EFFECT OF ARGININE-VASOPRESSIN ON THE RAT THYROID GLAND IN VITRO

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A. L. Polenov's hypothesis [3] relating to the possibility of a direct influence i.e., without the involvement of hormones of the anterior lobe of the pituitary gland, of hypothalamic nonapeptide neurohormones vasopressin (VP) and oxytocin (OT) on peripheral endocrine glands, has been confirmed in recent years by experiments on the isolated mammalian adrenal cortex [6, 14]. Confirmation also has been obtained of the possibility of a direct regulatory effect of VP and OT on the steroidogenic elements of the sex glands [12, 15]. So far as the thyroid gland (TG) is concerned, attempts to determine the possibility of this kind of effect of nonapeptide neurohormones were made before the 1960s [7, 8], but the existence of a truly direct para-adenohypophyseal influence of VP and OT on TG remained unproved.

In the investigation described below, it was shown that certain hypothalamic neurohormones can exert a direct effect on peripheral endocrine glands and, in particular, on TG. TRH has been shown to modify activity of secretion formation in TG in vitro [5, 10]. Growth hormone and melatonin in vitro can inhibit thyrocyte proliferation and reduce the intensity of thyroid hormone formation [11, 16]. There is abundant evidence which indirectly confirms the probable effect of the nonapeptidergic hypothalamo-hypophyseal neurosecretory system of TG [1, 2]. However, the results of experiments on animals with an intact pituitary gland, or even in the absence of the anterior lobe of the pituitary, have never entirely disapproved the hypothesis that certain unstudied agents are involved in the regulation of the target gland (catecholamines of the autonomic nervous system and chromaffin tissue, central and peripheral neurohormones such as TRH and growth hormone). Only the investigation of the effects of neurohormones on TG in vitro can therefore give a definite answer to the question of their possible direct on thyrocytes.

EXPERIMENTAL METHOD

TG was removed from young male Wistar rats weighing 100-120 g. Fragments not more than 300 μ thick were excised from the central part of each lobe of TG. All fragments of the gland were placed in medium 199 at 37°C. The fragments were preincubated for 1 h. All the material was then transferred for 30 min into medium containing ^{3}H-leucine (1.6 MBq/ml), as well as thyrotrophic hormone (TSH) in a concentration of 50 mIU/ml, or VP in various concentrations ($5\cdot10^{-11}$, $5\cdot10^{-10}$, $5\cdot10^{-9}$, and $5\cdot10^{-8}$ M). Pieces of the gland, incubated in medium not containing the hormones were used as the control. Part of the material was fixed 30 min after incubation in the medium with labeled leucine. The rest was transferred into solution with the same hormone, but not containing the isotope, and incubated for a further 90 min (total exposure to the hormone was 120 min). At the end of incubation the material was fixed in Bouin's fluid. ^{3}H-Leucine is usually used as an imino acid which is incorporated into the thyroglobulin molecule [13].

Serial paraffin sections of TG fragments 6 μ thick were coated with Ilford-400 photographic emulsion (Great Britain) and exposed for 2 weeks. The sections were then stained with Ehrlich's hematoxylin. The average number of autoradiographs above each thyrocyte, the number of autoradiographs above the follicular colloid, and the height of the thyrocytes were used as parameters of TG function and expressed as percentages of the corresponding

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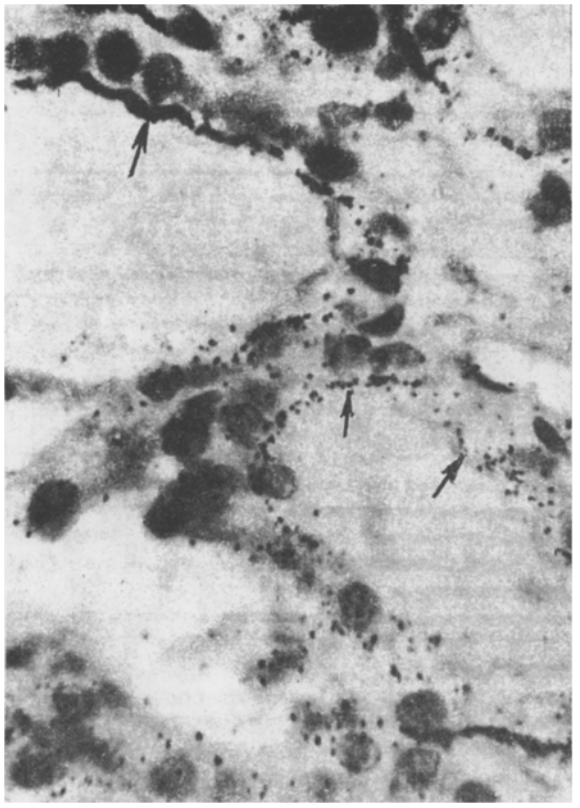

