RESTORATION OF DISTURBED RESPIRATION IN CATS BY HORMONALLY INACTIVE TRH ANALOG PR-546

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The presence of relatively high concentrations of thyrotrophin releasing hormone (TRH) in the region of the respiratory center and its marked activating effect on respiration in vertebrates of various classes in cases of exogenous administration [1-4, 8, 9] are evidence of the important role of this peptide in the regulation of respiration, and they indicate that endogenous TRH may perhaps perform the function of cotransmitter in the central stage of regulation of respiratory activity. Restoration of disturbed respiration by the action of this peptide under conditions of hemorrhagic shock is a particularly interesting fact [1, 7]. This makes TRH attractive for clinical use in order to stimulate respiration. However, because of the hormonal effects of TRH there is a need for its analogs, which will stimulate respiration without exhibiting its hormonal properties.

The aim of this investigation was to study the action of the synthetic TRH analog PR-546 on the regulation of respiration in cats with various disturbances of the respiratory rhythm: hyperventilation-induced respiratory arrest; slowing of respiration after bilateral vagotomy, and hypovolemic hemorrhagic shock. The TRH analog chosen for study was synthesized at the Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga, and preliminary tests showed that it does not induce secretion of either TSH or other pituitary hormones.

EXPERIMENTAL METHOD

Experiments were carried out on cats weighing 1.5-3 kg anesthetized with pentobarbital (50 mg/kg). Respiratory movements were recorded by means of a carbon transducer. The electromyogram (EMG) of the diaphragm was recorded with bipolar metal electrodes. A UBP 2-03 biopotentials analyzer and VITA-1 artificial ventilator were used. The PR-546 was injected in doses of 100-500 μg/kg into the femoral vein (in 2 ml of isotonic NaCl). In control experiments isotonic NaCl solution was injected in the same volume and by the same route. Against the background of normal respiration, hyperventilation of the lungs was carried out twice or three times with intervals of 15 min until activity of the diaphragm ceased. The duration of hyperventilation until cessation of the diaphragmatic EMG was constant in each separate experiment, but varied in different experiments from 2 to 8 min. After injection of physiological saline (control) and of PR-546, periods of hyperventilation of equal duration were instituted after 5, 15, 30, and 60 min. The cats were bled through a catheter in the femoral artery after vagotomy with denervation of the carotid bodies (by division of the carotid nerve). This operation prevented the quickening of respiration observed in the first period of moderate hypoxia after bleeding. The volume of blood removed was 40-70%, and the blood pressure fell after bleeding to 30-40 mm Hg. Under these conditions the amplitude and frequency of respiratory movements were reduced or complete respiratory arrest took place.

EXPERIMENTAL RESULTS

On the model of posthyperventilation respiratory arrest (6 experiments) a single injection of PR-546 in a dose of 100 μg/kg reduced the duration of the posthyperventilation pause by 40-60% in the first 10 min after injection, and if hyperventilation was carried out after 20-60 min it was ineffective: complete absence of the pauses was observed. In one typical

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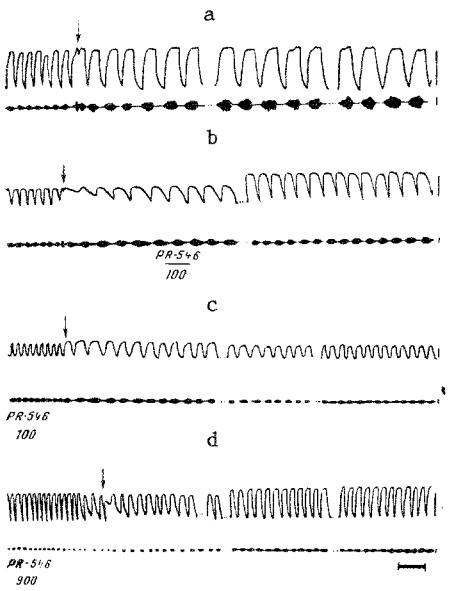

