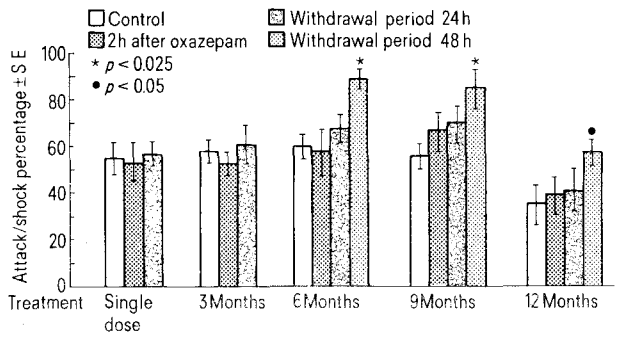
tween 11.00 h and 13.00 h. Animals were divided into groups: 1. 15 rats were treated with a single dose of oxazepam and aggressive behaviour was measured 2 and 24 h later. 2. 9 rats treated chronically with oxazepam were subjected to foot shock 2, 24 and 48 h after the last injection of the drug applied 3, 6, 9 or 12 month.

Corresponding control groups consisted of the same number of animals injected with 0.9% NaCl solution i.p. were handled identically. The extent of foot-shock-induced behaviour was calculated as an attack (shock percentage / the number of attacks divided by the number of shocks administered × 100) according to EICHELMAN[6]. Statistical significance was calculated using Student's *t*-test.



Effect of withdrawal of oxazepam (5 mg/kg i. p.) after the long-term treatment on the foot-shock-induced aggression in rats.

Results. Oxazepam, in a dose of 5 mg/kg i.p. administered only once or 6 times weekly for 3, 6, 9 or 12 months, had no influence on the shock-induced-aggressive behaviour 2 and 24 h after drug injection. The withdrawal of the drug for 48 h in rats treated for half 1 year and up to the one year caused an evident increase of foot-shock-induced aggression of rats (Figure).

Discussion. The antiaggressive action of oxazepam depends upon the used experimental model of aggression. Oxazepam did not reduce aggression elicited by grouping of male mice[7], but suppressed this phenomenon induced in mice by isolation[8], or by administration of D,L-DOPA[9]. Used in our experiments, shock-induced aggression is the most common model for irritable aggression. Oxazepam applied chronically did not affect this type of aggression in rats. The phenomenon which we observed of evident increase of foot-shock-induced aggression of rats during the period of withdrawal of oxazepam after the long-term treatment with the drug, we interprete as a sign of abstinence. This suggests that long-term treatment with oxazepam causes dependence on the drug in rats.

[7] K. A. FOX, E. J. GUERRIERO, D. C. ZANGHI, Pharmac. Res. Commun. *6*, 301 (1974).
[8] L. VALZELLI, in *The Benzodiazepines* (Eds. S. GARATTINI, E. MUSSINI and L. O. RANDALL; Raven Press, New York 1973), p. 405.
[9] H. C. Y. YEN, M. H. KATZ and S. KROP, Toxic. appl. Pharmac. *17*, 597 (1970).

Distribution of H^3-Dimetacrine in Rat Cerebral Cortex by Electron Microscopic Autoradiography

R. ISHITANI and T. IWAMOTO[1]

Department of Pharmacology, Faculty of Pharmaceutical Sciences, Josai University, 1076 Tawame, Sakado, Saitama 350–02 (Japan), 23 February 1976.

Summary. The cellular distribution of H^3-dimetacrine in rat cerebral cortex was studied by electron microscopic autoradiography. A considerable proportion of autoradiographic grains (40.6%) was located over the synaptic areas, while the other structures (i.e., dendrites, axons, glial and neuronal cells) contained less autoradiographic activity.

Dimetacrine[2], 10-[3-(dimethylamino)propyl]-9,9-dimethyl-acridan, is a tricyclic compound with a hexagonal ring instead of the heptagonal ring common to most antidepressants. Clinical superiorities of this drug in the treatment of depressive states and other psychic disorders have been well established[3]. Recent works[4,5] on the subcellular fractionation of brain tissue permitted the isolation of pinched-off nerve endings or synaptosomes, and it has been observed that the bound form of putative central transmitters (acetylcholine, noradrenaline, 5-hydroxytryptamine and dopamine) and the enzymes related to those transmitters are highly concentrated in the synaptosomal fraction. Previous paper[6] demonstrated that the highest concentration of dimetacrine consistently occurred in the synaptosomes-rich fraction. However, biochemical procedures used in the previous study were not sufficient to explain the subcellular distribution of dimetacrine in the central nervous system because of the contamination of each of the subcellular organelles. The objective of the present paper is to visualize the distribution of dimetacrine in undisrupted tissue by electron microscopic autoradiography.

