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Effect of Intermittent Electric Shock on Plasma Renin Activity in Rats[1] (38142)

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The effects of emotional stimuli on arterial pressure have been documented in humans (1, 2) and animals (3–5). Intermittent electric shock induces a moderate blood pressure elevation in rats (4), and increases in plasma catecholamines and corticosteroids in monkeys (6). Increase in sympathetic activity could be responsible for the rise in blood pressure but another potent pressor mechanism is the renin-angiotensin system. Since it is apparent that the sympathetic nervous system probably plays an important role in the regulation of renin release (7), the rise in blood pressure during exposure to emotional stimuli, therefore, may be at least partly due to an elevated renin release.

Emotional stimuli also induce ACTH release and consequent secretion of corticosteroids. Whereas ACTH stimulates renin release (8), administration of high doses of glucocorticosteroids blocks renin secretion (8, 9). The interaction of these 3 factors—sympathetic activity, ACTH release and steroid secretion—may determine the extent and direction which renin release changes during exposure to emotional stimuli.

Accordingly, in the present study plasma renin activity (PRA) and plasma corticosterone concentration were followed during exposure of individual rats to a 24 hr period of intermittent electric shock. The involvement of the above mentioned three factors was assessed by hypophysectomy or pretreatment with autonomic blocking agents and dexamethasone.

Materials and Methods. Male Sprague-Dawley rats, weighing 180–220 g, were housed in wire-mesh colony cages for 4–7 days prior to an experiment and maintained on Purina Chow pellets (containing 220 μEq Na^+/g) and tap water *ad libitum*. They remained in a temperature-controlled room (23°–25°), illuminated between 7 AM and 6 PM. No food and water were present during an experiment.

Rats were randomly divided into a "shock-group" (N=7–8), control-group (N=5–6) and in some experiments a group (N=4), which stayed on food and water in individual cages. The shock-group was placed in a separate room in a 240 × 20 × 35 cm wooden box with an electrified grid floor, divided into 8 compartments. Each rat was subjected to 3 sec of a 3 mA "scrambled" electric shock delivered every 24 sec. This intensity of shock initially caused the animals to jump about the cage and attempt to climb its walls; after 1–2 hr the rats became passive and inert. The shock did not cause convulsions or other evidence of physical harm. The control-group was placed in an identical box, without being exposed to an electric shock. Experiments were started in the mornings.

Animals were sacrificed by decapitation and approximately 3–4 ml of blood collected from the trunk within 30 sec. Plasma renin activity (PRA) was measured according to the method of Haber *et al.* (10), using a radioimmunoassay for angiotensin I. PRA was calculated as the amount of angiotensin I generated from endogenous renin substrate per

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ml of plasma during incubation of the sample at ambient pH (7.4) for 3 hrs at 37°. Within assay variation in duplicate samples averaged 7.7% (SE = ± 1.3; N = 25). Between-assays variation averaged 15.2% (SE = ± 1.9; N = 23).

Plasma corticosterone was estimated with a fluorimetric method (11). Hematocrit was measured in duplicate after centrifugation in standard heparinized capillary tubes (1.5–2.0 mm i.d., length 75 mm).

In the experiment in which hypophysectomized rats were used, the operation was performed under ether anesthesia, 3 days earlier, via the parapharyngeal route. These animals received 3 μg/kg/day of dexamethasone sodium phosphate (Decadron) sc.

To study pharmacological effects on renin release, dexamethasone (5 mg/kg, ip), propranolol hydrochloride (Inderal, 10 mg/kg, sc) or phentolamine mesylate (Regitine, 10 mg/kg, sc) were administered 30 min before onset of an experiment. Drugs were dissolved in 0.9% saline and injected in a volume of 1 ml per kg body wt. Control animals received 1 ml 0.9% saline/kg.

Results are expressed as means ± standard error of the mean (SE). Statistical analysis of the data was performed using Wilcoxon's 2 sample test.