Fig. 1

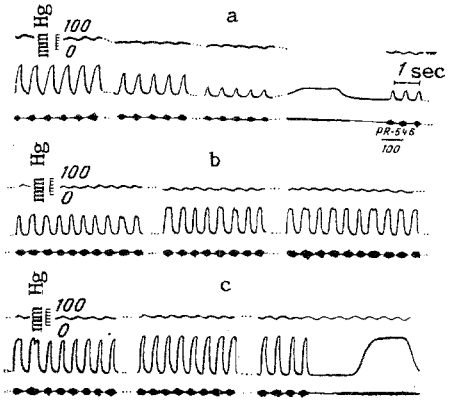

Fig. 2

Fig. 1. Action of PR-546 on respiration of vagotomized animals: a) respiratory activity immediately and 30 and 60 min after vagotomy; b) example of experiment with injection of PR-546 (100 μg/kg) after vagotomy (gaps in traces here and in c and d correspond to intervals of 30 min); c, d) examples of experiments with vagotomy preceded by injection of PR-546 (100-500 μg/kg respectively). Parallel lines indicate period of injection of peptide. Arrows indicate times of division of vagus nerves.

Fig. 2. Restoration of respiratory activity depressed as a result of blood loss. From top to bottom: BP, pneumogram, EMG of diaphragm. a) Decrease in respiratory activity due to bleeding (gaps in traces correspond to 15-min intervals); b) time course of recovery of respiratory activity after injection of PR-546 (100 μg/kg) for a period of 2 h. Time marker on expanded trace 1 sec.

experiment, for instance, the EMG of the diaphragm did not cease after hyperventilation starting with the 15th minute after injection of PR-546. The respiration rate also was doubled (from 10 to 20 cycles/min), to reach a maximum 60 min after injection of the compound. Similar results also were obtained in the other experiments of this series. These data are in agreement with the results of previous experiments to study the action of TRH under hyperventilation conditions [4].

In another series (6 experiments) the effect of PR-546 was studied on vagotomized cats in which the following frequency of the respiratory motor volleys was induced by 30-50% below normal. A single injection of PR-546 was given in doses of 100 and 500 μg/kg, either 20-30 min before vagotomy or 1-5 min thereafter. A stimulating action of the peptide was observed in all cases, however the experiment was modified. Injection of PR-546 either before or after vagotomy partially or completely prevented the decrease in frequency of the respiratory volleys. In individual experiments the respiration rate after vagotomy and under the influence of the peptide was on average 10-25% higher than before vagotomy. The activating effect developed with time and reached its peak 30-60 min after injection of PR-546. In two-thirds of the experiments the amplitude of the respiratory movements also increased. Examples of the action of PR-546 on vagotomized animals are given in Fig. 1.

A previous study using frogs showed that the stimulating action of TRH and its analog was manifested most strongly during slowing of respiration under hypoxic conditions after acute blood loss [2]. Other workers also have obtained similar results in relation to stimulation of respiration by TRH after acute blood loss in warm-blooded animals [1, 6]. The work was done on rats receiving TRH by systemic injection. We undertook a series of experiments (20)

with bleeding and injection of PR-536. In 10 experiments in which the extent of the blood loss was 60-70%, BP fell to 30-40 mm Hg and spontaneous activity of the respiratory center ceased completely, as was shown by disappearance of the EMG of the diaphragm. The animals were connected to the artificial ventilator. In five control experiments spontaneous recovery of respiratory activity was not observed under these conditions. Injection of PR-546 in a dose of 500 μg/kg or more led to restoration of breathing. The latent period of the reaction was 15-30 min and BP recovered to 60-80 mm Hg. Spontaneous activity of the respiratory center recovered in all cases, irrespective of the duration of the pause. In one experiment, for instance, arrest of spontaneous respiration lasted 5 h. The animal was kept alive by artificial ventilation. Spontaneous respiratory activity was restored 30 min after injection of the peptide. In another group of experiments (10 experiments) blood loss of 40-60% led to slowing of the respiratory movements and to reduction of their amplitude. BP fell to 40-50 mm Hg. Against this background restoration of breathing was observed after injection of PR-546 in a dose of 100 μg/kg. In this case the effect of stimulation of the respiration rate was exhibited in most experiments only after 4-5 min, and the greatest increase in the rate occurred after 30-40 min. An example of one such experiment is given in Fig. 2: 5 min after injection of the peptide the amplitude of the respiratory movements also began to increase, it reached its initial level after 30 min, and it continued to increase for 2 h.

