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On the Mechanism of Renin Release by Restraint Stress in Rats

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SIGG, E. B., K. L. KEIM AND T. D. SIGG. *On the mechanism of renin release by restraint stress in rats.* PHARMAC. BIOCHEM. BEHAV. 8(1) 47–50, 1978. – Restraint causes an increase in plasma renin activity (PRA) which is not affected by pretreatment with dl-propranolol (1 mg/kg IP) or sotalol (15 mg/kg IP). These doses of β-adrenergic blocking agents are effective in suppressing the stimulation of PRA by isoproterenol. Large doses of dl-propranolol (10 mg/kg IP) and d-propranolol (5 mg/kg IP) attenuate the restraint-induced PRA increase. Adrenal demedullectomy does not affect the PRA response to restraint. Renal denervation blunts the PRA rise due to restraint, but not to direct stimulation by the β-adrenergic agonist, isoproterenol. It is concluded that the increase in PRA during restraint stress in rats is not solely dependent on an intact renal sympathetic innervation. A significant portion of this stress-induced PRA increase appears to involve a non-adrenergic mechanism.

Renin release Restraint stress Plasma renin activity

MANY DIFFERENT mechanisms govern the release of renin from the kidney [8]. The most prominent factors involve the activation of the renal sympathetic nerves, β-adrenergic receptors in the kidney, renal baroreceptors and the macula densa. Humoral agents such as adrenal catecholamines, steroid hormones, antidiuretic hormone, adrenocorticotrophic hormone (ACTH), and especially electrolytes are known modulators of renin activity.

The role of the sympathetic nervous system [10] and the pituitary-adrenocortical system [11] in the release of renin strongly suggests that various stressors elevate circulating renin concentration. This has been documented in patients undergoing surgery [22], in rats subjected to intermittent electroshock [17], and monkeys exposed to a psychological stimulus [6]. The present experiments were designed to investigate whether restraint stress in rats increases plasma renin activity (PRA), and, if so, whether the principal mechanism involves the activation of the sympathetic-adrenal system. Plasma corticosterone (PCS) was measured to assess the magnitude of the pituitary-adrenal response to stress.

METHOD

General Experimental Procedure

Male Sprague-Dawley rats, received when 50 days old, were housed individually for 2 weeks prior to the experiment. The rats were kept in a room with constant humidity (45%) and temperature (23°C), illuminated from 6 a.m. to 6 p.m. Food and water ad lib was provided. All experiments were carried out between 8 and 11 a.m. with animals randomly assigned to experimental groups. In each experiment one unoperated or untreated group of 4–6 rats served to establish the unstressed, resting secretion of PCS and PRA. Each sham-operated or saline-injected group, and each operated or drug-treated group was divided into two subgroups of 5 to 8 rats each. One subgroup was sacrificed without being stressed. Each rat in the other subgroups was restrained for 30 min before being decapitated.

Biochemical Analyses

Blood and tissues were processed as previously described [15]. PCS and corticosterone from incubated, ACTH-stimulated adrenal fragments were determined fluorometrically [15,19]. PRA was determined by radioimmunoassay [23] and expressed as nanograms of angiotensin I formed per ml of plasma in 1 hr (ng AT I/ml/hr). Angiotensin I was generated during a 3 hr incubation period at pH 5.8–6.0. Norepinephrine and epinephrine were extracted from the adrenal glands and kidneys [12] and determined fluorometrically [16] as previously described [15].

Experiments

Time course of restraint-induced increase of PCS and PRA. Rats were restrained in a 7 cm diameter plastic cylinder (Plastic Labs, Lansing, MI) for periods of 30, 60, 120 and 240 min [15]. Immediately after each respective restraint period the rats were decapitated and trunk blood was collected for later determination of PCS and PRA.

Renal denervation. Under Equithesin^R anesthesia, a skin incision was made over the back. The kidneys were approached retroperitoneally and denervated bilaterally by cutting identifiable nerves, stripping the blood vessels and painting them with a solution of 5% phenol. Corresponding sham operations were also carried out. A recovery of 8 or 20 days was allowed before submitting the

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animals to restraint. The completeness of the denervation was checked by determining kidney norepinephrine (NE).

