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Possible Determinants of Plasma Renin Activity in Infant Rats (39535)

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In mature animals of a variety of species (1-6), plasma renin activity (PRA) is low as compared to neonates or during fetal development. In the rat, during the first 3 weeks of life, PRA is about five times as high as in the adult (5, 6). The high PRA probably results in part from a decreased rate of disappearance (6) as well as a possible increased rate of secretion. If, in fact, secretory rates are different from what one could expect, an explanation would fit into one of several general categories of possibilities: (a) A stimulus may be present disproportionally in the young to cause an increased rate of secretion; (b) a stimulus may be absent which normally suppresses renin secretion; or (c) the kidney is temporarily unresponsive to stimulus inputs and the new level represents a basal uncontrolled secretory rate. To test these possibilities, it was decided to analyze the responsiveness of the renin angiotensin system to a series of physiological states known to alter renin secretion. More specifically, we have studied the effect of increased salt intake, β-adrenergic blockade, antidiuretic hormone, and a competitive inhibitor of angiotensin (1-sarcosine-8-alanine-angiotensin II, hereafter given as P-113) on PRA in infant rats.

Materials and methods. Fourteen- to sixteen-day-old infant rats (14 litters, body weight 22-28 g; mothers, 300 g body weight) were usually used in these studies. This age was chosen because (i) it is well before the 3- to 4-week "critical period" when PRA decreases to mature levels (5, 6); (ii) such animals are large enough to allow for relatively easy venipuncture by way of the jugular vein; (iii) about 0.7 ml or more of blood can be collected, usually by free flow from the cut lower vena cava and abdominal aorta. In one series of studies, however, animals were investigated which ranged in age from birth to 14 days. All animals used in these studies were of the Charles River strain, obtained from a commercial supplier (Arie Lebenstein Laboratory Animals, Yokneam, Israel), or were born in our laboratory to pregnant dams obtained from the same supplier.

When necessary, animals were anesthetized with Nembutal by first administering two-thirds the normal adult dose intraperitoneally. If this amount of anesthetic was not adequate, supplementary small increments were administered. After an experimental maneuver (see below), a laparotomy was performed and the intestinal contents displaced so as to expose the inferior vena cava and aorta. The intestinal wall was quickly wiped dry and the major vessels were cut. The free-flowing blood was then collected in cold EDTA-containing syringes in order to inhibit angiotensinase activity. After transfer to cold tubes and centrifugation, plasma was stored at $-20°$ until analysis of PRA. Renin activity was determined by radioimmunoassay using a micromodification of the New England Nuclear kit. The procedure was in principle the same, but only 50 to 100 μl of plasma were incubated for 3 hr. Appropriate dilutional adjustments were made so that final concentrations of Ang I were appropriate for sensitive assay. The intra-assay coefficient of variation was found to be 7%, and inter-assay was 11%.

Significance of differences between means of treated and untreated animals was evaluated using a two-tailed t test. It was inferred that a difference existed when the likelihood of two means being the same was less than 0.5.

Control animals for most studies were untreated 14- to 16-day-old rats. There were several different experimental groups.

Group 1. Dams were obtained as soon as pregnancy was certain. This time was be-

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tween 2 and 3 weeks before delivery. The animals were given 1% saline exclusively for drinking water. At higher concentrations of salts, 2%, early abortion was observed or the infants died within 1 day of birth. On the 1% saline, however, about 50% or more of the pups survived. The survivors were sacrificed for blood collection at varying time intervals after birth.

Group 2. In this group, the effect of intraperitoneal salt administration on PRA was studied. Fourteen-day-old animals were injected with normal saline for 3 consecutive days at a dose of 10% body weight at each injection. One day later, blood was collected and examined for PRA.

Groups 3 and 4. The ability to suppress renin release by β-blockade was determined in these groups. The pups were injected subcutaneously with propranolol at a dose of 10 mg/kg. The propranolol solution (ABIC, Israel) had a concentration of 1 mg/ml. In group 3, blood samples were taken 2 hr after the injection, while in group 4, blood was collected 3 hr later. Controls for these groups consisted of pups injected with saline alone.

Groups 5 and 6. In these groups, the ability of antidiuretic hormone to suppress plasma renin activity was investigated. Since in the rat it had been suggested that only high doses of ADH lead to suppression of renin (8), two doses of Pitressin in oil (Parke-Davis) were used in these studies. In group 5, each animal received 200 mU, while in group 6, the dose was 2 mU/animal. The drug was administered im with controls being injected with oil alone.

Group 7. In these animals, the competitive inhibitor of angiotensin II, P-113, was administered via a jugular vein. The drug was given as an acute injection in a volume of 0.1 ml of saline containing a total of 20 mg of P-113. Fifteen minutes later, blood was sampled for PRA measurement.

