PHARMACOLOGY OF THYROTROPIN RELEASING HORMONE

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The tripeptide Glu-His-Pro-NH_2, a hypothalamic neurohormone (thyrotropin releasing hormone), sometimes called thyroliberin, according to observations made by many workers has a central stimulating antidepressant, analeptic action [8, 9]. This paper gives the results of a combined pharmacological study of thyrotropin releasing hormone (TRH). The effect of TRH was studied in spontaneous motor activity of mice and the avoidance reflex in rats, and interaction with opiates and cholinergic and adrenergic receptors. The hemodynamic effects of TRH were assessed on the basis of their effect on the blood pressure (BP) and cerebral blood flow of intact animals and in hemorrhagic shock.

EXPERIMENTAL METHOD

Changes in the motor activity of mice under the influence of TRH and a combination of TRH with amphetamine, apomorphine, or tetrabenazine were recorded by means of an Opto-Varimex actograph in 240 animals (10 mice at each time, for 15 min).

A conditioned avoidance reflex was formed in 10 rats by a modified method [5]. A photic stimulus was used as conditioned stimulus. The reflex was formed by 10 combinations of conditioned and reinforcing stimuli daily for 12-14 days. TRH (1 mg/kg) was injected intraperitoneally 15 min before the experiment. The conditioned passive avoidance reflex (CPAR) was assessed by the ability of the rat to avoid the dark compartment of the experimental chamber after a single electric shock reinforcement [4]. The difference in the length of stay of the animals (100 rats) in the dark compartment before and 24 h after formation of the CPAR was determined (Δt). Immediately after conditioning, the rats were subjected to electroconvulsive shock, evoking retrograde amnesia. The unconditioned-reflex response to pinching the base of the tail with special forceps (1 kg, 5 sec) was studied in the same animals.

The effect of TRH on the analgesic effect of morphine and the nitro-analog of the tetrapeptide [Tyr-D-Ala-Gly-Phe-$(NO_2)NH_2$] was investigaed by determining the vocalization threshold of rats in response to application of electric shocks to the tail (60 animals). The effect of TRH on inhibition of respiration by morphine and the nitro-analog of the tetrapeptide was observed in experiments on waking rabbits (10 animals). Besides the respiration rate, attention was paid also to the volume of expired air, as reflected in the volume of liquid displaced from the gas holder. At the same time, in experiments on 10 rats anesthetized with urethane (1.5 g/kg, intraperitoneally) the respiration rate and volume were estimated from the integrated myogram of the diaphragm.

Interaction between TRH and central cholinergic and adrenergic receptors was investigated by applying the test substances by microiontophoresis to single nerve cells in the ventrocaudal portion of the lateral hypothalamic region of a rabbit (AP +2.5, S 1.5, V −3.75 according to the stereotaxic atlas of Fifkova and Maršala [2]). In experiments on six unanesthetized, curarized (diplacin 5 mg/kg) rabbits, discharges of 34 neurons were recorded extra-

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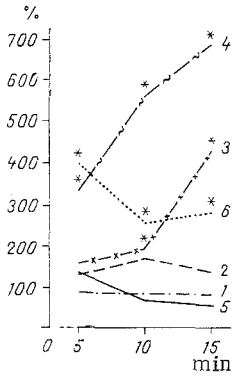

Fig. 1. Effect of TRH on motor activity of mice. Abscissa, time after injection of substances (in min); ordinate, per cent of change in motor activity relative to initial value, taken as 100. Each curve is the result of averaging data obtained on 30 mice (three groups, 10 mice in each group). *) Difference compared with control is significant ($P < 0.05$). 1) 0.9% NaCl solution, 0.2 ml (control); 2) TRH (5 mg/kg) and 0.9% NaCl; 3) 0.9% NaCl and amphetamine (5 mg/kg); 4) TRH (5 mg/kg) and amphetamine (5 mg/kg); 5) 0.9% NaCl and apomorphine (3 mg/kg); 6) TRH (5 mg/kg) and apomorphine (3 mg/kg). Aqueous solutions of amphetamine and apomorphine were injected subcutaneously, other solutions intraperitoneally. Two injections given with an interval of 1 min; recording of activity began 10 min after second injection.

