EFFECT OF A BENZOQUINOLIZINE (RO 4-1284) ON GRANULATED VESICLES OF THE RAT BRAIN

A. HALARIS, E. RÜTHER and N. MATUSSEK
Max-Planck-Institut für Psychiatrie,
Deutsche Forschungsanstalt für Psychiatrie, Biochemische Abteilung, München

Received August 11, 1966

Summary. Electron microscopical studies dealt with the reversible action of Ro4-1284 on norepinephrine and serotonin with respect to the granulated vesicles of the ventromedial hypothalamus and the pineal body of the rat. In the normal rat various types of vesicles were classified according to their size and form. In the hypothalamus two types of granulated vesicles were distinguished from the ungranulated ones. In the pineal gland granular and agranular vesicles have the same diameter; in addition to them large granular vesicles were also to be found.

After the application of Ro4-1284 the same types of vesicles appear, which are almost totally deprived of their osmiophilic content. Homogeneous vesicles partially containing membranous bodies remain. The results were interpreted as a visible depletion of norepinephrine and possibly serotonin from their pools which leave behind elements capable of becoming functionally active after the action of the depleting agent.

Introduction

In recent years biochemical and electron microscopical studies have indicated that osmiophilic granules in cells and nerve endings of specific brain regions contain catecholamines and also probably serotonin. (P. de Iraldi et al., 1963 and 1963a; S. Ishii et al., 1963; Y. Sano and T. Mashimo, 1966).

Unlike the acetylcholine containing vesicles, these granules cannot be found after the depleting of norepinephrine and serotonin by reserpine (P. de Iraldi et E. de Robertis, 1963; T. Hökfelt, 1966; I. Bak, 1965; F. Clementi, 1965). Similar to reserpine the benzoquinolizine derivative Ro 4-1284 depletes norepinephrine and serotonin from brain tissue.

In contrast to the long-lasting action of reserpine Ro 4-1284 is a short acting drug (F. Clementi, 1965; A. Pletscher, 1962). Our experiments deal with the morphological alterations detected in the osmiophilic granules of the central nervous system after norepinephrine and serotonin are depleted by Ro 4-1284.

Materials and Methods

Ten male Sprague-Dawley rats weighing 180—230 g were used to the experiment. Half received Ro4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydrobenzo-(a)-quinolizine, 15 mg/kg) intraperitoneally, while the rest were used as control animals. The animals were decapitated two hours after injection. After removal of the pineal body from the brain both were immediately immersed in glutaraldehyde at 0°C. The ventromedial part of the hypothalamus was then extirpated under the dissecting microscope and was additionally fixed with 1% osmic acid. The tissue was dehydrated in ethanol and embedded in Westopal. The sections were cut with an LKB microtome stained with lead citrate according to Reynolds (1963) and examined with a Zeiss electron microscope.
Results

Hypothalamus

We investigated the ventromedial part of the hypothalamus, limited dorsally
by the fornix and laterally by the tractus mamillo-thalamicus. Vesicular elements
were found in the axons and axonterminal structures. Three types of vesicles
were distinguishable in our control animals according to their diameter and con-
tent (Fig. 1).

<table>
<thead>
<tr>
<th>Type</th>
<th>Control</th>
<th>Hypothalamus</th>
<th>RO 4-1284</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>200-400 Å</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>●</td>
<td>560-830 Å</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>●</td>
<td>1200 Å</td>
<td></td>
</tr>
</tbody>
</table>

**Type A:** Vesicles with a diameter of 200—400 Å with a clear homogeneous
content.

**Type B:** Vesicles with a diameter of 550—830 Å whose content was fully or
partially occupied with a dense core placed either eccentrically or concentrically.

**Type C:** Vesicles with a diameter of about 1200 Å whose content resembled
that of type B (Fig. 2).

Two hours after the application of Ro 4-1284 (15 mg/kg) exactly the same
diameter of vesicle could be detected as in the control animals (Fig. 1). Only the
content of type B and C had changed. Entirely empty vesicles of type B were
often seen, and sometimes instead of the osmiophilic material there was a small
membranous ring placed concentrically or eccentrically within the lumen of the
vesicles (Fig. 3).

Pineal Gland

Besides the above described part of the hypothalamus we investigated the
pineal gland which was deprived of all neighboring tissue and embedded in toto.
In the sections of the pineal gland we found vesicular elements appearing in two
morphologically similar but histologically different structures, which are some-
times difficult to distinguish. In the pericapillar as well as in the extracellular
areas often lie axonterminals, of orthosympathetic origin, which contain mito-
chondria and neurofilaments and are sometimes surrounded by the cytoplasm of