Results. Plasma renin activity of group 1 animals (mothers on a high salt diet) at varying ages after birth are shown in Fig. 1. Using this method of attempting to increase body sodium of infants resulted in no decrease in circulating PRA during the first 14 days of life. In control animals at these ages, control PRA has been found to be in the

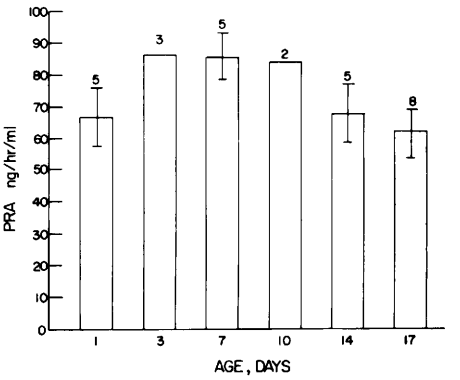

FIG. 1. Plasma renin activity (PRA) as a function of age in animals nursed by mothers receiving 1% NaCl for drinking water. Bars indicate standard error of the measurements, and numbers above the bars indicate the number of determinations at each age.

range of 50–80 ng/hr/ml of plasma and is constant with age. It is not possible to be certain, however, that the infants did in fact have a higher intake of sodium in these studies (10, see Discussion). As a result, we approached the problem in an alternate way. Pups were treated with saline as described in Methods, group 2. Results of these studies are shown in Table I. Saline administration resulted in no change in PRA. The value of 62.1 ± 6.3 ng/hr/ml is not significantly different from the control value.

β-Adrenergic blockade (groups 3 and 4) was induced by subcutaneous injection of propranolol. It has been shown in adults that this method of administration results in a maintained blood concentration, the plateau being reached in less than 30 min (9). In 2-week-old rats, no change in PRA of propranolol-treated animals was found at 2 hr after injection as compared to control animals injected with saline or to uninjected controls (Table I). Since, however, there may be a delay of absorption in infants (11), these studies were repeated with blood being sampled 3 hr after injection. In these animals, it was found that β-blockade also had no significant effect on PRA at this time.

In contrast to treatment by salt or by propranolol, injection of 0.2 unit of ADH in oil resulted in suppression of PRA. The dose was calculated from the data of Gut-

TABLE I. EFFECT OF VARIOUS AGENTS ON SUPPRESSING PRA IN 2-WEEK-OLD RATS.

Treatment	*n*	PRA	*P*
Controls	7	76.1 ± 7.8	–
Saline	8	62.1 ± 6.3	N.S.
Control	8	40.7 ± 5.0	–
Propranolol, 2 h	7	50.5 ± 12.1	N.S.
Propranolol, 3 h	18	45.6 ± 4.2	N.S.
Control	6	73.8 ± 8.2	–
ADH, 0.002 U	8	77.7 ± 14.5	N.S.
ADH, 0.2 U	8	38.9 ± 6.7	<0.01
Control	5	47.1 ± 3.4	–
P-113	6	278 ± 39.5	<0.001

man and Benzakein (8) who suggested that this was a very high dose at "pharmacological levels." A repeat of this study with administration of Pitressin at 1/100 of the original dose level did not suppress PRA.

The final treatment was with P-113. It is clear that administration of the antagonist to angiotensin II results in a marked increase in circulating PRA.

Discussion. The role of various regulatory agents altering renin secretion of mature animals is shown in Fig. 2. The question arises as to which of these may be physiologically significant in the infant. Accordingly, the following criteria would seem to be reasonable prerequisites to assigning a role for a regulatory agent as acting in a significant manner during the neonatal period. (i) Alteration of the quantity of the agent should produce predictable changes in PRA. (ii) During the postnatal period, changes should occur in the concentration or effectiveness of the agent. (iii) The time of change of the agent should coincide with the time of change in circulating PRA. (iv) The changes in the regulator should be in the appropriate direction to account for the change in PRA.

Plasma sodium concentration (P_{Na}) is low in the neonate, and in rats it shows a gradual increase in the postnatal period (Solomon and Bengele, in preparation). At 3 to 4 weeks of age, P_{Na} is still low relative to mature levels. Since this is the "critical period" of reduction of PRA to mature levels, it would not seem that the low P_{Na} by itself is responsible for the high PRA of the neonate. Since P_K is high in rat pups (12, Solomon and Bengele, in preparation), the action of this ion would be to decrease PRA. Potassium changes in the wrong direction to be a regulator of renin.

