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Effects of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on Passive and Active Avoidance Behavior and on Open-Field Activity of Rats

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H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH Avoidance behavior Open-field activity

THE recent identification of the structures of hypothalamic hypophysiotropic hormones [28, 29, 30] and the discovery that they are present in most parts of the brain lacking a hypophysis [3,9] has opened new avenues of investigation of brain function.

The first systemic [15, 16, 17, 18, 19] and cerebral ventricular [12,21] administration of hypothalamic peptides revealed a surprising variety of behavioral, electrophysiological and biochemical changes.

These results suggest that these hormones might play a role in the normal function of the central nervous system independent of their effects on the control of the adenohypophysis [24, 25, 26]. Chang *et al.* [4] purified a fraction from hypothalamic extracts of porcine origin which significantly stimulated the release of ACTH in vitro. Since the synthetic heptapeptide showed ACTH-releasing activity only at high doses in vitro, it is unlikely to be the physiological corticotropin releasing factor [4].

As the structure of this peptide is entirely different from those of other physiologically active peptides [4], it seemed to be worth while to investigate its action on avoidance behavior and open-field activity in several tests with different modes of treatment.

METHOD

Peptide

The heptapeptide was synthetized by a solid-phase method [4].

Subjects

Experiments were performed on male CFY rats weighing 180–220 g. They were kept under a standard 12 hr illumination schedule (light at 0600 a.m.). Food and water were available ad lib. All experiments were started at 0800 a.m.

Passive Avoidance Behavior

One-trial passive avoidance behavior was measured [1]. Briefly, the apparatus consisted of an illuminated platform, attached to a larger compartment. Rats were placed on the platform and allowed to enter the dark compartment. Since rats prefer dark to light, they normally entered within 15 sec. Two additional trials were given on the following day. After the second trial, unavoidable electric foot-shocks (0.5 mA, 3 sec) were delivered through the grid floor of the dark compartment. After this single learning trial, the rats were im-

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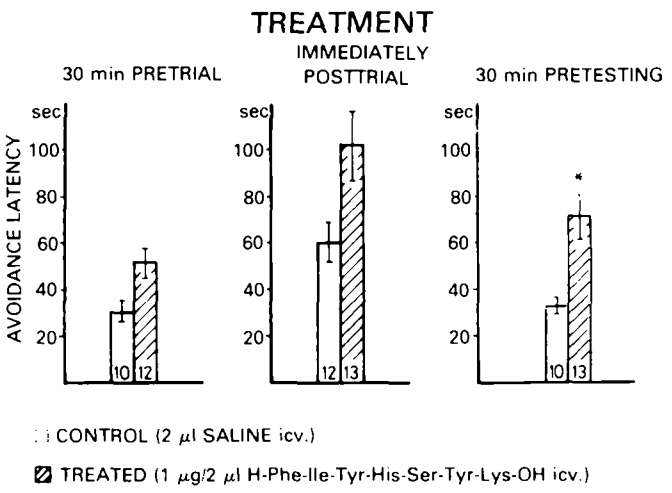

FIG. 1. Effect of intracerebroventricular administration of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on passive avoidance behavior. Number in bars represents the number of animals used. Point represents significant difference.

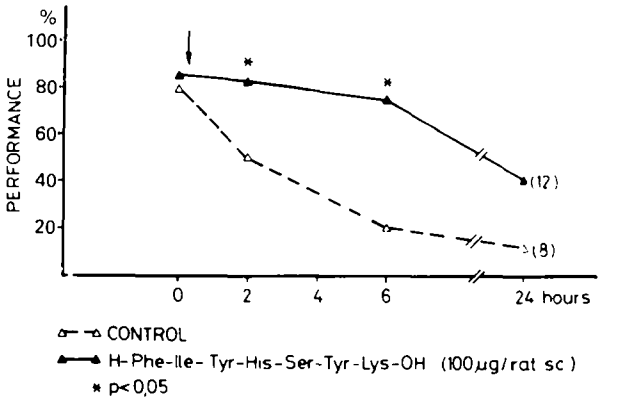

FIG. 2. Effect of subcutaneous administration of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on extinction of active avoidance behavior. Parentheses indicate number of animals used. Points represent significant difference. Arrow indicates time of administration of the peptide.

