Dopamine and Adenosinetriphosphatase Changes in the Corpus Striatum of the Rat

The corpus striatum of the rat contains significant amounts of the catecholamine dopamine (DA), while the presence of other biogenic amines (BAs) in this area is slight[1,2]. DA has been related to corpus striatum function in a number of animals and in man as well[1,3,4-6].

The enzyme adenosinetriphosphatase (ATPase) bears a constant relationship to norepinephrine (NE) containing cells in the adrenal medulla of a number of species of animals possessing NE cells[7-9]. The NE cells of the adrenal medulla can be at times typified as biogenic amine (BA) containing neurons. Reserpine injection produces a consistent increase in ATPase concentration along nerve fibers leading to NE containing cells of the adrenal medulla, as well as an increase in activity around the NE containing cells[8]. This concomitant rise in ATPase activity with a decrease in BA concentration after reserpine injection has been found to occur 2 h post-injection and persist for up to 48 h; thus indicating a possible functional relationship between BA containing cells and ATPase activity.

Not only has DA been determined quantitatively in the corpus striatum in a number of animals[1-6], but this BA has been localized by the histochemical fluorescent method[10-12] and has also been localized cytochemically using electron microscopy and heavy metal techniques[3,4,13].

Data obtained from rat corpus striatum following i.p. reserpine injection[a]

Reserpine dose (i.p.)	DA (μg/g)
Control	5.12 ± 0.59
5 mg/kg	1.58 ± 0.01
10 mg/kg	No reading – values at the blank level

[a] All animals weighed approximately 500 g. All were sacrified 24 h following injection.

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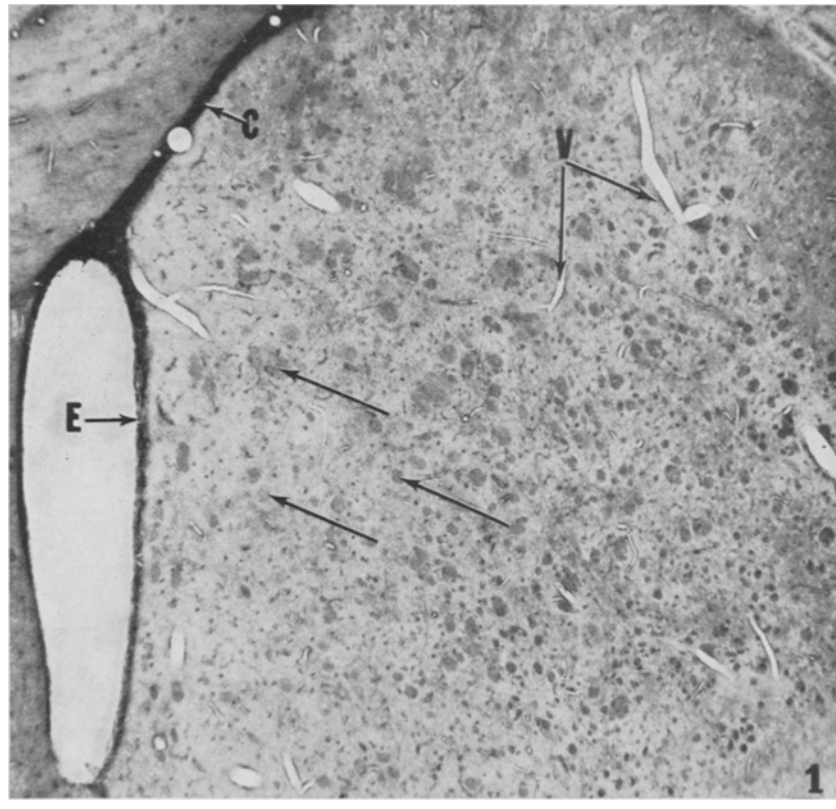

Fig. 1. Uninjected control art, perfused with glutaraldehyde and treated histochemically for ATPase activity. Fibre bundles of the corpus striatum are indicated by darker staining positive ATPase activity (unmarked arrows). The areas between these fibre bundles and arrows represent the neuron-neuropil which is relatively nonreactive and non-staining. The ependymal areas (E) indicate a high degree of precipitate for ATPase activity as does the external capsule (C) and the endothelial areas of the blood vessels (V) also represent a high degree of positive precipitate for ATPase activity.