Materials and methods. 3-H^3-dimetacrine (1.92 mCi/mg) was prepared from 3-bromo-dimetacrine. Male Wistar rats (200–250 g) were given 960 μCi/500 μg of H^3-dimetacrine by the direct lateral intraventricular injection method of NOBLE et al.[7]. The animals were sacrificed by decapitation 1 h after administration and cerebral cortices were fixed in cold 4% glutaraldehyde (MILLONIG's buf-

[1] Acknowledgments. The authors wish to thank Siegfried AG., Switzerland, for the generous supply of 3-bromo-dimetacrine. Dimetacrine was kindly donated by Nippon Chemiphar Co., Ltd., Tokyo, Japan.
[2] I. MOLNAR and TH. WAGNER-JAUREGG, Helv. chim. Acta *48*, 1782 (1965).
[3] S. TAEN and W. PÖLDINGER, Schweiz. med. Wschr. *96*, 1616 (1966).
[4] V. P. WHITTAKER, Progr. Biophys. molec. Biol. *15*, 39 (1965).
[5] E. DE ROBERTIS, Science *156*, 907 (1967).
[6] R. ISHITANI, T. SATOH, T. SUGA and H. KITAGAWA, Jap. J. Pharmac. *22*, 313 (1972).
[7] E. P. NOBLE, R. J. WURTMAN and J. AXELROD, Life Sci. *6*, 281 (1967).

fer[8]) for 2 h. The samples were postfixed for 1.5 h in cold 1.5% osmium tetroxide (CAULFIELD's buffer[9]), dehydrated in graded ethanols and embedded in Epoxy resin of LUFT[10]. For electron microscopic autoradiography, the method of SALPETER and BACHMANN[11] was used. Thin sections (pale gold to silver) were picked up on collodion-coated slides, double stained with uranyl acetate and lead monoxide, lightly coated with carbon and dipped in Sakura NR-H2 emulsion. After 10 to 20 weeks exposure at 5 °C, they were developed in Konidol-X at 15 °C for 4 min and examined with a Hitachi 12 electron microscope.

The cellular distribution of autoradiographic activity was quantitatively surveyed by the method of AGHAJANIAN and BLOOM[12]. The resulting distribution pattern was expressed as the percentage of total grains which occurred over particular cellular loci (e.g., synaptic areas, dendrites, axons, etc.). All of the grains which were applied to the cellular distribution study, were confirmed as the silver grains by an electron probe X-ray microanalysis[13]. By the calculation method of BACHMANN and SALPETER[14], the combination of Sakura NR-H2 emulsion (diameter of the silver halide crystal 800 Å, size of the developed silver grain 3050 Å, thickness of emulsion 960 Å) with thin sections of tissue permits a theoretical tissue-to-grain resolution of about 1690 Å.

[8] G. MILLONIG, Fifth Int. Congr. for Electron Microscopy (Academic Press, New York 1962), vol. 1, p. 8.
[9] J. B. CAULFIELD, J. biophys. biochem. Cytol. *3*, 827 (1957).
[10] J. H. LUFT, J. biophys. biochem. Cytol. *9*, 409 (1961).
[11] M. M. SALPETER and L. BACHMANN, J. cell. Biol. *22*, 469 (1964).
[12] G. K. AGHAJANIAN and F. E. BLOOM, J. Pharmac. exp. Ther. *156*, 23 (1967).
[13] R. ISHITANI, in preparation.
[14] L. BACHMANN and M. M. SALPETER, Lab. Invest. *14*, 1041 (1965).

