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ROLE OF MEDULLARY CHEMOSENSITIVE STRUCTURES IN BLOOD PRESSURE CONTROL AND THEIR MEMBERSHIP IN THE APUD SYSTEM

B. Ya. Peskov, I. M. Kvetnoi,
V. A. Kul'chitskii, and A. A. Nurmatov

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One of the functions of the medullary chemosensitive structures is not merely to organize and modify the respiratory drive from its ventral surface but also to take part in the formation of pressor reactions of the arterial pressure [2]. By central chemoreceptors are implied structures of nerve tissue located directly beneath the pia mater at a depth of under 150-200 μ from the ventral surface of the medulla [7, 8]. It has been suggested that these chemoreceptive structures determine the activity of sympathetic vasomotor neurons [9]. The problem of the nature of these formations is particularly interesting in connection with the extensive study of the apud system: This is a highly specialized system of cells which produce vitally important chemical agents — biogenic amines and peptide hormones [1, 5]. It has recently been shown that substances which also have been identified in the brain are synthesized in cells of the apud system (apud cells): gastrin, vasoactive intestinal peptide, substance P, somatostatin, and enkephalins [6]. These have been called neuropeptides.

Considering that the functional state of structures located within the central chemosensitive areas correlates clearly with the intensity of pressor reactions, it was decided to study whether biologically active substances (biogenic amines and their analogs) are present in these regions and to clarify the role of the chemosensitive input in blood pressure (BP) regulation.

EXPERIMENTAL METHOD

Acute experiments were carried out on 25 cats anesthetized by intravenous injection of a mixture of chloralose (40 mg/kg) and urethane (200 mg/kg). In a separate series of experiments the lungs were artificially ventilated, for which purpose the animals were relaxed with tubocurarine (0.5-1.0 mg/kg, intravenously for 1 h) and connected to an artificial respiration apparatus (AID). The medulla was exposed through a ventral approach from the

Department of Normal Physiology and Professorial Surgical Department, D. I. Ul'yanov Kuibyshev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR I. B. Soldatov.)

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lower boundary of the pons to the second cervical segment. The systemic BP was recorded on the N-327-3 automatic writer or SI-18 two-channel oscilloscope by means of a type DMI-03 miniature inductive pressure transducer, the catheter of which was introduced into the left common carotid artery. The transducer was connected to an ID-21 amplifier. To block the function of symmetrical structures of the intermediate chemosensitive area briefly (for 15 sec), they were subjected to bilateral local cooling to a temperature of 22-20°C by means of a thermode with two cooling surfaces, each 3 mm^2 in area. The parameters of the arterial blood gas composition of the animals were determined by means of the AZIV-2 apparatus. Frozen sections 5-10 μ thick were cut from the tissue of the ventral surface of the medulla. The sections were mounted on slides and fixed for 2-3 sec with cold acetone. An immunohistochemical investigation was carried out by the usual method [4], using specific antisera against melatonin, serotonin, N-acetylserotonin, and mexamine. Dry donkey serum against rabbit globulins, labeled with fluorescein isothiocyanate, was used as the luminescent serum. The preparations were examined in the ML-2A luminescence microscope (FS 1-2 and SZS 7-2 filters). To detect biogenic amines, the sections were stained by Sevki's method. When this method is used, cells containing serotonin and melatonin are stained orange, histamine red, adrenalin yellowish-green, and noradrenalin bluish-green.

EXPERIMENTAL RESULTS

Two series of experiments were carried out to study responses of BP during bilateral cold blocking of nerve tissue structures in the intermediate chemosensitive area on the ventral surface of the medulla.

In series I (15 experiments) responses of anesthesized, spontaneously breathing animals were investigated before and after blocking of the principal peripheral chemoreceptor zones. In animals with intact carotid sinus nerves and vagus nerves, the action of the cooling surfaces of the thermode on areas of the brain corresponding to the location of the intermediate chemosensitive area (2-4 mm above the middle of the roots of the hypoglossal nerves), led to a considerable depressor reaction. During cooling for 5 sec BP fell by 20-30 mm Hg (2.7-4.0 kPa, $P < 0.001$). In this case, the vasomotor reaction was preceded by respiratory arrest. Restoration of the initial BP level was observed 50-60 sec after cooling. The responses of BP were more marked after division of the carotid sinus nerves and vagus nerves. Cooling the same areas of the brain under these experimental conditions led to a fall in BP at the end of cooling by 40-50 mm Hg (5.3-6.7 kPa, $P < 0.001$). Restoration of the background level of BP took place 150-180 sec after the beginning of cooling.

