5. R. D. Nelson, P. G. Quie, and R. L. Simmons, J. Immunol., **115**, 11650 (1975).
6. S. H. Zigmond, Nature, **249**, 450 (1974).
7. S. H. Zigmund, J. Cell Biol., **75**, 606 (1977).

BINDING OF ^{3}H-DOPAMINE AND ^{3}H-QUINUCLIDINYL BENZYLATE BY TISSUE OF AUTONOMIC GANGLIA OF RATS OF DIFFERENT AGES

V. N. Yarygin, V. V. Glinkina, L. A. Knyazeva, F. N. Lagkueva, and A. S. Pylaev

UDC 616.834-008.935.835-092.9-07

KEY WORDS: receptors; ganglia; ganglion nodosum

Synaptic transmission in the autonomic ganglion, effected by acetylcholine, has several control systems. Some of the slow postsynaptic effects are mediated by muscarinic (M) acetylcholine receptors, some by receptors of transmission modulators. Of these, particular attention is being paid to the role of dopamine receptors as the most likely mediator secreted by the small intensively fluorescent cells of ganelia [1]. The system of M cholinergic reception in autonomic ganglia of adult animals and man have been characterized in detail with respect to parameters of binding of various ligands [6, 7]. Age changes in M cholinergic reception and dopamine reception for ganglion tissues have virtually not been studied.

The aim of the investigation was to study dependence of binding of labeled ligands with M acetcholine and dopamine receptors of ganglion cells on the period of postnatal development of the rat, chosen in accordance with the periods of formation, stable functioning, and age changes in the functional properties of nerve ganglia.

EXPERIMENTAL METHOD

Tissue of the inferior ganglion of the vagus nerve, the lumbar sympathetic ganglia, the great pelvic ganglion (in males), and the paracervical ganglion (in females) of rats aged 1, 7, 14, 28, and 60 days and 24-30 months, with 6 to 25 animals in the group, was used as the test object. Altogether 106 rats were used. Under pentobarbital anesthesia (40 mg/kg) the nerve ganglia of the animals were excised, weighed, and frozen. Serial frozen sections through the ganglia about 15 μm thick were mounted on slides and covered with gelatin. The mounted sections were kept for 24 h at −4°C and then preincubated in buffer solution for 15 min at room temperature. The incubation medium for binding of ^{3}H-quinuclidinyl benzylate (^{3}H-QNB, specific radioactivity 30 Ci/mmole) contained 0.9% NaCl in 15 mM phosphate buffer, pH 7.0. The duration of incubation was 2 h in darkness at 20°C and the concentration of the ligand was 1 nM. The medium for dopamine binding (^{3}H-DA, specific radioactivity 49.7 Ci/mmole) included 0.1% ascorbic acid, 0.1 pM pyrocatecol, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$ in 50 mM phosphate buffer, pH 7.7. The incubation time was 1 h at 20°C and the concentration of the ligand 1 nM. In preliminary experiments on membrane preprations of tissue from lumbar ganglia of the sympathetic trunk, the basic parameters of binding were established with the use of atropine (1 μM) and dopamine (1 μM) as displacing agents. The slides with the sections, after the binding procedure, were washed twice in cold buffer for 5 min each time, after which they were placed for a few seconds in distilled water, dried, and put into flasks with toluene scintillator. After 24 h the intensity of counting was recorded on an SL-30 scintillation counter. The results were expressed relative to wet weight of ganglion tissue. The results were subjected to statistical analysis by a standard program package.

Department of Biology and Department of Morphology, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 597-598, June, 1990. Original article submitted November 22, 1989.

TABLE 1. Level of Total Binding (cpm/mg tissue) of Tritium-Labeled Ligands of Rats Aged 2 Months ($M \pm m$)

Experimental conditions	Inferior ganglion of vagus nerve	Lumbar ganglion of sympathetic	Great pelvic ganglion	Paracervical ganglia
^{3}H-DA	128±5	152±9	146±17	135±21
^{3}H-QNB	451±28	534±35	472±33	468±36

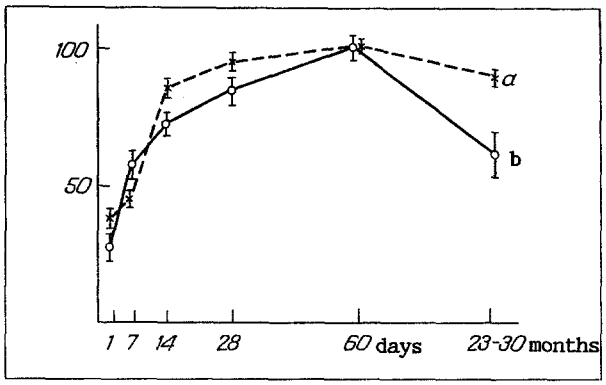

Fig. 1. Level of total binding of ^{3}H-DA (a) and ^{3}H-QNB (b) by tissue of autonomic ganglia of rats of different ages. Abscissa, animal's age (in months); ordinate, intensity of scintillation counting (in percent of maximal level).

EXPERIMENTAL RESULTS

Values of total binding of the ligand studied for the tissues of different ganglia of adult animals are given in Table 1. The level of binding of ^{3}H-QNB was more than three times higher than that for ^{3}H-DA, and this reflects to some degree the density of the corresponding receptors in the ganglion tissue. Meanwhile no significant differences could be found between the ganglia for reception of each labeled ligand. Values of binding of each of the ligands were virtually identical for homologous ganglia — the great pelvic and paracervical ganglia.

