Studies on Dipeptidyl(Amino)Peptidase IV (Glycyl-Proline Naphthylamidase)

II. Blood Vessels

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Summary. The activity of dipeptidyl(amino)peptidase IV (DAP IV, glycylproline naphthylamidase) was discovered in the endothelial cells of the venous part of capillary bed and of small venules of many organs of the rat, mini-pig, rabbit, cock as well as man. In aortae, large arteries and veins only a portion of vasa vasorum displays a positive reaction. Glycyl-proline-4-methoxy-2-naphthylamide (Gly-Pro-MNA) is the substrate of choice both from the viewpoint of enzyme kinetics as well as localization. Phenylalanyl-proline-4-methoxy-2-naphthylamide (Phe-Pro-MNA) is cleaved less easily, however, it enables a good localization. 1- and 2-naphthylamine derivatives of glycylproline display better kinetic properties than Phe-Pro-MNA, however they enable a satisfactory localization under special conditions only.

The recommended diazonium salt for the routine is Fast Blue B. The enzyme is quite firmly associated with the structure and chloroform-acetone preextraction of cryostat sections does not influence its activity significantly while improving the localization. Block fixation in aldehydes inhibits the enzyme activity (glutaraldehyde more than formaldehyde). The osmificated azo-dye originated of 4-methoxy-2-naphthylamine and Fast Blue B or hexazonium-p-rosaniline is still partially soluble in solvents used for the usual embedding in epoxyresins for electron microscopical examination. This is a drawback for a reliable demonstration of DAP IV in endothelial cells on the electronmicroscopical level using the epoxy-resin technique. DAP IV of the endothelium is inhibited totally by DFP (10^{-3}M), partially by E 600 (10^{-3}); and slightly by phenanthroline (10^{-3}M). It is unaffected by EDTA (10^{-3}M) and N-ethyl maleimide (10^{-3}M).

The combined demonstration of alkaline phosphatase and DAP IV in the same section renders a reliable demonstration of the capillary bed in many organs.

The contribution of DAP IV activity of the capillary endothelium to the total DAP IV activity in a particular organ is decisive in the myocardium, striated muscle, aorta and lung; it represents about one half of the total activity in spleen and pancreas and is less expressed in the liver, intestine and particularly in the kidney.
In the jejunum of patients suffering coeliac sprue the activity of capillary endothelium in the propria is decreased or not demonstrable in the acute stage. After a gluten-free diet it is restituted. The activity of DAP IV does not change significantly in aortae of the rabbit and man with atherosclerosis. In plaques of human aortae the capillary endothelium reacts at the most. Vasa vasorum in the adventitia overlying large plaques, which penetrate into the media, display a high DAP IV activity and their number can be increased. In plaques of arteries of cocks there is a positive DAP IV reaction in foam cells. DAP IV does not belong to the enzymes indicating early changes in atherogenesis.

The function of DAP IV in the endothelium is not known. It may be a part of the machinery influencing the protein part of the endothelial coat or may participate in the degradation of some vasoactive peptides.

**Introduction**

Dipeptidyl aminopeptidase IV (DAP IV, glycyl-proline-β-naphthylamidase, E.C. 3.4.15.4) is a peptidase discovered by Hopsu-Havu and Glenner (1966). Its biochemistry was thoroughly reviewed by Kenny (1977) and by McDonald and Schwabe (1977). The most important data can be also found in our previous reports (Lojda, 1977a, b).

DAP IV is a serine peptidase which removes N-terminal dipeptides from polypeptides with a penultimate proline residues. With alanine in this position the activity of DAP IV is much lower. The N-terminal aminocid is of lesser importance. Peptides with N-terminal glycine, phenylalanine and arginine are attacked most easily. Kenny et al. (1976) also described an endopeptidase activity of DAP IV. The pH optimum of DAP IV is between 7.4-8. The enzyme does not require the presence of easily dissociable cofactors or free SH-groups. It is inhibited by disopropyl-flurophosphate and salts of heavy metals.

The demonstration of DAP IV “in situ” was carried out first by Hopsu-Havu and Ekfors (1969) and by McDonald et al. (1971).

In our studies with improved methods (see Lojda, 1977a) a much better localization of DAP IV was achieved and the enzyme was demonstrated in many cells in which its presence was not known. This paper concerns the activity of DAP IV in blood vessels. Preliminary results were given in our previous communication (Lojda, 1977b).

**Material and Methods**

The following animals were used in our experiments: Twenty normal rats (males, strain Wistar, Konárovice, 190–220 g, fed a standard diet): aorta, myocardium, kidney, spleen, lung, striated muscle (M. biceps femoris, diaphragm), tongue, jejunum, liver, pancreas, brain. Fifteen normal mini-pigs (males and females, strain Göttingen from the research Institute of Veterinary Medicine, Brno, 35–45 kg, fed a standard food; we are indebted to MVDr. L. Lojda, CSc., for kindly providing us with this material): ascendent thoracic aorta, abdominal aorta (in the region of renal arteries),