Results. In unstressed rats PRA varied from 2 to 10 mμg A_I/ml/3 hr. Placing naive rats in the shockbox without exposing them to an electric shock (control-group) did not result in a rise in PRA during the first two hours (Fig. 1). However, rats exposed to the intermittent electric shock displayed a significant elevation of PRA to 43 ± 5 mμg/ml within 15 min and PRA remained at these levels after 30 and 60 min (Fig. 1). After 120 min PRA was still elevated above controls, but had dropped significantly (P = 0.01) from the level at 60 min. Following 8 and 24 hr of exposure to the intermittent electric shock, the difference between the shock-group and the control-group had disappeared. However, the level after 24 hr was significantly (P = 0.01) higher in both the control-group and the shock-group as compared with a "normal group" of rats who received food and water *ad lib* during this time. The hematocrit at 24 hr was increased (39.2 ± 0.6, 40.1 ± 0.7 and 35.2 ± 0.7% in the control-group, shock-group and "normal group" respectively, P = 0.01), suggesting that water deprivation probably caused

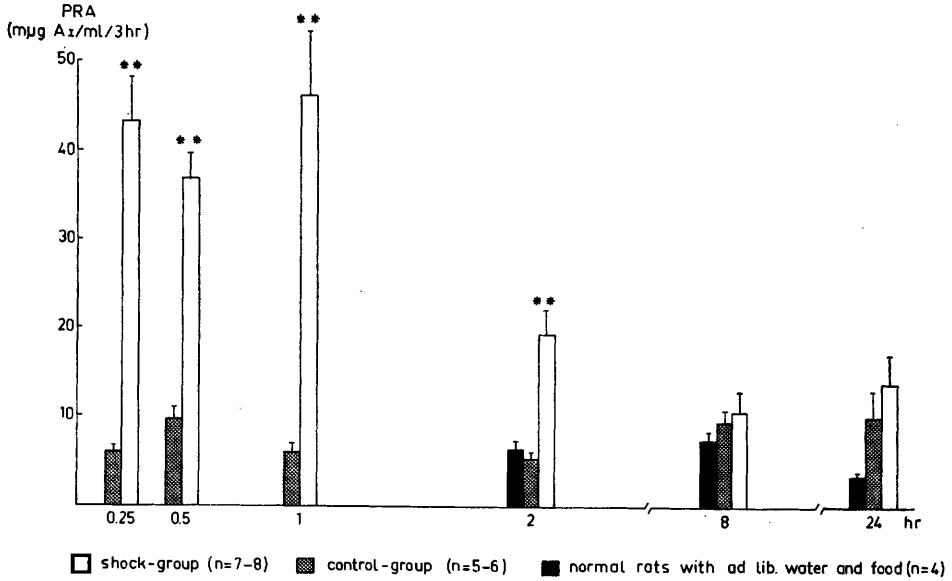

FIG. 1. Changes in plasma renin activity (PRA) during exposure of rats to an intermittent electric shock. Values are means (± S.E.) **P = 0.01.