Our own studies served further to eliminate sodium as a trigger for increased renin secretion. When the mothers were on a high salt diet, there was no change in PRA of the young (Fig. 1). Comparable results were obtained with fetal lambs, although salt restriction of the mother increased fetal PRA (4). In addition, fetal lambs respond to diuretics (5). It may be argued that the high salt diet has inadequate effect on fetal sodium or on milk sodium, since Dlouha and co-workers found only a small increase in rat milk sodium in mothers fed a high Na diet starting at birth (10). In the mature animal, it is well known that salt loading suppresses PRA. To insure that salt intake was in fact increased, we administered isotonic saline by the intraperitoneal route. PRA was found to be the same as in controls. It does not appear, therefore, that increasing body sodium in the neonate is able to suppress PRA. In newborn dogs, however, peritoneal dialysis produces a significant increase in PRA. As a result, the possibility must be considered that offsetting effects are produced in this experiment, a reduction in PRA by the salt and an increase because of the route of administration.

The results with propranolol are similar in being ineffective in suppressing PRA. One may argue that we did not utilize a high enough dose, but at this level we have always observed bradycardia (unpublished). In addition, we have found that propranolol prevents or reduces the rise in PRA secondary to a stimulus in mature animals (13). In agreement with Dornier *et al.*, we found little suppression of basal PRA in mature animals (14). It has been shown that plasma

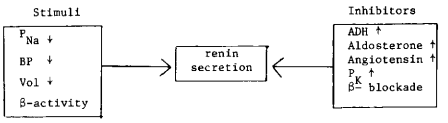

FIG. 2. Diagrammatic representation of some known factors affecting renin secretion. Arrows indicate direction of change of factor.

propranolol reaches a steady-state level 10 min after subcutaneous injection. If one assumes that propranolol would be effective 30 min after injection, one can estimate that if β-blockade reduced renin secretion, one would expect some reduction at 2 hr after injection, since the half-time of destruction is about 26 min in neonates with high PRA (7). At this time, no significant reduction in PRA was found. Since it is known, however, that absorption in the neonate from several sites can be slower than in the mature animals (11), we repeated these studies with blood collections made 3 hr after injection. Again, no significant depression of PRA was observed. As a result, one can suggest that the high PRA of the neonate is not a result of high levels of autonomic stimulation.

In contrast to the failure of salt or β-blockade to affect PRA, 0.2 unit of ADH suppressed renin levels 4 hr after injection. Although this dose has been labeled "pharmacological," to us such characterization of the dose is not appropriate at this time. The hormone was injected in oil and intramuscularly, and one does not know either the rate of absorption or the blood levels attained in our studies as well as in those by others (8). It is of interest that in the dog, "physiological" doses of ADH can suppress renin (15) when given intravenously. It is, however, possible that species differences exist, and, if the effective dose is in fact excessive, this result may have little bearing on a possible role of ADH in suppressing PRA.

With respect to the possibility that ADH is involved in the regulation of PRA, it should be pointed out that it meets criteria presented at the beginning of this discussion. (i) The change in PRA is in the direction predicted for its playing a regulatory role. (ii) Rats are unable to produce a concentrated urine in response to ADH until the third week of life. Thus, the time of ADH effectiveness coincides with the time of onset of mature levels of PRA. Although part of the change in neonatal responsiveness to ADH is a result of anatomical maturation of the thin loops of Henle (16), part of the failure to produce a concentrated urine is the result of a lack of ADH (17). (iii) The changes in the hormone increase during neonatal development are accordingly in the appropriate direction to assign ADH a regulatory role. In part, then, the high PRA may possibly be related to the failure of ADH to suppress renin secretion.

It is not possible, however, to say that ADH ineffectiveness and reduced metabolic destruction of renin are the only factors responsible for the high PRA of neonates. It is known that the angiotensin II antagonist, P-113, does not affect basal secretion in adults. If, however, a stimulus to renin secretion exists, it has been found that the inhibitor interferes with biological feedback and renin activity increases. Such an effect has been observed with varied stimuli as adrenalectomy (18), vena caval constriction (19), reduction of hypertension of renal origin (20) and following renal ischemia and subsequent production of acute renal failure (13). In the infant, P-113 administration also markedly increases the level of renin. One possible source of the stimulus in these animals is anesthesia (21). It has been found, however, in control animals in other studies that P-113 alone does not raise PRA in anesthetized mature rats (21). In addition, in mature animals we have never found PRA levels as high as in infants with comparable anesthesia (11). It would, therefore, seem that a stimulus exists to cause secretion of renin but that the nature of that stimulus has not been elaborated by these studies.

Summary. The effect of various experimental maneuvers in altering PRA of infant rats was studied. In 2-week-old animals, no suppression of PRA is found after saline loading or β-blockade. Infants nursed by mothers receiving 1% saline for drinking water show no changes in PRA from control levels. Intramuscular administration of ADH (0.2 U) in 2-week-old animals suppressed PRA, while P-113 resulted in a marked increase in PRA. These latter data suggest that some stimulus is causing inappropriate secretion of renin, and, in addition, there was some failure of feedback suppression by ADH.

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