in the intensity of fluorescence, evidence of a fall in NA concentration, proportional to the degree of desympathization. After 4 months, in all experimental groups a significant decrease was observed in the changes in the parameter observed, caused by desympathization (Fig. 3).

Similarity of the trend in changes in template activity and NA content in the desympathized animals suggests that reduction of the latter may be due to depression of the transcription function of the genetic apparatus. Death of some sympathetic nerve cells at the periphery thus is reflected in the level of specific functioning of central NA neurons of LC, leading to a fall in the corresponding parameters both in the nucleus and in the cytoplasm. The absence of change in the type of nuclear staining of neurons with AS indicates the quantitative character, i.e., not connected with a change in spectrum of transcribed genes, of the structural changes in chromatin.

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IMMUNOHISTOCHEMICAL STUDY OF INSULIN-SENSITIVE CELLS OF THE MEDIAN EMINENCE OF THE HYPOTHALAMUS

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KEY WORDS: insulin; insulin-like growth factor; receptors; hypothalamus.

There is much experimental evidence that insulin affects physiological processes of the brain and of the body as a whole [7, 9]. Nevertheless, information on the distribution of insulin receptors in the CNS is contradictory [4, 13]. Besides insulin, the attention of research workers is currently drawn to a group of peptides known as insulin-like growth factors (ILGF), which can also activate growth processes in various tissues of the body. In turn, injection of ILGF-1 into the cerebrospinal fluid causes a decrease in the secretion of somatotrophic hormone in the adenohypophysis [12]. Considering the ability of these peptides to induce DNA synthesis in a culture of fibroblasts [10], it has been suggested that specific receptors exist to ILGF-1. Such receptors have now been discovered not only in actively proliferating cells, but also in brain homogenates from adult animals [6], the formation of which is largely complete during the prenatal period of development. On the basis of investigations [2] showing that the distribution of receptors for ILGF-1 in the brain is limited to the outer zone of the median eminence of the hypothalamus, it can be postulated that this peptide has a regulatory function in the CNS.

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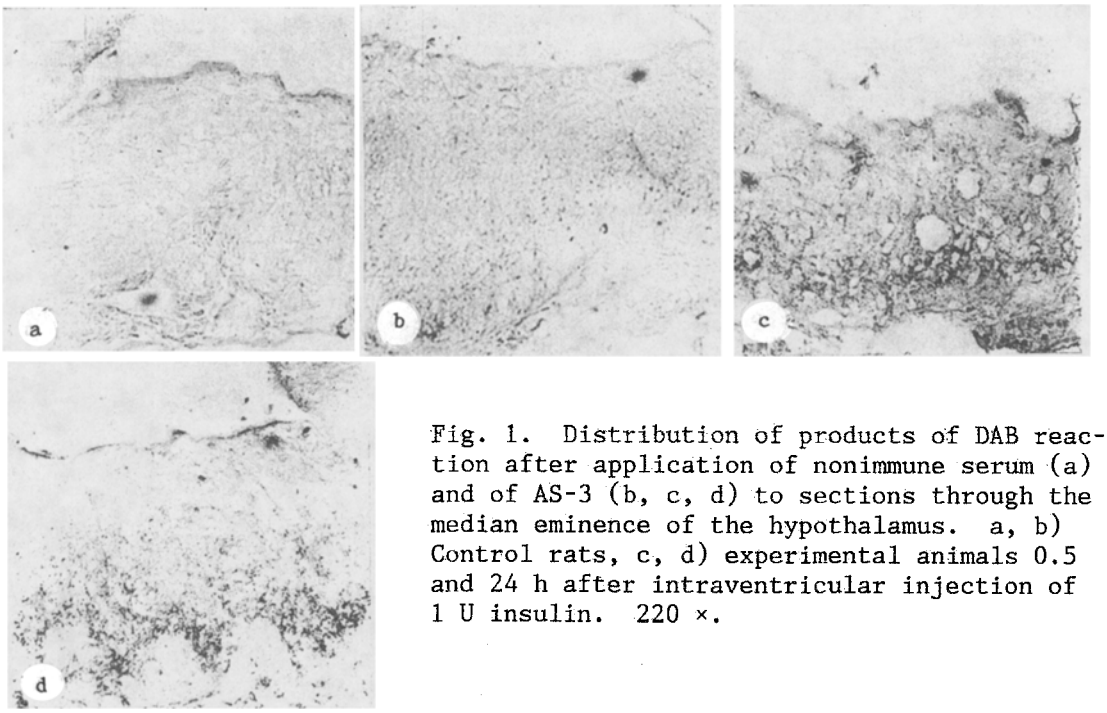

Fig. 1. Distribution of products of DAB reaction after application of nonimmune serum (a) and of AS-3 (b, c, d) to sections through the median eminence of the hypothalamus. a, b) Control rats, c, d) experimental animals 0.5 and 24 h after intraventricular injection of 1 U insulin. 220 ×.

