MODULATION OF ORNITHINE DECARBOXYLASE ACTIVITY IN THE NORMAL AND REGENERATING RAT LIVER BY VARIOUS DOSES OF THE PEPTIDE MORPHOGEN OF *HYDRA*

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Several factors of peptide nature capable of inducing growth of various tissues have recently been isolated, and this suggests that oligopeptides may participate in the regulation of anabolic processes. The undecapeptide pGlu-Pro-Pro-Gly-Gly-Ser-Lys-Val-Ile-Leu-Phe, isolated from *Hydra* and sea anemones, accelerates regeneration of injured organs of the Coelenterata and Polycheta [2, 3]. Recently this peptide morphogen, which has been called *Hydra* head activator, or a substance similar to it in its immunoreactivity, has been found in the mammalian brain [4] and in human blood plasma [8], but no data on the biolgoical action of this peptide have yet been obtained in experiments on mammals. The writers have studied the effect of *Hydra* head activator on ornithine decarboxylase (ODC) activity in the intact and regenerating rat liver. ODC is an enzyme which limits the velocity of polyamine synthesis. Its activity rises under the influence of various agents stimulating proliferation [6]. This enzyme can therefore be used as a marker of the anabolic activity of a tissue.

In the present investigation changes in ODC activity were studied in the normal and regenerating liver of rats receiving injections of various doses of *Hydra* peptide morphogen (HPM).

RESULTS

All the experiments were conducted on 30 male Wistar rats weighing 160-180 g. HPM was synthesized in the Laboratory of Peptide Synthesis, research Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. The peptide was dissolved in physiological saline and injected intraperitoneally into the animals in a volume of 1 ml. Animals of one group were injected with a solution of the peptide. Animals of the other group were previously subjected to partial hepatectomy [1] under ether anesthesia, in the course of which the central and left lateral lobes of the liver were removed. The peptide was injected into the animals of this group 10 days after the operation. In both groups, rats which received an injection of physiological saline instead of the paptide served as the control. Since ODC activity in the liver undergoes circadian fluctuations [5], the injections of the peptide were always given and the operations always performed at the same time of day, from 9:30 to 10 a.m. ODC activity was determined in liver homogenate and in the supernatant obtained by centrifugation of the homogenate at 105,000 g for 70 min 6 h after injection of physiological saline or of the peptide. To obtain the homogenate, the animals were decapitated, after which 1 g of the minced right lateral lobe of the liver was homogenized in 5 ml of buffer solution in a Polytron homogenizer (position 5) for 20 sec at 4°C. The composition of the buffer solution was: HEPES 25 mM, dithiothreitol 2 mM, pyridoxal phosphate 100 μM, EDTA 100 μM, pH 7.2. Activity of ODC was deter-

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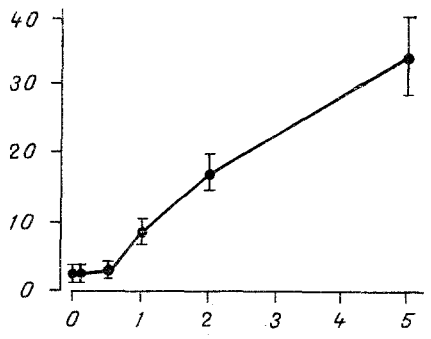

Fig. 1

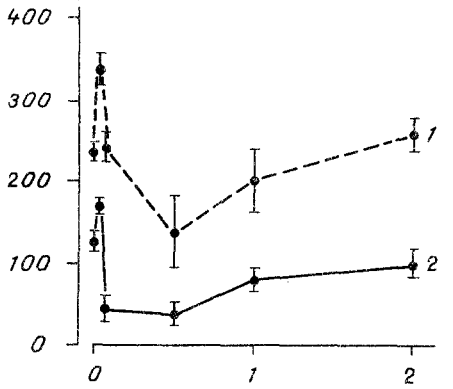

Fig. 2

Fig. 1. Dependence of activity of soluble form of ODC in intact rat liver on dose of HPM injected. Here and in Fig. 2: abscissa, dose of peptide (in mg/kg); ordinate; specific ODC activity (in pmoles CO_2/mg protein/h).

