IMMUNOCYTOCHEMICAL LOCALIZATION OF CATECHOL-O-METHYLTRANSFERASE IN LYMPHOID TISSUE AND BONE MARROW OF RAT

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Catechol-O-methyltransferase (COMT) was localized in the spleen, thymus, and lymph node of the rat using the unlabeled antibody enzyme technique. Immunoreactive deposits were found in the cells of the white pulp, marginal zone, and perinodular ring between the white pulp and marginal zone of the spleen and the sinus, germinal center and cortex of the lymph node. These cells appeared to be reticular cells and macrophages on the basis of their shape and distribution. COMT was present in epithelial reticular cells of the thymus. No COMT reaction product was found in bone marrow.

Catechol-O-methyltransferase (COMT) (E. C. 2.1.1.6) catalyzes the transfer of the activated methyl group from S-adenosyl-L-methionine to one of the phenolic groups of a variety of catechols (1, 10, 11, 13). COMT is important in the enzymatic inactivation of circulating catecholamines (27, 28), catecholamines released as neurotransmitters from both central and peripheral catecholamine containing neurons (9, 18, 24), xenobiotic catechols (3, 14), and catecholestrogens (6, 15). COMT activity is widely distributed in mammalian tissues (17, 18) and has been clearly demonstrated in splenic tissue (1, 2, 19) and in erythrocytes (32, 42). Biochemical studies indicate that COMT is present almost exclusively as a soluble cytoplasmic protein with a molecular size of 23000 Daltons (7, 8) although several investigators have reported the presence of small amounts of membrane-bound COMT activity (7).

Until recently little information has been available with regard to the specific cellular localization of COMT. However, in 1977, Inoue *et al.* reported a survey of the immunocytochemical localization of COMT in the peripheral tissues of rat (20). Subsequent reports have appeared on the immunocytochemical localization of COMT: in mammalian brain (25); in the cardiovascular tissues of rat (29); and in the pregnant rat uterus (22). An unexpected observation was the immunocytochemical demonstration of COMT in reticular endothelial cells of the lymphatic system (20). In the present paper we report a detailed study of the localization of COMT in lymphoid tissue and bone marrow of the rat using a specific rat liver-COMT antibody (12) in conjunction with periodic sodium borohydride inhibition

of endogenous peroxidase (23) and peroxidase-antiperoxidase (PAP) with diaminobenzidine to visualize the immunoreactive COMT (38). A preliminary report of these results has been published (21).

MATERIALS AND METHOD

Young adult Wistar rats were anesthetized with Nembutal and perfused through the heart with 100 ml of periodate-lysine-paraformaldehyde (PLP) (30). After perfusion tissues were rapidly removed, cut into small pieces and fixed for 3 hr in PLP at 4°C, dehydrated through graded alcohols and embedded in paraffin. Sections (8 μ m) were cut, mounted on glass slides with egg albumin and glycerine and dried overnight at 37°C. For immunocytochemical staining paraffin sections were deparaffinized with xylene and brought to water through graded alcohols. Sections were washed with phosphate-buffered saline (PBS), treated with 0.005 M periodic acid and 0.003 M sodium borohydride to inhibit endogenous peroxidase (23), and rinsed with three changes of PBS for a total of 30 min. Sections were incubated with rabbit antisera to rat-COMT (12) (1 : 500) at 4°C for 24 hr, goat anti-rabbit serum (1 : 10) (Microanalytical Associates Inc., Warrington, PA) for 30 min and PAP (1 : 10) (Microanalytical Associates Inc., Warrington, PA) for 30 min and PAP (1 : 10) (polyscience, Inc., Warrington, PA) for 45 min at room temperature. All dilutions were made with PBS. Sections were washed for 15 min each in 3 changes of PBS between each step. The final peroxidase reaction product was developed by exposing sections for 5 min to 3,3'-diaminobenzidine (0.05%) in 0.05 M Tris buffer, pH 7.6, containing 0.01% H₂O₂. This resulted in the formation of an insoluble, dark brown polymer at sites of peroxidase activity. After a final wash in distilled water sections were dehydrated through graded alcohols, cleared with xylene and mounted in Canada Balsam. Controls consisted of omission of the goat antirabbit serum or the substitution of normal, non-immuno rabbit sera for the specific rabbit anti-rat COMT sera.

RESULTS

The sites of positive immunocytochemical reaction indicated by the dark brown diaminobenzidine polymer indicate the localization of the interaction of the specific antiserum with tissue COMT and will be referred to as a COMT-positive reaction. The COMT-positive reaction was clearly distinguishable from the pale yellow to colorless background observed in both types of control staining procedure. COMT-positive reaction product in reactive cells was diffusely present throughout their cytoplasm.

In the three zones in rat spleen, the red pulp, the white pulp, and the marginal zone, COMT was present in some cells of the white pulp and marginal zone, and in some cells which formed a dense ring around the margin of the white pulp (Fig. 1).

As shown in Fig. 2, no specific COMT reaction product occurred in the white pulp and marginal zone in control sections. In the red pulp, a light brown peroxidase reaction product was equally distributed in red blood cells in both control and experimental sections due to endogenous peroxidase activity. In the central areas of the white pulp, clusters of COMT-positive cells were present (Fig. 1). These

FIG. 1.~4.

FIG. 5.~8.

FIG. 9.~12.

cells are usually characterized by irregular cytoplasmic process extending into the surrounding lymphoid tissue (Fig. 3). Similar COMT-positive cells were distributed throughout the marginal zone and the perinodular ring between the white pulp and marginal zone (Fig. 4). These cells were irregular in both shape and size and not highly branched. On the basis of their shape and distribution the COMT-positive cells in rat spleen appeared to be reticular cells and macrophages. No clearly COMT-positive reaction was associated with lymphocytes (Figs. 3 and 4).

