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EFFECT OF THE N-TERMINAL FRAGMENT OF SUBSTANCE P_{1-4} ON SOMATIC STRESS RESPONSE AND CATECHOLAMINE LEVELS IN RAT ADRENALS

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Investigations have shown [4-6] that the endogeneous neuropeptide substance P_{1-11} has an antistressor effect. Its normalizing, regulatory action has been observed on certain functional changes induced by chronic stress. According to Selye [8], a principal role in the development of the stress response of the body is played by catecholamines (CA) and, in particular, by CA of the adrenal medulla. Substance P also has been shown to be present in adrenal tissue, in the so-called S-ergic cells [3]. Substance P, released along with CA, can participate both in the regulation of certain physiological processes and in the maintenance of homeostasis during the development of stress [4, 6]. Oehme suggested that the main contribution to the antistressor effect of substance P belongs to the N-terminal end of the molecule of this neuropeptide [5].

This paper gives the results of a study of the somatic manifestations of the stress reaction in its dynamic course, and changes in the CA levels in the adrenals after preliminary injection of substance P_{1-4}.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 200 ± 20 g, immobilized in special frames which fixed the cervical and lumbar regions [2]. Preliminary experiments were carried out to study different stages of development of the general adaptation symdrome (GAS) under the specific conditions of this model of stress. The effect of substance P_{1-4} on the stage of alarm (immobilization for 1 h) and the stage of exhaustion (48 h) was investigated.

After decapitation of the animals the lymphoid organs (thymus and spleen) and the adrenals were weighed and the state of the gastric mucosa determined. The concentrations of adrenalin (A) and noradrenalin (NA) also were determined spectrofluorometrically by Euler and Lisjajko's method in the adrenals. CA levels in the adrenocytes and noradrenocytes of the adrenal medulla were studied by cytochemical electron microscopy, using the JEM-100B electron microscope ("Jeol") and the method of Tranzer and co-workers, in our own modification [1, 9], suitable for estimating the CA concentration in chromaffin granules.

The N-terminal fragment of substance P_{1-4} (Arg-Pro-Lys-Pro) was injected intraperitoneally once a day in a dose of 100 μg/kg; to study the stage of alarm it was injected 3 times preliminarily and once immediately before the experiment; when the stage of exhaustion was studied it was given twice preliminarily and twice during stress.

The results were subjected to statistical analysis by Student's t test.

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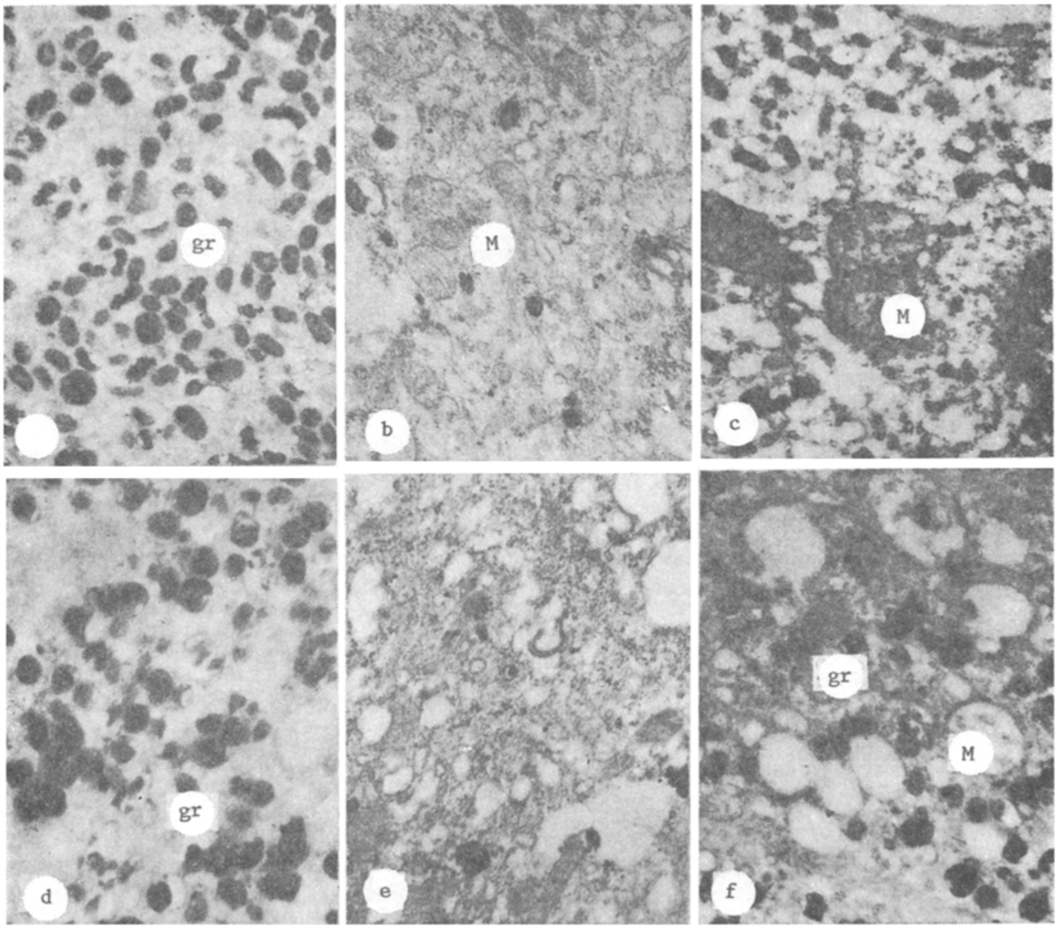