Demedullectomy. Under Equithesin^R anesthesia, the paravertebral musculature was dissected to expose the adrenal glands. The glands were lifted from the peritoneal cavity, their capsule slit with a sharp blade, and the contents were gently squeezed out. Appropriate sham operations were carried out. For 5 days postoperatively, all rats received 5% glucose in physiological saline as drinking fluid before being switched back to tap water. Two to three weeks were permitted for recovery. The success of the operation was tested by analyzing adrenal catecholamines by the histochemical fluorescent technique [9] or biochemically (see above). Also, the regeneration of cortical tissue was assessed by determining in vitro reactivity of the adrenal cortex to exogenous ACTH [15].

Renal denervation and demedullectomy. Both procedures described above were combined in a single stage operation.

Time course and dose-response of isoproterenol-induced increase in PRA. An intraperitoneal injection of isoproterenol (ISP) was used to evaluate the responsiveness of the renal adrenergic receptor in rats with denervated kidneys and to determine the effectiveness of β-blockade. Following ISP administration the rats were grouped in a carrying cage and killed after five minutes. The effectiveness and duration of the ISP dose on PRA was verified in a preliminary experiment.

Administration of adrenergic blocking agents. To assess the involvement of an adrenergically mediated release of renin in restraint stress, various adrenergic blocking agents were administered intraperitoneally, one hour prior to restraint. The drugs used were: phenoxybenzamine (10 mg/kg); dl-propranolol (1, 5 and 10 mg/kg); d-propranolol (1, 2.5 and 5 mg/kg); and sotalol (15 mg/kg). All drugs were dissolved in saline and the solutions were adjusted to a pH of 5 to 6. Appropriate acidified saline injections served as controls. The injected volume was 1 ml/kg.

STATISTICS

Statistical differences between the experimental means were compared by the Newman-Keuls test following a one way analysis of variance and where indicated in the text by Student's *t*-test for nonpaired samples.

RESULTS

The average basal PRA, determined from 45 animals was 6.1 ± 0.4 ng AT I/ml/hr. Thirty min of restraint stress increased this value to 23.3 ± 2.4 ng AT I/ml/hr, (n = 45; $p<0.001$, Student's *t*-test). While PCS rose in all rats exposed to the stressor, PRA remained at basal levels during stress in approximately 6% of this population. The rise of PCS and PRA in rats reached a maximum after one hour of restraint and declined when immobilization was extended to 4 hr (Table 1).

To determine if the increase in PRA was due to an activation of renal sympathetic nerve activity rats with denervated kidneys were subjected to restraint. Eight days after the operation the resting level of both PCS and PRA from nonstressed, kidney-denervated rats was comparable to that of sham-operated controls. The marked reduction of renal NE, from 0.11 ± 0.1 μg/g in controls to 0.02 ± 0.08 μg/g (−82%) in the denervated animal indicated that the sympathetic nerve supply to the kidney was largely eliminated. Restraint stress elicited a rise in PCS which was of similar magnitude in both the sham-operated and kidney-denervated rat (Table 2, Group B vs. D). PRA in response to restraint in sham-operated rats 8 days after surgical intervention was significantly higher ($p<0.001$, Student's *t*-test) when compared to unoperated, stressed animals (i.e., 17.7 ng AT I/ml/hr; Table 1, 30 min value). However, 20 days after operation there was no difference between the sham-operated and unoperated controls in regard to stress-induced increases in PRA. The stress-induced PRA increase observed 8 days (Group D, Table 2) or 20 days after renal nerve section was significantly lower (49% and 41%, respectively, $p<0.01$) than that of sham-operated rats (Group B, Table 2). Direct release of renin by 30μg/kg ISP IP was the same in sham-operated and renal-denervated rats (41.2 ± 10.5 and 40.4 ± 6.5 AT I/ml/hr, respectively).