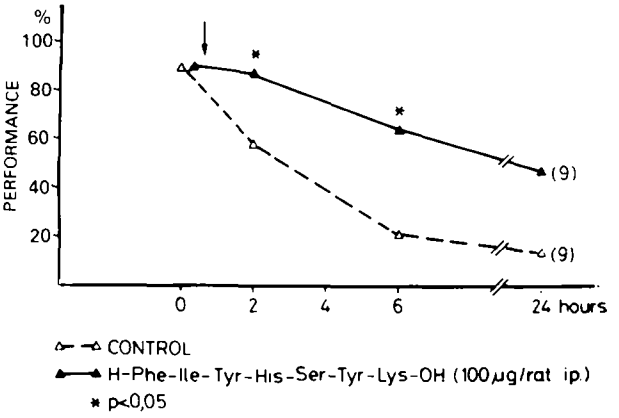

FIG. 3. Effect of intraperitoneal administration of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on extinction of active avoidance behavior. Parentheses indicate number of animals used. Points represent significant difference. Arrow indicates time of administration of the peptide.

mediately removed from the apparatus. Passive avoidance behavior was tested 24 hr after the learning trial: the rats were placed on the platform and the latency to enter the dark compartment was measured up to a maximum of 5 min.

In passive avoidance behavior 1 μg H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH, or physiological saline for controls, in a volume of 2 μl was administered intracerebroventricularly 30 min pretrial, immediately posttrial or 30 min before the testing.

Active Avoidance Behavior

Active avoidance conditioning was performed in a platform jumping conditioning apparatus [32,33]. The conditional stimulus (CS) was the light of a 40 W electric bulb, while the unconditional stimulus (US) was an electric shock of 0.2 mA delivered through the grid floor of the apparatus to the paws of the rat. Each day for three consecutive days, 10 trials were performed, with an intertrial interval of 60 sec. On the fourth day, extinction trials were run and the US was no longer applied. The CS was presented for a maximum of 10 sec, or it was terminated as soon as the animal made the response. Animals which made at least 8 conditional avoidance responses out of 10 trials in the first extinction session were used for further experiments. These animals were allocated to different groups (control and treated), and immediately after the first extinction session were treated by subcutaneous, intraperitoneal (100 μg/0.5 ml) or intracerebroventricular (1 μg/2 μl) administration. In one experimental session the intracerebroventricular administration was repeated 30 min before the last test (24 hr). Second and third extinction sessions were performed 2 hr and 6 hr after treatment on day 4. A fourth extinction session was run on day 5, 24 hr after the single injection of the peptide or saline.

Exploratory Activity

The animals were placed in an open-field box, consisting of 36 squares measuring 10×10 cm each. Activity was characterized by the total number of squares explored, the total numbers of rearings and groomings and the defecation boluses produced during a 3 min session immediately, 30 min and 24 hr after intracerebroventricular treatment (1 μg/2 μl) and 30 min and 3 hr after subcutaneous (100 μg/0.5 ml) treatment.

Surgical Method

The animals were anesthetized with pentobarbital-Na (Nembutal, 35 mg/kg IP) and a cannula was placed into the lateral cerebroventricle and fixed to the skull with dental cement. The rats were used after a recovery period of 7 days. The correct positioning of the cannula was checked by dissection of the brain.

Statistical Analysis

Student's *t*-test (two-tailed) was used in passive

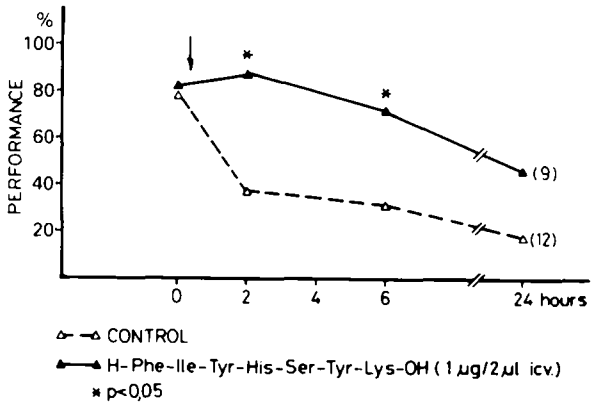

FIG. 4. Effect of intracerebroventricular administration of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on extinction of active avoidance behavior. Parentheses indicate number of animals used. Points represent significant difference. Arrow indicates the time of administration of the peptide.

