drawn and the kidneys were removed. Thus, each experimental period was flanked by a control and recovery period. Statistical analyses (paired *t* test) were done using only control and experimental periods.

Immediately after removal, the kidneys were flushed with physiological saline solution (PSS) until the effluent from the renal vein was clear. Cortical cubes approximately 1 cm on each side were cut in small thin slices with a Stadie Riggs microtome. Slices were placed in flasks containing 5 ml of PSS. The slices were incubated at 37° for 1 hr, during which the medium was changed three times. They were then transferred to other flasks and incubated for 20 min, an aliquot of medium (200 μl) was removed for renin assay, and one of three drugs was added (isoproterenol (ISP), angiotensin II, or vasopressin). Additional 200-μl aliquots were taken at 40, 60, and 80 min. Each experiment was done in quadruplicate, i.e., four flasks were used for each drug and each flask contained six slices. Renin analyses were done as reported previously (5). Renin release (Figs. 1 and 2) is plotted as a percentage change from control flasks. Experimental flasks were compared with control ones so that the value on the ordinate represents [80-min value − 20-min value/20-min value] experimental [80-min value − 20-min value/20-min value] control. This ratio corrects for a nonlinear secretory rate in control slices. A ratio of 1.0 would indicate no effect of drug. Values deviating from 1.0 indicate drug-induced changes in release rates of renin. The results are plotted as a percentage change in the ratio. PRA was measured using the New England Nuclear radioimmunoassay kit. Dog substrate was used for renin analysis in the *in vitro* experiments. The composition of the artificial CSF (m*M*) was as follows: Na, 150; Cl, 133; Mg, 2.0; Ca, 3.4; HCO_3, 25; PO_4, 0.5; glucose, 2.5. The CSF was prepared at the University of Michigan pharmacy and was sterile and nonpyrogenic. The composition of the PSS (m*M*) was: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$ 25; glucose, 10. The medium was gassed with 95% O_2 and 5% CO_2.

Results. Incubation of kidney slices with isoproterenol from 10^{-8} to 10^{-5} *M* resulted in increased release of renin at all doses tested, with an apparent maximum at 10^{-7} *M* (Fig. 1). In contrast to these data, the

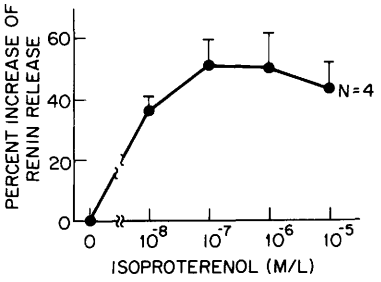

FIG. 1. Dose–response curve for isoproterenol. Each point represents the average of three or four flasks (six slices per flask) per animal. Results are expressed as the release rate during 20 min following addition of ISP compared to a 20-min period preceding addition of drug. Each point is significantly different from control ($P < 0.01$, paired *t* test).

addition of commercial vasopressin (Parke Davis) or AII caused a suppression in renin release (Fig. 2). The reduction in renin release was approximately 20% with angiotensin and 30% with vasopressin (VP). Thus, both of these hormones inhibited the *in vitro* release of renin from baboon kidney slices.

Basal release rates of renin were directly proportional to the renin content (Fig. 3), an observation comparable to that shown previously in kidney slices from dogs (6).

The results of intraventricular infusion of AII, SQ20881, and P-113 are shown in Fig. 4. AII infusion reduced the circulating levels of PRA by approximately 27%. In the four animals infused with SQ20881, PRA increased by approximately 50%. The single animal that received P-113 also showed an increase in PRA of about 50%. If the results of the four animals given SQ20881 and single animals given P-113 are combined, the results are significant at the 0.03 level (paired *t* test). No significant changes in blood pressure were seen in any of the groups. MBP for control and experimental groups were as follows: AII, 125 ± 8 and 131 ± 14 mm Hg; P-113, 104 and 103 mm Hg; SQ20881, 123 ± 12 and 119 ± 8 mm Hg.

