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The Interaction Between β-[TYR^9]Melanotropin-(9–18), Haloperidol and Amphetamine in Different Behavior Tests of Rats

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TELEGDY, G., L. VÉCSEI, A. V. SCHALLY AND D. H. COY. *The interaction between β-[Tyr^9]melanotropin-(9–18), haloperidol and amphetamine in different behavior tests of rats.* PHARMAC. BIOCHEM. BEHAV. 17(1) 15–18, 1982.—The effects of β-[Tyr^9]melanotropin-(9–18) on the extinction of the active avoidance reflex in (dopamine receptor blocker) haloperidol-treated animals, and on the open-field activity in haloperidol and amphetamine-treated rats were studied. It was shown that a systemically given 100 μg dose of the peptide, which had no action on the ambulation and rearing activity in the open-field test, could still delay the extinction of the active avoidance reflex. Haloperidol treatment was able to partially block the effects of the ICV administered β-[Tyr^9]melanotropin-(9–18) on both the extinction and open-field activity. After intracerebroventricular administration, the effect of the peptide on the open-field test was partially similar to that of amphetamine: it facilitated the ambulation and rearing activity, and (in contrast with amphetamine) was able to facilitate the grooming activity, even in the presence of amphetamine. The results suggest that dopaminergic innervation might play a mediating role in the effect of β-[Tyr^9]melanotropin-(9–18) on the extinction of the active avoidance reflex and open-field activity. The effect on the open-field activity differens in part from that of amphetamine.

β-[Tyr^9]melanotropin-(9–18) Haloperidol Amphetamine Avoidance behavior Open-field activity

β-[Tyr^9]melanotropin-(9–18) (H-Tyr-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH) is a naturally-occurring peptide which was isolated by Schally and coworkers from pig hypothalami [11]. This peptide has an MSH-like activity in the frog skin test, but it was ineffective in pituitary quarters on the release of ACTH, GH, FSH and TSH [11].

It was earlier demonstrated that after intracerebroventricular administration this peptide inhibited the extinction of the active avoidance behavior, increased the avoidance latency in the passive avoidance test and increased the ambulation and rearing activity of rats in an open-field test 30 min after treatment [16]. It has also been shown that intracerebroventricular administration of β-[Tyr^9]melanotropin-(9–18) in the same dose as in the behavioral experiment affected the dopaminergic, noradrenergic and serotoninergic activities in different brain areas [4]. On the basis of these findings, the following problems were studied in the present investigation:

(1) What is the role of the increased open-field activity in the delayed action of the peptide on the extinction of the active avoidance behavior?

(2) What is the role of the dopaminergic system in the action of the peptide in the extinction of the active avoidance and open-field behavior?

(3) How would the amphetamine-like action of the peptide on the open-field behavior change when it is given in combination with amphetamine?

METHOD

Peptide

The β-[Tyr^9]melanotropin-(9–18) was synthetized by a solid-phase method [11].

Animals

Adult male albino rats of an inbred CFY strain, weighing 180±35 g, were used. The animals were kept on a standard illumination schedule, with the light period beginning at 6 a.m. Food and water were available to the animals ad lib. Behavioral observations were made daily at 8 a.m. and 2 p.m.

Active Avoidance Behavior

Avoidance behavior was investigated in a platform jumping apparatus described in detail earlier [13,15]. The rats were trained to avoid electric footshock by jumping onto a

TABLE 1

EFFECTS OF SUBCUTANEOUS ADMINISTRATION OF Tyr-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-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
30 min	94.6 ± 7.3* (10)	81.5 ± 6.5 (10)	25.4 ± 1.8 (10)	27.4 ± 2.6 (10)	23.0 ± 2.4 (10)	30.6 ± 5.2 (10)	4.7 ± 0.4 (10)	4.0 ± 0.6 (10)
180–183 min	39.6 ± 4.3 (10)	33.5 ± 7.1 (10)	10.5 ± 1.7 (10)	9.8 ± 2.6 (10)	28.1 ± 4.9 (10)	35.4 ± 4.9 (10)	3.4 ± 0.8 (10)	2.3 ± 0.5 (10)
24 hr	76.1 ± 11.8 (10)	60.6 ± 7.7 (10)	30.5 ± 4.6 (10)	21.6 ± 3.5 (10)	24.4 ± 2.6 (10)	32.2 ± 2.9† (10)	2.4 ± 0.4 (10)	3.2 ± 0.6 (10)