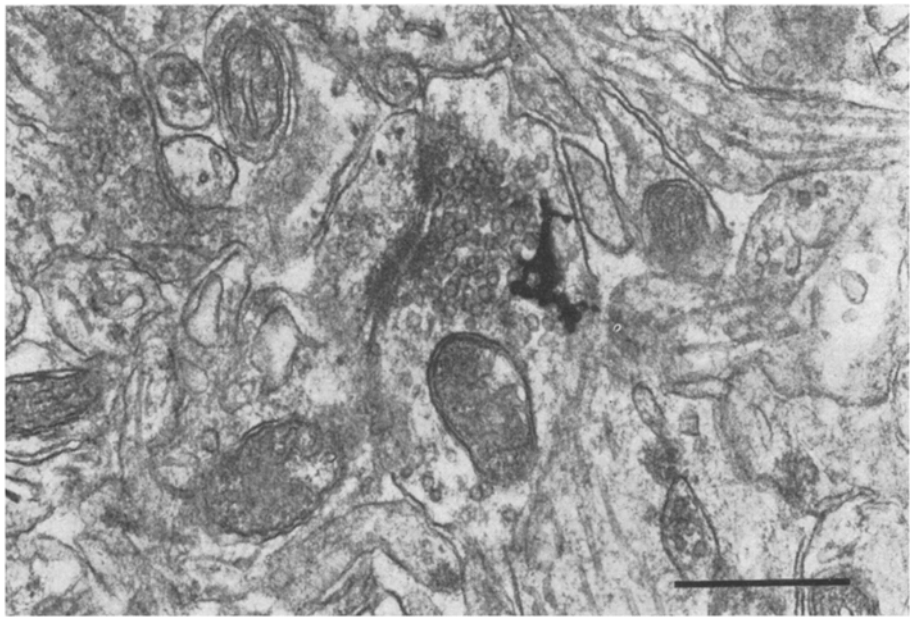


Fig. 1. Autoradiogram of synaptic area in cerebral cortex after lateral intraventricular injection of H^3-dimetacrine. A cluster of grains is seen over the pre-synaptic process. Bar equals 0.5 μm.

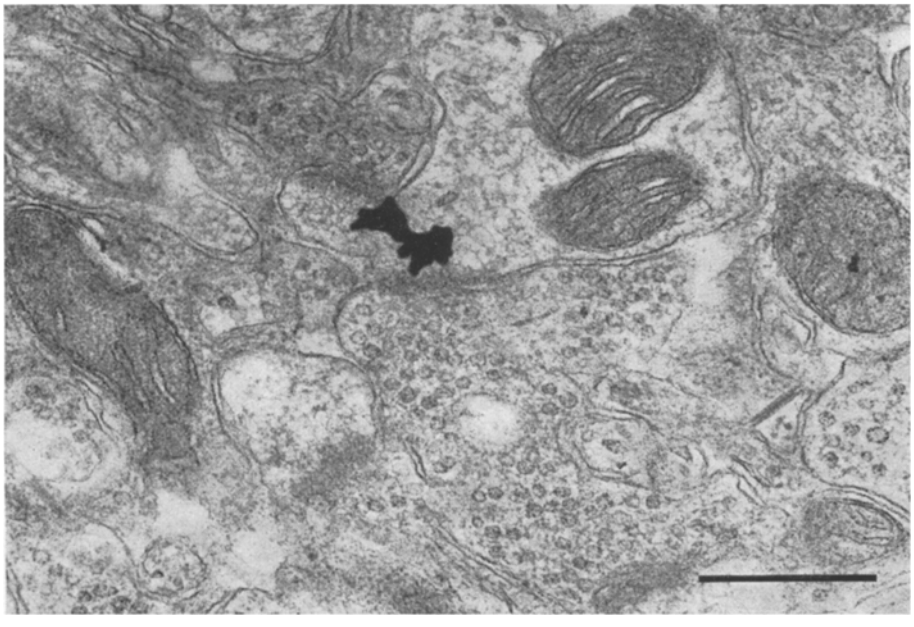


Fig. 2. Autoradiogram from cerebral cortex after lateral intraventricular injection of H^3-dimetacrine. Grain (dense, irregular coil) can be seen over the post-synaptic process. Bar equals 0.5 μm.

Cellular distribution of autoradiographic grains in the cerebral cortex after intraventricular injection of H^3-dimetacrine

Cellular components	No. of grains	Percentage
Synaptic areas	130	40.6
Dendrites	60	18.8
Axons	52	16.2
Glial cells[a]	38	11.9
Neuronal cells[a]	26	8.1
Unknown	14	4.4
Total	320	

The synaptic areas contain the pre-synaptic processes, synaptic junctions and post-synaptic processes. [a]Both components include each of the processes.

Results and discussion. To determine the elution of H^3-dimetacrine during the histological processing, radioactivity in the various processing fluids and in an alkaline hydrolysate of the final tissue blocks was counted in a tT-21 emulsion phosphor[15]. Only 23.4% of radioactivity was eluted during preparative steps. We are therefore studying the distribution of a large proportion of the total radioactivity taken up by the tissue. The retained radioactivity presumably represents the firmly bound H^3-dimetacrine, since in the previous study[6] about 76% of the intraventricular injected H^3-dimetacrine was recovered in the crude mitochondrial fraction as the unchanged bound form. However, the exact chemical nature of the beta-emitter in the final autoradiograph cannot be known with complete certainty.