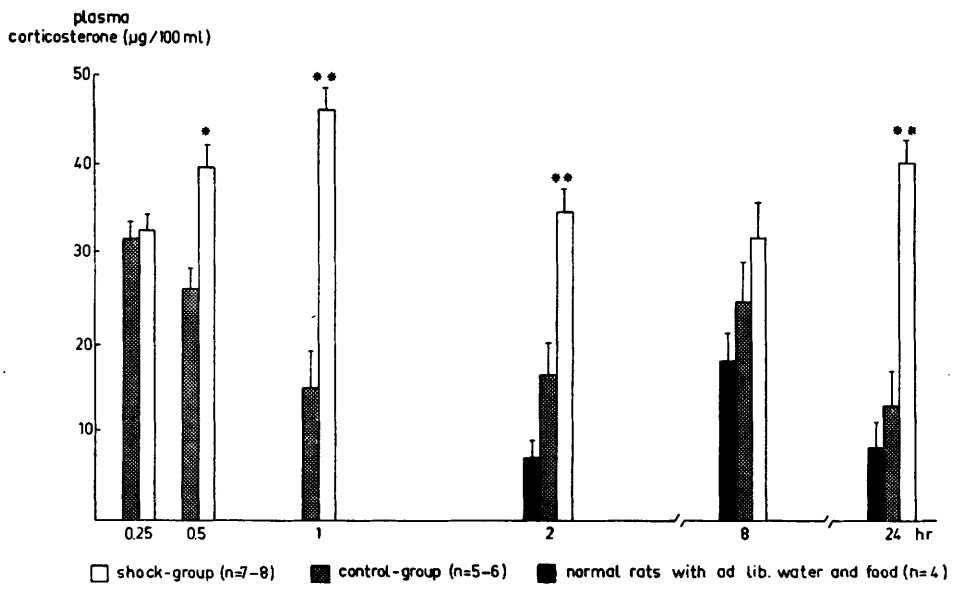

FIG. 2. Changes in plasma corticosterone concentration during exposure of rats to an intermittent electric shock. Values are means (± S.E.) *$P = 0.05$; **$P = 0.01$.

the increase in PRA at this point.

In unstressed rats plasma corticosterone concentration varied from 5 to 15 µg/100 ml. In contrast with PRA, corticosterone was increased significantly, 15 min after placing the rats in the strange environment of the box in both the control-group and the shock-group (Fig. 2). However at 30 min, the levels in the shock-group had risen further while those in the control-group had dropped, a difference which was maintained throughout the 24 hr period. By this time the corticosterone in the control-group had returned to essentially normal levels, while the levels in the shock-group remained markedly elevated. Due to the diurnal variation in adrenocortical secretion and PRA the 8 hr values in normal rats were significantly ($P = 0.01$) higher than the 24 hr value.

To assess the role of ACTH in the initial increase in PRA and the role of the ACTH-dependent corticosteroids in the subsequent disappearance of this response, hypophysectomized rats and rats receiving pretreatment with dexamethasone were studied. As shown in Table I, 30 min of intermittent shock caused a greater increase in PRA in hypophysectomized rats than in intact rats although to a questionably significant degree ($P = .10$), but PRA was still elevated after 8 hr ($P = .05$). Plasma corticosterone showed the expected changes. By contrast after 30 min of intermittent electric shock no increase in PRA was observed in intact rats, pretreated with dexamethasone.

In order to assess the importance of the sympathetic nervous system, rats were pretreated with propranolol or phentolamine (Table I). Propranolol prevented the increase in PRA, without blocking the rise in plasma corticosterone. Pretreatment with phentolamine induced a significant elevation of PRA and of plasma corticosterone ($P = 0.01$). When these rats were exposed to electric shock, PRA increased further and was significantly elevated above that of both untreated shock rats and treated unshocked rats ($P = 0.05$). The elevation in plasma corticosterone was the same in treated as in untreated rats.

Discussion. The present study demonstrates an acute effect on renin release during exposure of rats to the stress of intermittent electric shock. Plasma renin activity increased rapidly, stayed elevated for 1–2 hr but was returned to normal after 8–24 hr. Plasma

TABLE I. Effect of Hypophysectomy or Pretreatment with Dexamethasone, Propranolol or Phentolamine on the Changes in PRA and Plasma Corticosterone Concentration during Exposure of Rats to Intermittent Electric Shock.[a]

