A METHOD OF QUANTITATIVE ANALYSIS OF MONOAMINES IN THE ARCUATE NUCLEUS OF THE HYPOTHALAMUS

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A simple and reliable method of detecting monoamines in the arcuate nucleus of the rat hypothalamus is described and the criteria enabling changes in the content of monoamines in this nucleus are established. The need for a differential approach to the cell composition of the arcuate nucleus of the hypothalamus when its functional state is assessed is demonstrated.

The level of the monoamine content is one of the most important indices of activity of the hypothalamic nuclei. Determination of changes in the monoamine level in the nerve cells of the parvocellular hypothalamic nuclei is particularly interesting because of the difficulty in estimating their activity as the result of the absence of suitably refined techniques.

The histochemical method of detection of monoamines suggested by Falck and Hillarp [7] is highly complicated [4]. The simplified method developed by Sakharova and Sakharov [2, 3] cannot be used to detect monoamines in the mammalian brain. Methods by which changes in the level of the monoamine content can be determined are not yet at a sufficiently high level of development. For instance, the method based on counting the total number of luminescent cells of the arcuate nucleus does not allow changes in the monoamine content to be determined accurately [9]. Quantitative microphotometry of luminescent compounds formed by condensation of monoamines with formaldehyde is restricted, as several workers [8, 10, 11] have pointed out, by the relatively short interval during which the content of monoamines is directly proportional to the intensity of their fluorescence.

The object of the investigation described below was, first, to develop a simple and reliable histochemical method of detecting monoamines in the hypothalamus and, second, to use it to determine changes in the monoamine level in the arcuate nucleus of the hypothalamus.

EXPERIMENTAL METHOD

The method is based on the principle suggested by Eränkö [5, 6] – on the possibility of formation of luminescent compounds by monoamines if the tissue is treated with aqueous formaldehyde solution. Attention was concentrated on the choice of optimal conditions of the histochemical reaction, for they have a decisive effect on the quality of luminescence. Rat hypothalamus was used as the test object. The animals were decapitated, the hypothalamic region of the brain was quickly removed, and the isolated fragment was immersed for 20-25 sec in freon cooled to the temperature of liquid nitrogen (−210°C). The frozen blocks were then cut into sections on a cryostat between −12 and −15°C (when the thickness of the 10−μ serial sections was verified on the interference microscope the difference between them was ±10%, and this was allowed for during photometric analysis), and the sections were mounted on coverslips and treated with 10% formalin solution made up in Ringer−Locke solution (pH to adjusted to 7.4) for 15 min at 30°C. The sections were then placed in an exsiccator with a known weight of phosphorus pentoxide and dried in an incubator at 40°C for 45 min. After drying and subsequent mounting in nonluminescent immersion oil the sections were examined in the ML-4 luminescence microscope and then photographed under standard conditions.

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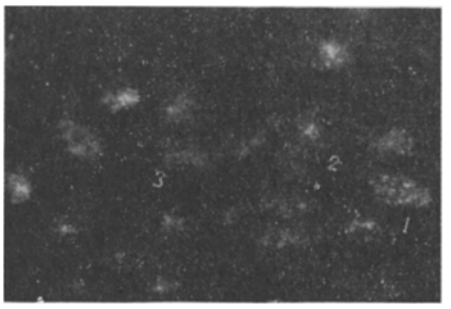

Fig. 1

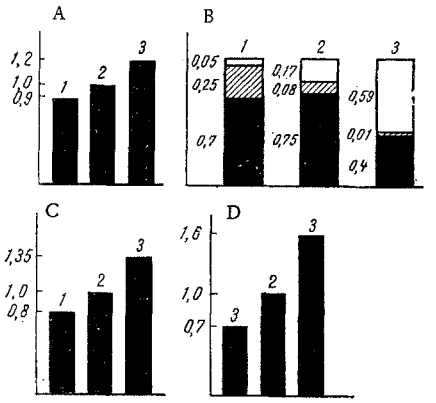

Fig. 2

Fig. 1. Photomicrograph of arcuate nucleus of hypothalamus (90×). Modified method of Falck and Hillarp: 1) cells with intensity of luminescence of 30-40 microphotometer scale units; 2) cells with intensity of luminescence of 20-30 units; 3) cells with intensity of luminescence of under 20 units.