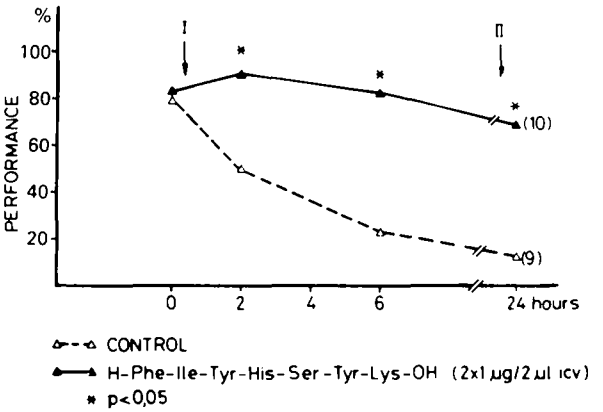

FIG. 5. Effect of repeated intracerebroventricular administration of H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH on extinction of active avoidance behavior. Parentheses indicate number of animals used. Points represent significant difference. Arrow indicates time of administration of the peptide.

TABLE 1

EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF H-PHE-ILE-TYR-HIS-SER-TYR-LYS-OH ON OPEN-FIELD ACTIVITY OF RATS

Time after Treatment	Total Number of Squares		Total Number of Rearings		Total Number of Groomings		Defecation Boluses	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
30–33 min	69.8 ± 6.2* (20)	79.2 ± 9.1 (20)	14.5 ± 1.7 (20)	10.7 ± 1.3 (20)	8.2 ± 1.8 (20)	13.3 ± 2.3 (20)	3.6 ± 0.6 (20)	2.6 ± 0.7 (20)
180–183 min	46.4 ± 7.0 (19)	43.6 ± 6.5 (19)	6.9 ± 1.7 (19)	5.9 ± 0.8 (19)	6.1 ± 2.3 (19)	15.0 ± 3.7† (19)	2.1 ± 0.6 (19)	1.3 ± 0.6 (19)

Rats received saline or peptide (100.0 µg dissolved in 500 µl saline SC) 30 min or 3 hr before the open-field test (for details see text).
*Mean ± SEM (standard error of the mean).
†$p<0.05$ versus control (Student's t-test).
Parentheses indicate number of animals used.

avoidance behavior and in open-field activity, with the analysis of variance in the active avoidance response [31].

RESULTS

In passive avoidance behavior the peptide showed a tendency to increase the avoidance latency after pretrial and immediately posttrial administration, while when the administration was performed 30 min pretesting the avoidance latency was significantly increased, $t(21)=2.25$, $p<0.05$ (Fig. 1).

Following subcutaneous (100 µg/0.5 ml) (2 hr: $F(3,49)=3.12$, $p<0.05$; 6 hr: $F(3,49)=3.20$, $p<0.05$) (Fig. 2), intraperitoneal (100 µg/0.5 ml) (2 hr: $F(3,56)=2.92$, $p<0.05$; 6 hr: $F(3,56)=2.85$, $p<0.05$) (Fig. 3) and intracerebroventricular (1 µg/2 µl) (2 hr: $F(3,56)=3.05$, $p<0.05$; 6 hr: $F(3,56)=3.12$, $p<0.05$) (Fig. 4) administration the extinction of the avoidance reflex was significantly inhibited. When the material was administered intracerebroventricularly (1 µg/2 µl), not only immediately after the first extinction sessions (2 hr: $F(3,56)=3.05$, $p<0.05$; 6 hr: $F(3,56)=3.12$, $p<0.05$) but also 30 min before the last session, the performance (24 hr) was significantly greater, $F(3,56)=3.12$, $p<0.05$, than that of the control animals (Fig. 5).

H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH increased the grooming activity, $t(36)=2.11$, $p<0.05$, 3 hr after subcutaneous administration (100 µg/0.5 ml) (Table 1). A similar increase, $t(26)=2.21$, $p<0.05$, in grooming activity could be observed 30 min after intracerebroventricular administration (1 µg/2 µl). The ambulation and rearing activity were not changed following subcutaneous or intracerebroventricular administration (Table 2).