The aim of this investigation was to study the ultrastructural localization of receptors for insulin and ILGF-1 with the aid of an indirect immunohistochemical method [8].

EXPERIMENTAL METHOD

Rabbit antisera (AS) in a dilution of 1:400 against synthetic peptides with an amino-acid sequence corresponding to regions 9-25 (AS-1), 48-77 (AS-3) of the α-subunit and region 736-760 (AS-31) of the β-subunit of the insulin receptor (IR) molecule from human placenta, were used as primary antisera. The sera were obtained from Professor Janaihara and co-workers [14]. Secondary antibodies consisted of Fab-fragments of donkey antirabbit immunoglobulins in a dilution of 1:200, conjugated with horseradish peroxidase ("Sigma VI," USA) by the periodate method. Preliminary experiments showed that AS-3 can interact not only with peripheral insulin receptors, but also with receptors for ILGF-1. In the present investigation, to discover neutral receptors for insulin and ILGF-1, we used the property of an increase in the number of central receptors on the cell surface after addition of insulin [5]. It can thus be expected that after intraventricular injection of insulin, an increase in the number of IR would be observed in the experimental animals on the surface of the cells, sufficient for their detection by immunohistochemical methods. Experiments were carried out on three experimental and three control 21-day-old Wistar rats and on five experimental and three control adult Wistar rats. Six 21-day-old rats (three experimental and three control) in which neurons of the arcuate neuclei of the hypothalamus had been destroyed by injections of sodium glutamate in the early postnatal period of development [3], also were used in the experiments; destruction of this kind leads to an increase in functional activity of the tanycytes of the median eminence of the hypothalamus [1]. Injection of 1 U of insulin in 10 μliters of solution (Humalin RV-100, Sinogi, Japan) was given stereotaxically into the right lateral cerebral ventricle of all the experimental rats, whereas control animals were given a corresponding injection of 10 μliters of physiological saline. The immunohistochemical activity of the sera was studied at the light-optical level, using the liver of the experimental animals as the positive control; the negative control was provided by brain and liver slices, treated with nonimmune rabbit sera. Ultrathin sections were prepared from frozen sections of the median eminence with high immunohistochemical reactivity, after appropriate treatment [8], and these were examined without any additional contrasting.

EXPERIMENTAL RESULTS

Light-optical analysis of frozen sections from control and experimental animals with all the AS tested showed that in the case of a positive diaminobenzidine (DAB) reaction in the

