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Hyperresponsivity to Angiotensin Induced in Rats by Behavioral Stimulation.* (31399)

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Hyperreactivity to pressor stimuli is a characteristic of the hypertensive organism. Among the various pressor stimuli, those of psychological origin play a prominent role(1). It was of interest to us to determine whether psychological stimuli, which generally are studied in regard to their direct pressor effects, might also induce the hyperreactive state.

We have used previously the pressor response to angiotensin§ infusion in patients with benign essential hypertension as well as in hypertensive rats to demonstrate hyperreactivity(2,3). In the present experiment angiotensin was employed to study reactivity in normotensive animals after exposure to a noxious behavioral stimulus.

Materials and methods. Thirty-four male albino rats of the Holtzman strain weighing 175 to 400 g were fed standard rat chow, allowed tap water *ad libitum* and studied in groups of 4 to 5 experimental and control animals of the same weight. Systolic pressor response to intravenous injection of 0.1 μg of angiotensin was determined in each animal during light ether anaesthesia by an indirect technique which permits repeated determinations of the magnitude and duration of response, as described previously(3). Three to four responses were determined for each rat at each session and these were averaged. Immediately after the initial (pre-shock) determination, the animals were placed in an 18″ × 18″ × 18″ wooden box with an electric grid flooring. The experimental rats were exposed for 24 hours to a 25 milliampere electric shock paired in presentation with a bright light attached to the cage. This light-shock combination was delivered every 24 seconds for 3 seconds duration. Control animals were placed in the same box for 24 hours, but received no stimulation. Neither group received food or water while in the box. Immediately after the 24-hour period of shock, a second set of angiotensin responses was determined in both experimental and control groups. The rats then were returned to their colony cages and given food and water. A third set of angiotensin responses was determined 24 hours later.

Data on catecholamine excretion and organ content were obtained from an additional

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§ All references to angiotensin refer to Angiotensin II kindly supplied by Ciba Pharmaceutical Co., Summit, N. J.

TABLE I. Pressor Response to Angiotensin II Infusion (Average ± Standard Error).

Group		Pre-stimulus	Immediate post-stimulus	24 hr post-stimulus
Shock	No. of rats	24	24	18
	Baseline (mm Hg)	108.0 ± 3.2	107.9 ± 3.4	108.9 ± 4.0
	Response (mm Hg)	18.5 ± 1.4	31.0 ± 1.8	16.7 ± 1.0
	Duration (sec)	34.1 ± 3.3	35.7 ± 2.4	33.2 ± 2.8
Control	No. of rats	10	10	6
	Baseline (mm Hg)	116.6 ± 3.5	104.2 ± 3.6	118.3 ±10.0
	Response (mm Hg)	23.4 ± 2.1	22.9 ± 2.0	16.7 ± 2.0
	Duration (sec)	34.9 ± 4.0	33.1 ± 2.7	25.8 ± 5.0
Analyses of variance				
Baseline	$F = 1.08$ Not significant			
Response	$F = 12.46$ $p < .01$			
Duration	$F = .06$ Not significant			
t Tests				
Response; shock × control		$t = 1.94$, not significant	$t = 2.99$, $p < .01$	$t = .25$, not significant

group of 46 male albino Holtzman rats in which the pressor responses were not measured. The same protocol was used as in the first experiment except that during the shock (or control) periods, urine was collected through a stainless steel funnel-shaped cage bottom placed just below the electric grid and connected to a jar containing 1 ml of 6 N HCl. After 24 hours of shock (or control) half of the rats from each group were selected randomly and sacrificed; their hearts were individually weighed and ground in 5 ml of 6 N HCl for catecholamine determinations. The remaining rats in each group were then placed in individual metabolic cages and given tap water, but, no food. Urines were collected for 24 hours and pooled for each sub-group of rats; all were then sacrificed and their hearts treated as above.

The free urinary catecholamines were separated by alkalinization, batch adsorption on alumina, and acid elution. The heart catecholamines were determined by filtering the acid homogenate, alkalinizing the filtrate and then absorbing on, and eluting from, alumina, as for the urines. The catecholamines in the eluates were determined by a modification of the method of Cohen and Goldenberg(4). Urinary creatinine was measured by the picric acid method(5) and the urine catecholamines expressed as ng catecholamine/mg creatinine; heart catecholamines were expressed as ng/mg of wet weight.

In a third experiment 20 male albino Holtzman rats were assigned randomly to either group or to an additional control consisting of fed animals not removed from their cages. Immediately after 24 hours of shock (or fasting or normal feeding for the first and second control groups respectively) they were sacrificed by decapitation and blood collected for determination of corticosterone(6).

Results were subjected to an analysis of variance for randomized groups(7). When significant F values were obtained ($p = .05$ or less), t tests between individual groups were performed.

Results. Comparison of the pressor responses after the injection of 0.1 μg of angiotensin in shocked and control animals is given in Table I. There was no significant difference ($F = 1.08$, $p > .05$) among the baseline pressures either prior to the shock, immediately after, or 24 hours after shock. However, analysis of variance of the angiotensin responses indicated highly significant differences ($F = 12.45$, $p < .01$) and t tests done between the control and shocked groups for each period demonstrated that this result was due to a greater response in the shocked group when tested immediately after the stimulus. Before and 24 hours after the shock, the responses in the two groups were not different from each other. Analysis of variance of the duration of pressor responses revealed no significant differences ($F = .06$, $p > .05$).