Fig. 1

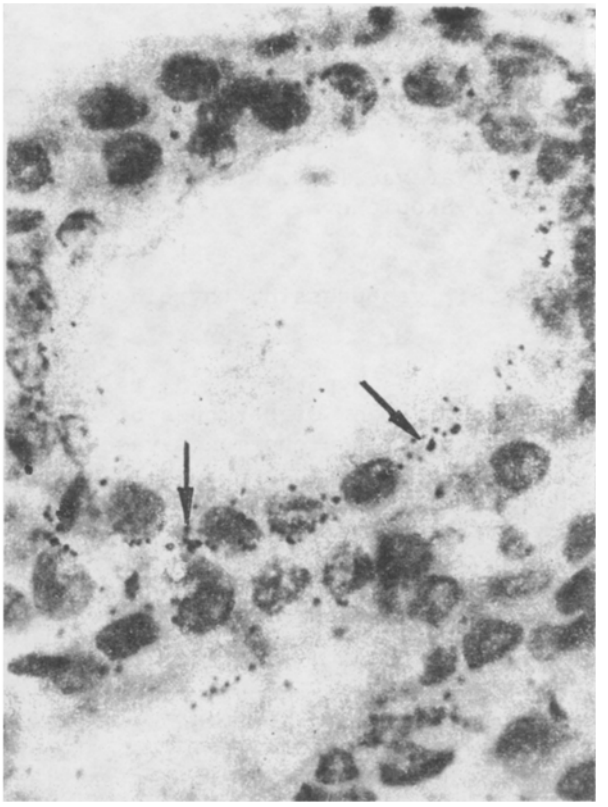

Fig. 2

Fig. 1. Thyroid gland. Control. Arrows indicate concentration of grains of silver near apical surface of thyrocytes. Here and in Fig. 2, magnification 3.2 × 63. Hematoxylin.

Fig. 2. Thyroid gland. Incubation with VP ($5 \cdot 10^{-11}$ M) for 2 h. Grains of silver concentrated in basal part of thyrocytes (arrows).

control. From four to six animals were used in each experimental group. The significance of differences between the groups was determined by the nonparametric Wilcoxon–Mann–Whitney U test.

EXPERIMENTAL RESULTS

After incubation for 30 min, TG of the control group was in a state of moderate activity: the thyrocytes wre cubical in shape and their nucleus occupied a central position in the cell. Relatively many grains of silver were counted above each thyrocyte, especially near the apical surface, and toward the end of the experiment (2 h) the number increased, although the height of the thyrocytes remained constant (Fig. 1).

TSH has a marked stimulating action of TG. The nucleus most frequently occupied a central position in the cells, the colloid contained no grains of silver, and the thyrocytes became prismatic in shape. After incubation with TSH for 30 min an increase in height of the thyroid epithelium (123%, $p < 0.01$) and a very small increase in the number of grains of silver above the thyrocytes (113%, $p > 0.05$) were observed. Further incubation with TSH led mainly to increased secretion of thyroid hormones, for the number of grains of silver accumulating in the course of incubation for 30 min with ^{3}H-leucine subsequently fell to 59% ($p < 0.05$), the epithelial cells remaining high (121%, $p < 0.01$).

After the action of VP on the thyrocytes for 30 min in a concentration of $5 \cdot 10^{-11}$ M (corresponding to the peripheral blood level of the hormone in stressed animals [9]) significant accumulation of autoradiographs was observed above the thyrocytes (165%, $p < 0.05$) with an increase in height of the thyroid epithelium (120%, $p < 0.05$). These changes indicate activation of thyroid hormone formation. If incubation of TG continued to 2 h in medium with VP in the same concentration, but without ^{3}H-leucine, the number of grains of silver above the thyrocytes decreased (63%, $p < 0.05$) and the height of the epithelium continued

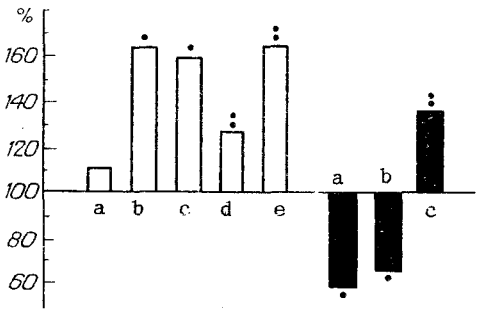

Fig. 3. Number of autoradiographs above each thyrocyte (in % of control) on incubation of fragments of TG in medium containing VP in different concentrations: a) TSH (50 mIU/ml), b) $5 \cdot 10^{-11}$ M, c) $5 \cdot 10^{-10}$ M, d) $5 \cdot 10^{-9}$ M, e) $5 \cdot 10^{-8}$ M. Unshaded columns denote incubation for 30 min in medium with addition of ^{3}H-leucine up to 120 min. Content of label in control fragments taken as 100%. .$p < 0.05$; :$p < 0.01$.

to increase (135%, $p < 0.05$) (Fig. 2). Such a sharp decrease in the quantity of label (Fig. 3) indicates intensive processes of release of thyroid hormones from incubated fragments of the gland.