TABLE 1

THE EFFECT OF RESTRAINT DURATION ON PLASMA CORTICOSTERONE (PCS) AND RENIN ACTIVITY (PRA)

Restraint duration	No. of rats	Plasma CS μg/100 ml	PRA ng AT I/ml/hr
Control (no restraint)	4	5.5 ± 0.3	7.5 ± 0.46
30 min	6	39.9 ± 3.0	17.7 ± 2.77
60 min	6	42.7 ± 2.1	20.8 ± 2.11
120 min	6	29.8 ± 5.1	17.5 ± 3.05
240 min	6	20.2 ± 3.3	8.7 ± 2.49

To test the possibility of receptor activation by adrenergic neurohumors released by the adrenal medulla, PRA was measured in demedullectomized rats. Although the nonstress PCS concentration was slightly diminished in demedullectomized rats, the resting PRA was not affected. The stress-induced increase in PRA was not altered significantly in animals prepared in this manner; the PCS response to 30 min of restraint was blunted in the demedullectomized rat when compared to sham-operated controls (Table 2, Group B vs C). However, the in vitro reactivity to ACTH of adrenal fragments from demedullectomized rats was decreased by 50% (from 27.3 ± 4.2 to 13.7 ± 1.5 ng CS/mg adrenal/3 hr). When demedullectomy was combined with renal nerve section, the attenuating effect of denervation upon stress-induced PRA was equal to the rise in the kidney-denervated rat with intact adrenal medullae (Table 2).

The effects of dl-propranolol, d-propranolol and sotalol on restraint-induced increases of PCS and PRA are summarized in Table 3. Only large doses of dl-propranolol (5 and 10 mg/kg) and of d-propranolol (5 mg/kg) reduced the stress-induced rise of PRA significantly. Sotalol (15 mg/kg) was ineffective. The PCS response to restraint was diminished by dl-propranolol (all three doses) and by the largest dose (5 mg/kg) of d-propranolol. Phenoxybenzamine (10 mg/kg IP) had no effect on stress-induced PCS and enhanced PRA variably.

Direct β-adrenergic-receptor stimulation with ISP (10 μg/kg IP) caused a marked rise in PRA (Table 4). Pretreatment with dl-propranolol (1 mg/kg) or sotalol (15 mg/kg) prevented the isoproterenol-induced enhancement of PRA, whereas, d-propranolol was ineffective.

TABLE 2

EFFECT OF RESTRAINT ON PLASMA CORTICOSTERONE (PCS) AND RENIN ACTIVITY (PRA) IN DEMEDULLECTOMIZED AND KIDNEY-DENERVATED RATS*

	Treatment	Number of Rats	PCS μg/100 ml	PRA ng AT I/ml/hr
A	Sham-operated, non-stressed	5	5.3 ± 0.9	4.1 ± 0.4
B	Sham-operated, plus restraint	6	55.0 ± 2.6	48.1 ± 4.5
C	Demedullectomy, plus restraint	7	36.4 ± 2.0	43.7 ± 5.8
D	Renal denervation, plus restraint	6	56.5 ± 1.7	24.5 ± 4.2
E	Demedullectomy plus renal denervation plus restraint	8	41.8 ± 1.3	21.0 ± 2.8

*eight days after operation.

Significance of differences for PCS: B-C $p<0.01$; B-E $p<0.01$; C-D $p<0.01$; D-E $p<0.01$.

Significance of differences for PRA: B-E $p<0.01$; B-D $p<0.01$; C-D $p<0.05$; C-E $p<0.01$.

TABLE 3

THE EFFECT OF β-ADRENERGIC BLOCKING AGENTS ON RESTRAINT STRESS-INDUCED INCREASES IN PLASMA CORTICOSTERONE (PCS) AND RENIN ACTIVITY (PRA)

	Treatment	PCS μg/100 ml	PRA ng AT I/ml/hr
A	Control (non-stressed)	4.8 ± 0.3(20)	6.9 ± 0.8(21)
	30 min Restraint following:		
B	Vehicle	45.8 ± 1.5(21)	18.7 ± 1.9(20)
C	1 mg/kg dl-propranolol	29.5 ± 4.8(5)	17.8 ± 1.6(5)
D	5 mg/kg dl-propranolol	32.6 ± 3.3(11)	12.0 ± 2.6(11)
E	10 mg/kg dl-propranolol	34.6 ± 3.5(10)	7.3 ± 1.2(10)
F	1 mg/kg d-propranolol	40.1 ± 2.5(5)	26.4 ± 4.9(4)
G	2.5 mg/kg d-propranolol	47.0 ± 4.3(6)	12.6 ± 2.1(5)
H	5.0 mg/kg d-propranolol	30.5 ± 2.6(5)	5.2 ± 1.0(5)
I	15 mg/kg sotalol	43.7 ± 5.0(11)	16.8 ± 3.0(11)

In parentheses: number of rats.