Fig. 2. The same area of tissue taken from an animal sacrificed 4-h post-reserpine, 10 mg/kg, and treated for ATPase activity. The fibre bundles of the corpus striatum which had previously stained positive and dark in Figure 1 (control) are not now stainable by the ATPase reaction (unmarked arrows). The areas between the fibre bundles and arrows which represent the neuron-neuropil areas, now are highly positive and represent an increase in ATPase activity above that seen in similar areas in the control areas (CF 1 to 2). This is a significant shift, not only from fibre bundle to neuron-neuropil areas, but a significant shift histochemically in levels of ATPase activity from control, thus representing a decrease in fibre bundle activity and an increase in the cellular area activity. ATPase activity ependymal area (E) and the external capsule (C) and in the vascular area (V) are unchanged.

Reserpine, a known BA depleting agent, depletes DA in the corpus striatum in the rat[3] and the caudate nucleus of primates[4,13]. This has also been determined cytochemically[3,4,13].

Changes in caudate nucleus DA concentration and ATPase activity in the squirrel monkey have been presented in a preliminary report[3] which is in the final stages of preparation. However, the present study using rat tissue sheds considerable light on the activities of the DA containing areas of the corpus striatum.

Material and methods. Male, 500 g rats were divided into 3 groups. (All animals were anesthetized with pentobarbital prior to sacrifice.) One group served as uninjected controls. The animals in this group were decapitated and the corpus striatum dissected and analyzed biochemically for DA according to the method of ANTON and SAYER[14]. Comparable animals were perfused with 3% glutaraldehyde, tissue fixed for $^1/_2$ h and incubated in the Wachstein-Meisel medium for ATPase activity[15]. A second group of animals received i.p. 5 mg/kg of reserpine. These animals were sacrificed 24 h following injection and analyzed for DA and ATPase as indicated above. Animals in a third group received 10 mg/kg of reserpine i.p. and were processed similarly to the groups above. Animals were sacrificed as late as 48 h following reserpine injection. The spectrofluorometric analyses were performed on a Zeiss PMQ-2 spectrophotofluorometer and the light micrographs were taken on a Zeiss Ultraphot.

Results and comments. Quantitative determinations are presented in the Table. The normal or control content of DA in µg/g of wet tissue is approximately 5.1 which agrees in essence with information presented in the literature[1,6]. 24 h after a 5 mg/kg dose of reserpine the DA content was reduced to approximately 1.6 µg/g, and a 10 mg/kg level dose of reserpine reduces the quantitative determination to the level of the blank, the latter indicating a non-detectable amount of DA in the corpus striatum of the rat. This dose-response relationship coincides with previous work done in this laboratory[3,4,8]. This also indicates the high level of tolerance of the rat to reserpine as compared to other animals, e.g. squirrel monkey, 1 mg/kg; Syrian hamster, 0.1 mg/kg on which work has been done in this laboratory.

Figure 1 illustrates a control rat corpus striatum showing dark ATPase activity in fibre bundles (arrows); in the area of the ependyma (E) of the lateral ventricle; and in the external capsule area (C). Nerve cell body and neuropil areas are light and much less reactive than the fibre bundles. Control ATPase reactions were performed using unfixed tissues. Although the reaction product is greater in unfixed tissue, the morphology is less preserved. The densities of the reactive sites are relatively constant when fixed and unfixed tissues are compared.

Histochemical determinations on corpus striatum in this study were done on at least 6 animals to maintain a system of control for the reaction. ATPase activity is also, as indicated by other investigators[15], localized in the vascular structures which due to the perfusion technique in these particular animals is shown as open spaces (V). The fibre bundles of the corpus striatum (arrows) are dark and strongly positive, while the cellular and neuropil areas show less reaction. Figure 2 illustrates changes occurring in the ATPase activity in the corpus striatum of the rat following reserpine injection. This photograph was taken from a series of animals which were sacrificed by perfusion 4 h post-reserpine injection. This area of the corpus striatum when compared with Figure 1 indicates

