Effects of psychosocial stimuli on plasma renin activity in rats

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NUMEROUS EXPERIMENTS (11, 13, 28, 31, 32) have documented the fact that renin secretion can be stimulated by the sympathetic nervous system. However, there are few studies of the effect on renin secretion of psychosocial stimulation, one of the important types of input controlling the sympathetic nervous system (24). Esler and Nestel (12) reported that forced problem solving did not produce any change in plasma renin activity (PRA) in humans despite significant increases in urinary catecholamine excretion. In contrast, Blair et al. (3) have reported that PRA is increased within 1 h during Sidman avoidance conditioning in baboons. There are several other studies that describe increases in PRA as a result of painful stimuli in rats (4, 9, 23) or performance of the cold-pressor test in humans (8, 14, 27), but these stimuli involve physical as well as psychosocial components.

We have chosen to investigate this question in the rat using the open-field (or novel-environment) procedure. This technique provides a simple standardized psychosocial stimulus that consistently has been shown to elevate the secretion rates of other hormones (2, 6). We have also utilized the presence of a hungry cat as a second type of psychosocial stimulus to the rats. Experiments were performed with the rats on either a sodium-free diet or a standard (1% NaCl) rat chow since a low-sodium diet may enhance the renin response to sympathetic stimulation (32).

METHODS

All experiments were performed on male Sprague-Dawley albino rats (200-250 g), maintained on either Teklad mouse and rat diet (1% NaCl) or Nutritional Biochemicals Corporation sodium-free diet. Because isolation increases emotionality during open-field testing (1, 22, 30), rats were housed in individual metal cages; experiments were performed in an adjacent workroom separate from the housing room. The housing room was on a 6:00 A.M. - 6:00 P.M. light cycle. Experiments were performed in the morning or in the afternoon, as detailed below.

Open-field exposure. All animals were handled once daily on at least 4 days prior to use. This consisted of removing each rat from his cage and carrying him to the workroom door. On the morning or afternoon (see below) of experimental days, open-field animals were then placed individually in a 4 foot x 4 foot x 2 foot plywood box, which was painted white on the inside, lined with brown paper, and illuminated by a 200-W white light suspended from above. For each exposure, note was made of the amount of time spent in the workroom and the degree of exploring behavior. After open-field exposure, the animal was removed and decapitated for blood collection. Ten- and 30-min exposures were alternated. Control animals were simply removed from their cages, carried to the workroom, and immediately decapitated; one control animal was decapitated 15 min into each 30-min open-field exposure, with note made of the amount of time spent in the workroom (usually 5-10 s, with a maximum of 30 s) and the time of day. After decapitation, blood was collected for 30 s, with a wide-mouth plastic funnel used to transfer the blood to a 10-ml polystyrene tube in an ice-filled beaker. Each tube contained 100 µl of (NH₄)₂EDTA (7.5 g/100 ml). All blood was centrifuged for 20 min within 2.5 h after collection.

Open-field exposure and propranolol. Animals maintained on the sodium-free diet for 5-10 days were divided into four groups, as shown in Table 1. All injections were administered subcutaneously in the nape of the neck 1 h before decapitation. A control animal was
TABLE 1. Groups in open field propranolol experiment

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<th>Vehicle Only*</th>
<th>Propranolol†</th>
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<tr>
<td>Open-field exposure, 30 min</td>
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* Injection vehicle was 0.1 ml of either isotonic saline or glucose. † Propranolol dose was 0.5 mg/rat or approximately 2 mg/kg (total injection volume 0.1 ml).

decapitated halfway through each 30-min open-field exposure, with blood collection and handling as described above. The experiments were performed from 8:00 A.M. to noon. A pilot study performed prior to undertaking these experiments demonstrated that the propranolol dose used, 0.5 mg/rat, completely blocked the cardiovascular responses to isoproterenol; a second study demonstrated that subcutaneous injection of the vehicle per se does not alter PRA 1 h later.

Exposure to a cat. Eight rats maintained on the sodium-free diet for 4 days were transferred to a large box at the same time, all left in their individual cages. A cat, fasted for 24 h, was then placed in the box, and a wire-mesh roof was lowered over the entire box. The animals were left for 30 min, after which the rats were decapitated. Another group of control rats were subjected to the same procedure except that a cat was not placed in the box with them. Prior to the experimental day, all control and experimental animals had been handled once daily for 4 days as follows: rats were placed in their cages to the workroom, and the cages were placed in the experiment box for 15 min; after this, each animal was removed from his cage and held for approximately 30-60 s.

Chemical methods. Plasma renin activity was determined by a modification of the method of Haber et al. (16). Angiotensin I was generated in vitro during a 1-h incubation at a pH of 6.5 and a temperature of 37°C; the incubation mixture contained 500 µl test plasma, 100 µl 2 M maleic acid buffer (pH adjusted to 6.5 with NH₄OH), 10 µl BAL (1.7 g/100 ml), and 10 µl 8-hydroxyquinoline (6.6 g/100 ml). The angiotensin generated was then measured with the 125I radioimmunoassay kits supplied by New England Nuclear. Plasma renin substrate was measured only in experiment A and was not elevated in open-field rats (control mean ± 1 SE = 1,791 ± 86 ng/ml, open field = 1,760 ± 87 ng/ml).

