EFFECT OF SHORT-TERM EXPOSURE TO COLD ON SOME HYPOTHALAMIC CENTERS IN RATS

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Cooling causes rapid and significant changes in metabolism and in the state of the endocrine system [6]. The study of the functional state of the hypothalamic neurosecretory centers during short-term exposure to cold can give valuable information about their role in the regulation of the functions of peripheral endocrine glands and, primarily, of the thyroid gland, for it is this gland which responds most constantly and consistently to exposure to cold by activation [1, 13]. Several investigations by Hefco et al. [8, 9] have been devoted to the study of the influence of hypothalamic centers on thyroid function. These workers concluded that the controlling influence on the level of thyrotrophic hormone originates from the anterior hypothalamus, but they do not specify the precise location of the centers producing thyrotrophin releasing factor (TRF). This conclusion is contradicted by the results of investigations indicating that TRF is found in the arcuate (AN), ventromedial (VMN), paraventricular (PVN), and other nuclei of the middle hypothalamus [4, 10, 14]. Data on morphological and functional changes in the hypothalamic nuclei, especially in mammals, under conditions of increased synthesis of TRF, are still incomplete [1, 7, 13, 14].

EXPERIMENTAL METHOD

Male Wistar rats weighing 200-230 g were kept in individual cages for 1 h (four rats) and 2 h (eight rats) at 4°C. The eight control rats were kept at a temperature of 20°C.

Material (brain, pituitary and thyroid glands) were fixed in Bouin's fluid and with a mixture of picric acid and formalin [5]. Sections were stained with paraldehyde-fuchsin by the Gomori–Gabe method, and in some cases counterstained with Heidenhain's azan.

The area of cross-section of the cell nuclei and nucleoli in the supraoptic nucleus (SON) and also in PVN, VMN, and AN, was measured photographically with an overall magnification of the picture of 9600 ×. All measurements were made on material fixed with Bouin's fluid. In PVN cells of the ventral magnocellular part were measured, in VMN and AN cells in the rostromedial portions. The quantity of Gomori-positive neurosecretory material (NSM) in the posterior lobe of the pituitary (PP) was estimated visually on a conventional 5-point scale.

Every 30 min the animals' body temperature was measured by a thermometer inserted into the rectum to a depth of 1.5 cm.

EXPERIMENTAL RESULTS

The rectal temperature 30 min after the beginning of the experiment was lowered by 1°C, and after 2 h by 2-2.5°C, at which level it remained during the next 2 h of observation.

After cooling of the rats for 2 h the number of cells with a low content of NSM granules in SON and PVN of the rats was appreciably increased (Fig. 1). The Nissl's substance was represented by a wider and denser rim at the periphery of the perikaryon than in the control animals. The thinnest neurosecretory fibrils, penetrating throughout the region of PVN and SON, were clearly observed in the control rats in preparations stained with paraldehyde-fuchsin without counterstaining (Fig. 1a). The neurosecretory fibers in the experimental rats had lost their NSM and it was almost impossible to observe them (Fig. 1b). In the region of PVN

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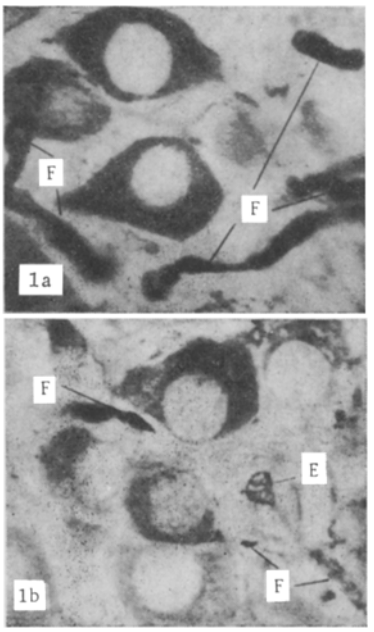


Fig. 1. Paraventricular nucleus of hypothalamus. a) Control; large quantity of NSM in neurosecretory cells and fibers (F); b) after 2 h at 4°C: emptied neurosecretory fibers (F) and their expansions (E). Fixation with picric acid and formalin. Stained with paraldehyde-fuchsin by Gomori–Gabe method. 900×.

