Demonstration of Endogenous Inhibitors of Monoamine Oxidase in Dog Cerebrospinal Fluid

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Abstract—Cerebrospinal fluid (CSF) from dogs competitively inhibited A-form MAO, but was non-competitive with B-form MAO. Heat treatment of CSF (90°C, 20 min) had no effect on the inhibition. Digestion with trypsin and chymotrypsin reduced the MAO inhibitory activity. After ultrafiltration of the CSF through a membrane to remove substances of >5,000 M.W., significant inhibitory activity persisted. These results suggest that CSF contains endogenous substances that act like MAO inhibitor to inhibit A and B-form MAO, and these substances are peptides of less than 5,000 M.W.

Generally, it has been well observed that mean monoamine oxidase (MAO) activity is usually lower in the homogenate of various tissues of animals than in mitochondrial preparations, even though these differences are not always statistically significant. Therefore, MAO inhibitors may be present which regulate MAO activity in the soluble fractions. Although some endogenous MAO modulators have been found in the cytosol of various tissues (1-3), in plasma (4, 5) and urine (6, 7), there have been few studies on their possible role as physiological regulators of MAO activity.

Following an earlier report that normal human cerebrospinal fluid (CSF) contains endogenous substances of different molecular weights (3,000 to more than 35,000) which act like MAO inhibitors (8), we report in this paper that dog CSF also contains an inhibitor or inhibitors of both A and B-form MAO. We also report on the inhibiting mechanism of the dog CSF on dog brain mitochondrial MAO.

Dogs and monkeys were anesthetized with ketalar given s.c., and the brains were quickly removed after withdrawing CSF by cisterna cerebellomedullaris puncture. Mice and rats were also anesthetized with sodium pentobarbital given i.p., and the brains were quickly removed. These brains and CSF samples were stored at -60°C until used. The brains were homogenized in 10 volumes of 0.32 M sucrose (previously adjusted to pH 7.2 with 0.5 M NaHCO₃). The crude mitochondrial fractions were prepared by differential centrifugation as described earlier (9). The mitochondria were washed once by resuspension in 0.32 M sucrose solution and used as the enzyme preparations. These operations were carried out at 4°C. The protein contents of the enzyme preparations were measured by the method of Lowry et al. (10) with bovine serum albumin as the standard; preparations containing 2 mg/ml of protein were used for the enzyme assay. MAO activity was estimated by radioisotopic assay with [¹⁴C]-5-HT (substrate for A-form MAO) and [¹⁴C]-β-phenylethylamine (β-PEA) (substrate for B-form MAO) as described earlier (9). The incubation medium contained suitable amounts of the enzyme to give a linear reaction for at least 40 min in the total volume of 225 µl of 0.1 M phosphate buffer, pH 7.2. The reaction was started by adding 25 µl of labelled substrate; incubation was carried out for 20 min at 37°C. Then the reaction was stopped by adding 2 N HCl (200 µl). The reaction products were extracted with ethyl acetate-benzene (1:1, v/v). Samples of the extracts were mixed with Triton X-100-toluene
scintillation fluid, and their radioactivities were measured by liquid scintillation spectrometry. MAO activity was expressed as nmol of products formed/min/mg of protein.

The effects of adding varying amounts of dog CSF to mouse, rat, dog and monkey brain mitochondrial MAO, in vitro, were studied using 5-HT and β-PEA as substrates (Fig. 1). When the brain mitochondria of various animals were incubated with increasing amounts of dog CSF, a linear increase in the inhibition of MAO activity with 5-HT as substrate was observed. Dog CSF inhibited both A-form and B-form MAO in dog brain mitochondria. However, a pronounced activation of MAO in monkey brain mitochondria by dog CSF has been observed with β-PEA as a substrate. Dog CSF may act as an activator or inhibitor of MAO depending on the substrates used for assay as well as on different animal species.