Fig. 2. Dependence of total ODC activity (1) and activity of soluble form of ODC (2) in liver of partially hepatectomized rats on dose of HPM injected.

mined by a radioisotope method based on liberation of $^{14}CO_2$ from L-(1-^{14}C)-ornithine. Each 1 ml sample consisted of 200 µl of biological material (homogenate or suspension after centrifugation of the homogenate at 105,000 g for 70 min), 700 µl of buffer solution, and 100 µl of buffer solution containing of mixture of labeled and unlabeled ornithine. The L-(1-^{14}C)-ornithine-HCl (56 µCi/mmole) was obtained from Amersham Corporation, England, and the L-ornithine·HCl from Calbiochem, USA. The final concentration of L-(1-^{14}C)-ornithine in the sample was 8 µM and of L-ornithine 24 µM; the specific radioactivity of the added substrate was 0.5 µCi. The reaction was triggered by addition of the substrate and by incubation of the sample at 37°C. After incubation for 1 h the reaction was stopped by the addition of 100 µl of concentrated $HClO_4$. CO_2 liberated in the course of incubation was absorbed by 200 µl of 4M NaOH, with which the glass fiber filter, fitted into the lid of the test tube, was soaked. After the reaction had stopped the covered samples were reincubated for 1 h at 37°C to absorb the residues of liberated CO_2. At the end of incubation the filters were removed and placed in 10 ml of scintillation mixture of the following composition: POPOP 0.2 g, PPO 4 g, naphthalene 60 g, methanol 100 ml, ethylene-glycol 20 ml, and 1,4-dioxan to 1 liter. Radioactivity was measured on a RackBeta-1215 counter (LKB, Sweden). Preliminary experiments showed that the quantity of CO_2 liberated in the course of the reaction was proportional to the volume of biological material added to the sample. The results were subjected to statistical analysis by Student's t test for small samples ($P < 0.01$). Protein was determined by Sedmak's method [9].

RESULTS

In the experiments of series I changes in ODC activity in the intact rat liver under the influence of various doses of HPM were studied. Most of the enzyme activity was concentrated in the homogenate, and only about 1% of the total enzyme activity was accounted for by soluble ODC. The initial level of activity of the soluble enzyme under these experimental conditions was 2.75 ± 0.24 pmoles CO_2/mg protein/h, in agreement with data in the literature [7]. With an increase in the dose of peptide injected, activity of soluble ODC increased, and with doses of 1, 2, and 5 mg/kg of peptide, enzyme activity was increased by 2.9, 6.4, and 11.9 times (Fig. 1). ODC activity in the homogenate was virtually unchanged after administration of HPM to intact rats.

In the experiments of series II changes in IDC activity were studied in the regenerating rat liver under the influence of different doses of HPM (Fig. 2). Activity of the soluble form of ODC in the regenerating rat liver under these experimental conditions was 48 times higher than that in intact animals. A small dose of the peptide (20 µg/kg) caused a significant increase of enzyme activity in the homogenate by 1.4 times, and in the supernatant by 1.3 times compared with the control (partially hepatectomized rats, not receiving an in-

jection of peptide). Higher doses caused a decrease of enzyme activity in both fractions; the decrease was more marked, moreover, for the soluble enzyme. For instance, a dose of peptide of 500 μg/kg lowered ODC activity in the supernatant by 3.7 times, and in the homogenate by 1.7 times compared with the control. With a further increase in the dose of HPM, gradual weakening of the inhibitory effect of the peptpide on ODC activity, stimulated by hepatectomy, was observed. Thus the effect of HPM on ODC activity in the regenerating liver exhibits complicated dose-dependence: small doses stimulate the enzyme, somewhat larger doses inhibit activity induced by hepatectomy, and in even larger doses still, the inhibitory action disappears.

The results, in the writers' view, indicate that HPM may have a role in the regulation of anabolic processes and, in particular, of regenerative processes in mammals. This means that the physiological role of this peptide, or of compounds similar in structure to it, in higher animals may be the same as in invertebrates. The mechanism of action of HPM is not clear; however, the fact that some of its effects (for example, stimulation of ODC in the regenerating liver) are manifested in very small dose s indicates that it may act through specific mechanisms, including the presence of high-affinity receptors in target organs.

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INTERACTION OF α-TOCOPHEROL WITH FREE FATTY ACIDS AND STEREOCONFIGURATION OF THE COMPLEX

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It has recently been suggested that one of the molecular mechanisms of the action of vitamin E is by stabilization of biological membranes against the harmful action of free fatty acids (FFA) [1]. This hypothesis is based on the following facts: First, α-tocopherol (TP) can protect membranes of the sarcoplasmic reticulum against disturbances of structural and functional organization due to the action of FFA (inhibition of Ca^{++}-transporting function, lowering of the temperature resistance of Ca^{++}-dependent ATPase, lowering the temperature of thermotrophic phase transitions of Ca^{++}-dependent ATPase) [2, 3], and second, TP in solution can form complexes with FAA [1]. The formation of these complexes takes place through interactions of two types: 1) polar interaction of the OH-group of the chromane nucleus of TP with the C=O-group of fatty acids (FA); 2) nonpolar interaction of acyl chains of FA with methyl groups of the chromane nucleus of TP [4].

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