Rats received saline or peptide (100 μg dissolved in 500 μl saline SC) 30 min, 3 hr and 24 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).
( )Number of animals used.

platform during the 10 sec of a conditional stimulus, the light of a 45 W bulb. The lack of performance during this time was associated with an electric footshock of 0.2 mA. Ten trials were given daily, in a fixed intertrial interval of 55 sec (range 50–60 sec). On the fourth day extinction trials were run and unconditional stimulus was no longer applied. Animals which made at least 8 conditioned avoidance responses out of 10 trials in the first extinction session were used for further experiments.

These animals were allocated to different treatment groups and treated immediately after the session.

In the first experiment 100 μg/500 μl β-[Tyr^9]-melanotropin-(9–18), or saline for controls, was administered subcutaneously in the neck of the animal immediately after the session. In the second experiment haloperidol (Gedeon Richter, Budapest) in physiological saline (20 μg/kg IP), or saline as a control, was injected (Fig. 2, I) immediately after the extinction session. This was followed 30 min later by the intracerebroventricular administration of β-[Tyr^9]melanotropin-(9–18) (1 μg/2 μl) or saline (2 μl) (Fig. 2, II).

Exploratory Activity

The animals were placed in an open-field box, which consisted of 36 squares measuring 10×10 cm each. The activity was characterized by the total number of squares explored, and the total numbers of rearings and groomings during 3 min session. In the first experiment 100 μg β-[Tyr^9]melanotropin-(9–18), or physiological saline for controls, was administered subcutaneously in a volume of 500 μl. Open-field activity was measured 30 min, 3 hr and 24 hr after treatment with the peptide. In the second and third experiments 1 μg peptide in a volume of 2 μl was injected intracerebroventricularly and the animals were tested 30 min following injection. d,l-Amphetamine phosphate (CHINOIN, Budapest) was injected intraperitoneally in a dose of 0.5 mg/kg 15 min before administration of the peptide. Haloperidol was administered intraperitoneally in a dose of 20 μg/kg 30 min before injection of the peptide.

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 and screws. The rats were used after a recovery period of 7 days. The correct positioning of the cannula was checked by dissection of the brain.

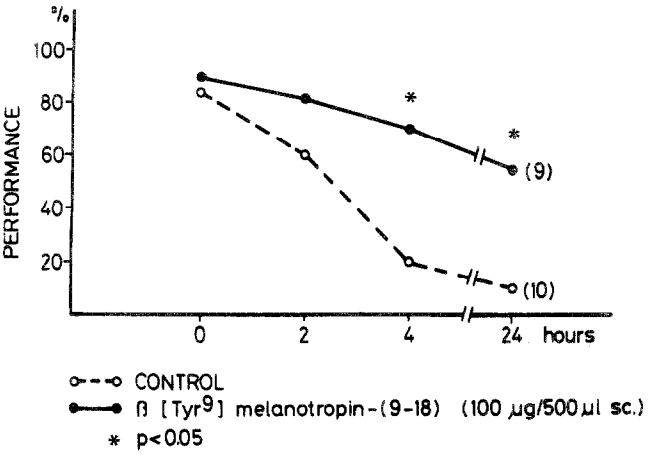

FIG. 1. Effect of β-[Tyr^9]melanotropin-(9–18) on extinction of active avoidance behavior. ( ) Number of animals used. Asterisks represent significant difference.

Statistical Analysis

In the active avoidance response the U-test of Mann-Whitney was used, with the analysis of variance (intracerebroventricular administration) and Student's t-test (two-tailed) (subcutaneous administration) in the open-field behavior. A probability level of 0.05 was accepted as a significant difference.

