EXCITATORY ACTION OF THE TETRAPEPTIDE TUFTSIN ON ACTIVITY OF ALBINO RATS

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Oligopeptides are widely occuring regulatory factors acting at the cell and tissue levels. Special attention has been paid in recent years to neuroactive oligopeptides. However, many peptides, hitherto regarded as peripheral factors, are now known to be found in considerable amounts in the brain (bradykinins, gastrins, cholecystokinins, etc.).

The study of the central effects of peripheral peptides is thus of great interest. These include the tetrapeptide tuftsin, known as a regulator of phagocytosis and of certain functions of lymphocytes [5, 6]. The more detailed study of its pharmacological properties is important also because, among the regulators of functions of relatively independent cells such as lymphocytes, it can be expected that the most universal and the phylogenetically oldest regulators will be discovered.

EXPERIMENTAL METHOD

Tuftsin (thr-lys-pro-arg) was injected intraperitoneally, in aqueous solution, into male rats weighing 170-220 g (the peptide was synthesized in the Laboratory of Biopolymers, Institute of Heteroorganic Compounds, Academy of Sciences of the USSR, by the method of hydrophilic poly-N-hydroxysuccinimide reagents [3].

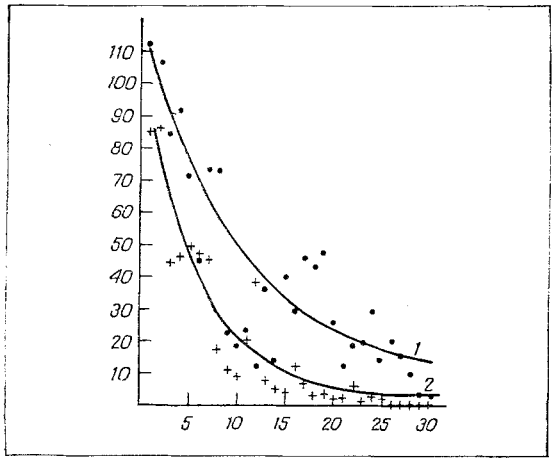

Fig. 1. Changes in motor activity of rats in "Animex" under the influence of tuftsin in a dose of 300 μg/kg (1) and in control animals (2). Abscissa, time of observation (in min); ordinate, number of movements per minute (in conventional units).

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TABLE 1. Changes in Indices Recorded in "Open Field" under the Influence of Tuftsin in a Dose of 300 μg/kg, Injected 5 Min Before Testing

Experimental conditions	Duration of run, sec				"Standings" (2 min)	"Washings"	Defecations (2 min)
	0—30	31—60	61—90	91—120			
"Nonstressor" modification	126	133	159*	202*	117	100	33
"Stressor" modification	130*	100	135	130	113	240*	57

Legend. Data given as percentages of corresponding controls. Length of run recorded in successive 30-sec intervals. Asterisk) differences significant at the $P < 0.05$ level.

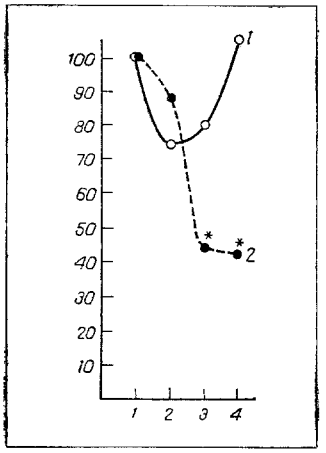

Fig. 2. Changes in latent period of response of control (1) animals and animals receiving tuftsin (2) during training. Abscissa, days of training; ordinate, latent period (in percent of that on first day of training). Asterisk) $P < 0.05$.

The animals' motor activity was recorded for 30 min by means of the "Animex" apparatus (from LKB, Sweden), in which an inductive-capacitive circuit was used as the detector; data were obtained on 43 animals.

The "open field" method in two modifications was used for observation on the orienting behavior and for assessing the rats' response to stress-producing factors: In the first or "nonstressor" method the animals were observed under conditions of weak illumination, in silence; in the second or "stressor" modification they were observed under conditions of bright illumination plus acoustic stimulation (the ringing of a bell). The length of the run, the number of times the animal stood up on its hind limbs ("standings"), the number of times it touched its mouth with its forelimb ("washings") and the number of defecations were recorded for a period of 2 min. In these experiments 89 animals were used.

Total oxygen consumption (TOC) was measured by the method in [2] for 1 h after injection of the peptides or water. Altogether 52 rats were used.

The electrocardiogram (EKG) was recorded in the freely moving animals by means of two nichrome electrodes implanted beneath the skin (one in the region of the cervical, the other in the region of the last thoracic vertebrae). Data were obtained on 36 animals.

Training of the animals in a food-getting reflex took place in a T-maze. The rats were placed in the maze five times in succession, for 3 min daily for 4 days; during these exposures the number of absent responses, the latent period, reaction time, and the number of mistakes were recorded. Tuftsin was injected daily 5 min before the training session. In this series 70 animals were used.

All the data were averaged and subjected to statistical analysis. The significance of differences between the mean was assessed by the U-criterion [1].

EXPERIMENTAL RESULTS

Injection of tuftsin in a dose of 50 μg/kg did not cause any changes in the parameters studied. Increasing the dose to 150 μg/kg led to brief excitation of the animals, as could be judged by the increased intensity of their motor activity, measured by means of the "Animex" instrument. The experimental animals moved about 5-12 min after injection of the tetrapeptide significantly more ($P < 0.05$) than the control. The cardiac frequency in the animals receiving this dose of the peptide also was a little higher (by 4-10%) than in the control for 1 h. In the other tests used, tuftsin in a dose of 150 μg/kg was ineffective. A further increase in the dose to 300 μg/kg led to an even more marked increase in the animals' motor activity in the "Animex" for 30 min after injection of the peptide (Fig. 1). In addition, rats receiving tuftsin in the above dose 5 min before exposure in the "open field" exhibited greater investigative activity in the "nonstressor" modification of the experiment than the control animals, as shown by an increase in the length of the run (Table 1).

