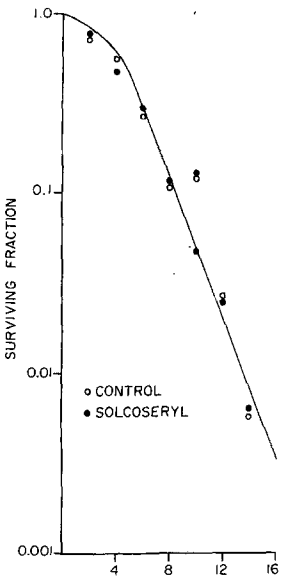

Figure 1. Survival of V79 Chinese hamster cells in vitro after cobalt-60 gamma irradiation. Closed circles represent cells treated with solcoseryl (13.3 mg/ml) prior to irradiation. Open circles represent untreated control cells.

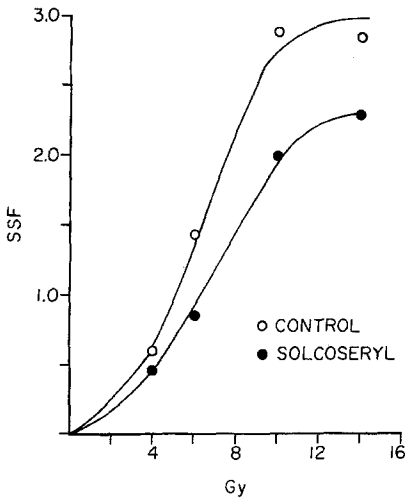

Figure 2. DNA single-strand breaks in V79 cells treated with solcoseryl (13.3 mg/ml); closed circles or untreated control cells (open circles). See text for description of Strand-Scission Factor (SSF).

tor[13]. The lack of correlation between this protection and cell survival is not surprising, inasmuch as it has been shown that double-strand DNA breaks apparently correlate better with cell lethality than do SSB[14]. The protective effect on SSB is not insignificant, however, because DNA damage of this type has been correlated with mutagenesis[15]. Further studies will be required to determine if the protection from SSB in DNA results in a modification of mutagenesis induced by radiation.

1 Solcoseryl is a registered trademark of Solco Basle Ltd, CH-4127 Birsfelden, Switzerland and was kindly supplied by Drs Haigis and Nasrin. In scientific German literature this material is referred to as 'Actihaemyl'.

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Fine structure histochemical study of the distribution of dipeptidylpeptidase IV (DPP IV) in the meningeal lamellae of the rat

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Summary. DPP IV was localized in the meningeal lamellae of the spinal cord sheaths of the rat by light and electron microscopy. A membrane-bound reaction product of DPP IV was found in the internal, intermediate and external meningeal lamellae which delineated the CSF-filled meningeal spaces. The cells of the marginal glia displayed heterogeneous localization of the reaction product for DPP IV. DPP IV distribution in the spinal cord sheaths suggests its possible participation in the interactions of the meningeal cells with the neuropeptides in cerebrospinal fluid.
Key words. Dipeptidylpeptidase IV; histochemical localization; meningeal cells.

DPP IV is a membrane-bound serine exopeptidase splitting N-terminal dipeptides with proline in the penultimate position. Using histochemical and immunohistochemical techniques, this enzyme was found in several tissues including structures of the nervous system [1-7]. DPP IV was also detected in spinal cord sheaths [6], though there the light microscopy was not sufficient for precise localization of DPP IV, particularly in the pia mater interna and pia mater externa cells. At the ultrastructural level, this study [6] was concerned with detection of DPP IV positivity only in the external meningeal lamella.

The purpose of the present study was to define the exact localization of DPP IV in the spinal cord sheaths of the rat in relation to the meningeal spaces filled with CSF. At the same time, our attention was drawn to the heterogeneous distribution of the enzyme in the marginal glia with respect to possible function of DPP IV.

Material and methods. Histochemical localization of DPP IV activity was carried out in spinal cord sheaths of the rat under ether anesthesia. Sections of fresh and paraformaldehyde-fixed segments of the spinal cord with their sheaths, 15-µm and 100-µm-thick, cut transversely, were incubated as described previously for light and electron microscopic localization of DPP IV activity [3, 8]. L-glycyl-prolyl-4-methoxy-2-naphthylamide (Bachem, Bubendorf, Switzerland) was used as substrate and either Fast Blue B or hexazotized new fuchsine (Merck, FRG) were added to the medium as coupling agents for light and electron microscopy, respectively. Control sections were incubated in substrate-free medium. For description of meningeal strata the following terminology according to Krisch et al. [9] was used. Briefly, the layers of CNS sheaths were divided into 3 lamellae: a) internal meningeal lamella made up of internal pia mater cells with the subpial space inside; b) intermediate meningeal lamella made up of cells of pia mater externa and internal arachnoid with the pial space situated between internal and intermediate lamella; c) external meningeal lamella made up of cells of the external arachnoid, neurothelium and internal layer of dura mater. The external and intermediate lamella delineated the arachnoid space. The subpial, pial and arachnoid spaces are filled by CSF.