RESULTS

In the active avoidance behavior the extinction was de-

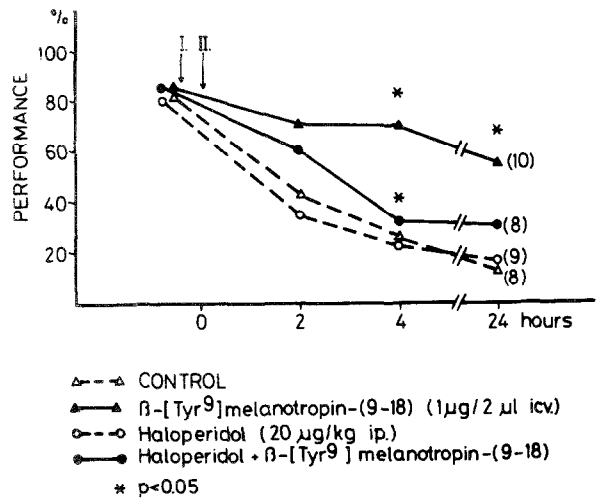

FIG. 2. Effect of β-[Tyr⁹]melanotropin-(9–18) and haloperidol on the extinction of active avoidance behavior. ( ) Number of animals used. Asterisks represent significant difference.

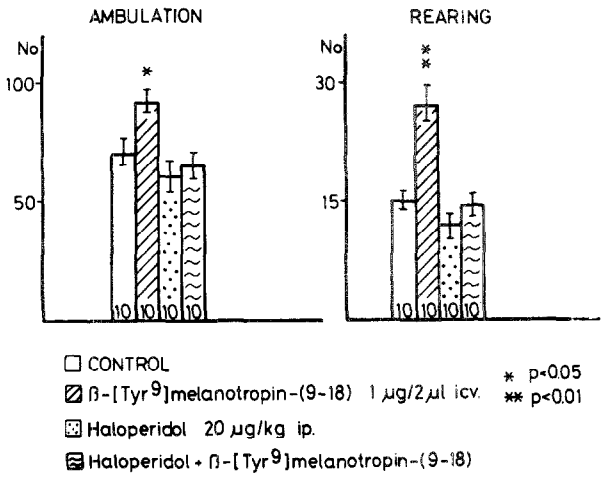

FIG. 3. Effect of β-[Tyr⁹]melanotropin-(9–18) and haloperidol on ambulation and rearing activity. Number in bars represents the number of animals used. Asterisks represent significant difference.

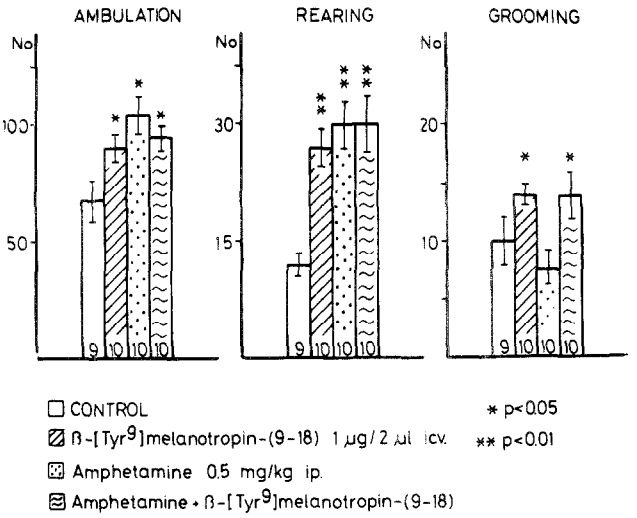

FIG. 4. The action of β-[Tyr⁹]melanotropin-(9–18) and amphetamine on open-field activity. Number in bars represents the number of animals used. Asterisks represent significant difference.

layed ($p<0.05$) when the material was injected subcutaneously and testing was performed 4 and 24 hr later (Fig. 1).

In the open-field behavior when the material was given subcutaneously 30 min, 3 hr and 24 hr before testing, no effect on the ambulation and rearing activity was observed. The grooming activity showed a tendency to increase both 30 min and 3 hr after the administration of the peptide. Twenty-four hr later it was significantly greater than that of the saline-treated group ($p<0.05$) (Table 1). β-[Tyr⁹]melanotropin-(9–18) inhibited the extinction of the active avoidance behavior 4 and 24 hr after intracerebroventricular administration ($p<0.05$). In each of the sessions haloperidol showed a tendency to decrease the peptide action. This effect was significant four hours after the administration of the drug ($p<0.05$) (Fig. 2).

