Morphologic and electrophoretic findings in mice bearing transplanted HIPA tumor

Generations	Histologic form	Virus-like particles A	C	Electrophoretic pattern
1st to 2nd	Undiff. sarcoma	0	0	normal
3rd	Differentation into Plasmocytoma	0	0	normal
4th	Differentation into Plasmocytoma	0	0	α-2-globulin peak
5th to 26th	Differentation into Plasmocytoma	0	+++	α-2-globulin peak
27th to 37th	Plasmocytoma	0	+	normal
38th to 70th	Plasmocytoma	0	0	normal
71th to 78th	Plasmocytoma	+	+	normal

Zusammenfassung. Untersuchungen an einem Plasmocytom von BALB/c-Mäusen ergaben keine Beziehungen zwischen dem Vorkommen von virusähnlichen Partikeln des Typus «A» und «C» und der Sekretion von Immunoglobulin.

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Immunohistochemical Studies on Phenylethanolamine-N-Methyltransferase, Dopa-Decarboxylase and Dopamine-β-hydroxylase

With the use of specific antibodies towards dopamine-β-hydroxylase (DBH) the localization of this enzyme in the adrenal medulla and in the peripheral nervous system was described[1–4]. In addition we also investigated the cellular localization of DBH in the central nervous system[3,4]. We extended these studies, and the localization of other catecholamine synthesizing enzymes was investigated in various tissues. The present paper describes the cellular localization of dopa-decarboxylase (DCA), phenylethanolamine-N-methyltransferase (PNMT) and DBH in peripheral and central tissues of mammals. A preliminary report on the cellular localization of PNMT in the adrenal medulla was previously presented[4].

Male Sprague Dawley rats (body wt. 150–170 g) were used in these studies. In some experiments guinea-pigs, hamsters and mice were also studied.

PNMT was purified from the supernatant fraction of bovine adrenal medulla by the previously reported procedure[5,6]. The ultracentrifuge pattern reveals that the enzyme is homogenous and a molecular weight of 31,000–33,000 was estimated by the meniscus depletion method of Yphantis[7]. The purified enzymes show several bands on disc gel electrophoresis, but all the protein stained bands are enzymatically active, indicating the possible presence of conformational PNMT isoenzymes[6].

DCA was purified from bovine adrenal glands by a recently described procedure[8,9].

DBH was purified from bovine adrenal gland as described previously[10].

Immunization and testing of antibodies was performed as previously described[3,4].

The immunohistofluorescent procedure has previously been described in detail[4]. Cryostate sections from unfixed tissue were used. The sections were usually post-fixed in acetone for 10 min. The indirect method was used for the staining (see[11]), which involved the use of fluoresceine-isothiocyanate (FITC) labelled sheep anti-rabbit immunoglobuline. Before the use of FITC labelled immunoglobuline it was shaken with mouse liver powder or brain powder (Miles Laboratories, USA) at room temperature for 2 h in order to remove as much as possible of the FITC labelled proteins not related to the specific antigens under investigation. Furthermore, the specific antibodies against DCA, PNMT and DBH were incubated with serum from the species studied overnight at room temperature, in order to remove unspecific antibodies. As a specificity test, preimmune serum was used instead of the specific antibody serum.

PNMT. *Adrenal medulla.* In rat and mouse most of the gland cells showed a specific greenish immunofluorescence of moderate intensity which was localized to the cytoplasm of the cells (Figure 1). The outlines of these fluorescent cells were somewhat indistinct, probably due to some diffusion of the PNMT. In guinea-pigs practically all of the gland cells showed a specific immunofluorescence. The cells of the adrenal cortex did not show any specific fluorescence.

Peripheral and central nervous system. No specific immunofluorescence was observed in the sympathetic ganglia or in the CA cell bodies of the central nervous system. Furthermore, no specific immunofluorescence was observed cranial of a transection of the sciatic nerve, which contains many adrenergic axons[12,13]. – In all sections of the brain there appeared a strong unspecific fluorescence in the apical parts of the ependyma outlining the ventricles and in the glial cells, especially the astroglia.

DCA. *Adrenal medulla.* A specific immunofluorescence of weak intensity was observed in the cytoplasm of

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practically all gland cells of hamster (Figure 2), mouse and guinea-pig. No specific immunofluorescence was observed in the adrenal cortex.

Kidney. A strong immunofluorescence was observed in the distal and proximal tubuli.

Peripheral and central nervous system. A specific immunofluorescence of weak to moderate intensity was observed in the NA cell bodies of the sympathetic ganglia (Figure 3) and the DA cell bodies of the substantia nigra and the NA cell bodies of locus coeruleus. Also the 5-HT cell bodies exhibited a weak specific immunofluorescence. No specific fluorescence was observed in the CA nerve terminals. Unspecific fluorescence appeared in the ependyma and the glial cells as for PNMT.

DBH. Adrenal medulla. In rat the gland cells exhibited a green specific immunofluorescence which in most cells was of weak to moderate intensity. Several islands of cells, however, contained a strong specific immunofluorescence.