Dog CSF was treated in various ways such as heating it in a boiling water bath (90°C, 20 min) and digestion by incubation with crude pepsin, trypsin and chymotrypsin. Heat treatment exhibited no effect on the inhibition. Digestions with trypsin and chymotrypsin reduced the MAO inhibitory activity to 40 or 5% of that originally present, respectively (data not shown). The inhibitors in CSF were heat stable, but labile to treatment with trypsin and chymotrypsin as well as plasma MAO modulator (4). After ultrafiltration of the dog CSF through Centrisart I Sartorius membranes to remove substances of >5,000 M.W., significant inhibitory activity persisted. This suggested that the MAO inhibitors in CSF may be peptides of less than 5,000 M.W. Becker et al. (8) have reported the isolation of endogenous MAO inhibitors from human CSF of one or more proteins with low molecular weight (3,000 to more than 35,000). In the present study, these MAO inhibitors in dog CSF may be similar substances to that of human CSF (~3,000), although the properties of endogenous MAO inhibitors in human CSF have not been investigated; and moreover, the bovine brain mitochondria and human platelets are used as the enzyme source of A-form MAO and B-form MAO, respectively. A kinetic study of the inhibition of MAO by CSF addition was investigated with Lineweaver-Burk double reciprocal plots. The result with 5-HT and β-PEA as substrates are shown in Fig. 2. The inhibition activity to 40 or 5% of that originally present, respectively (data not shown). The inhibitors in CSF were heat stable, but labile to treatment with trypsin and chymotrypsin as well as plasma MAO modulator (4). After ultrafiltration of the dog CSF through Centrisart I Sartorius membranes to remove substances of >5,000 M.W., significant inhibitory activity persisted. This suggested that the MAO inhibitors in CSF may be peptides of less than 5,000 M.W. Becker et al. (8) have reported the isolation of endogenous MAO inhibitors from human CSF of one or more proteins with low molecular weight (3,000 to more than 35,000). In the present study, these MAO inhibitors in dog CSF may be similar substances to that of human CSF (~3,000), although the properties of endogenous MAO inhibitors in human CSF have not been investigated; and moreover, the bovine brain mitochondria and human platelets are used as the enzyme source of A-form MAO and B-form MAO, respectively. A kinetic study of the inhibition of MAO by CSF addition was investigated with Lineweaver-Burk double reciprocal plots. The result with 5-HT and β-PEA as substrates are shown in Fig. 2. The inhibition

Fig. 1. Effects of dog CSF on MAO activity in brain mitochondria of several animals. After each of the mitochondrial preparations was incubated at 37°C for 20 min in the presence of varying amounts of dog CSF, MAO activity was determined with 5-HT (■) and β-PEA (□) as substrates at 37°C for 20 min. The mean±S.E. control values for MAO activity were 0.57±0.01 and 1.75±0.07 in mouse, 1.75±0.05 and 2.23±0.03 in rat, 0.88±0.03 and 1.15±0.03 in dog, and 0.98±0.02 and 1.49±0.03 nmol/min/mg of protein in monkey with 5-HT and β-PEA as substrates, respectively. The results are the mean of triplicate assays. Values are percentages of the control MAO activity without dog CSF.
was competitive at least toward 5-HT (Fig. 2, upper). With β-PEA as substrate, the inhibition was non-competitive with a decrease in the \( V_{max} \) and no change in the \( K_m \) (Fig. 2, bottom). Similar results have been reported using endogenous MAO modulators: an inhibitor or activator of MAO in plasma (4, 5), urine (6, 7) and in the cytosol of heart (3); these modulators exhibited competitive inhibition or activation of A-form MAO, but were non-competitive with B-form MAO. Moreover, the effects of imipramine, known as a tricyclic antidepressant and a weak MAO inhibitor, and clorgyline and deprenyl, known as a selective A-form and B-form MAO inhibitor, on dog brain mitochondrial MAO, respectively, were also investigated (Fig. 2). Imipramine and clorgyline exhibited competitive inhibition to A-form MAO with 5-HT as substrate. However, imipramine and deprenyl each showed non-competitive inhibition towards B-form MAO when β-PEA was the substrate. From these results, it may be presumed that the endogenous materials in the dog CSF also possess a MAO inhibiting effect which is similar to the existing MAO inhibitors.

In this present study, the presence of these inhibitors in CSF indicate that a CSF MAO modulator could affect the central nervous system (CNS) MAO, providing a link between CNS MAO activity and CNS mechanisms and underlying symptoms or behavior associated with MAO activity.

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