Age changes in binding of labeled QNB and DA demonstrate a marked rise of the chest parameter during the first two weeks of the animal's postnatal development, relative stability in the adult state, and a certain decline with age (Fig. 1). The closeness of the binding levels in different ganglia enabled these values to be averaged for each ligand for the tissues of all the nerve ganglia studied. Comparison of polygons of values for binding of ^{3}H-QNB and ^{3}H-DA leads to the conclusion that changes in M acetylcholine reception in the ganglia were less marked both in early ontogeny and, especially, in the zone of peak values of the rat's age.

The investigation showed that with respect to the basic parameters of binding of ^{3}H-QNB, tissue of the autonomic nerve ganglia closely resembles the tissues of several regions of the brain [8]. The level of binding of ^{3}H-QNB changes similarly during the periods of postnatal development in autonomic ganglia and in tissue of the whole brain [5], the cerebral cortex [4], and the cerebellum [3]. A very small decrease also was observed in the level of labeling with ^{3}H-QNB of the brain structures of rats toward the age of 31 months, compared with the state achieved at 4 months [2].

Comparison of the development of dopamine reception and M acetylcholine reception in autonomic ganglia may be evidence of the rather greater functional stability of the latter, for the range of age changes in the level of binding of ^{3}H-QNB was significantly less than for ^{3}H-DA. However, the distinct parallel between the early postnatal development of the two receptor systems studied must be noted. The sharpest changes during the development of both M acetylcholine and dopamine reception evidently take place in the perinatal period and during the first two postnatal weeks, reflecting general rules for the differentiation of ganglion cells.

LITERATURE CITED

1. J. H. Ashe and B. Libet, Brain Res., **217**, 93 (1981).
2. A. Biegon, R. Duvdevali, V. Greenberger, and M. Segal, J. Neurochem., **51**, 1381 (1988).
3. J. M. East and J. R. Dutton, J. Neurochem., **34**, 657 (1980).
4. R. Q. Evans, M. Watson, H. I. Jamamura, and W. R. Roeske, J. Pharmacol. Exp. Ther., **235**, 612 (1985).
5. E.P. Fiedler, if.J. Marks, and A.C. Collins, J. Neurochem., 49, 983 (1987).
6. H. I. Jamamura, M. Watson, J. K. Wamsley, et al., Life Sci., **35**, 753 (1984).
7. L. M. Koval, V. A. Derkach, A. A. Selyanko, et al., J. Auton Nerv. Syst., **6**, 37 (1982).
8. J. O. Rinne, Brain Res., **404**, 162 (1987).

EFFECT OF DIRECT EXPOSURE TO LOW-INTENSITY LASER RADIATION ON MORPHOLOGY AND FUNCTION OF THE ZONA FASCICULATA OF THE ALBINO RAT ADRENAL CORTEX

V. I. Kozlov, M. K. Pugachev, and O. A. Terman

UDC 612.453.014.46:615.849.19].019.086

KEY WORDS: blood capillaries; adrenal cortex; laser radiation; microcirculation

The action of a laser on biological tissue [2], and of skin wound surfaces [3] is known to promote healing of the damage tissue by intensifying repair processes. It has also been shown that the microcirculation in organs is increased under the influence of low-intensity laser irradiation [4]. It has been shown experimentally that with an increase in the rate of flow of the Ringer's solution used to perfuse the vascular system of the adrenal gland the function of its cortex is enhanced, and this is accompanied by corresponding morphological changes and by the release of corticosteroid hormones into the blood stream [7, 8]. These results served as the basis for the suggestion that local application of laser radiation to the adrenals would stimulate the microcirculation in them and would also bring about corresponding morphological and functional changes in the adrenal cortex.

The aim of this investigation was to study structural and functional changes in the zona fasciculata of the adrenal cortex in response to the direct action of low-intensity laser radiation on them.

EXPERIMENTAL METHOD

The zona fasciculata of the adrenal cortex of 30 noninbred female albino rats weighing about 200 g was studied. In the experiments of series I, under hexobarbital anesthesia, laparotomy was performed on 20 of the animals and the region of the left adrenal gland was subjected to superficial irradiation from a helium–neon laser (LG-75) for 30 min (total dose 11 J/cm^2, λ = 363 nm). The right adrenal was left intact and served as the control. In series II, 10 rats were anesthetized and subjected to direct irradiation of one adrenal gland from the dorsal aspect by means of an "Uzor" gallium arsenide laser for 8 min 32 sec (total dose $3.5 \cdot 10^{-2}$ J/cm^2, frequency 80 Hz, λ = 890 nm). The adrenals were fixed in a mixture of chromium salts and formalin. After fixation the gland was divided into two equal parts. One half was used to prepare frozen sections, 30 μm thick, in which the degree of congestion of the capillaries was deterlnined by the chromium method, and frozen sections 20 μm thick, in which

Medico-Biological Department, Research Institute of Laser Surgery, Moscow. Department of Histology, Smolensk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. V. Kupriyanov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 6, pp. 598-600, June, 1990. Original article submitted June 20, 1989.

