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AUTORADIOGRAPHIC STUDY OF ONTOGENY OF CEREBRAL CORTICAL IMIPRAMINE RECEPTORS IN RATS

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UDC 612.825.014.467:615.214.32].088.6

KEY WORDS: imipramine; cerebral cortex; receptors; ontogeny.

Imipramine receptors, found initially in the rat brain, have subsequently been discovered in human brain tissue and classed with the "drug receptors" [2]. Their number has been determined in different parts of the brain and the highest density of imipramine receptors has been shown to be in the hypothalamic zone and cerebral cortex [3]. The need for an ontogenetic approach to the study of drug receptors of this type is determined by the increasingly wide use of imipramine in the clinical treatment of depressive states.

This paper describes an analysis of the number and distribution of imipramine receptors in the rat cerebral cortex during normal ontogeny and antenatal exposure to imipramine.

EXPERIMENTAL METHOD

Wistar rats were used. Imipramine was injected subcutaneously in a dose of 5 mg/kg into pregnant rats on the 17th, 18th, and 19th days of embryonic development. Three age groups were studied: 19th day of embryonic development and 3rd and 14th days of postnatal development (three animals in each group). Under pentobarbital anesthesia (60 mg/kg) the rats were perfused through the left ventricle with 0.1% paraformaldehyde solution in 0.1 M phosphate buffer, pH 7.4. This procedure does not affect the binding level, while improving the histologic integrity of the preparation a little [5]. After removal of the brain, frontal slices were cut to a thickness of 2-3 mm, frozen in liquid nitrogen, and placed on the stage of a freezing microscope. Frozen sections about 25 μ thick were mounted on gelatin-coated slides and dried at 4°C. The finished preparations were kept at −20°C. A whole series of preparations was incubated simultaneously in medium containing 50 mM Tris-HCl (pH 7.4), 100 mM NaCl, 5 mM KCl, and 10 mM [^{3}H]imipramine (814 Tbq/millimole). The sections were incubated for 2 h at room temperature, washed with cold (4°C) buffer, and dried at 4°C. Strips of lavsan film, coated with emulsion, were glued to the slides with the sections, covered with teflon film

Department of Morphology and Cytology, 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. 99, No. 6, pp. 752-754, June, 1985. Original article submitted August 17, 1984.

 0007-4888/85/9906-0820 $09.50

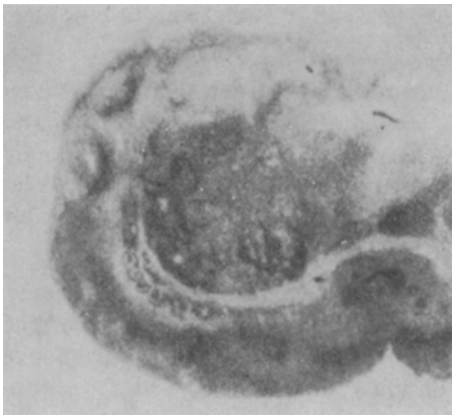

Fig. 1. Autoradiograph of frontal section of rat brain.

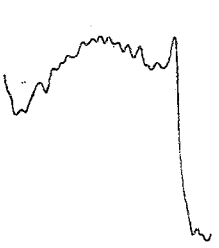

Fig. 2

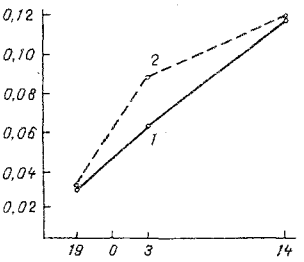

Fig. 3

Fig. 2. Distribution of optical density in autoradiograph of parietal region of rat cerebral cortex in direction from surface (right) to deep brain structures.

Fig. 3. Mean values of optical density of autoradiographs of rat cerebral cortex in pre- and postnatal period under normal conditions (1) and with antenatal exposure to imipramine (2). Abscissa, time of embryonic and fetal development (in days); ordinate, optical density.

and another slide, and fixed together by a clamp. The duration of exposure of the preparations was 7-12 months. After exposure the second slide and the teflon film were removed, the film coated with emulsion was folded back, and the photographic layer was developed for 5 min in D-19 developer. The sections were then fixed in hypo solution and washed. They were then fixed in Carnoy's fluid, stained with pyronine, dehydrated, and mounted in synthetic resin, joining them with the autograft. Autoradiographs intended for quantitative analysis were processed separately from the preparation. The intensity of labeling was estimated by measuring the optical density of the autograft. The distribution of labeling density by thickness of the cerebral cortex was established by scanning with the probe of the MF-4 microphotometer in a direction perpendicular to the cortical surface.

EXPERIMENTAL RESULTS

Frozen sections through the cerebral hemispheres of the rats, after incorporating [^{3}H]imipramine, gave autoradiographs of moderate optical density after the chosen exposure times (Fig. 1).

Visual analysis of the autoradiographs shows heterogeneity of labeling of the brain structures, including the different zones of the cortex. Besides regions with a high density of labeling (cortex, some subcortical structures) there is also a series of zones with background values of intensity of labeling. Repetitive struc-

tures were visible in the cortex, reflecting regular variations in labeling density along the cortical layers (Fig. 1).

The mean optical density of the autoradiographs of the adult rat cerebral cortex was 0.12 ± 0.02. A scanogram, showing the distribution of optical density of the autoradiograph along the layers of the parietal cortex of an adult rat is shown in Fig. 2. Marked variability of the densitograms of these regions between animals in the group, whereas the general character of labeling was preserved within each cortical zone, was observed. The density of distribution of imipramine receptors rose to an ill-defined maximum at the level of cortical layers IV-V (Fig. 2).

Binding of labeled imipramine by preparations of the cerebral cortex was observed in embryos as early as at the 19th day of intrauterine development. After 3 and 14 days of postnatal development a gradual increase was observed in the mean optical density recorded in autoradiographs (Fig. 3). Three injections of imipramine into pregnant rats, given on the 16th, 17th, and 18th days of pregnancy, led to a marked rise in labeling intensity of the brain tissue of the 3-day-old rats. At earlier and later stages of development the level of [^{3}H]imipramine binding did not differ significantly from that in intact animals of the corresponding age (Fig. 3).

By this method it is thus possible to reveal not only the average level of binding of labeled imipramine, but also the pattern of distribution of imipramine receptors in the brain tissue. The results of the present investigation confirm data showing a relatively high content of imipramine receptors in the cerebral cortex [3]. Analysis of the distribution of serotonin receptors [1] gave results closely similar to those obtained in the present investigation as regards the labeling density of the cortical layers.

The discovery of a raised level of binding of labeled imipramine by preparations of the cerebral cortex of animals aged 3 days, born to rats receiving imipramine on the 16th to the 18th days of pregnancy, is evidence of marked stimulation of receptor formation by injections of imipramine. Meanwhile the effect of stimulation of imipramine binding by the experimental scheme chosen was transient, possible evidence of the relative stability of the definitive level of imipramine reception in cortical tissue.

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