TABLE 1. Area of Nuclei and Nucleoli of Some Hypothalamic Neurosecretory Centers of Rats Exposed to Short-Term Cooling (M ± mt; in conventional units of area)

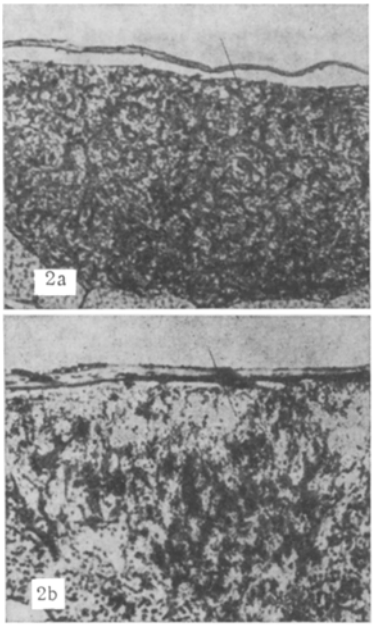
Group of investigation	SON		PVN		AN		VMN	
	nucleus	nucleolus	nucleus	nucleolus	nucleus	nucleolus	nucleus	nucleolus
Control	2372±29,6	156±3,7	2536±24	158±3,4	1769±87	81,3±3,2	2164±63	82,7±4,1
After 2 h at 4°C	2389±14,1 $P>0,05$	153±3,1 $P>0,05$	2748±32 $P<0,05$	155±2,5 $P>0,05$	1732±101 $P>0,05$	73,5±3,8 $P<0,05$	2677±120 $P<0,05$	99,7±3,6 $P<0,05$

numerous neurosecretory fibers appeared with large, bead-like expansions, filled with NSM, or almost empty (Fig. 1b). The region of PVN was appreciably hyperemic. The nuclei in the cells of PVN were significantly enlarged, whereas in SON there was no change in the dimensions of the nuclei (Table 1).

In the region of the median eminence in the experimental animals the NSM content in fibers of the hypothalamo-hypophyseal tract was reduced, and no large expansions filled with NSM could be seen. The outer zone of the median eminence was sharply hyperemic (Fig. 2). After exposure of the animals to cold for 1 h the quantity of NSM in PP was appreciably reduced, and after 2 h of the experiment it was estimated at only 3 points (compared with 5 points in the control). The distribution of NSM within the lobe had become irregular, with a characteristic concentration around the dilated blood vessels. Hyperemia of PP was conspicuous (Fig. 3).

Changes in the "classical" hypothalamo-hypophyseal neurosecretory system thus point to an increase in its functional activity. Transport of neurohormones along fibers in the region of SON, PVN, and the median eminence and liberation of neurohormones into the blood stream in PP and also, possibly, in the region of the median eminence were intensified. In PVN, additionally, the intensity of formation of neurohormones was increased: The cell nuclei were significantly enlarged in PVN. These results agree basically with those obtained by Sarajas et al. [12] but not with the observations of Palkovits et al. [11].

From ellipsoidal in the control, the shape of the cell nuclei in VMN under the experimental conditions became round, and they were appreciably larger in size. The nucleoli in the cells also were enlarged (Table 1), evidence of marked activation of VMN. No visible changes were observed in the structure of AN, but measurements showed a decrease in size of the nucleoli (Table 1). The number of cells containing chromatoid bodies



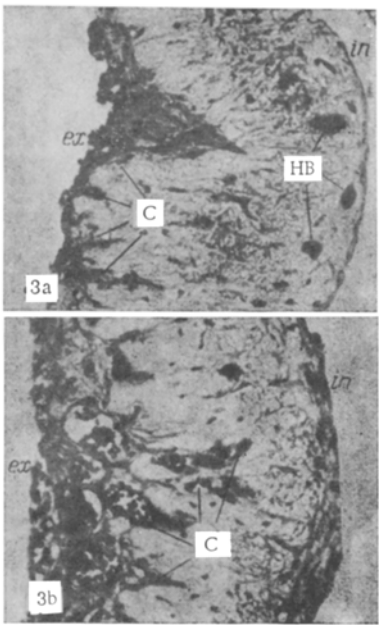


Fig. 2 Fig. 3

Fig. 2. Posterior lobe of pituitary. a) Control: uniform distribution of NSM; b) after 2 h at 4°C; quantity of NSM reduced, distributed irregularly, concentrated around dilated capillaries. Fixation with Bouin's fluid, staining with paraldehyde-fuchsin by Gomori–Gabe method and counterstaining with Heidenhain's azan. 90×.

