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.

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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

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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.

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Group	n	Prolactin level (OD±SE)	Adenohypophyseal prolactin level IU/mg	μg/mg	Weight of adenohypophysis (mg±SE)
Control	29	0.226±0.007	0.378	34.4	8.03±0.35
V FB-10726					
0.05 mg	14	0.124±0.015[a]	0.275	25.0	7.57±0.41
0.5 mg	21	0.096±0.007[a]	0.242	22.0	6.19±0.37[a]
1.0 mg	17	0.072±0.006[a]	0.209	19.0	6.00±0.56[a]

[a] Statistically significant difference from control value (p 0,95).
OD, optical density.

The adenohypophyseal prolactin levels were estimated by the disk electrophoretic method[2,3]. The prolactin zones were subjected to photodensitometry (Densitometer TLD-100, Vitatron). The standard curve was obtained from measurements of samples containing 10–40 μg of rat prolactin NIAMDD-RP-1 (11 IU/mg)[4].

For histological examinations of the hypophyses, samples were fixed with formol-mercuric chloride, sliced to sections 3–4 μm thick and stained according to El Etreby[5]. This method makes possible a discrimination of eosinophillic elements from the other as well as the cell type of I-cells, producing STH (accepting yellow colour), and the cell type of II-cells, producing LTH (accepting orange to purple colour).

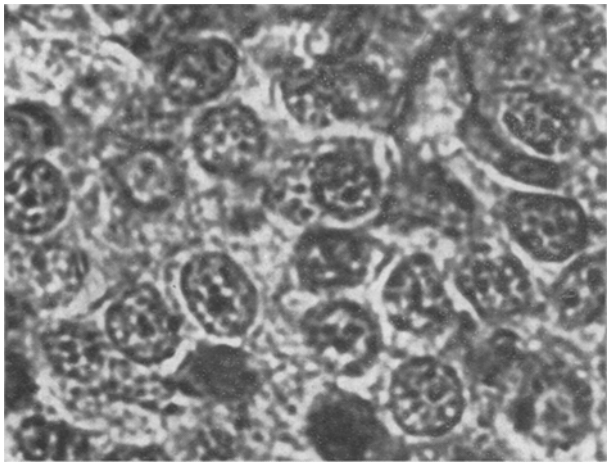

Fig. 1. Median section of adenohypophysis of lactating rat. Control group.

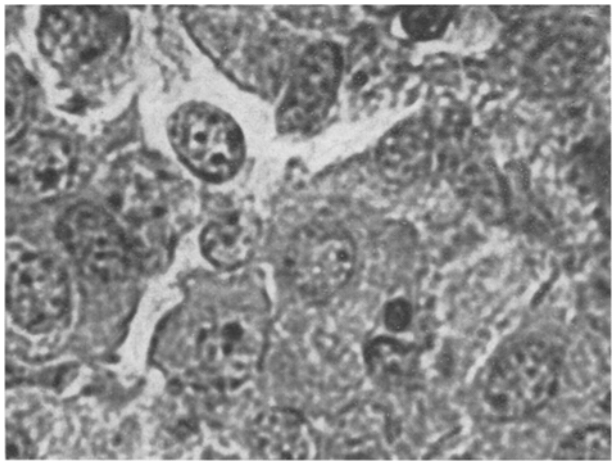

Fig. 2. Median section of adenohypophysis of lactating rat after administration of VUFB-10726 dosed 4×1.0 mg/kg. Evident degranulation in LTH-producing cells.

Results and discussion. The adenohypophyseal prolactin levels found after administration of the compound VUFB-10726 are presented in the Table. After all applied doses of the compound the prolactin content of the pituitories was reduced statistically significantly. After the higher daily dosages of 0.5 and 1.0 mg/kg, the weights of the adenohypophyses decreased statistically significantly.

In the histological preparations of the adenohypophyses there is evidence in rats treated with the compound of a decrease in the specific stainability of the cytoplasm of the LTH-producing cells with cytoplasmic degranulation (Figures 1 and 2). The reduced granulation indicates a reduced hormone-producing function of the cells.

The control of lactation showed that this was reduced by 50% with the lowest dose and fully stopped with the two higher doses.

From the results reported, it ensues that the ergoline derivative tested, administered repetitively, reduces both the release of prolactin and its production in the hypophysis and decrease in weight of the adenohypophyses.

Similar decreases in the hypophyseal prolactin contents were also observed in our tests with repetitive administration of D-6-methyl-8-ergoline-I-ylacetamide (VUFB-6683)[6] and of N-(D-6-methyl-8-isoergoline-I-yl)-N′,N′-diethylurea (VUFB-6638)[7].

These results are not contradictory to those described by Flückiger and Kovács[8], who found in acute experiment that a single dose of 2-bromo-α-ergokryptine inhibited the acute decrease in hypophyseal prolactin, physiologically evoked in lactating rats during suckling of milk by the puppies.

Compounds from this chemical group evidently interfer with the function of neuroendocrine mechanisms which control the prolactin secretion so that initially the hypophyseal prolactin release due to a physiological stimulus is suppressed, and later, after a longer treatment, the hypophyseal production and storage of prolactin are reduced.

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