Fig. 2. Change in content of monoamines in nerve cells of parvocellular hypothalamic nuclei: A) change in total number of cells (in conventional units); B) change in relative proportions of groups of cells with different intensities of luminescence (ordinate, number of cells in conventional units). Shaded parts of columns denote intensity of luminescence of 10-20 microphotometer scale units, black areas represent intensity of luminescence of 20-30 units; white areas intensity of luminescence of 30-40 units; C) change in mean intensity of luminescence of an individual cell of the nucleus (in conventional units); D) change in mean intensity of luminescence of the whole nucleus (in conventional units). 1) Reserpine (50% of monoamines); 2) control (100% of monoamines); 3) nialamide (150% of monoamines).

To study the level of monoamines in the arcuate nucleus, substances giving rise to predictable changes in the monoamine content in the tissue were injected into the rats. Nialamide, in a dose of 50 mg/kg, increases the monoamine content after 4 h by 50%, while reserpine, in a dose of 2 mg/kg, reduces the monoamine concentration by 50% over the same period [1, 12].

For each series of experiments five animals were taken and sacrificed when the specified period had elapsed after injection of these drugs. After treatment of the sections of the hypothalamus, the arcuate nucleus was photographed under standard conditions. The films were then examined on the MF-4 microphotometer, when the intensity of the background and of all cells of the frame, i.e., of virtually the whole arcuate nucleus, was measured. The numerical results were subjected to statistical analysis in order to determine the significance of the difference between the mean values, using the standard value of Student's criterion.

EXPERIMENTAL RESULTS

By treatment of the material as described above, sections of high quality were obtained, not inferior to those treated by the method of Falck and Hillarp (Fig. 1). By analysis of the results it was possible to detect changes in the total content of luminescent cells in the nucleus depending on their monoamine content but degree of these changes was very small, not more than ± 15% (Fig. 2A).

Photometric analysis of the cell composition of the nucleus showed that the intensity of luminescence differed in all the cells (Fig. 2). It was accordingly possible to subdivide the cells of the nucleus into three groups, between 20 and 50 units on the logarithmic scale of the microphotometer: cells with a low intensity of luminescence (under 20 units), those with average intensity (20-30 units) and those with a high intensity of luminescence (over 30 units). Most cells belong to the group with luminescence of average intensity; this group can be defined as basal, for the number of cells in it showed relatively little change to correspond with changes in the content of monoamines (the number of cells was slightly reduced only after injection of nialamide). In the groups with a low and high intensity of luminescence, there were considerable

changes in the numbers of cells. For instance, if the monoamine content in the nucleus was increased, the number of cells with a high intensity of luminescence was increased sharply, and there was a corresponding decrease in the number of cells with a low intensity of luminescence. Conversely, if the monoamine content was reduced, the number of weakly luminescent cells was sharply increased and the number of strongly luminescent cells was reduced. The total number of weakly and strongly luminescent cells remained relatively unchanged from the initial value, despite the changes in the individual groups (Fig. 2B).

The mean intensity of luminescence of the individual cell varied within relatively narrow limits (Fig. 2C), but the change in the intensity of luminescence of the whole nucleus was more marked and corresponded approximately to the change in the monoamine content in the hypothalamus following the injection of reserpine or nialamide (Fig. 2D). The mean intensity of luminescence of the cell thus cannot reflect the true change in the content of monoamines in the hypothalamus sufficiently accurately: the intensity of luminescence of the whole nucleus is the reliable criterion. Statistical analysis confirmed the difference between the quantitative indices of the functional state of the arcuate nucleus in each of the three series of experiments (nialamide, reserpine, intact animals).

A relatively simple and reliable histochemical method of detecting monoamines in the arcuate nucleus of the hypothalamus has thus been developed. It has been established that the principal histochemical index of the monoamine content is the total intensity of luminescence of all cells of the nucleus. It has also been shown that during a change in the monoamine content in the arcuate nucleus, there is a definite change in its cell composition. The change in the level of the monoamine content in the nucleus was mainly reflected by a change in the number of strongly and weakly luminescent cells, while the number of cells with an average intensity of luminescence (known as the basal cell) remained relatively unchanged. When the functional state of the arcuate nucleus of the hypothalamus is assessed, a differential approach to its cell composition is therefore necessary.

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