ACTION OF FEMTO- AND PICOMOLAR CONCENTRATIONS OF THYROTROPHIN RELEASING HORMONE AND TUFTSIN ON CONTRACTILE ACTIVITY

T. V. Lelekova, P. Ya. Romanovskii,
P. N. Aleksandrov, and I. P. Ashmarin*

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The principal factor involved in the movement of lymph along the lymphatics in warm-blooded animals is the rhythmic contractile activity of the lymphangions, which are segments of lymphatics which contain all the necessary elements for effective transport of lymph [7]. Special myogenic pacemakers, located in the circular layer of muscle cells of the lymphangion in the immediate vicinity of a valve, are found in the lymphatics of warm-blooded animals [6]. The system of humoral regulation of functions of lymphatics has received little study. The main obstacle in the way of such investigations is technical difficulties. In particular, only the first steps have been taken toward the study of the corresponding role of regulatory peptides (RP). Peptides of opioid nature have been observed to act on contractile activity and permeability of the walls of lymphatics [2, 8, 9]. The aim was to make a systematic search for and study of RP which modulate the functions of lymphatics. With this aim, we first considered RP with a particularly broad spectrum of action on different systems and RP connected with functions of white blood cells, namely thyrotrophin releasing hormone (TRH) and tuftsin. TRH can be regarded as a substance modulating the activity of many systems of the body [4]. TRH is an immunomodulating peptide [4, 10]. Tuftsin, the other representative of the peptides, activates phagocytic cells, is involved in triggering of the immune response, stimulates systems of antitumor immunity, has a myotropic action, and can also regulate the relationship between universal intracellular mediators of excitability [11-13].

The action of TRH and tuftsin was investigated in the present study in relation to contractility of lymphatics.

EXPERIMENTAL METHOD

Mesenteric microlymphatics of the rat small intestine, 60-100 μ in diameter, were used as the test object. Contractions of the microvessels were recorded by intravital photometry [1]. The frequency of contractions of the vessel was recorded quantitatively and the amplitude of the contractions qualitatively. The mesentery of the rat, anesthetized with pentobarbital, was irrigated with physiological saline from a pipet to prevent it from drying. The test substances were dissolved in physiological saline. When the solutions were prepared and applied to the mesentery, replaceable tips of automatic pipets (new ones for each separate manipulation) were used. A 30× water immersion objective was lowered on to the surface of the chosen segment of the vessel (preferably a single, spontaneously active vessel). A special kind of humid chamber was formed, with an area of contact with the mesentery of about 1.5 cm^2. The test substance was diluted on introduction (the error of dilution did not exceed 15%). TRH was obtained from "Sigma," and was synthetized at the Institute of Organic Synthesis, Latvian Academy of Sciences; tuftsin was obtained from "Sigma."

EXPERIMENTAL RESULTS

TRH was tested in concentrations of between 1 and 10^{-10} μg/ml on the mesenteries of 15 rats. The main effect of TRH in all concentrations tested was an increase in the frequency of contraction of the lymphatics and, consequently, an increase in the flow rate of the lymph. A typical effect of TRH is illustrated in Fig. 1. In a concentration of 10^{-8} μg/ml and in the

*Academician of the Academy of Medical Sciences of the USSR.

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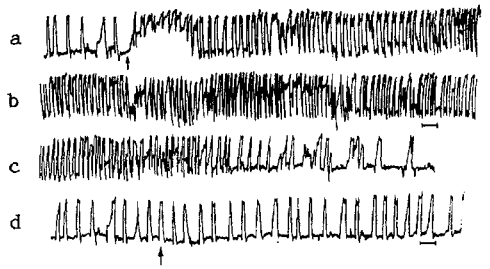

Fig. 1

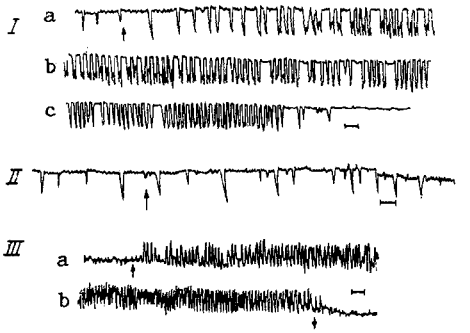

Fig. 2

Fig. 1. Action of TRH (10^{-8} μg/ml) on contractility of lymphatics: a) after 1-4 min of action of TRH; b) after 5-9 min of action of TRH; c) after 41-45 min of action of TRH; d) control (application of physiological saline). Here and in Figs. 2 and 3; arrow pointing upward — time of application of substance, arrow pointing downward — time of rinsing out with physiological saline. Calibration 10 sec.