In open-field behavior β-[Tyr⁹]melanotropin-(9–18) increased significantly the ambulation, $F(3,34)=3.02$, $p<0.05$ and rearing activity, $F(3,34)=4.52$, $p<0.01$ after intracerebroventricular administration. Haloperidol (20 µg/kg) alone did not change the behavior of the animals, but decreased the peptide action (Fig. 3). β-[Tyr⁹]melanotropin-(9–18) after intracerebroventricular administration increased the ambulation, $F(3,36)=3.13$, $p<0.05$ and rearing activity, $F(3,36)=4.56$, $p<0.01$. Similar action could be produced by amphetamine after intraperitoneal administration, $F(3,36)=3.02$, $p<0.05$; $F(3,36)=4.52$, $p<0.01$. The grooming activities of the rats treated with the peptide alone, $F(3,36)=3.15$, $p<0.05$ and with the combination of the peptide and amphetamine, $F(3,36)=3.42$, $p<0.05$ were higher than that of the amphetamine-treated group (Fig. 4).

DISCUSSION

In our previous experiments it was demonstrated that the intracerebroventricularly administered β-[Tyr⁹]melanotropin-(9–18) was able to delay the extinction of the active avoidance reflex. However, since this peptide also facilitated the open-field activity, the experiment could not solve the problem of whether the delayed extinction was a result of facilitated arousal or not [16]. In the present experiments a dose of 100 µg/SC, which had no action on the ambulation and rearing activity, normally responsible for the increased arousal, could still delay the extinction, indicating that the action was not brought about by increased motor activity of the animals. One possible explanation for the difference between the results of ICV and SC administration could be that the primary action of the peptide is exerted on avoidance behavior and the secondary one is on locomotor activity. The dose used (100 µg SC) in this experiment was

not enough to elicit the secondary action on the open-field activity.

Regarding the mechanism of action of the peptide, previous experiments indicated that the activity of the dopaminergic system was increased in the septum and impeded in the striatum, while the norepinephrine disappearance was lowered in the striatum, and the serotoninergic system was inhibited in the hypothalamus and facilitated in the septum [4]. β-[Tyr⁹]melanotropin-(9–18) in many respects has action on behavior similar to those of MSH and ACTH [1, 2, 3, 6, 8, 9, 12], which might be a result of the common tetrapeptide sequence (Phe-Arg-Trp-Gly) present in MSH and ACTH. Furthermore, the fact that MSH shows a DOPA potentiating effect [7], while ACTH influences mainly the dopaminergic system [14], suggests the possibility of a dopamine-mediated effect of β-[Tyr⁹]melanotropin-(9–18) on behavior. Indeed, haloperidol, a dopamine receptor blocking drug [10], which in a 20 μg/kg dose per se could not influence the extinction of the active avoidance reflex, partly blocked the effect of β-[Tyr⁹]melanotropin-(9–18). This suggests that the action of β-[Tyr⁹]melanotropin-(9–18) on the extinction of the active avoidance reflex was mediated at least in part by dopaminergic pathways. A similar effect was seen on open-field activity, too. Given intracerebroventricularly in a 1 μg dose, the peptide facilitated ambulation and rearing, which could be blocked by haloperidol. Haloperidol treatment alone in the same dose had no effect.

The second part of our experiments indicated that the dopaminergic system was involved in both types of behavior, though the first part of the experiments suggested that these two types of behavior are not interrelated.

It was shown earlier that amphetamine could increase both ambulation and rearing [5]; this action is very similar to that of β-[Tyr⁹]melanotropin-(9–18) [16]. Combination of amphetamine with β-[Tyr⁹]melanotropin-(9–18) indicated an important difference: amphetamine alone could not increase the grooming activity, whereas β-[Tyr⁹]melanotropin-(9–18) and the peptide in combination with amphetamine increased it. This suggests that the mechanism which facilitates the grooming activity is different from the action of amphetamine. Since the grooming activity can also be influenced by β-MSH [3], it is possible that the common sequence present in these peptides is responsible for this action.

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