tion are central or peripheral (*21*), the concentration of CSF serotonin at night exceeds that of melatonin 50-fold and might contribute to some of the effects attributed to melatonin. Serotonin and related indoleamines (*21*) may thus contribute to diurnal alterations in physiological functions, a suggestion in keeping with the possible role of the cerebroventricular system as a conduit for neurohormonal integration of brain function (*22*).

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Endogenous Inhibitors of Monoamine Oxidase Present in Human Cerebrospinal Fluid

Abstract. *Inhibitory activity against the enzyme monoamine oxidase is present in low molecular weight fractions (less than 100,000) of human cerebrospinal fluid. These endogenous substances of different molecular weights (3000 to more than 35,000) act like monoamine oxidase inhibitor drugs to inhibit both type A and type B monoamine oxidase.*

There has been intense interest in monoamine oxidase (MAO) activity since the reports by Murphy *et al.* and others, which linked low platelet MAO activity to schizophrenia and schizophrenic symptoms (*1*). Inhibitors of MAO have been identified in blood (*2*) and urine (*3*). We report the isolation from human cerebrospinal fluid (CSF) of low molecular weight substances that inhibit both type A and type B MAO. The pharmacological effects of these endogenous inhibitors and of the MAO inhibitor drugs are similar: both reduce the activity of MAO toward its substrates. The presence of these inhibitors in CSF indicates that there may be a naturally occurring endogenous mechanism for the regulation of MAO activity in the brain.

The oxidation of monoamine neurotransmitters by MAO is one of the brain's major metabolic mechanisms for the degradation and inactivation of these neurotransmitters (*4*). In the brain, MAO exists in two forms, type A and type B (*5*). Both type A MAO, which deaminates dopamine, norepinephrine, and serotonin, and type B MAO, which also deaminates dopamine and norepinephrine but not serotonin, can be inhibited by antidepressant drugs of the MAO inhibitor type (*6*). The MAO inhibitor drugs are psychotogenic in schizophrenia, presumably as a result of increased dopamine concentrations after MAO inhibition. Schizophrenia has been related to increased brain concentrations of dopamine (*7*). Conversely, MAO inhibitor drugs are therapeutically effective against depressive diseases; these diseases have been linked to decreased brain concentrations of norepinephrine, serotonin, and dopamine (*8*). The MAO inhibitor drugs increase brain concentrations of these neurotransmitters by decreasing their inactivation by MAO (*9*). The therapeutic effects of these drugs have been postulated to derive from the increased neurotransmitter concentrations.

There may be an association between low concentrations of plasma MAO inhibitors and depressive symptoms that are responsive to MAO inhibitor drugs. Persons with high concentrations of platelet type B MAO activity have been shown to have lower concentrations of endogenous MAO inhibitors in their plasma than persons with midrange or low concentrations of platelet type B MAO activity (*2*). High concentrations of platelet type B MAO activity have

been reported in patients with depressive and phobic symptoms (*10*). These symptoms are particularly responsive to MAO inhibitor drugs (*11*).

Inhibitory activity was determined in excess human CSF samples obtained from 64 patients in the course of routine diagnostic lumbar punctures. Samples were discarded if they contained blood, other cells, or abnormal concentrations of protein. Samples were stored for 1 to 4 weeks at −20°C.

Since the quantity of CSF available from an individual sample (0.25 to 3 ml) was too small to permit separate purification, the CSF from individuals was combined to obtain six pooled test samples of 20 ml each. Pooled CSF was lyophilized, redissolved in a small volume of water, and loaded into a Sephadex G-50 column (1.5 by 50 cm) that had been calibrated with markers of known molecular weight: glucagon, 3,500; ribonuclease, 13,700; trypsin, 24,000; pepsin, 35,000; and bovine serum albumin, 67,000. The column was eluted with 50 m*M* K_3PO_4 buffer that contained 0.1 *M* KCl and was adjusted to *p*H 7.4. Effluent fractions (3 ml) were collected and monitored for ultraviolet absorbance at 280 nm to determine the protein concentration. Aliquots (400 μl) of each fraction were assayed for MAO inhibitory activity. For the assay of inhibitory activity against type A and type B MAO we used published methods (*4*, *5*). We determined type A MAO activity by using a standard preparation of bovine striatum as the source of MAO and ^{14}C-labeled serotonin, a type A MAO substrate. We determined type B MAO activity by using pooled blood bank platelets as the source of MAO type B and ^{14}C-labeled tryptamine as substrate.