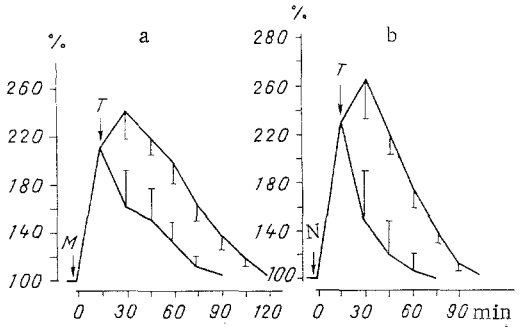

Fig. 2. Effect of TRH on analgesic effect of morphine (a) and of nitro-analog of tetrapeptide [Tyr-D-Ala-Gly-Phe-$(NO_2)NH_2$] (b), Abscissa, time (in min); ordinate, threshold of rat's vocalization response to electric shock stimulation of tail (in %, threshold of response before injection of substances taken as 100). Each curve plotted from averaged data for group of 10 rats. T) TRH (10 mg/kg, intravenously); M) morphine (2.5 mg/kg, intravenously); N) nitro-analog of tetrapeptide (2.5 mg/kg, intravenously). Arrows indicate time of injection of substance.

cellularly through one barrel of a seven-barrel microelectrode, filled with 3 M NaCl. The remaining barrels of the microelectrode were filled with aqueous solutions of the following substances: TRH (0.03 M, pH 6.0), acetylcholine chloride (0.3 M, pH 4.0), atropine sulfate (0.3 M, pH 4.5), propranolol hydrochloride (0.03 M, pH 3.5), and phentolamine hydrochloride (0.03 M, pH 3.0).

The hemodyanmic effects of TRH were studied in experiments on cats under general anesthesia (urethane 400 mg/kg, chloralose 40 mg/kg) with artifical ventilation of the lungs. The cerebral blood flow in the carotid system was determined by means of an electromagnetic meter (Nihon Kohden, Japan). To produce hemorrhagic shock in the rabbits, BP was lowered to 40 mm Hg by bleeding.

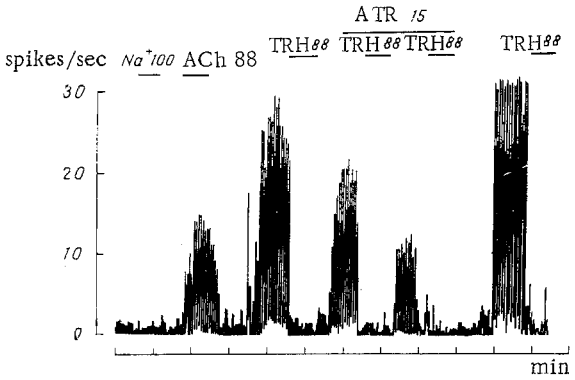

Fig. 3. Excitation of cholinergic neuron of lateral hypothalamic region by microiontophoretic application of TRH and reduction of this excitation by atropine. Abscissa, time (in min); ordinate, spontaneous firing rate of neuron (spikes/sec). Na^+ 100) control passage of outward microiontophoretic current, definitely of great strength. ACh) Acetylcholine; TRH) thyrotropin releasing hormone; ATR) atropine. Numbers accompanying abbreviations of names of drugs indicate microiontophoretic current (in nA).

EXPERIMENTAL RESULTS

TRH, injected intravenously in a dose of 5 mg/kg, increased the spontaneous motor activity of the mice. When injected intraperitoneally, TRH to the same dose had a weaker effect. Under these conditions the ability of TRH to potentiate the stimulating effect of amphetamine (5 mg/kg, subcutaneously) and apomorphine (3 mg/kg, subcutaneously; Fig. 1) on motor activity was clearly manifested. The depriming effect of tetrabenazine (40 mg/kg, intraperitoneally) on motor activity was reduced by TRH.

The time of formation of the conditioned avoidance reflex was reduced by TRH from 10-14 to 3 days. The number of conditioned-reflex responses realized by the rats with an established conditioned reflex was significantly increased ($P < 0.05$). The latent period of the reflexes was reduced from 5 to 1-2 sec.

The established CPAR was characterized by a decrease in the length of stay of the rats in the dark compartment from 102.2 to 19.6 sec ($\Delta t = 82.4$ sec). Electric shock induced retrograde amnesia ($\Delta t = 24.9$ sec). TRH in a dose of 1.0 mg/kg, injected intraperitoneally 15 min before testing the preservation of CPAR, gave an antiamnesic effect; Δt was increased to 71.1 sec ($P < 0.05$). When TRH was injected 15 min before electric shock and immediately after it, no antiamnesic effect was observed.

In the tail pinching test the rats usually gave a vocalization response, twisted the body toward the base of the tail, and bit the forceps. After electric shock these reactions were not observed. TRH (1 mg/kg) if injected intraperitoneally 15 min before electric shock or immediately thereafter, restored the above-mentioned responses of the animals ($P < 0.05$).