Results and discussion. Light microscopy demonstrated that the reaction product corresponding to DPP IV activity was localized in the external meningeal lamella lining the arachnoid space on the outside. DPP IV activity was discernible also in the meningeal cells on the inside of the arachnoid space. Using cryostat sections, however, it proved not to be feasible to make a precise distinction between the intermediate and internal meningeal lamellae. Immediately on the surface of the spinal cord the reaction product exhibited heterogeneous distribution (fig. 1). The light microscopic results were consistent with previous observations [6].

Electron microscopy revealed the presence of the fine membrane-bound reaction product in the plasma membranes of the internal pia mater, external pia mater, and internal arachnoid cells (figs 2 and 3). These plasma membranes with DPP IV activity were seen lining the pial and arachnoid spaces. Endothelial cells in some segments of blood vessels within the intermediate meningeal lamella displayed a membrane-bound positivity for DPP IV, too (not shown here). DPP IV activity was irregularly localized in cells of the marginal glia (figs 2 and 3). Therefore, the subpial space was delineated only in some places by cells exhibiting a membrane-bound electron-dense DPP IV reaction product, in plasma membranes of both pia mater interna cells and of marginal glia cells.

Previous histochemical studies demonstrated DPP IV activity as a membrane-bound enzyme [2, 8]. The results presented here confirmed the membrane-bound localization of DPP IV in cells of spinal cord sheaths as well. Comparison of control sections (incubated in substrate-free medium) with those incubated in the substrate medium clearly showed that the increased density of plasma membranes of meningeal cells was caused by the enzyme activity of DPP IV.

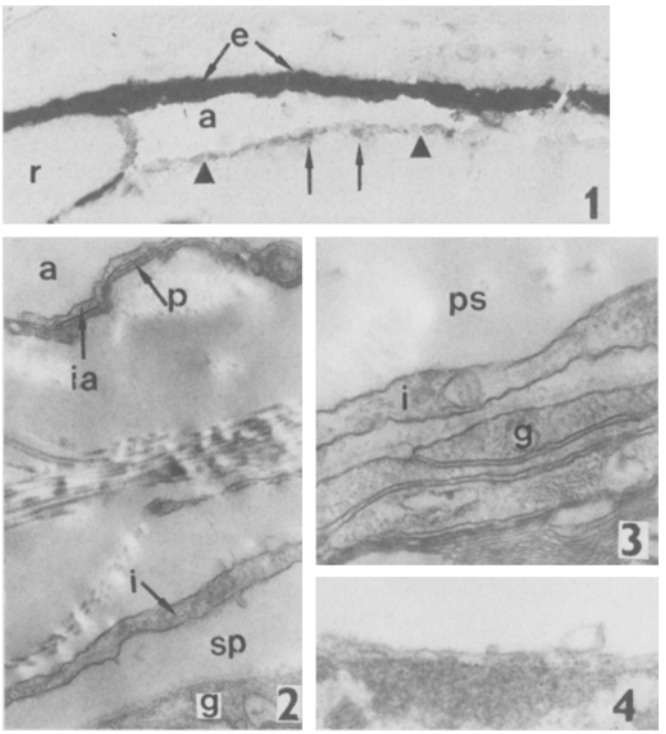

Figure 1. A transverse cryostat section of dorsolateral region of the cervical spinal cord and its sheaths incubated for DPP IV activity. External meningeal lamella (e) as well as the inner delineation of the arachnoid space (a) made up of intermediate and inner meningeal lamellae (arrowheads), showing the distinct positivity for DPP IV. The marginal glia cells are heterogeneously DPP IV positive (arrows). r, dorsal root. ×200.

Figure 2. An ultrathin section of the marginal glia (g) displaying no DPP IV reactivity; internal pia mater (i), external pia mater (p) and internal arachnoid (ia) cells with conspicuous DPP IV activity. sp, subpial space; a, arachnoid space. ×20,000.

Figure 3. An ultrathin section of the marginal glia (g) and internal pia mater cell (i) with the membrane-bound reaction product for DPP IV at higher magnification. ps, pial space. ×37,000.

Figure 4. A control ultrathin section incubated in substrate-free medium. The plasma membrane of the pia mater interna cell does not exhibit distinct electrondensity. ×37,000.

The marginal glia cells exhibited a heterogeneous distribution of DPP IV activity, the functional significance of which still remains a matter of conjecture. However, the same pattern of DPP IV distribution has been seen in other structures, too, e.g., taste bud cells [10]. It seems to be likely that the presence or absence of DPP IV activity on the cell surface may determine whether or not a particular biologically active peptide will act effectively on target cells [2, 10].