Shock Time	PRA (mμg A_I/ml/3hr) ½ hr	PRA (mμg A_I/ml/3hr) 8 hr	Plasma corticosterone (μg/100ml) ½ hr	Plasma corticosterone (μg/100ml) 8 hr
Effect of Hypophysectomy				
Intact	7.4 ± 0.6	14.8 ± 5.6		21.4 ± 4.0
Intact-Shock	33.7 ± 6.6**	16.8 ± 2.4		33.6 ± 2.5*
Hypox		23.7 ± 3.1		unmeasurable
Hypox-Shock	55.4 ± 8.5	62.0 ± 21.2*		unmeasurable
Effect of dexamethasone				
Control-Shock	31.4 ± 4.5			
Dexamethasone-Shock	8.3 ± 2.0**			
Effect of β-blockade				
Control	9.8 ± 1.3			
Control-Shock	31.4 ± 3.2*		40.9 ± 3.4	
Propranolol	9.3 ± 1.5			
Propranolol-Shock	13.1 ± 3.4		52.5 ± 2.7	
Effect of α-blockade				
Control	4.7 ± 1.2		4.9 ± 1.1	
Control-Shock	34.5 ± 9.9*		63.2 ± 0.9**	
Phentolamine	28.1 ± 5.8		30.4 ± 5.9	
Phentolamine-Shock	89.6 ± 15.6*		59.1 ± 5.9**	

[a] Values represent means ± S.E. of 4–6 rats per group.

* $P = .05$.

** $P = .01$.

corticosterone concentration remained elevated throughout the entire 24 hr period.

The initial rise in PRA probably is due to an activation of the sympathetic nervous system and appears to be mediated via β-adrenergic receptors, because propranolol blocked the response. However propranolol may have other actions which could influence renin release. One example is the as yet unconfirmed report that d-propranolol (with only slight β-blocking activity) also blocks renin release due to isoproterenol (12). It is of interest that exposure of phentolamine-pretreated rats to electric shock resulted in a higher renin release than in untreated rats, suggesting that activation of the system—probably related to the blood pressure lowering effect of phentolamine—makes it more sensitive for another stimulus. This has been shown in renal hypertensive rats (13) and in rats on sodium-deficient diet (14).

Although ACTH is capable of inducing renin release (8, 14) it would not seem that it played an essential role in the stress-induced renin release, since hypophysectomy potentiated the effect. A high dose of dexamethasone (presumably fully inhibiting ACTH release) blocked the response completely. It has been reported to block the stimulatory effect of exogenous ACTH or cyclic AMP (8, 14), while adrenalectomy or pretreatment with aminogluthethimide potentiate the stimulatory effect of ACTH on renin release (8, 14).

Considering these data it can be argued that the stimulatory effect of ACTH on renin release was blocked rapidly by the simultaneous ACTH-induced increase in corticosterone

secretion. These high circulating levels of corticosterone also may have blunted and subsequently blocked the effect of the sympathetic nervous system, since prevention of the rise in the endogenous corticosteroids by prior hypophysectomy not only potentiated the response, but also prolonged it. It appears that the blocking effect of endogenous or exogenous corticosteroids on renin release overrides the stimulatory action of ACTH and is independent of inhibition of the ACTH-release (cf. 8, 14). It could be related to a shift of intracellular sodium into the extracellular fluid, thereby increasing plasma sodium and plasma volume (15), but the increase in hematocrit in both the shock-group and the control-group, militates against this explanation. The blocking effect also has been related to a direct action on the juxtaglomerular cells producing changes in RNA synthesis (14).

To summarize, the increase in renin release with stress seems to derive primarily from sympathetic arousal, probably with an additional ACTH-stimulatory component, is soon terminated by a rise in adrenal cortical secretion, can be blunted by β-receptor blockade, and is exaggerated by blood pressure reduction with α-receptor blockade. In any case, the response of PRA with stress and the magnitude of this response as demonstrated in our study, provides another physiological explanation for observed variation and lability of the renin-angiotensin system in the intact organism, and possibly for acute elevations in blood pressure which occur with stress.

Summary. During exposure of rats to an intermittent electric shock PRA increased initially by about 500%. After 1–2 hr the PRA response decreased and after 8–24 hr PRA was no longer different from that of control-rats. Plasma corticosterone concentration stayed elevated during the whole 24 hr period.

Following hypophysectomy the increase of PRA was not inhibited and occurred also after 8 hr. Both dexamethasone and propranolol blocked completely the initial rise in PRA. Phentolamine potentiated the response.

These results suggest that stress-induced release of renin is mediated via β-adrenergic receptors and that endogenous corticosteroids modify this response.

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