Inhibitory activity is expressed as units of inhibition: one unit represents 1 percent inhibition of control MAO activity. Specific inhibitory activity (SIA) is the total area under the inhibitory peak curve divided by the total area under the protein curve for those fractions included in the inhibitory peak. Total inhibitory activity (TIA) is the total area under the inhibitory peak curve divided by the total protein loaded into the column; TIA expresses MAO inhibitory activity per milligram of CSF protein and therefore reflects the actual inhibition present in the CSF.

Fractions containing peak MAO inhibitory activity were added to varying concentrations of substrates to determine their effects on the K_m (Michaelis constant) and V_{max} (maximum velocity when the enzyme is saturated with substrate) of type A and type B MAO as determined by published methods (*4*, *5*).

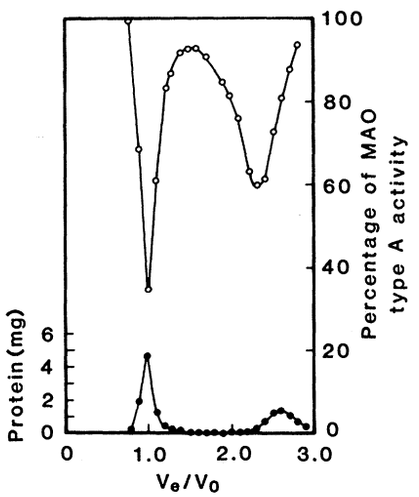
Table 1. Inhibitor activity of CSF fractions eluted from Sephadex columns.

V_e/V_0	SIA	TIA	*N*
	MAO type A		
1.00 ± 0.00	97 ± 6	39 ± 11	3
2.32 ± 0.04	205 ± 37	78 ± 16	3
	MAO type B		
1.00 ± 0.00	19 ± 2	6.5 ± 1.6	3
1.53 ± 0.03	448 ± 53	6.2 ± 3.2	3
1.93 ± 0.03	417 ± 42	10.3 ± 4.8	3

Elution profiles from the Sephadex column are shown in Fig. 1 and 2. Using the type A MAO inhibitory assay preparation, we found two inhibitory peaks (Fig. 1): one at the ratio of elution volume to void volume (V_e/V_0) of 1.0 corresponding to a molecular weight greater than 35,000 and another at a V_e/V_0 of 2.3 corresponding to a molecular weight of ~ 3000. Assaying for type B MAO activity inhibition, we found three inhibitory peaks (Fig. 2): one at a V_e/V_0 of 1.0 with molecular weight greater than 35,000, a second at a V_e/V_0 of 1.5 with a molecular weight of ~ 14,000, and a third at a V_e/V_0 of 1.9 with a molecular weight of ~ 7000. The profiles for MAO type A and MAO type B inhibition were replicated three times (Table 1).

The effects of these inhibitors on the kinetic properties of MAO are shown in Table 2. The fractions that inhibited type A MAO (V_e/V_0 = 1.0 and 2.3) and the fractions that inhibited type B MAO (V_e/V_0 = 1.0, 1.5, and 1.9) increased the K_m of MAO for its substrate. The type A MAO fractions also decreased the V_{max}.

We have evidence that these are endogenous substances and not an artifact of our procedures. Blanks treated with each assay as an internal control are



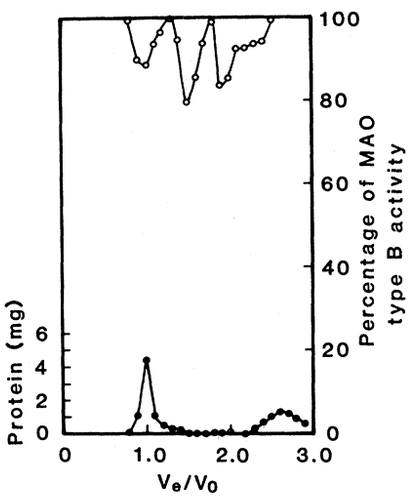


Fig. 1 (left). Elution profile of CSF from Sephadex columns: effect on MAO type A activity.
Fig. 2 (right). Elution profile of CSF from Sephadex columns: effect on MAO type B activity.

Table 2. Effects of CSF fractions on the kinetic parameters of MAO.