Fig. 3. Median eminence. a) Control: capillaries (C) of outer zone (ex) constricted, in inner zone (in) Herring's bodies (HB) are visible; b) after 2 h at 4°C: dilatation of capillaries (C) in outer zone, decrease in content of NSM. Fixation and staining the same as in Fig. 2. 250×.

in the cytoplasm or nucleus was unchanged both in AN and in VMN. Changes in the size of the nucleoli of the cells in AN can be interpreted as a sign of depression of functional activity.

In the thyroid of the rats distinct signs of activation of function could be seen as early as 1 h after the beginning of cooling, and more distinctly after 2 h: an increase in height of the follicular epithelium, some increase in size of the cell nuclei, and the presence of numerous granules of intrafollicular colloid. Although the mean diameter of the follicles was not significantly changed, the homogeneity of the follicular structures and the liquefaction and vacuolation of the juxtamural colloid were noteworthy features. All these observations are evidence of activation of the synthesis and liberation of thyroid hormone.

The results can be summarized by the statement that depression of the functional activity of AN under conditions requiring increased synthesis of TRF makes it seem doubtful that AN may be the source of this neurohormone or may have a stimulating effect on its synthesis and liberation. The participation of SON in the regulation of thyroid function also is unlikely, for under the experimental conditions used no clear changes were discovered in it. The simultaneous activation of the thyroid gland and of VMN and PVN suggests that it is these nuclei which produce TRF. However, this coincidence alone is insufficient, for the response of these centers could be secondary and induced by an increase in the TRF content in the body from other sources. The response of PP can be explained in a similar way [15].

Depression of the functional activity of PVN, correlating with depression of thyroid activity, demonstrated by the writers previously [2, 3], and the simultaneous increase in PVN and thyroid activity, established in the present experiment, together suggest that relationships of direct functional dependence and, possibly, positive feedback also, exist between PVN and the thyroid gland.

Although some workers [4, 10, 14] have found that TRF is present in AN, VMN, and PVN, the different response of these nuclei during activation of the thyroid gland thus makes it unlikely that they are all TRF-producing centers.

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ULTRASTRUCTURAL CHANGES IN THE LUNGS DURING EXPERIMENTAL INFECTION BY *Mycoplasma pneumoniae*

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The important role of *Mycoplasma pneumoniae* in the etiology of acute respiratory diseases and pneumonias in children and adults has now been explained [4, 8, 11]. Morphological investigations have demonstrated the particular features of *M. pneumoniae* infection in man and in experimental infection of animals [1, 2, 5-7, 10, 14]. Meanwhile, very few investigations have so far been devoted to the analysis of fine structural changes in the trachea and lungs in mycoplasmosis. Some of them were undertaken on organ cultures of the trachea of Syrian hamsters [1, 9, 13]. It has been shown that *M. pneumoniae* causes metabolic and ultrastructural changes in cells of the ciliated epithelium, leading to ciliostasis and death of the cells. It has also been found that the ATP and RNA content is reduced in tracheal explants exposed to the action of *M. pneumoniae* cells or their membranes [12]. Electron-microscopic studies of the lungs of Syrian hamsters infected intranasally with a culture of *M. pneumoniae* have shown adhesion of mycoplasmas to the surface of the cells of the ciliated epithelium of the bronchi and their ingestion by alveolar macrophages [3, 10, 15].

The object of this investigation was to make a dynamic study of interaction between mycoplasmas and lung cells in experimental aerosol infection (i.e., under conditions close to those of natural infection), in an inhalation chamber, with a culture of *M. pneumoniae*.

EXPERIMENTAL METHOD

The experimental material consisted of lungs of 36 female Syrian hamsters weighing 60-80 g, infected in an inhalation chamber with a culture of *M. pneumoniae* (titer 10^8-10^9 CFU/ml), in a dose of 0.1 ml per animal

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