In high concentrations ($5 \cdot 10^{-9}$ and $5 \cdot 10^{-8}$ M), after incubation for only 30 min, VP led to accumulation of the label above the apical surface of the thyrocytes compared with the control (128%, $p < 0.01$ and 164%, $p < 0.01$ respectively; Fig. 3). However, the height of the thyrocytes under these circumstances was not increased, indicating activation of the synthesis of thyroglobulin but without intensification of its release.

The changes described above suggest that on incubation of TG in medium not containing hormones, mainly thyroglobulin formation and accumulation in the follicles takes place. Under the influence of TSh, resorption of colloid and release of thyroid hormones are accelerated, as shown by absence of accumulation of labeled thyroglobulin in the colloid and an increase in ehight of the thyrocytes. VP in a concentration of $5 \cdot 10^{-11}$ M, corresponding to the blood level of this hormone in stressed animals, the formation and release of thyroid hormones are activated. The effect of VP varies depending on the concentration used. Under the influence of concentrations of VP 2-4 orders of magnitude higher than the physiological level, hormone formation was activated but release of thyroid hormones from the thyrocytes was probably inhibited. These results confirm Polenov's hypothesis that relatively small doses of VP stimulate, whereas large doses inhibit, peripheral endocrine glands [4]. This investigation in vitro thus confirms that VP may have a direct para-adenohypophyseal [3] action on the rat TG and it demonstrates that the effect of VP depends on its concentration in the incubation medium.

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EFFECT OF HYDRA UNDECAPEPTIDE MORPHOGEN ON CELL DIVISION IN THE ALBINO RAT CORNEAL AND LINGUAL EPITHELIUM

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Hydra peptide morphogen (HPM) is an undecapeptide Glp-Pro-Pro-Gly-Gly-Ser-Lys-Val-Lle-Leu-Phe. HPM was first isolated from Hydra and the sea anemone, in which it has a marked stimulating action on reparative regeneration [5].

HPM has been found in the mammalian brain [6] and also in human blood serum and in neurons of the cranial part of the human brain [1]. The role of the peptide in regulation of physiological functions in mammals remains largely unclear. There is information in the literature on the role of HPM in the activation of anabolic processes. HPM significantly increases activity of ornithine decarboxylase, an enzyme limiting polyamine synthesis in the rat liver [4]. No information could be found on the effect of HPM on cell renewal processes.

The aim of this investigation was to study the character of the effect of HPM on cell division in the albino rat corneal and lingual epithelium.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150-190 g. HPM was injected intraperitoneally in a dose of 10 μg/kg. Control animals received an injection of the same volume of isotonic sodium chloride solution. An intraperitoneal injection of ^{3}H-thymidine (specific radioactivity 0.6 μCi/g) was given to the rats 1 h before sacrifice, and in addition, 5 μCi of ^{3}H-thymidine was applied to the cornea. To estimate the rate of mitosis, some animals were given an intraperitoneal injection of colchicine (0.2 μg/100 g) 2 h before sacrifice. Cell division was studied 4 and 24 h after injection of colchicine. In the experiments of group 2, HPM was injected in the same dose in the course of 5 days and the animals were decapitated 24 h after the last injection of the peptide. Preparation of autoradiographs, determination of the index of labeled nuclei (ILN, %), obtaining total preparations, and determining the mitotic index (MI, %) were undertaken by methods described previously [3]. The mitotic index of blocked metaphases (MI_{col}) and the duration of mitosis t_m were determined by the usual method [2] in the lingual epithelium only, for complete blocking of mitosis did not take place in the cornea with the dose of colchicine used. Altogether 110 animals took part in the experiments. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The results are evidence that 4 h after injection of HPM an increase took place in the number of dividing cells in the lingual epithelium: MI_{col} was increased by 1.8 times. MI_{col} in the tongue 24 h after injection of colchicine was 1.9 times higher than in the control (Table 1). Together with an increase in the number of dividing cells in the lingual epithelium, injection of HPM led to acceleration of mitosis itself: t_m in the control was 116 min, whereas 4 and 24 h after injection of HPM it was reduced to 75 and 85 min respectively. Evi-

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