V_e/V_o	K_m* (× 10^{-5}*M*)	K_m (% of control)	V_{max}* (mmole per mg protein per hour)	V_{max} (% of control)	K_m/V_{max} (% of control)
		MAO type A			
Control	168 ± 14	100	67 ± 2	100	1.00
0.50	180 ± 19	107	71 ± 3	105	1.02
0.70	202 ± 33	120	71 ± 3	105	1.14
1.00	251 ± 68	149	45 ± 5	67	2.20
2.50	300 ± 30	179	47 ± 2	70	2.56
2.75	261 ± 67	155	44 ± 5	66	2.34
		MAO type B			
Control	14.3 ± 1.6	100	16.4 ± 0.7	100	1.00
1.0	20.0 ± 1.1	140	17.3 ± 0.4	105	1.33
1.6	21.1 ± 1.1	147	18.4 ± 0.4	112	1.31
1.9	16.8 ± 1.7	117	18.0 ± 0.8	109	1.07

*The kinetic determinations on MAO type B were repeated four times on four sets of column effluents. The results showed the following changes in K_m and V_{max} (expressed as the percentage of the control mean ± standard error of the mean): for V_e/V_o = 1.0, 136 ± 6 and 100 ± 3 percent of control K_m and V_{max}, respectively; for V_e/V_o = 1.6, 127 ± 10 and 106 ± 47 percent of control K_m and V_{max}, respectively; and for V_e/V_o = 1.9, 115 ± 1 and 105 ± 2 percent of control K_m and V_{max}, respectively.

devoid of MAO inhibitory activity. We have isolated from human blood, human brain, and rat brain substances of the same molecular weights that selectively inhibit either type A or type B MAO (*12*). In addition, if these CSF, blood, and brain samples are stored at room temperature for 72 hours prior to Sephadex separation, they no longer yield active fractions in the eluate.

These findings demonstrate that human CSF contains endogenous substances that act like MAO inhibitor drugs to inhibit both type A and type B MAO. These endogenous inhibitors of type A and type B MAO have different molecular weights and therefore probably are different substances. The type A MAO total inhibitory activity in CSF is six times as great as the type B MAO total inhibitory activity. These substances may have different degrees of control over type A MAO as compared with type B MAO.

As far as we know, this is the first isolation of endogenous inhibitors of MAO from the central nervous system. There is at present no evidence that links the inhibitory substances we have isolated to any clinical condition or to a dynamic regulation of MAO activity; only by direct study of the effects of these substances in animals and man will their physiological significance be determined.

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Evidence for Olfactory Function in Utero

Abstract. *Pregnant rats received 2-[^{14}C]deoxy-D-glucose (2DG) intravenously on the last day of gestation, and their fetuses were delivered 1 hour later by cesarean section. Fetal brains showed high 2DG uptake spread throughout the accessory olfactory bulb and little or no differential uptake in the main olfactory bulb. These findings demonstrate that functional activity occurs in the accessory olfactory bulb in utero and suggest that the accessory olfactory system may be the pathway by which fetal rats detect the odor quality of their intrauterine milieu.*

Our understanding of the main olfactory system has been increasing rapidly in recent years through the use of a variety of experimental techniques (*1*). Knowledge of the accessory olfactory system is much more limited. Although behavioral studies have implicated the accessory olfactory system in several important vertebrate behaviors (*2–4*), there have been few investigations of the physiological properties of this system (*5*). In the course of a developmental study of the rat olfactory system, we have used the 2-deoxy-D-glucose (2DG) method in utero and have obtained evidence for functional activity in the accessory olfactory bulb before birth.

The accessory olfactory pathway arises from receptors in the vomeronasal organ, which lies within a cartilaginous cavity in the fetal rat and is situated in the ventral portion of the nasal cavity. These receptors have an adult appearance at birth (*6*). This accessory pathway is anatomically distinct from the main olfactory pathway (*7*), which originates from receptors in the olfactory epithelium lining the nasal cavity; this epithelium also appears mature at birth (*8*). Figure 1 illustrates a frontal section of a newborn rat's main and accessory olfactory bulbs, where synapses of the main olfactory and vomeronasal nerves, respectively, are made. Although the laminae are not as distinct as in the mature olfactory bulb, the characteristic laminar pattern of each bulb is apparent. At birth, all the major cell types of the main and accessory olfactory bulbs are present, including mitral, tufted, granule, and periglomerular cells (*9*). Cortical projections of the main and accessory olfactory bulbs develop earlier in the rat (*10*) than the corresponding projections in hamsters (*11*). Therefore, anatomical considerations in rats indicate that each pathway has structural features that may allow them to function early in development.

Recent behavioral studies suggest that fetal rats are sensitive to the odor quality of amniotic fluid (*12*). Odor molecules dissolved in amniotic fluid could reach main olfactory and vomeronasal receptors by fetal respiratory movements, fetal swallowing, or passive diffusion. To test whether one or both of these pathways are stimulated in utero, we used