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LEUCOCYTE RESPONSE TO SOUND STRESS IN RATS: ROLE OF THE ADRENAL GLAND

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CHANGES in blood leucocytes—lymphocytopenia, eosinopenia and neutrophilia—follow many forms of stress (Selye, 1950), including exposure to loud noise (Jensen and Rasmussen, 1963). Adrenal cortex participation in such reactions has been proposed because the administration of large doses of glucocorticoids produces leucocyte changes similar to those of the stress reaction (Nelson *et al.*, 1952). Furthermore, the leucocyte reaction to sound and other stresses can be prevented by total adrenalectomy (Elmadjian and Pincus, 1945; Jensen and Rasmussen). However, in adrenalectomised animals, many of the metabolic and circulatory responses to stress can occur in the presence of minimal amounts of glucocorticoid (Campbell *et al.*, 1954; Papacostas, Kanis and Reed, 1964); this suggests that the glucocorticoids have only a " permissive " action (Ingle, 1954; Selye, 1954). The present experiments were designed to confirm the leucocyte response to sound stress and to examine the role of the glucocorticoids as " permissive " or " direct " agents in that response.

METHODS

Fifty-eight male hooded rats aged about 16 wk and weighing 200–300 g. were divided into 4 groups. Each group was housed in a wire cage in a quiet air-conditioned room at about 20°C and fed on " Barastoc " dog cubes (Barastoc Products, Melbourne) and water *ad libitum*. The animals were handled by no one but the writer during the experiments. Two groups of animals were totally adrenalectomised by a transabdominal approach under ether anaesthesia and, to maintain mineralocorticoid activity, a 35 mg. tablet of deoxycorticosterone acetate was implanted subcutaneously over the right shoulder. Post-operatively, 1 per cent. saline was given instead of drinking water.

Blood was collected by slicing off the tip of the tail while the rat was comfortably restrained in a holding device, and leucocyte counting was carried out within 30 min. with a Neubauer chamber and Türk's diluting fluid. A differential count was made on a Leishman-stained smear, in which only mononuclear and polymorphonuclear leucocytes were distinguished. The significance of changes in leucocyte counts was examined by the t-test and the Mann-Whitney U-test.

Animals were subjected for 1½ hr to intense noise (112 decibels), roughly comparable to that heard a few yards away from a jet engine, emitted from 2 loudspeakers mounted in a " sound-proof " box, 1·5 × 0·9 × 0·6 m. in size. The animals were placed in the centre of the box in the customary type of cage, ventilation being maintained by means of a forced draught of air. They huddled in the corners, but I did not observe any audiogenic seizures though these occur in certain strains of rat (Biró, Szokolai and Kovách, 1959). Sound-stressing was carried out from 11 a.m. to 12·30 p.m. in each experiment.

RESULTS

Effect of sound stress on leucocyte counts in normal and adrenalectomised rats

The results are summarised in table I.

Normal rats were stressed with sound for 1½ hr and their leucocyte counts compared at approximately the same times with those of control rats, which were

disturbed only for a few minutes in order to collect blood samples. Blood was collected from groups of the sound-stressed rats 1½, 4 and 6 hr after the start of the stress. There was no significant variation in leucocyte counts in the control rats from 11 a.m. to 5 p.m., the hours of the experiment. In the sound-stressed rats the mononuclear cells, predominantly lymphocytes, decreased by 43 per cent. after 1½ hr, but the polymorphonuclear leucocytes were virtually unaffected. The mononuclear cell counts were still less than the control values 4 hr after the start of the stress; the increase in the polymorphonuclear leucocyte count at this time was not significant. After 6 hr the number of polymorphonuclear leucocytes was normal, but the mononuclear cells were still 19 per cent. below the control levels. The changes were not followed for any longer period.

On the other hand, when adrenalectomised rats were stressed for 1½ hr, neither their total nor their differential leucocyte counts differed significantly from those of unstressed adrenalectomised rats.

TABLE I

Effect of sound stress on leucocyte counts in normal and adrenalectomised rats

Leucocytes counted	Mean count in thousands per c.mm. ± standard deviation in					
	intact animals				adrenalectomised animals	
	controls (11)	at hr after start of sound stress			controls (4)	1½ hr after start of sound stress (6)
		1½ (5)	4 (5)	6 (4)		
Total	14·3 ± 2·0	9·0* ± 1·2	12·7 ± 0·9	12·2* ± 0·9	14·4 ± 3·8	12·2 ± 1·5
Mononuclears	11·2 ± 1·5	6·3* ± 2·1	9·0 ± 2·4	9·1* ± 1·0	10·5 ± 2·7	9·5 ± 1·0
Polymorpho-nuclears	3·1 ± 0·7	2·7 ± 1·3	3·7 ± 1·7	3·1 ± 1·0	3·9 ± 1·3	2·7 ± 0·4

* Significantly different from corresponding control figures ($P < 0.01$).
Number of animals studied is shown in brackets.