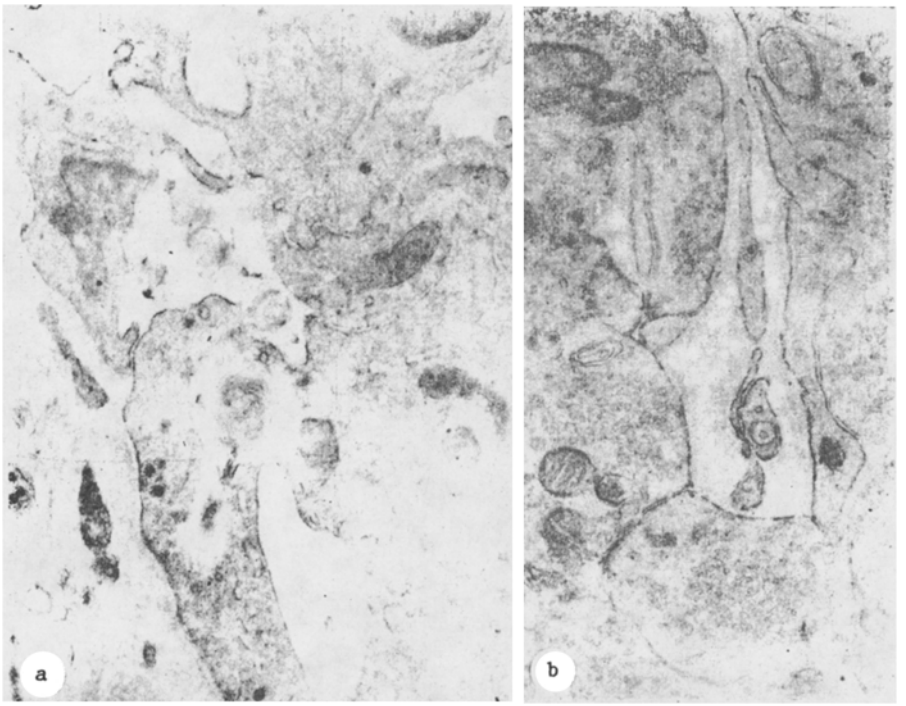

Fig. 2. Distribution of electron-dense products of immunohistochemical reaction with AS-3 in outer zone of median eminence of hypothalamus 0.5 h (a) and 24 h (b) after intraventricular injection of 1 U insulin. a) On membranes of processes of tanycytes. 30,000 ×; b) On membranes on axon endings and fine processes of tanycytes. 26,000 ×.

liver and cerebral vessels, products of the immunohistochemical reaction were found in the outer zone of the median eminence of the hypothalamus only with AS-3 (Fig. 1a, b). An increase in the intensity of the immunohistochemical reaction and widening of the region of its detection were found in the median eminence of the hypothalamus of the experimental rats 30 min after injection of insulin (Fig. 1c). Products of the DAB reaction in the form of small, radially arranged bands, 24 h after injection of insulin were distributed in the periventricular region of the median eminence and the arcuate nuclei of the hypothalamus (Fig. 1d). Electron-microscopic investigations of the median eminence of the hypothalamus of the control group of rats revealed electron-dense DAB reaction products on membranes of the rough endoplasmic reticulum and cytoplasmic membranes of fibroblasts located in the pericapillary space of the sinusoidal capillaries.

Ultrastructural study of sections through the median eminence of the experimental animals 30 days after intraventricular injection of 1 U insulin, treated with AS-3 showed, besides DAB reaction products localized as described above, similar products on the surface of the processes of tanycytes (Fig. 2a). Immunohistochemical reaction products were distributed in these structures on the surface membranes and ω-like formations of cytoplasmic membranes after intraventricular injection of insulin into the rats (Fig. 2a).

Electron-dense immunohistochemical reaction products also were located 24 h after intraventricular injection of insulin on cytoplasmic membranes of axon endings in the outer zone of the median eminence of the hypothalamus (Fig. 2b).

A fundamental property of central receptors for insulin and ILGF-1 is an increase in their number on the cell membranes in the presence of the ligand, whereas peripheral proteins of the same group are characterized by predominance of internalization processes [11]. The increase in the amount of receptor proteins on membranes of axon endings and processes of tanycytes in the present investigation after intraventricular injection of insulin confirms the fact that the proteins discovered belong to the central receptor family.

The primary structure of central receptor proteins for insulin and ILGF-1 has not yet been decoded. However, since in the present investigation on sections through the median eminence of the hypothalamus of experimental animals no distribution of products of the immunohistochemical reaction with AS-1 and AS-31 were found, which would reveal regions of the

α- and β-subunits of the peripheral insulin receptors of the liver and blood vessels, respectively, proteins localized with the aid of AS-3 corresponded more closely in their immunocytochemical properties to ILGF-1 than to insulin receptors.

By contrast with concepts put forward previously on the effect of insulin on functional activity of the neurosecretory cells of the median eminence through the intermediary of collaterals from a special type of insulin-sensitive neurons to central formations of the hypothalamus [13], the results of the present experiments are evidence of direct interaction of the peptides insulin and ILGF-1 with neurosecretory cells and ependymal elements of the median eminence of the hypothalamus, located on axon endings, and central receptors for ILGF-1. The results support the hypothesis that insulin and ILGF-1 peptides play a regulatory role in the CNS.

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DISTRIBUTION OF COLLAGEN OF TYPES III AND IV IN VILLI OF THE HUMAN PLACENTA

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Despite intensive biochemical reseach on the human placenta, little is still known about the distribution of the main components of the intercellular matrix in its structures. It was found previously that type IV collagen is located not only in the basement membrane, but also in the stroma of the villi of the human placenta [2]. To verify and clarify this observation an investigation was carried out with the use of immunofluorescence and immunoelectron-microscopic methods.

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