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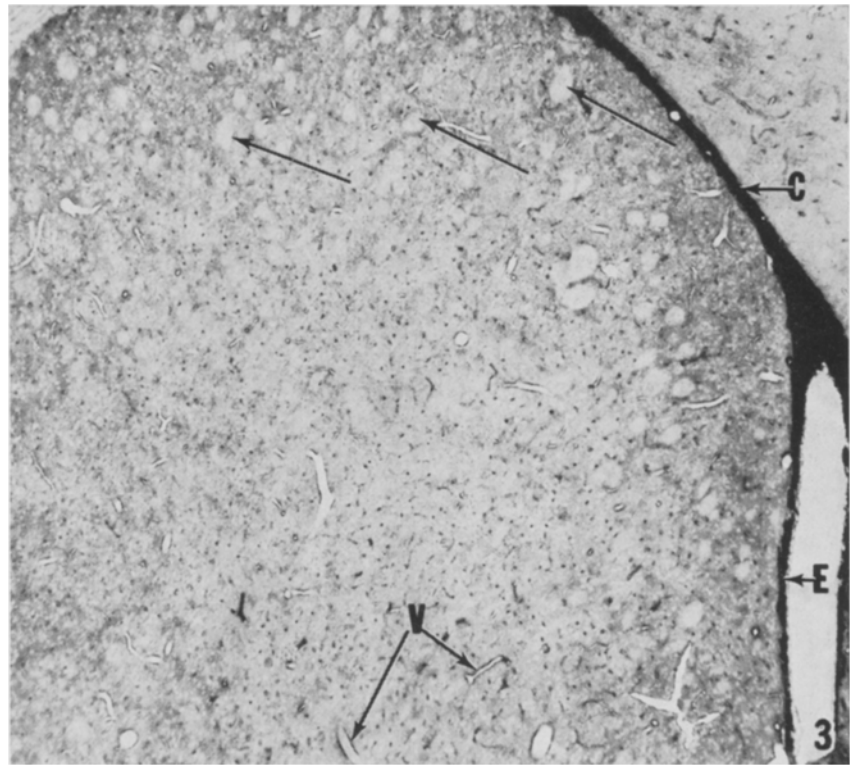

Fig. 3. A 48-h, post-reserpine injection animal sacrificed as those in Figures 1 and 2, the ATPase activity has not returned to normal in the fibre bundles (unmarked arrows) which are still unreactive for ATPase activity in that they appear light, whereas there remains some increased activity indicated by the darker staining areas in the neuropil areas between the fibre bundles. The ependymal area (E), external capsule (C), and vascular endothelial areas remain unchanged for ATPase acrivity. (Note: the marked differences in the 3 photographs represent a change from positive reactivity for ATPase in the fibre bundles in the control animals to non-reactivity in Figures 2 and 3 in the reserpine injected animals. The other significant feature is the marked increased enzymatic (ATPase) activity from the control animals (Figure 1) to markedly increased neuron-neuropil ATPase present in Figures 2 and 3.)

the maintenance of ATPase activity in the external capsule (C) and in the ependymal areas (E). The vascular enzymatic activity (V) appears unchanged by reserpine. BUCKLEY and WOOD[3] have reported a shift in ATPase toward the internal capsule in monkeys. However, the marked change in the current group of animals is a shift of ATPase activity from the fibre bundles of the corpus striatum (arrows) into the cellular and neuropil areas producing an almost negative image when compared with Figure 1. Thus, the fibre bundles (arrows) are lighter than the neuron and neuropil areas which are now dark. Also, the fibre bundles (arrows) of Figure 2 are lighter and less reactive than those of Figure 1 (arrows) while the increased ATPase activity of the neuron-neuropil area Figure 2 (between the arrows) is much darker following reserpine injection when compared to similar, but lighter, areas in Figure 1. This shift to increased activity in the cellular areas is similar to that reported by WOOD, BENJAMIN and BOGY[8] for the NE cells of the adrenal medulla. This shift in ATPase activity persists through the 24 h post-injection period when DA quantitations were done and into the 48-h post-injection period which is illustrated in Figure 3. At 48 h, there is beginning of a return to relatively normal ATPase; however, there is still decreased activity in the fibre bundles (arrows) while the interfibre areas of the striatum appear to be at higher enzymatic activity level than that in the control (Figure 3 compared with Figure 1).

The information from this study indicates that several events may be occurring: 1. There is a reasonable amount of ATPase activity which remains in the brain of animals following glutaraldehyde fixation and this enzymatic activity is sufficiently high to be localized at the light microscopic level. 2. Graded doses of reserpine produce changes in the DA content in the rat brain and a relatively maximum depleting dose of reserpine in the rat adult is 10 mg/kg. 3. The concomitant changes, i.e., decrease in DA content and increase in ATPase activity are constant and repeatable. At least 6 animals in each experimental group were used in this series of experiments. 4. This shift in ATPase with a decrease in DA content can be correlated with the increased ATPase related to NE decrease in the adrenal medulla. This latter was found to be a nerve dependent activity, i.e., the increased ATPase activity and decreased NE content was abolished by cutting the nerve supply to the adrenal medulla[8].