Seven of the shocked animals and 4 of the controls died or were otherwise unable

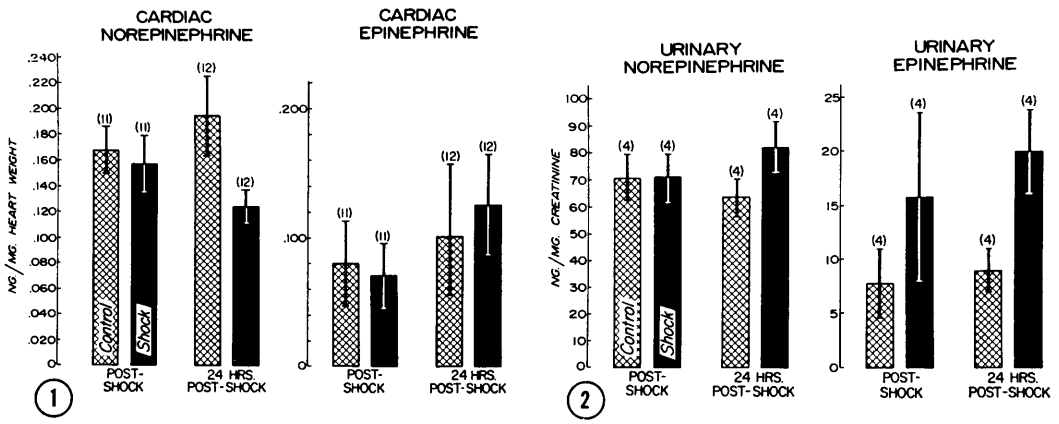

FIG. 1. The cardiac catecholamines are shown for animals sacrificed either immediately after shock or 24 hr later. None of the differences are significant.

FIG. 2. Urinary catecholamines collected during stress are labelled "Post-Shock." Those collected during the 24 hr recovery period are labeled "24 hr Post-Shock." None of the differences are significant.

to be assayed through all 3 periods. Block analysis of variance using *only the completed pairs* again revealed significant differences in pressor responses between the immediate "post-shock" and the immediate "post-fast only" groups and, in addition, the responses of the group immediately after the shock were significantly greater than the responses of the same group 24 hours earlier or later.

The catecholamine data are summarized in Fig. 1 and 2. No significant differences between any of the groups for either the heart or the urinary norepinephrine were noted. Urinary epinephrine appeared slightly higher during shock, but the difference failed to achieve significant levels.

The plasma corticosterone levels from the third experiment are shown in Table II. Levels in the shocked group immediately after this stimulus were significantly higher than in each of the control groups.

TABLE II. Plasma Corticosterone Levels in Rats Exposed to 24 Hours of Shock.

Group	No. of rats	Avg ± S.E. (μg/100 ml)
Shocked	6	28.3 ± 1.8
Control (fasted)	6	11.2 ± 4.6
" (fed)	8	13.3 ± 4.8
$F = 4.52$; $p = .05$		
	t	p
Shock × control (fasted)	3.43	<.01
" " " (fed)	2.97	<.01
Control (fed) × (fasted)	.30	Not significant

Discussion. The data offer evidence that an acute behavioral stimulus in a normotensive animal can result in an increased pressor response to exogenous angiotensin and that this increased response is not related to a change in baseline pressure. Furthermore, the hyperresponsiveness to angiotensin is a transient phenomenon, not being present 24 hours after termination of the stimulus. As in other studies, a sustained blood pressure elevation was not produced by the noxious stimulus alone(8).

Since noxious stimuli are known to release epinephrine and possibly norepinephrine(9), the catecholamine assays were performed to determine if the noradrenergic nervous system was involved in the increased responsivity to angiotensin. It was postulated that the catecholamines might enhance responsivity by a direct action on the vascular system or conversely that norepinephrine might be depleted from nerve endings resulting in a chemically denervated animal rendered more susceptible to a directly acting humoral agent. Since changes in norepinephrine excretion or tissue content were not demonstrated it seems unlikely that either of these mechanisms mediate the phenomenon. The suggestion of an increase in epinephrine excretion in the shocked animals is not surprising and probably indicates that the shock was an effective acute stimulus.

The elevation of corticosterone levels in the animals receiving shock might provide the answer to the increased pressor response. Elevation of cortical steroids with environmental stimuli has been reported(10), while increase in reactivity to renin with large doses of exogenous steroids has been noted(11).

The significance of these experiments lies in the production of an altered physiologic response by a prior behavioral stimulus. The possible role of this type of change in the development of psychosomatic disease states has been discussed by Engel(12). We have reported alterations in metabolic responses after behavioral stimuli(13,14), and the present data provide evidence of change in reactivity by such stimuli in still another area.

Summary. It has been demonstrated that angiotensin hyperresponsivity can be induced in the normotensive animal by a behavioral stimulus. This hyperresponsivity is limited to the period immediately following application of the stimulus. It is not associated with a change in baseline pressure and does not appear to be mediated through the autonomic nervous system, but it is accompanied by increased levels of plasma corticosterone.

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Function of Transcobalamin II: A B_{12} Binding Protein in Human Plasma.* (31400)

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Early studies in vitamin B_{12} binding by plasma showed that more than one protein could participate(1). B_{12} naturally present was bound to an alpha globulin while part of that which was added to plasma *in vitro* was bound to a protein with the mobility of a beta globulin. Subsequently these observations were confirmed by several investigators and by a variety of techniques of protein separation. The significance of the beta globulin in binding has been the subject of much controversy but a physiologic role for this binder usually has been considered unlikely since no one has shown that it bound B_{12} which had been taken into plasma by natural means.

In 1963(2) we found that when vit B_{12} was either injected or taken by mouth it was initially bound to a protein which we subsequently called transcobalamin II (TC II) (3). By conventional electrophoresis, the mobility of TC II is close to that of the beta

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