Peripheral and central nervous system. The NA cell bodies of the sympathetic ganglia (Figure 4) and of the pons and medulla oblongata, *e.g.* those in the locus coeruleus, exhibited a specific immunofluorescence of moderate intensity. The DA cell bodies showed no specific immunofluorescence. Furthermore, after transection of the central ascending NA axons, a strong specific immunofluorescence appeared in the NA axons of the cell body side but not in the DA axons. Unspecific fluorescence appeared in the ependyma and the glial cells as for PNMT and DCA.

The present paper gives the first demonstration of the cellular localization of PNMT and of DCA in the adrenal medulla and of DCA in the peripheral and central nervous system of mammals.

PNMT was found to be localized to the cytoplasm of most of the adrenal medullary glands in rats and mice and in all medullary cells in the guinea-pig. These results support the view that the PNMT is localized in the adrenaline-containing gland cells, since these cells are in majority in rats and mice and constitute practically all gland cells in the guinea-pig. The failure of demonstrating PNMT in the peripheral and central CA containing cell bodies and fibres is in agreement with the concept that the peripheral sympathetic autonomic nervous system of mammals is entirely built up of NA neurons; also there is very little adrenaline found in the mammalian brain (see [14]).

DCA was found to have a widespread distribution in the mammals studied. Thus, it could be localized to practically all adrenal medullary cells and to both peripheral and central CA cells bodies. The failure to demonstrate DCA and DBH in the CA nerve terminals can probably be accounted for by the relatively low sensitivity of the technique due to low penetration of the antibodies into the nerve terminals and/or to diffusion of the CA synthesizing enzymes out of the terminals.

Not only was DCA found in the DA and NA cell bodies but also in the 5-HT cell bodies, which suggests that the dopadecarboxylases of these neurons are similar or identical. The kidney decarboxylase also seems to be similar.

With the present immunofluorescent technique the strongest immunofluorescence was obtained with the DBH antibodies. In contrast to DCA, DBH was only localized in the NA neurons, not in the DA neurons[15].

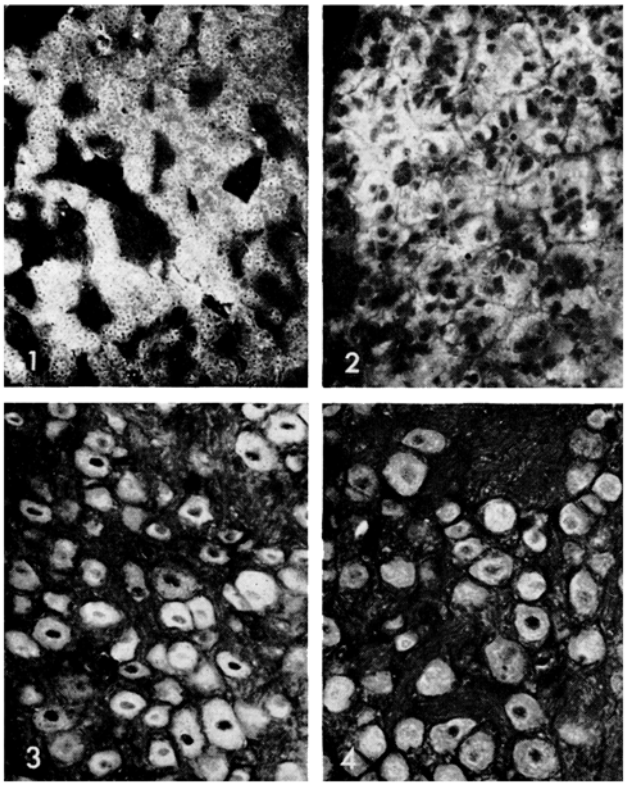

Fig. 1. Rat adrenal medulla. PNMT. A specific immunofluorescence of medium to strong intensity is observed in most of the adrenal medullary gland cells. The rest of the gland cells show no immunofluorescence at all. ×160.

Fig. 2. Hamster adrenal medulla. Dopa-decarboxylase. A specific immunofluorescence of moderate intensity is observed in practically all the adrenal medullary gland cells. ×250.

Fig. 3. Rat superior cervical ganglion. Dopa-decarboxylase. A specific immunofluorescence of weak to strong intensity is observed in the cytoplasm of the vast majority of the ganglion cells. ×250.

Fig. 4. Rat superior cervical ganglion. Dopamine-β-hydroxylase. A specific immunofluorescence of weak to strong intensity is observed in the vast majority of the ganglion cells. ×250.

Zusammenfassung. PNMT konnte mit Hilfe einer immunohistochemischen Fluoreszenzmethode ausschliesslich in bestimmten Markzellen der Nebenniere nachgewiesen werden. DDK wurde in Markzellen der Nebenniere, in peripheren und zentralen Katecholamin- und Serotoninneuronen gefunden. DBH kam in Markzellen der Nebenniere und nur in peripheren und zentralen Noradrenalinneuronen vor.

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