It can be concluded, therefore, that there is a concomitant increase in ATPase activity along with a decrease in DA content in the corpus striatum of the rat following reserpine injection. These enzymatic-BA changes in all probability indicate a functional relationship between this particular BA and the enzyme concerned. The exact interaction of membrane permeability along with DA release or possibly reuptake of DA by the amine pump cannot fully be determined at this time but the amine pump may be related to the increased enzymatic activity. This information appears significantly important in that it establishes a drug-BA-enzymatic activity relationship which has been constant in a number of species of animals in various biogenic amine areas. Further studies

are in progress at the electron microscopic and biochemical levels in order to determine the mechanism and fine structural foci of activity[16,17].

Zusammenfassung. Nachweis, dass im Ratten-Striatum die Reserpin-induzierte Dopamin-Depletion mit einer Erhöhung der ATPase-Aktivität verbunden ist.

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The Histology of the Retina of *Pomatoschistus* (Gobius) *microps* (Krøyer)

In nearly all members of the teleosts there are 3 types of visual cells: rods, single cones and twin cones. The twin cones are found only in teleosts. In 1911 Hess[1], having studied different retinae, noted that in no other vertebrate class is there such a variety in structure and distribution of rods and cones. Subsequently, the teleost retina has received only scant attention[2–4].

Various teleostean retinae are currently being studied both histologically (light and electron microscope) and histochemically in this department. In this paper the structure of the retina, particularly of the photoreceptors, of *Pomatoschistus microps* is given.

Material and methods. *Pomatoschistus microps* is a katadromous fish, found from October to March in the Salt Marsh, Bull Island, Dublin[5]. The fish were decapitat-

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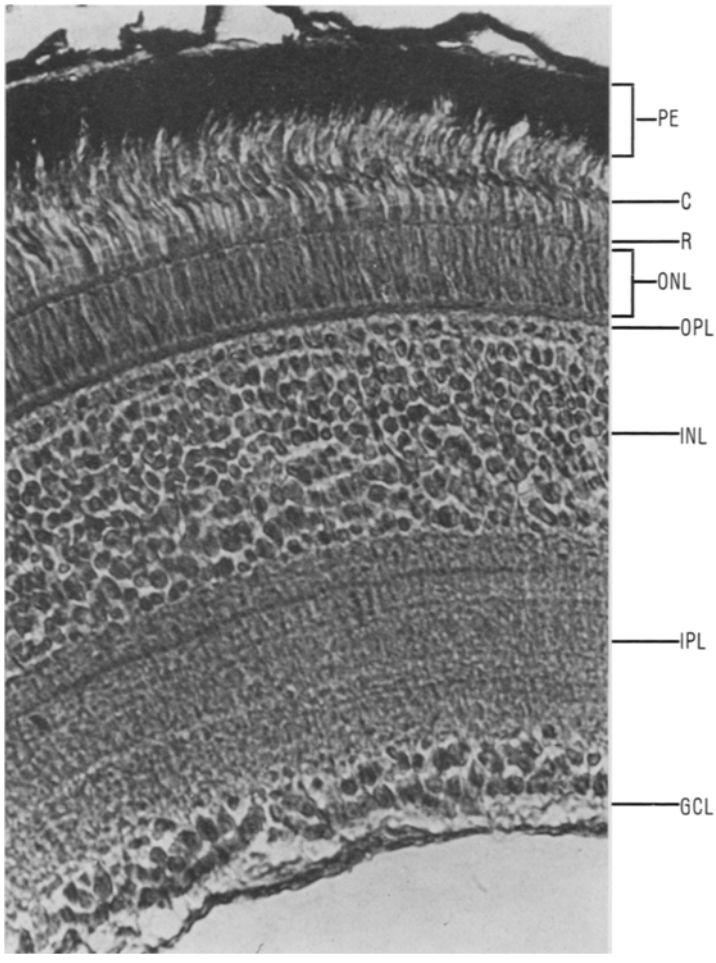

Fig. 1. Retina of *Pomatoschistus microps* (dark adapted). C, cone; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer; PE, pigment epithelium; R, rod.