Fig. 1. Electron-microscopic cytochemical demonstration of catecholamines in adrenocytes and noradrenocytes of the adrenal medulla of rats with immobilization stress and after preliminary injection of substance P_{1-4}. a) Catecholamines in granule form in adrenocytes of intact rat; d) catecholamines in granule form in noradrenocytes of intact rat; b) adrenocytes and e) noradrenocytes after 48 h of immobilization stress; c) adrenocytes and f) noradrenocytes after 48 h of immobilization stress preceded by injection of substance P_{1-4}. M) Mitochondria, gr) granules containing catecholamines. Magnification ×18,000.

TABLE 1. Effect of Preliminary Injection of Substance P_{1-4} on Somatic Manifestations of Stress Response in Rats at Different Periods of Immobilization

Conditions	Weight of thymus	Weight of spleen	Weight of adrenal	State of gastric mucosa
Normal	137,6±15,5	199,8±25,8	4,7±0,4	No change
Stress for 1 h (control)	97,4±16,5*	122,7±18,4*	4,1±0,6	» »
Stress for 1 h + SP_{1-4}	105,9±21,7	158,0±27,6	5,3±0,7	» »
Stress for 48 h (control)	77,4±9,9*	98,5±20,6*	10,5±1,5*	Multiple hemorrhages, two to four ulcers (6.0 ± 3.0 mm²)
Stress for 48 h + SP_{1-4}	81,5±19,3*	142,2±33,1**	9,8±1,9*	Single hemorrhage; no ulcers, cyanosis

Legend. Weight of thymus, spleen, and adrenals given in mg/50 g body weight. Here and in Table 2: *) difference from normal significant at the $p \le 0.05$ level; **) difference from control significant at the $p \le 0.05$ level.

EXPERIMENTAL RESULTS

Data on the effect of the N-terminal fragment of substance P_{1-4} on somatic manifestations of the stress response are given in Table 1. In the case of stress lasting 1 h (the alarm stage of the GAS), following preliminary injection of the substance, the weight of

TABLE 2. CA Concentration in Adrenals of Rats after Preliminary Injection of Substance P_{1-4} in the Course of Immobilization Stress

	A, μg/g	NA, μg/g
Normal	837±100	643±90
Stress for 1 h	715±128	625±105
Stress for 1 h + SP_{1-4}	757±138	743±95
Stress for 48 h	95±22*	132±23*
Stress for 48 h + SP_{1-4}	176±31**	240±47**

the lymphoid organs showed a smaller decrease than in the control animals. Reduction of the weight of the thymus and spleen in animals not receiving the substance was statistically significant, whereas in the case of stress after injection of substance P_{1-4} these parameters had only a tendency to fall compared with intact rats. The weight of the adrenals in the alarm stage of the GAS was virtually indistinguishable from the control. Changes in the state of the gastric mucosa likewise did not differ from the control.

The normalizing effect of the N-terminal fragment of substance P_{1-4} was observed to a greater degree after 48 h of stress, i.e., at the stage of exhaustion of the GAS. It was expressed as prevention of the decrease in weight of the spleen and as a decrease in the intensity of ulcer formation in the gastric mucosa compared with control animals (Table 1).

The data in Table 2 show that the effect of substance P_{1-4} on the CA reserves in the adrenal tissue is manifested only during long-term stress. For instance, after immobilization for 48 h the concentration of both A and NA were almost twice as high as in the control.

The ultrastructural and cytochemical study of electron-dense granules in the adrenocytes and noradrenocytes of the adrenal also showed an increase in their number after injection of substance P_{1-4} but only at the exhaustion stage, as is clear from the electron micrographs (Fig. 1).

The facts described above are evidence that the N-terminal fragment of substance P_{1-4} has an antistressor action, as may be judged by normalization of the somatic manifestations induced by stress. These data are in agreement with results obtained by other workers, pointing to the antistressor effect of substance P_{1-4} [6, 7]. Oehme and co-workers suggest that the antistressor effect of this fragment of the neuropeptide may be linked with a direct effect on the adrenal medulla, which has receptors specific for substance P [6]. This part of the molecule, exhibiting its physiological action, evidently does so through its action not only on specific SP-receptors of the adrenal, but also through the CA-ergic component of the same gland.

The results described above show that the N-terminal fragment of substance P_{1-4} led to normalization of the CA concentration in the rat adrenals. This effect may perhaps be connected with suppression of CA release from the adrenals. We know from data in the literature that substance P_{1-4} inhibits release of NA, activated by nicotinic receptors located on the chromaffin cells of the adrenal [4]. This evidently helps to maintain the CA reserves in the adrenals, which, in turn, play an important role in the maintenance of homeostasis during development of the stress response.

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