To avoid the depressor reaction arising as a result of respiratory arrest during cooling of the intermediate chemosensitive area, the experiments of series II were carried out with artificial ventilation of the lungs. The reactions of the artificially ventilated animals before and after blocking of the principal peripheral chemoreceptor zones were studied in 10 experiments. Artificial ventilation of the lungs enabled the parameters of the arterial blood gas composition to be kept within normal limits: actual pH = 7.38, pO_2 = 100 mm Hg (13.3 kPa), pCO_2 = 40 mm Hg (5.3 kPa). Experiments on animals with intact peripheral chemoreceptors showed that, as before, the most marked fall in BP took place during cooling of areas corresponding to the location of the intermediate chemosensitive area. Division of carotid sinus nerves and of the vagus nerves caused no significant change in the direction of the responses of BP during cold block of the nerve tissue structures mentioned above. Bilateral cooling of areas located 2 and 4 mm above the middle of the roots of the hypoglossal nerves reduced BP toward the end of the procedure by 25-35 mm Hg (3.3-4.7 kPa, $P < 0.001$). The initial pressure was restored 35-40 sec after discontinuation of the cold block.

Histochemical and immunohistochemical investigations showed that the region of the medulla studied contains two types of cells which can be classed as apud cells. Cells of the first type, in their morphological structure (shape, size, presence of outgrowths), are typical nerve cells, and exist in two versions: 1) cells containing many secretory granules reacting positively for adrenalin; 2) empty cells, either with a few isolated noradrenergic granules or with none whatever.

They are arranged in groups of 6-8 cells, and for every two or three "empty" cells there are four or five cells with abundant granules (Fig. 1).

Cells of the second type do not contain catecholamines but give a strong positive reaction with antisera against serotonin, N-acetylserotonin, mexamine, and melatonin, i.e., with all the successive precursors of melatonin in the course of its synthesis from sero-

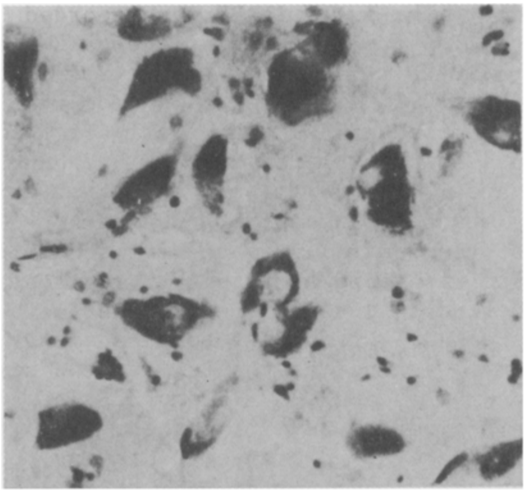

Fig. 1

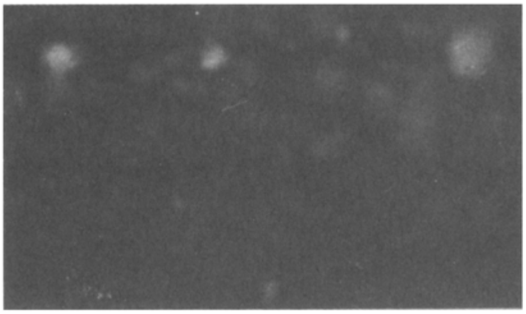

Fig. 2

Fig. 1. Two versions of apud cells from the cat medulla: cells containing many secretory granules reacting positively for noradrenalin (darkly stained) and cells with solitary noradrenergic granules (more palely stained). Method, 240 ×.

Fig. 2. Melatonin-producing cells in the cat medulla. Positive reaction with antiserum against melatonin, 240 ×.

tonin, so that melatonin synthesis can be assumed to take place in these cell populations in the medulla (Fig. 2).

Unlike true noradrenergic neurons, cells of the second type are small, round or club-shaped, and have a distinctly perivascular distribution. In some sections cells of this type were directly against the wall of the microvessels, and in the character of their distribution they resemble neurons found previously in this same region of the brain by intravital contact microscopy [2].

Two types of cells, possessing the morphological and histochemical characteristics of the apud system, were thus found in the ventral part of the medulla. The fact that noradrenalin is contained in neurons, whereas melatonin is present in cells whose categorization as nerve cells is doubtful, confirms the view [3, 6] that besides their endocrine function, cells of the apud system can also perform neurocrine and paracrine functions. In our observations the noradrenalin of nerve cells evidently plays the role of mediator (neurocrine function), whereas melatonin-producing cells, located near blood vessels, perform paracrine (action on structures of the vessel wall) and endocrine (secretion of melatonin into the blood stream) functions. These findings are in good agreement with the observations described above showing that structures of the intermediate chemosensitive area participate in BP regulation.

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