In the "stressor" modification of the experiment this dose of the peptide also increased the length of the run compared with the control, and an indicator such as "washing," reflecting the animal's emotional state, also was significantly increased. Meanwhile tuftsin slightly reduced the number of defecations and increased the number of "standings," although not significantly (Table 1). The cardiac frequency in rats receiving peptides in this dose was a little higher than in the control animals, but the differences likewise were not significant. TOC in this case did not differ in the experimental and control animals. Injection of tuftsin in a dose of 300 μg/kg led to some changes in the rats' behavior in the maze: The number of missed responses was the same as in the control but the latent periods on the 3rd and 4th days of training were reduced (Fig. 2). The reaction time of the experimental animals also was significantly shorter on the 4th day.

The results show that tuftsin evoked a marked behavioral effect, manifested as an increase in the animals' motor activity. Under these circumstances there was also an increase in the indices reflecting the animal's emotional state (the number of "washings," the cardiac frequency). All this is evidence of excitation of the animals receiving the peptide. The doses inducing these effects were comparable with those of several other neuropharmacological agents, namely 150-300 μg/kg body weight. It is an interesting fact that no changes were observed under these circumstances in the energy metabolism of the body as a whole: Tuftsin had no effect on the level of TOC.

On presentation of frightening stimuli in an "open field" rodents respond by hiding, with a reduction in motor activity [4]. In that case the increase in the length of the run observed under the influence of tuftsin under stressor conditions may be the result of a decrease in the response associated with fear, a conclusion also supported by the evidence of a reduction in the number of defecations observed with rats in this situation. However, the increase in motor activity of the animals under the influence of this peptide and in the absence of stressogenic factors (in an "open field," in the "Animex" chamber), and also the decrease in the latent period and reaction times without any change in the number of animals trained in the maze points more to an excitatory action of the peptide, whereas abolition of the response connected with fear may perhaps be a result of this excitation. The data described above cannot, of course, provide a sufficiently solid basis for application of the effects of tuftsin thus revealed, still less for it to be regarded as belonging to the neuropeptide category. It will, however, be evident that tuftsin, like other regulatory peptides hitherto regarded as purely peripheral, deserve close study from neurophysiological and neuropharmacological aspects. It would also be very interesting to attempt to discover the biological significance of the width of the spectrum of observed effects of tuftsin, including its action on phagocytosis and on antibody production and also, as follows from the data presented above, on the CNS.

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EFFECT OF NONACHLAZINE AND OXYFEDRINE ON CENTRAL REGULATION OF VASOMOTOR TONE

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KEY WORDS: vasomotor reflexes; sympathetic activity; nonachlazine; oxyfedrine; reserpine; propranolol.

The peripheral effects of the new Soviet antianginal drug nonachlazine are largely determined by its effect on adrenergic structures of the myocardium and blood vessels [4, 5, 9]. Nonachlazine increases the noradrenalin concentration in myocardial tissue and blocks the reverse transport mechanism in adrenergic neurons [2, 4]. Its effects are not manifested after preliminary injection of α- and β-blockers [5, 9]. It is well known that drugs affecting peripheral adrenergic processes of regulation of the circulation can induce changes also through central processes of regulation of sympathetic vascular tone [8]. There have been reports that a large dose of nonachlazine has a central inhibitory action [1, 3].

The object of this investigation was to compare the central effects of two modern antianginal drugs [7, 12] with a β-stimulating component of their action on adrenergic structures of the myocardium, namely nonachlazine and oxyfedrine (Ildamen). The central effects of the drugs were judged by their effect on tonic and reflex activity in the sympathetic nerves of the kidney and also on vasomotor reflexes evoked by stimulation of different groups of afferent fibers of a somatic nerve in anesthetized animals with an intact brain.

EXPERIMENTAL METHOD

Experiments were carried out on cats weighing 2.5-4 kg anesthetized with urethane and chloralose (300 and 40 mg/kg respectively, intravenously). The animals were artificially ventilated and immobilized with myorelaxin (succinylcholine, 0.1 mg/kg/min, intravenously). The body temperature (rectal) was maintained at 36-37° by artificial external heating. The systemic arterial pressure (BP) in the femoral artery was measured by means of an electromanometer. Electrical activity in the renal nerve was recorded by buried platinum electrodes with an interelectrode distance of 3-4 mm. Reflex responses in the renal nerve and vasomotor reflexes were evoked by electrical stimulation of n. tibialis (NT) by stimuli of different parameters. In other series of experiments, reflex responses detected by a monopolar technique were averaged by means of the ATAK-350 analyzer (Japan).

Nonachlazine was injected intravenously in doses of 1 and 6 mg/kg and intra-arterially (a. carotis com.) in a dose of 1 mg/kg. Oxyfedrine was injected in a dose of 0.3 and 1 mg/kg intravenously or in a dose of 0.3 mg/kg intra-arterially. To study the role of adrenergic mechanisms in the central effects of nonachlazine and oxyfedrine the β-adrenoblocker propranolol was used in a dose of 3 mg/kg (intravenously) and reserpine (Rausedil – 0.25%) in a dose of 1 mg/kg.

The results were subjected to statistical analysis.

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