In the thymus, cells yielding a COMT-positive response were present only in the cortex (Fig. 5). These cells were branched and tended to form a meshwork. COMT-positive reaction was present in their cytoplasmic processes (Fig. 6). On the basis of their shape, distribution and their presence in a meshwork, these cells appeared to be epithelial reticular cells. Lymphocytes lying in the interstices of this meshwork were COMT-negative (Fig. 6).

In mesenteric lymph nodes, COMT-positive reaction was present in sinusoidal macrophages (Figs. 7 and 9). In the germinal centers the COMT-positive cells were arranged in circular patterns which corresponded in shape and size to the periphery of the center (Figs. 8, 10 and 11). These cells were irregular in shape and larger than the stellate cells in the cortex and appeared to be reticular cells and macrophages. The COMT-positive reaction was not evident in lymphocytes (Figs. 7-11).

Attempts to demonstrate specific COMT-positive reaction in sections from bone marrow were negative (Fig. 12).

DISCUSSION

The presence of COMT activity has been demonstrated in the spleen of most vertebrates by direct enzymatic assay or indirectly through the release of O-methylated metabolites from intact, perfused splenic preparations (1, 18). Studies in immunosympathectomized rodents (24) indicate that COMT is predominately

FIG. 1. Rat spleen. COMT-positive cells in the white pulp and marginal zone. Arrows point to the ring of perinodular COMT-positive cells. Mz; marginal zone, Rp; red pulp, Wp; white pulp. $\times 50$

FIG. 2. Control rat spleen treated with normal rabbit serum. Cells in the red pulp are lightly stained. Mz; marginal zone, Rp; red pulp, Wp; white pulp. $\times 50$

FIG. 3. Rat spleen of the COMT-positive macrophages in the white pulp. $\times 570$

FIG. 4. Rat spleen. Perinodular reticular cells (arrows) and macrophages in the marginal zone contain COMT. Mz; marginal zone, Wp; white pulp. $\times 580$

FIG. 5. Rat thymus. COMT-positive cells in the cortex. Co; cortex, Me; medulla. $\times 125$

FIG. 6. Rat thymus. Detail of COMT-positive epithelial reticular cells at the cortex. Arrows point to the process of the epithelial reticular cells. $\times 550$

FIG. 7. Rat lymph node. COMT-positive cells in the sinuses. S; sinus. $\times 120$

FIG. 8. Rat lymph node. COMT-positive cells in the cortex and large COMT-positive cells associated with the germinal center. Co; cortex, G; germinal center. $\times 120$

FIG. 9. Rat lymph node. COMT in sinusoidal macrophages and the cells lining the sinus (arrows). S; sinus. $\times 560$

FIG. 10. Rat lymph node of the stellate reticular cells of the cortex containing COMT. $\times 560$

FIG. 11. Rat lymph node. Macrophages in the germinal centre containing COMT. $\times 500$

FIG. 12. Rat bone marrow. No apparent COMT-positive cells are seen. $\times 140$

localized at extraneuronal sites within the spleen. Our results provide a direct demonstration of an extraneuronal localization for COMT in rat spleen as well as in rat thymus and lymph nodes.

In the rat spleen, COMT-positive cells were found in the white pulp, marginal zone and the perinodular ring between the white pulp and the marginal zone. This distribution, especially that of the COMT-positive cells forming the perinodular ring, corresponds closely to the metalophil cells or marginal metalophils described by Snook (36). Cytochemical studies indicate that the marginal metalophils may differ somewhat from other metalophil cells in that they show very weak phagocytic (36) and non-specific esterase activity (31), a constant level of acid phosphatase (31) and stain specifically for monoamine oxidase (39). However, despite these differences, the metalophil cells of the white pulp and marginal zone as well as those in the perinodular ring appear to be reticular cells (31). Our results suggest that the COMT-positive cells in rat spleen are reticular endothelial cells.

In rat lymph node the distribution of COMT is similar to the distribution of reticular endothelial cells stained by the silver impregnation method of Turner (40). Three different cell groups of this type have been defined morphologically: phagocytic cells in the sinusoids and at the periphery of the germinal centers, stellate reticular cells, and sinusoidal endothelial cells (40). The COMT-positive cells in the sinusoids and at the germinal centers in the lymph node accumulated carbon black and thus can be tentatively classified as macrophages (data not shown).

In the rat thymus the COMT-positive cells appeared to be epithelial reticular cells. However, the question of COMT-positive macrophages in the rat thymus has yet to be determined. Further, the absence of apparent COMT-positive cells in bone marrow is unclear since according to the mononuclear phagocyte concept, macrophages in both bone marrow and lymphoid tissue are derived from monocytes in bone marrow.

The distribution of COMT reported here raises an interesting question regarding the function of COMT in lymphoid tissue. There is increasing evidence that the immuno system is intimately associated with adrenergic receptors in the plasma membrane of lymphocytes (16, 26, 33, 34, 35). It is possible that reticuloendothelial cells may serve as barriers restricting the free diffusion of catecholamines by providing a mechanism for the enzymatic inactivation of catecholamines. A similar role has been postulated for the function of COMT in glial cells and ventricular ependymal cells in the central nervous system (25) and for COMT in the endothelial cells of rat aorta (29). Another possible function to be considered is the control of catecholesterogen levels. This would appear to be an important consideration since lymphoid tissue is estrogen sensitive (37, 41), estrogen-2-hydroxylase has been demonstrated in spleen (4) and catecholesterogen has a higher affinity for COMT than catecholamines (5, 6).

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