The ultrahistochemical technique helped to detect DPP IV activity in structures surrounding CSF-filled meningeal spaces. The functional relevance of DPP IV in those structures is a matter of discussion. There is evidence that DPP IV is involved in splitting of substance P [11, 12] and other biologically active peptides [7, 13]. It is known that DPP IV is not a specialized enzyme for the degradation of one specific neuropeptide, rather it is an enzyme designated to split the respective dipeptides from various oligopeptides [2]. Interestingly enough, some neuropeptides transported by CSF [14]

possess at their N terminus sequences which might be split by DPP IV and may, therefore, serve as a natural substrate for this enzyme (e.g., substance P, growth hormone, thyreoliberin).

In their recent communication, Zajac et al. [15] report that the possible role of enkephalin-degrading enzymes and of angiotensin-converting enzyme in meninges could be to maintain the homeostatic concentration of neuropeptides in the CNS and that these enzymes could ensure complete hydrolysis of peptides filtered by the meninges. It seems that DPP IV has a similar functional significance in meninges.

Furthermore, it has been suggested that DPP IV on the brush border of enterocytes and in the proximal tubulus cells of the kidney might constitute the channel and play a significant role in the reabsorption of the dipeptides [16, 17]. It could be deduced that in meningeal cells also, DPP IV may be involved in the digestion of proline-containing peptides, thus making it possible for X-Pro dipeptides to be transported through meningeal cells into the capillaries of the intermediate meningeal lamella.

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Monoamine oxidase activity in single nerve cell bodies from substantia nigra of rat and man

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Summary. In single nerve cell bodies isolated from the substantia nigra of man and rat the active forms of MAO A and MAO B were found by the use of the microdiver technique and specific inhibitors.
Key words. MAO A; MAO B; substantia nigra; rat; man; MPTP.

It has been suggested that MAO B (MAO, monoamine oxidase – monoamine:oxygen oxidoreductase:EC 1.4.3.4.) of the pars compacta of the substantia nigra in humans and primates is responsible for the transformation of MPTP into MPP^+, which is neurotoxic to nerve cells of the pars compacta, producing a Parkinson's disease-like effect [1–5]. Therefore it is possible that rats, which are resistant to MPTP, possess a different pattern of MAO A and MAO B in their substantia nigra as compared to susceptible species, such as man and primates. The microgasometric technique [6] allows the measurement of MAO activity in a single nerve cell body isolated from the pars compacta of the substantia nigra, and by the use of selective inhibitors the activity of both molecular forms of MAO can be determined separately [7].

Methods and materials. Normal albino rats of both sexes, weighing 150–200 g, were used. Rats were decapitated under light ether anesthesia, the brain was dissected and used immediately. Human brain tissue, from patients who died from causes other than neurological ones, was taken at autopsy and kept at 4 °C. The time interval between the death and the MAO assay was 26–30 h. Single nerve cell bodies were identified [8, 9], visualized by lightly staining the cut surface of the brain with 0.5 mM methylene blue, which leaves the glial cells unstained, isolated under a stereomicroscope and dissected free-hand using glass rods with tips of a thin (∅ 15 µm) stainless steel wire [10]. MAO activity was measured microgasometrically at 30 °C using the electromagnetic diver technique [6]. The nerve cell body floating in a droplet of reaction medium was sucked into the diver ampulla, containing a small air bubble, and the microgasometric measurement was performed (for details about the methods [11–16]). The magnetic diver technique allows easier manipulation but yields the same sensitivity and accuracy as the classical microdiver technique (1×10^{-6} µl gas/h) [6, 7]. The enzyme reaction rate was measured by recording oxygen consumption. The reaction medium contained: 1/15 M phosphate buffer pH 7.0, 10 mM tyramine and 10 mM semicarbazide. KCN was omitted from the reaction medium [7]. The total MAO activity was measured in the absence of inhibitors, whereas the activities of the two isoenzymes of MAO were determined in the presence of different concentrations of clorgyline and deprenyl, selective inhibitors of MAO A and MAO B, respectively. In these experiments, an isolated nerve cell body was first preincubated at room temperature for 40 min prior to assay in the medium containing the appropriate inhibitor without the substrate. Readings were taken every 10 min for 1 h. Cell free controls were used to correct for nonenzymic reactions. The changes of gas volume were then converted into pmols of substrate used per h and expressed as the MAO activity per single nerve cell body. The substances used were: tyramine hydrochloride (Hoffmann-La Roche, Basel, Switzerland); semicarbazide hydrochloride (Riedel-De-Haën, Hannover, FRG); clorgyline hydrochloride (May and Baker, Dagenham, UK); l-deprenyl hydrochloride was a gift from Prof. J. Knoll, Department of Pharmacology, Semmelweiss University of Medicine, Budapest, Hungary.

Results and discussion. In single nerve cell bodies isolated from the pars compacta of the substantia nigra in rats and