Figure 2 summarizes the grouped data of the two open-field experiments involving rats on the standard (1% NaCl) diet. Experiment A was performed between 8:30 A.M. and 11:30 A.M. and experiment B between 2:00 P.M. and 5:00 P.M. It is evident that open-field exposure tends to increase PRA in these standard-sodium ani-

RESULTS

Open-field exposure. Figure 1 summarizes the results of the three separate open-field experiments involving animals on a sodium-free diet. These experiments were performed over a 6-mo period and demonstrate that a 30-min exposure to a novel environment (open field) significantly increased PRA. We have shown the results of these three experiments separately in order to demonstrate the range of responses seen. The response to a 10-minute exposure (performed only in experiment A) was not statistically significant. In no experiment did PRA values correlate with the amount of defecation, urination, or exploration in the box. All experiments were performed between 8:00 A.M. and noon; there was no correlation between PRA and time of decapitation in these rats maintained on the sodium-free diet. Plasma sodium concentrations were measured for all animals and were not different for control and open-field rats. Plasma renin substrate was measured only in experiment A and was not elevated in open-field rats (control mean ± 1 SE = 1,791 ± 86 ng/ml, open field = 1,760 ± 87 ng/ml).

FIG. 1. Effects on plasma renin activity of a novel environment (open field) in rats maintained on a sodium-free diet for 5–10 days. A, B, and C represent 3 separate experiments performed over a 6-mo period.

FIG. 2. Effects on plasma renin activity of a novel environment (open field) in rats maintained on a standard (1% NaCl) diet. Experiment A was performed in morning and B in afternoon.
mals, but in neither experiment was the difference in group means statistically significant. However, we subsequently demonstrated a large circadian variation of PRA in rats ingesting a standard-sodium diet (unpublished observations). Variation is most prominent over the period of time covered by this experiment and contributes strongly to the variance of the present results. In order to minimize this component of the variance, we employed paired-sample analysis of the data. This was possible because, as described above, the experimental protocols were constructed to ensure that each 30-min open-field rat had a matched control decapitated at the midpoint of the open-field exposure: i.e., matched control and 30-min open-field animals were decapitated within 15 min of each other. Figure 3 summarizes this paired-sample analysis, which brings out the significant difference ($P < 0.01$) between control and 30-min open-field animals. Plasma sodium concentrations were not different; plasma renin substrate was not measured.

Open-field exposure and propranolol. The effect of propranolol on the renin response to open-field exposure is summarized in Fig. 4. Open-field exposure again produced a significant increase in PRA in sodium-deprived rats (group $B$ vs. group $A$). After propranolol injection, PRA was still increased after open-field exposure (group $D$ vs. group $C$), but the response was only approximately 50% of that seen in the absence of propranolol and is of borderline statistical significance. This same phenomenon is demonstrated by the fact that the mean PRA of group $D$ (propranolol) was significantly lower than that of group $B$ (vehicle). In contrast, the lack of a significant difference between the two control groups ($A$ and $C$) demonstrates that propranolol in this dose did not alter basal PRA in salt-deprived rats.

Exposure to a cat. Figure 5 summarizes the PRA data for sodium-deprived rats exposed to the presence of a hungry cat. This stimulus caused a significant elevation of PRA comparable to that seen with open-field exposure. Control PRA was lower in this series because the animals were deprived of sodium for only 4 days.

**DISCUSSION**

These experiments demonstrate that psychosocial stimuli can acutely increase PRA in unanesthetized rats maintained on either a standard or a sodium-free diet. Plasma renin substrate concentration did not change, indicating that the PRA increase reflects increased plasma renin concentration. Although it is possible that this change in concentration might reflect decreased hepatic catabolism of renin, it is far more likely to be the result of an increased rate of renin secretion, given the half-life of plasma renin (15-30 min).

Given the ability of psychosocial stimuli to increase the activity of the sympathetic nervous system (11, 13, 31, 32), it is logical to postulate that the increased renin...
secretion is mediated by increases in circulating catecholamines or enhanced activity of the renal sympathetic nerves. The fact that propranolol partially blocked the response is consistent with this hypothesis, since a large number of studies have demonstrated that β-adrenergic receptors are the major intrarenal adrenergic receptors mediating the renin response to sympathetic stimulation (13).

There are several possible explanations for the failure of propranolol to block the renin response completely. 1) The dose of propranolol may have been inadequate to block completely the renal β-adrenergic receptors; this dose completely blocked the cardiovascular response to isoproterenol but the dose-response characteristics of the cardiovascular and renin-releasing receptors may differ. 2) Part of the renin response to psychosocial stimuli may be due to renal vasoconstriction mediated by α-adrenergic activation, resulting in stimulation of the intrarenal baroreceptor or macula densa (32). Psychosocial stimuli have been shown to induce such renal vasoconstriction (5, 25, 29), and several studies have indicated that part of the renin response to direct renal nerve stimulation, catecholamines, or pain is eliminated by drugs that block α-adrenergic receptors (10, 33). 3) Part of the renin response to psychosocial stimuli may be mediated by a nonadrenergic hormonal pathway. Our experiments do not suggest a choice among these alternative explanations.

The finding that psychosocial stimuli increase PRA may have implications for the pathophysiology of hypertension. Several animal models of chronic hypertension induced by psychosocial stimuli have been reported (18–21), and it has been theorized that certain types of human hypertension may result from multiple bouts of psychosocial stress (5, 17, 19). The possible role of renin in hypertension related to psychosocial stress has not been investigated, to our knowledge.

Finally, an incidental finding in these experiments is that propranolol does not reduce the elevated renin secretion associated with chronic sodium deprivation in rats. This had previously been shown for dogs (7) and is additional evidence that the renal nerves are not an essential pathway for stimulating renin secretion in this situation (7, 15).

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REFERENCES