The cellular distribution of autoradiographic activity was surveyed by consecutively tabulating the location of developed grains in random grid squares. The results are based upon several hundred electron micrographs of specimens, prepared after varying periods of autoradiographic exposure but otherwise handled identically. In agreement with previous biochemical results[6], i.e., synaptosomes-rich fraction contained 46.8% of radioactivity, 40.6% of autoradiographic grains in the cerebral cortex were located over the synaptic areas. Developed grains were also scattered over the other structures, such as dendrites, axons, glial and neuronal cells with the following percentage: 18.8, 16.2, 11.9 and 8.1%, respectively. In addition, about 12% of total synaptic areas examined contained the deposits of silver grains. Within synaptic areas, the developed grains were located over the pre-synaptic processes and post-synaptic processes or synaptic junctions (Figures 1 and 2). From the preliminary grain analysis, it was found that about 77% of total grains which were present within synaptic areas, were located over the pre-synaptic processes. However, the resolution of the present autoradiographic method is not sufficient to distinguish between these ultrastructural components as the possible sources of the emission. For the same reason, it was also impossible to distinguish between the pre-synaptic membranes and synaptic vesicles (or matrix).

The present results demonstrate that the autoradiographic grains show a higher probability of association with the synaptic areas. In a preliminary study[16], we found that H^3-dimetacrine was specifically bound to synaptosomes similar to H^3-imipramine[17], and furthermore this drug inhibited the 5-hydroxytryptamine binding to synaptosomes as well as desmethylimipramine[18]. These observations indicate that dimetacrine is definitely associated with the nerve endings-function. It is plausible to presume that those specific interactions may affect synaptic transmission and thus bring about the remarkable physiological and behavioral effects of this drug.

[15] M. S. PATTERSON and R. C. GREENE, Analyt. Chem. *37*, 854 (1965).
[16] R. ISHITANI, R. SAITO and T. IWAMOTO, Jap. J. Pharmac. Suppl. *24*, 98 (1974).
[17] P. F. HUNT, M. H. KANNENGIESSER and J. P. RAYNAUD, Biochem. Pharmac. *24*, 681 (1975).
[18] S. FISZER and E. DE ROBERTIS, J. Neurochem. *16*, 1201 (1969).

Changes in the Concentration of Adenohypophyseal Prolactin and Morphological Manifestations in the Adenohypophysis of Lactating Rats after Administration of D-6-Methyl-8-[β-isopropylaminoethyl] ergoline-I

P. KREJČÍ, O. MARHAN, M. AUŠKOVÁ, K. ŘEŽÁBEK, J. BÍLEK, M. SEMONSKÝ[1]

Institute of Physiology and Genetics of Animals of the Czechoslovak Academy of Sciences, Praha-Uhříněves (Czechoslovakia); and Research Institute for Pharmacy and Biochemistry, Kourimska 17, CS-130 60 Praha 3 (Czechoslovakia), 1 March 1976.

Summary. The four days administration of D-6-methyl-8-[β-isopropylaminoethyl]ergoline-I (VÚFB-10726) to nursing rats decreases adenohypophyseal prolactin as determined with disc electrophoresis, and produces changes in the histological appearance of adenohypophyses, which indicate the inhibition of prolaction production and secretion.

Many derivatives of ergoline and ergolene, for example, ergotoxine-type alkaloids, 2-bromo-α-ergokryptine, D-6-methyl-8-cyanomethylergoline-I, D-6-methyl-8-ergoline-I-ylacetamide, and others, inhibit prolactin release. With a view to a prospective therapeutic exploitation of this effect in both clinical and veterinary medicine, the search continues for new compounds with better pharmacological properties. In this program, there was synthesized, among other compounds, the D-6-methyl-8-[β-isopropylaminoethyl]ergoline-I *bis*-(hydrogen maleate), compound VUFB-10726. This compound, showing high inhibitory activity on prolactin-dependent processes, was investigated for its effects on hypophyseal prolactin.

Material and methods. The experiments were performed in lactating rats (Wistar strain, Konárovice breed, 200–220 g, 6 young with each mother). The compound VUFB-10726 was administered by gastric tube on the 4th to 7th day after delivery in daily doses of 0.05, 0.5 or 1.0 mg/kg in 5 ml/kg. The controls received corresponding volumes of water. Throughout the duration of the experiment, the lactation was observed. On the 5th day the rats were killed by decapitation 10 h after weaning of the young.

[1] The authors thank Dr. I. J. SKÁLA, Mrs. J. BOUBÍNOVÁ, Mrs. J. EMANUELOVÁ and Mrs. V. MAŠITOVÁ for technical assistence.