The analgesic activity of morphine (2.5 mg/kg, intravenously) and of the nitro-analog of the tetrapeptide (2.5 mg/kg, intravenously) was unchanged by injection of TRH in a dose of 1 mg/kg (intravenously), it was very slightly reduced by TRH in a dose of 5 mg/kg, and substantially reduced by injection of larger doses of TRH: 10-20 mg/kg (Fig. 2).

In experiments on rabbits TRH in a dose of 1.5 mg/kg (intravenously) completely abolished the inhibition of respiration caused by morphine (2-5 mg/kg, intravenously) or fentanyl (0.01-0.05 mg/kg, intravenously). Nalorphine (1 mg/kg, intravenously), a specific blocker of opiate receptors, had a similar action. In experiments on rats morphine and the nitro-analog of the tetrapeptide (1 mg/kg, intravenously) reduced the rate and volume of respiration by 50-70%. TRH in a dose of 0.3-0.6 mg/kg (intravenously) completely abolished the inhibitory effect of the analgesics on respiration. To inhibit respiration after preliminary injection of TRH, the nitro-analog of the tetrapeptide had to be given in a dose 10 to 12 times larger than usual (the dose inhibiting respiration).

Microiontophoretic investigation of neurons in the ventrocaudal portion of the lateral hypothalamus of the rabbit showed that TRH excited those neurons which are excited by acetyl-

choline (12 of the 17 neurons excited by acetylcholine, against two of 17 neurons not responding by excitation to acetylcholine; the muscarinic cholinolytic atropine reduced the excitatory effect of acetylcholine and TRH on five of the seven neurons tested (Fig. 3). Phentolamine and propranolol, which block adrenoreceptors, did not reduce the excitatory effect of TRH.

In experiments on cats TRH (1 mg/kg, intravenously) increased BP (by 49 ± 11.6%) and the volume velocity of the cerebral blood flow (by 61 ± 16%). During blockade of peripheral α-adrenoreceptors by dihydroergotoxin (1 mg/kg, intravenously) TRH increased the blood supply to the brain without affecting BP. After the sharp fall in BP and the cerebral blood flow due to hemorrhagic shock, TRH (1 mg/kg, intravenously) caused BP to rise to its initial level. The volume velocity of the cerebral blood flow rose considerably under these conditions under the influence of the drugs and in some experiments reached the original value.

The results demonstrate that TRH combines a unique range of neurotropic properties. Its central stimulating effect (increased motor activity) is combined with so-called nootropic activity (improvement of learning and memory processes). However, the well-known correlation between nootropic activity and the antihypoxic effect could not be found in the case of TRH (experiments on mice in a pressure chamber). TRH also possesses characteristic properties of antidepressants. This is shown by potentiation by TRH of the stimulating effect of amphetamine and apomorphine on spontaneous motor activity of animals, and also by weakening of the inhibitory effect of tetrabenazine on it. Nevertheless, TRH cannot be classed as a typical antidepressant because of the antagonism which it exhibits with barbiturates and ethanol [3, 6].

The antagonism of TRH with opiates and opioid peptides discovered in these experiments deserves special attention. The antagonistic action of TRH is exhibited mainly against the inhibitory effect of opiates and opioids on respiration rather than on their analgesic effect. The differences mentioned above evidently depend on the type of opiate receptors.

During the study of the effect of TRH on brain mediator systems with the aid of microiontophoresis, interaction of TRH with the central muscarinic cholinergic receptors of the hypothalamus was found. This suggests that the excitation of rabbit cerebral cortical neurons discovered by the writers previously after intravenous injection of TRH [1] is due to an increase in efficiency of the muscarinic choline-sensitive nerve cells of the subcortical structures, which have an activating influence on the neocortex.

Investigation of the effect of TRH on the hemodynamics revealed an increase in BP and the cerebral blood flow. By contrast with the cerebrovascular effect, the hypertensive effect of TRH is realized through the α-adrenoreceptors of the blood vessels.

One of the most significant results of this investigation is the fact that TRH restores hemodynamic parameters in animals with marked hypotension (hemorrhagic shock). TRH may thus be interesting as a subject for clinical trials as a central stimulator with certain antidepressant and nootropic properties. TRH can be used in anesthesiology, especially if respiration is inhibited by opiates. Finally, the hypertensive and cerebrovascular effects of TRH encourage the hope of its successful use in the treatment of various types of hypotension and, in particular, the hypotension due to acute blood loss.

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