DISCUSSION

Although corticotropin releasing factor (CRF) was the first hypothalamic hormone to be demonstrated [22, 23, 27], attempts to isolate it in the pure state were hampered by the loss of activity during purification and by other reasons. The CRF activity of our preparation also decreased significantly during the final stages of purification. H-Phe-Ile-

TABLE 2

EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF H-PHE-ILE-TYR-HIS-SER-TYR-LYS-OH ON OPEN-FIELD ACTIVITY OF RATS

Time after Treatment	Total Number of Squares		Total Number of Rearings		Total Number of Groomings		Defecation Boluses	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
0–3 min	62.6 ± 9.4* (18)	49.8 ± 6.4 (10)	15.4 ± 3.0 (18)	14.6 ± 3.3 (10)	12.9 ± 2.6 (18)	17.4 ± 2.3 (10)	3.9 ± 0.4 (18)	2.4 ± 0.4† (10)
30–33 min	43.1 ± 9.1 (18)	50.7 ± 7.8 (10)	18.1 ± 2.6 (18)	14.2 ± 2.3 (10)	9.3 ± 2.8 (18)	17.3 ± 1.1† (10)	2.8 ± 0.6 (18)	1.4 ± 0.4 (10)
24 hr	62.9 ± 10.0 (18)	46.5 ± 3.8 (10)	12.9 ± 2.6 (18)	12.2 ± 2.5 (10)	16.6 ± 3.7 (18)	14.4 ± 2.1 (10)	4.3 ± 0.7 (18)	4.0 ± 0.3 (10)

Rats received saline or peptide (1.0 μg dissolved in 2 μl saline ICV) 30 min or 24 hr before the open-field test (for details see text).
*Mean ± SEM (standard error of the mean).
†$p<0.01$ versus control (Student's t-test).
Parentheses indicate number of animals used.

Tyr-His-Ser-Tyr-Lys-OH is unlikely to be the physiological corticotropin releasing factor as it displays ACTH releasing activity only at high doses [4]. Neither the synthetic nor the natural peptide stimulated the release of TSH, GH, PRL, LH and FSH in vitro from rat pituitary quarters [4].

A possible explanation for the decrease in CRF activity of the hypothalamic extract during the purification procedure is the removal of some unknown factor which facilitates the CRF activity. However, in vivo application allows the peptide H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH to recombine with this unknown factor, thereby exerting considerable CRF activity and causing ACTH release. The investigations indicating that there are some similarities between the action of this heptapeptide and ACTH in behavior tests, strengthen this possibility. Furthermore, Chang *et al.* [4] reported that this peptide stimulated ACTH release not only in vitro, however, in vivo as well.

ACTH affects acquisition of passive avoidance behavior in rats [7,10]. In a single step-through, one-trial passive avoidance procedure [1]. $ACTH_{1-24}$ administered 1 hr prior to the 24 hr retention trial markedly facilitated passive avoidance behavior. Other authors reported that the effects of ACTH depended on the dose used [11]. Low doses induced latencies higher than that of saline-treated rats, while high doses had the reverse effect. Our material increased the latency in all three sessions, but this was significant only when the administration was carried out 30 min pretesting. The extinction of active avoidance behavior can be delayed by ACTH [6, 13, 14]. When applied by different routes, our material inhibited the extinction of active avoidance behavior, but this action ceased 24 hr later. When it was repeatedly administered intracerebroventricularly, not only after the first extinction session, but also 30 min before the last session, the extinction was delayed.

The avoidance behavior data obtained with H-Phe-Ile-Tyr-His- Ser-Tyr-Lys-OH indicate that the action is probably exerted mainly on the retrieval processes, however this phenomenon has to be investigated in a dose-related manner also. In passive avoidance the action was most marked when the material was given pretesting, and in the active avoidance situation when the treatment was repeated before the 24 hr test (see Fig. 5), in contrast to the data obtained when the animals were treated only once and tested 24 hr later (see Fig. 4). This effect is very similar to that of ACTH [7].

In open-field behavior intramuscular injection of $ACTH_{1-24}$ did not produce any difference in the rearing and grooming of mice [2]. A similar lack of influence of two $ACTH_{1-10}$'s on motor activity has been described in the rat [34]. Though peripheral administration of our material does not affect the ambulation and rearing activity, it does increase the grooming activity, indicating an endogenous action of the liberated ACTH, probably directly on the brain. It is well known from earlier data that, after intracerebroventricular administration. ACTH can induce excessive grooming [5, 8, 20].

H-Phe-Ile-Tyr-His-Ser-Tyr-Lys-OH exhibits very similar action to that of ACTH in the different tests we used, indicating that one possibility is that the effects described are due to ACTH release. However, it cannot be excluded that the peptide itself could also have a direct action on the brain. This question can be answered only in repeated experiments on hypophysectomized animals.

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