Discussion. It has been demonstrated that vasopressin and AII inhibit *in vivo* renin secretion in dogs (3, 4). The *in vitro* results presented here indicate that both drugs are active inhibitors of renin release. Although no dose–response curves were obtained, these data are in agreement with those obtained *in vivo* from nonprimate mammals. The doses of AII and ADH were high; but, it is not possible to estimate the effective concentration in the slice during the entire incubation period. Since it is likely that the slices degrade both drugs, the effective concentration must have been considerably less than the starting concentration of the medium. To our knowledge, this is the first demonstration that both angiotensin and vasopressin are effective inhibitors of renin release *in vitro*.

It is evident that ISP is a potent stimulator of renin release in the same system. Furthermore, the basal release rate of renin is a direct function of that which is stored in the kidney, or of the renal renin

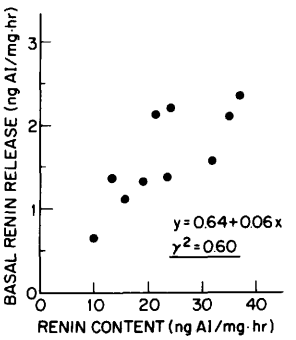

FIG. 3. Relation between renal renin content and basal release rates of renin. Results are based upon the release rate during the initial 20-min incubation. Each point represents the results from one kidney of a baboon.

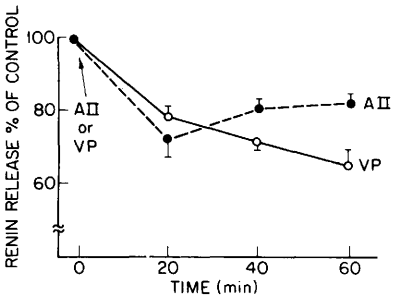

FIG. 2. Suppression of renin release by AII and VP. Results are expressed as in Fig. 1, as a function of time after addition of drug. The initial concentration of VP and AII was 10^{-6} *M*, $N = 4$. All values were significantly different from control ($P < 0.01$, paired *t* test).

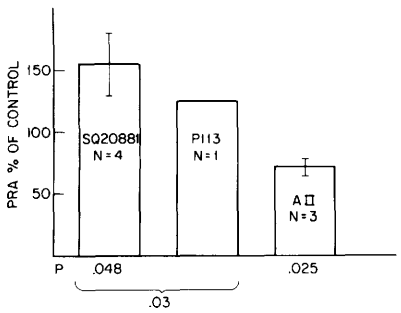

FIG. 4. Effect of central infusion of AII, P113, and SQ208811 on PRA. Control PRA values for the SQ20881, P-113, and AII groups were 6.3 ± 2.3, 6.5, and 11.8 ± 6.5 ng/ml·hr, respectively.

content. Taken together, these data indicate that renin release in kidney slices of primates is affected by the same stimuli and inhibitors that have been demonstrated to work *in vivo* and *in vitro* in the dog (6).

The data relating to PRA in response to alterations of the CSF demonstrate that the results obtained in goats and cats (7, 8) are applicable to a primate species. Central administration of angiotensin causes a significant reduction in PRA. The same phenomenon now has been demonstrated in a primate species. Although the mechanism for the AII-induced decrease in PRA is not established by these experiments, increased release of ADH most likely is responsible. Malayan *et al.* (9) showed that central administration of AII reduced PRA by increasing peripheral ADH levels. It should be noted that in contrast to the work reported by others (7, 8) this effect was obtained without any increase in blood pressure. Perhaps of greater significance, the converting enzyme inhibitor, SQ20881, had an effect opposite to that of AII. Since the converting enzyme inhibitor must work in the interstitium of the brain, the results suggest that endogenously generated AII, in local brain areas, can affect plasma renin activity. These data are consistent with the concept that a brain–renin–angiotensin system plays a role in the regulation of PRA.

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