Effect of cortisone, and of cortisone with sound stress, on leucocyte counts in adrenalectomised rats

Two weeks after operation, aqueous suspensions of 0·8, 1·6 or 4·0 mg. of cortisone acetate per 100 g. body weight were injected subcutaneously into adrenalectomised rats. Half the rats receiving the lowest dose of cortisone, which is roughly equivalent to estimates of the normal minimum daily glucocorticoid production (Bush, 1953), also received sound stress beginning 2 hr after the injection. Blood samples were collected from most of the animals about 4 hr after the injection, as this is the time-interval for the leucocyte response to occur after glucocorticoid administration (Nelson *et al.*). Blood from some of the animals receiving cortisone plus sound stress was collected 4 hr after the start of the stress, that is 6 hr after the injection.

In adrenalectomised rats, the mononuclear leucocyte counts were not significantly affected 4 hr after 0·8 mg. cortisone acetate per 100 g. body weight, but with higher doses a significant lymphocytopenia was found (table II). In the rats receiving 0·8 mg. cortisone acetate per 100 g. body weight and subjected to sound stress, the mononuclear leucocyte count at 1¾ hr after the start of the sound stress was 33 per cent. lower than that of unstressed rats receiving the same dose of cortisone. The mononuclear leucocyte count had returned to the same level as

in unstressed controls 4 hr after the start of the stress. The polymorphonuclear leucocyte counts were not significantly affected in any of these groups of animals, although the eosinophils would presumably have been found to be decreased if they had been counted separately.

Discussion

A marked leucopenia, mainly affecting lymphocytes, occurred 1½ hr after the start of sound stress in normal rats and persisted for at least 4½ hr after its cessation: this response was prevented by adrenalectomy. In adrenalectomised rats, cortisone acetate injected in amounts greater than the normal minimal 24-hr glucocorticoid secretion caused a significant lymphocytopenia within 4 hr in the absence of sound stress. It has previously been shown that 2-hr sound stress produces a three-fold increase in the concentration of steroids in adrenal-vein blood in normal rats (Henkin and Knigge, 1963). This increased secretion of glucocorticoids in sound

Table II

Effect of cortisone, and of cortisone with sound stress, on leucocyte counts of adrenalectomised rats

Leucocytes counted	Mean count in thousands per c.mm.±standard deviation in adrenalectomised rats					
	untreated (8)	given cortisone (mg. per 100 g. body weight)				
		4·0 (3)	1·6 (4)	0·8, and		
				unstressed (12)	bled at hr after start of sound stress	
					1¾ (8)	4 (4)
Total	16·0 ± 3·4	13·5 ± 2·5	17·9 ± 6·2	15·0 ± 4·0	11·6* ± 1·9	14·6 ± 1·4
Mononuclears	11·4 ± 2·4	7·6* ± 0·9	8·7* ± 1·1	10·1 ± 1·9	6·8* ± 1·3	10·6 ± 2·5
Polymorphonuclears	4·6 ± 1·3	5·9 ± 2·2	9·2 ± 5·7	4·9 ± 3·4	4·8 ± 1·4	4·0 ± 1·1

* Significantly different from corresponding figures for untreated animals ($P<0.05$).
Number of animals studied is shown in brackets.

stress may, therefore, play a " direct " role in the blood leucocyte response. However, the present experiments suggested that at least 75 per cent. of the lymphocytopenia observed after sound stress in normal rats also occurred in adrenalectomised rats in the presence of a small amount of glucocorticoid which, by itself, did not affect the leucocyte counts. Here, the glucocorticoid appeared to have a " permissive " role as it did not directly cause the leucocyte changes.

The counts of polymorphonuclear leucocytes, predominantly neutrophils, were not affected consistently by the sound stress or the different doses of cortisone administered in any of the experiments. Neutrophilia often accompanies lymphocytopenia and eosinopenia in reactions to stresses such as severe muscular exercise or subcutaneous injection of formalin (Harlow and Selye, 1937), but it shows poor correlation with the other leucocyte changes. It can occur in the absence of lymphocytopenia or eosinopenia after injection of deoxycorticosterone acetate in normal or adrenalectomised mice (Dougherty and White, 1944), and is probably mediated by mechanisms different from those that cause the other leucocyte changes.

SUMMARY

Intense sound stress (112 decibels) for 1½ hr produced marked lymphocytopenia in normal, but not in adrenalectomised rats. In the latter, lymphocytopenia was produced by the injection of cortisone acetate in doses greater than the normal minimum daily glucocorticoid production. In adrenalectomised rats given cortisone acetate in amounts that by themselves produced no change in leucocyte counts, sound stress led to a lymphocytopenia; this response was about 75 per cent. of that observed in normal rats subjected to the same stress. Thus, the glucocorticoids appear to have a "permissive" as well as a "direct" role in the lymphocyte response to sound stress. Neutrophilia, which has been observed in the reaction to other stresses, did not occur.

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