Fig. 2. Action of TRH on contractile activity of vessel with regular or zero spontaneous activity. I) Against the background of irregular spontaneous activity: a) after 1-5 min of action of TRH, b) after 5-10 min of action of TRH, c) after 10-13 min of action of TRH (10^{-1} μg/ml); II) control (application of physiological saline); III) TRH (10^{-4} μg/ml) applied to lymphatic with no spontaneous activity; a) after 1-4 min of action of TRH, b) after 4-7 min of action of TRH.

course of about 40 min TRH induced and maintained an increased frequency of contraction of the vessel. The highest frequency of contractions was 22/min, compared with the normal value of 8/min, i.e., the increase was almost threefold. As a rule, the effect of TRH developed in the following order. The vessel responded to application of TRH with small and frequent contractions of its walls (Fig. 1), and this period lasted from 10 to 70 sec in different experiments. This initial brief vasodilatation and primary "hyperactivation" of the vessel wall was followed by active constriction of the lumen of the vessel with a simultaneous increase in the power and, in particular, of the frequency of contractions of the vessel wall. Periods of increased frequency of contractions of the vessel alternated term by term with periods of relative slowing. The duration of the effect of TRH in different concentrations varied from 1.5 min to 1 h, and was frequently limited by the duration of observation. The action of TRH was seen particularly clearly on vessels with a low or zero initial frequency of spontaneous activity. It will be clear from Fig. 2a that the irregular and low initial spontaneous rhythmic activity (only about 6 contractions per minute) reached a maximum after the action of TRH, with an increase by 2.5 times after 10 min of action of the hormone. The effect lasted 13 min. An example in which contractions of an initially inactive vessel were recorded is given in Fig. 2b. TRH (10^{-4} μg/ml) was able to evoke contractile activity of such a vessel and to maintain it for 7 min. In general, vessels with an initial frequency of contraction of their walls of 3-8/min, after exposure to TRH were able to increase their normal frequency by 2.5-3 times. On vessels whose frequency of contractions was higher than 10/min, values of this kind were found less frequently. Assuming that TRH, by raising the catecholamine level [4, 5], changes the state of the pacemaker structures, it can be postulated that for each pacemaker structure there exists an optimal schedule of frequency of action potential generation. If the pacemaker works at the limit of this schedule, the frequency can be raised only very slightly, and this we observed in experiments with preparations in which the initial frequency of contractions of the vessel was 10-12/min.

Tuftsin was used in concentrations of between 10 and 10^{-6} μg/ml. Experiments were carried out on 14 preparations of mesentery. The main action of all the concentrations studied was vasodilator — by up to 30% of the normal lumen of the vessel. A typical effect of application of tuftsin in a concentration of 10^{-3} μg/ml is shown in Fig. 3a: during the first 23 sec vasodilatation takes place, the vessel walls carry out frequent and low-amplitude contractions, just as during the action of TRH, next follows several strong but infrequent contractions and constriction of the vessel for a few seconds, and finally, quite prolonged vasodilatation takes place again. The duration of observation was 250 sec. Only in some cases was

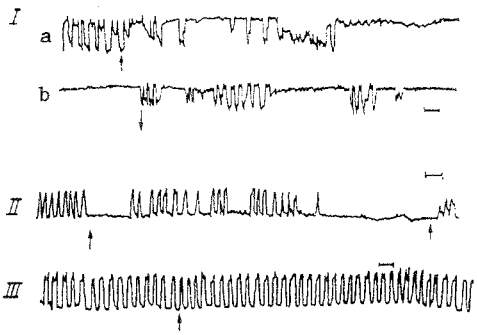

Fig. 3. Action of tuftsin on contractile activity of lymphatics. I) application of tuftsin (10^{-3} μg/ml): a) after 1-220 min of action, b) restoration of rhythm after removal of tuftsin (10^{-3} μg/ml) with physiological saline; II) application of tuftsin in a concentration of 1 μg/ml; III) control (application of physiological saline).

the effect terminated by rinsing the substance out with physiological saline. In 50% of cases the action of tuftsin could not be abolished for 15-30 min by rinsing out. The irreversibility of the action of tuftsin was not evidently connected with the concentration tested. The effect of tuftsin in a high concentration (1 μg/ml) also is demonstrated in Fig. 3b. It leads to vasodilatation, and against this background the vessel continues to contract, initially for 130 sec, after which dilatation of the lumen of the vessel becomes stabilized and its rhythmic activity ceased. The low level of effective concentration (doses) of RP is not surprising. However, the ability of TRH, discovered by the present investigation, to stimulate contractile activity of lymphatics in a concentration of 10^{-10} μg/ml, i.e., less than 10^{-15} M, is remarkable even for bioregulators of this type. Effectiveness in femtomolar concentrations has so far been described only for a few RP (for example, dilatation of the skin vessels under the influence of cocalcigenin, stimulation of killer lymphocytes by β-endorphin, increased permeability of venules by bradykinin and substance P, and certain other cases [3]). Effective concentrations of tuftsin also were found to be low, though not as low as those for TRH. The important point is that minimal active concentrations of the two peptides are closely similar to or even below their average levels in the body fluids — plasma, CSF [4, 11]. There is thus a firm basis for the view that the effects of TRH and tuftsin revealed in these experiments reflect their physiological function, namely their regulatory role relative to the lymphatic system.

There is an evident need for further study of the characteristics and mechanisms of these powerful effects of TRH and tuftsin.

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