Significance of differences for PCS: B-C $p<0.001$; B-D $p<0.05$; B-E $p<0.05$;B-H $p<0.01$; G-H $p<0.01$.

Significance of differences for PRA: B-E $p<0.05$; B-H $p<0.05$; C-E $p<0.05$; F-H $p<0.01$; F-G $p<0.05$.

TABLE 4

EFFECT OF ISOPROTERENOL (ISP) AND β-ADRENERGIC BLOCKING AGENTS ON RENIN ACTIVITY (PRA)*

Group	Treatment	Number of Rats	PRA ng AT I/ml/hr
A	Control	5	11.3 ± 1.2
B	Isoproterenol (ISP) (10 μg/kg IP)	5	60.7 ± 7.3
C	dl-propranolol + ISP (1 mg/kg)	5	29.7 ± 1.5
D	d-propranolol + ISP (1 mg/kg)	5	52.8 ± 8.0
E	sotalol + ISP (15 mg/kg)	5	14.3 ± 1.7

*The rats were pretreated 1 hr before sacrifice with the different β-adrenergic blocking agents, and 5 min before decapitation with ISP. Significance of differences for PRA: B-C $p<0.01$; B-E $p<0.01$; C-D $p<0.01$; D-E $p<0.01$.

DISCUSSION

Our findings demonstrate that restraint stress increases PRA. Various other stressors, e.g., cold, exercise, hemorrhage, postural change, and electroshock are also known to increase PRA [5, 17, 21, 26], presumably by enhancing sympathetic nerve activity. A relationship between sympathetic nerve activity and PRA is suggested by histological association of sympathetic nerve fibers with the juxtaglomerular apparatus [25]. More directly, electrical stimulation of the renal nerves increases PRA [18]. Conversely, renal sympathectomy [4,24] and ganglionic blocking agents [7] lower PRA. There is good evidence that the PRA response to NE released at the renal nerve endings and to circulating catecholamines is mediated by β-adrenergic receptors [3] and is independent of hemodynamic and ionic influences [1]. The effect of many different, adrenergically mediated stimuli releasing renin are attenuated by β-adrenergic blocking agents [2, 14, 18].

The rise of PRA in response to restraint is, however, only partially due to an activation of intrarenal adrenergic receptors since kidney denervation fails to completely prevent a stress-induced increase in PRA. However, it is possible that the denervation was not complete, and that a depletion of NE by 82% left a significant pool for activation. On the other hand, the decreased PRA to restraint stress in kidney denervated rats may be due to long lasting, ill defined postoperative changes unrelated to the interference with the renal nerve supply. This argument is supported by the abnormally large rise in PRA to restraint in sham-operated rats.

The failure of adrenergic β-blockade to prevent the restraint-evoked PRA rise, supports the notion that the renin released during restraint does not involve intrarenal β-adrenergic mechanisms to any significant extent. The decrease of restraint-induced PRA rise by large doses (10 mg/kg) of dl-propranolol must be considered as unrelated to β-blockade, inasmuch as d-propranolol has the same effect. This interpretation would also apply to the finding that 10 mg/kg dl-propranolol s.c., 30 min prior to electric foot shock blocked the stress-induced increase in PRA [17]. Even when most of the indirect and direct adrenergic stimulation is removed by demedullectomy combined with renal denervation, the stress-induced renin response is not abolished and must, therefore, be considered largely non-adrenergic in nature. This is concordant with the observation that, at least in cats, renin released by non-adrenergic mechanisms is not blocked by propranolol [13].

The variable enhancement of PRA after pretreatment

with phenoxybenzamine is in agreement with the finding that phentolamine, another α-adrenergic blocker, induces renin release [20].

It is concluded that restraint stress causes an increase in PRA. This increase is not completely accounted for by an activation of renal sympathetic nerves and/or catecholamines liberated from the adrenal gland since, in spite of a partial reduction after the renal denervation, administration of β-adrenergic-blocking drugs (except in large doses) or adrenal medullectomy do not affect the response. Although neither systolic arterial blood pressure [15] nor plasma sodium or potassium concentrations (unpublished observation) are affected by 30 min restraint, it is possible that hemodynamic changes in the kidney occurring during stress or hormonal factors (e.g., release of pituitary hormones such as ACTH) contribute to the homeostatic release of renin.

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