Analysis of the results relating to the action of PR-546 on models of experimental disturbance of the rhythm of respiration shows that this peptide stimulates respiration in cats, increasing both the frequency and the amplitude of the respiratory movements. For instance, hyperventilation, after administration of the peptide, did not inhibit spontaneous respiratory activity, which always occurred in the two groups of control experiments. In the first group hyperventilation was preceded by normal breathing, in the second group by injection of isotonic NaCl solution. Enhancement of the stimulating action of PR-546 also was expressed as an increase in the respiration rate after disconnection of the ventilator. TRH also had a similar action on this model [4]. The activating effect of PR-546, like that of TRH, also was preserved after bilateral vagotomy, which rules out the possibility that vagal afferents are involved in the realization of this effect. The use of a model of respiratory arrest against the background of hypovolemic hemorrhagic shock revealed the activating action of the peptide on respiration in acute hypoxia. The character of responses to injection of the analog recorded in these experiments on cats is similar to the effects of TRH described elsewhere [1, 6, 7]. However, unlike TRH, which led to a marked rise of BP, PR-546 had a weaker action on the BP level.

Comparison of our results with data in the literature on the action of TRH on respiration suggests a similar mechanism of action of the two compounds. On the basis of brain transection experiments most workers consider that stimulation of respiration by TRH is realized through extrahypothalamic mechanisms [5, 6]. Meanwhile the high concentration of endogenous TRH in the region of the bulbar respiratory center [8] indicates that TRH (or its derivatives) acts directly on structures of the respiratory center. Since PR-546 has no hormonal activity, it can be tentatively suggested that its stimulating effect on respiration is not the result of endocrine activity of the peptide, which also points to a direct role of endogenous TRH in the activity of the central components of the mechanism controlling respiration. In particular [3], in a study of activity of respiratory neurons during the action of TRH on structures of the ventral surface of the medulla, in which the central chemosensory drive is formed, it was suggested that these structures play a leading role in the realization of the effect of TRH on respiratory responses.

The TRH analog PR-546, which is hormonally inactive but, under certain experimental conditions, has a stimulating action on respiration in warm-blooded animals, may be used in clinical practice as one of a group of resuscitation measures for restoring respiratory activity.

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EXPERIMENTAL ANTIOXIDANT THERAPY OF PURULENT WOUNDS

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During the development of suppurative inflammation in wounds a series of complex and interconnected morphological, biochemical, immunological, and other changes takes place both in the pathological focus and in the body as a whole [3, 8-10]. As we showed previously [2, 4, 7] the most significant of these changes, of pathogenetic importance, are: a) increased formation of superoxide anion-radicals of oxygen, which have a damaging action on tissues and thus promote intensive development and spread of suppurative inflammatory processes; b) a profound disturbance of the balance between the pro- and antioxidant systems; c) disturbance of biogenic amine metabolism; and d) lowering of the pH of the blood and wound medium.

It is thus evident that the use of preparations capable of "quenching" free oxygen radicals will have an antioxidant action, stimulate biogenic amine metabolism, normalize the pH and suppress growth of the microflora, and ultimately improve the results of treatment of purulent wounds greatly. This paper describes an investigation in which ionol, nialamide, and urotropine were used for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on 22 chinchilla rabbits weighing 2.5-3 kg. The experimental model consisted of abscesses, created by the method described by the writers previously [5]. Seven days after the operation (i.e., after an abscess had formed), the abscess was opened and sutures removed from the skin wounds and subjacent tissues. After surgical toilet of the wounds thus formed the animals were divided into three groups: 1) 6 untreated animals (control), 2) 6 rabbits treated for 7 days by the usual methods (irrigation with antiseptics, hypertonic and medicated dressings), 3) 10 rabbits treated by a combined method: immediately after opening of the abscess a single dose (2 mg/kg) of nialamide was given per os through a special tube; surgical toilet of the wound was carried out, followed by irrigation with a 10% aqueous solution of urotropine, a pack soaked with the same solution was left in the wound, and a gauze dressing with a 2% alcoholic solution (70°) of ionol was applied over it; this treatment was carried out twice a day for 7 days.

The experimental results were evaluated by clinical observation of the state of the wounds with determination of the time of their cleansing and complete covering, and biochemically with a spectrophotometric study [12] of the relative serum superoxide dismutase (SOD) activity of the animals before the operation (basic) and on the 1st and 3rd days of treatment.

Painful manipulations (surgical toilet, blood sampling) were performed under intravenous hexobarbital anesthesia (1% solution, 9.0 mg/kg body weight, intravenously).

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