in a progressive delay of fade, until the potentiation process itself becomes saturated.

The potentiating effect of dipyridamole (Fig. 3), which cannot be ascribed in the present case to blockade of adenosine uptake, may have resulted from the sensitization of one set of adenosine-binding sites, but with the distinction that dipyridamole was more potent in this respect than adenosine. We have no proof that they share the same binding site that is involved in sensitizing the muscle, but this is not unlikely in view of their structural similarity and the documented evidence on the affinity of dipyridamole to the adenosine carrier in cell membranes.

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Type B monoamine oxidase activities toward β-phenylethylamine in discrete hypothalamic and circumventricular nuclei of the rat

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Monoamine oxidase (MAO; EC1.4.3.4) plays an important role in brain function by catalyzing the deamination of various monoamines, and it is designated type A and type B MAO based on substrate specificity and inhibitor sensitivity [1, 2]. Some investigators have demonstrated the differential effects of hormonal manipulations [3, 4] and emotional behaviors [5, 6] on each type of MAO, which led us to postulate that the two types of MAO may play different physiological roles in the regulation of several neuroendocrine secretions and some emotional behaviors. It was, therefore, of interest to study further the physiological roles of these enzymes in discrete brain nuclei.

We first showed that MAO catalyzing serotonin oxidation (5-HT–MAO; type A MAO) and MAO catalyzing tyramine oxidation (type A + B MAO) were unevenly distributed in various brain nuclei [7, 8] and that high proportions of type B MAO were found in discrete circumventricular regions of the rat when using the specific type A MAO inhibitor clorgyline [9].

It was shown recently by several authors [10–12] that the substrate specificity of β-phenylethylamine (PEA), which has long been regarded as a specific substrate for type B MAO [13], changed dramatically with changes in PEA concentration and pH of the reaction medium and that PEA was oxidized by either type of MAO at relatively high concentrations, based on inhibition studies with clorgyline and the specific type B MAO inhibitor deprenyl. On the basis of these findings, it was suggested that a low concentration of PEA as substrate is needed to assay type B MAO activities exclusively. In the present experiment, therfore, we determined MAO activities toward a low concentration of PEA (10 μM; PEA–MAO; type B MAO) in discrete hypothalamic nuclei and in some circumventricular regions of the rat by applying radiochemical micromethods to freeze-dried sections [14].

Male Wistar–King rats weighing 250–350 g were used. The animals were decapitated, and the brains were removed rapidly and placed on ice. The parts containing the preoptic area and hypothalamus and that of the lower brain stem were isolated and frozen in liquid nitrogen. Frontal sections of 200 μm thickness were made in a cryostat at −13°. The sections were freeze-dried overnight at −30° and 10^{-3} mm Hg and stored in evacuated tubes at −20° until use.

The individual preoptic and hypothalamic nuclei, or areas, were dissected carefully freehand under a steromicroscope, according to the atlas of König and Klippel [15]. The area postrema (coordinate, p 7.0 mm) was dissected also, according to the atlas of Palkovits and Jacobowitz [16]. The schematic drawings of the dissected nuclei are shown elsewhere except for the area postrema [7, 8]. Each sample (2–8 μg) was weighed using an electronic microbalance (Type EO-12, Eto. Co.). The sensitivity of this balance is 0.1 μg.

MAO activity was determined by a modification of the radiochemical methods of Wurtman and Axelrod [17] and White and Wu [18]. Ice-cold buffer–substrate (15 μl) was added to each tube containing tissue samples in an ice-bath (final concentration, 0.05 M sodium phosphate buffer (pH 7.4) and 10 μM β-[ethyl-1-^{14}C]phenylethylamine hydrochloride, 64.16 mCi/mmole, New England Nuclear Corp., Boston, MA). After preincubation for 15 min at 0°, the experimental and blank (containing no enzyme) tubes were mixed without warming and incubated at 37° for 10 min. The reaction was stopped by the addition of 3 N HCl. The radiolabeled products were extracted by 50 μl of toluene, and the toluene layer was washed once with 30 μl of 0.3 N HCl. After centrifugation, 20 μl of the organic layer was transferred to a counting vial, to which 10 ml of scintillator toluene solution was added. Radioactivity was determined by liquid scintillation spectrometry. The counting efficiency was 78 per cent. The preliminary experiments demonstrated that the reaction was linear with respect to

Table 1. Monoamine oxidase activities toward β-phenylethylamine and serotonin (5-HT) in discrete hypothalamic and circumventricular nuclei of the rat*

Nucleus and area	PEA–MAO (type B MAO)	5-HT–MAO† (type A MAO)
Preoptic area		
Organon vasculosum laminae terminalis	28.3 ± 2.2 (4)	42.9 ± 3.3 (4)
Nucleus preopticus suprachiasmaticus	29.0 ± 2.5 (5)	40.1 ± 1.3 (3)
Anterior part of the hypothalamus		
Nucleus anterior	9.1 ± 1.0 (4)	69.3 ± 4.7 (5)
Nucleus paraventricularis	20.8 ± 1.7 (6)	85.1 ± 6.8 (5)
Medial part of hypothalamus		
Nucleus ventromedialis	15.2 ± 1.1 (4)	91.9 ± 10.7 (4)
Nucleus dorsomedialis	14.5 ± 0.8 (4)	79.8 ± 9.6 (4)
Area lateralis	10.3 ± 0.8 (4)	55.0 ± 3.3 (4)
Nucleus periventricularis	32.6 ± 2.0 (6)	72.8 ± 9.3 (4)
Nucleus arcuatus	29.7 ± 3.3 (6)	53.4 ± 6.6 (5)
Median eminence	24.6 ± 2.1 (6)	48.4 ± 4.2 (4)
Posterior part of hypothalamus		
Nucleus posterior	11.9 ± 0.8 (6)	68.2 ± 5.9 (4)
Nucleus premammillaris ventralis	22.8 ± 2.6 (5)	88.1 ± 12.1 (4)
Nucleus premammillaris dorsalis	22.6 ± 1.4 (5)	47.2 ± 4.5 (4)
Nucleus arcuatus	43.2 ± 3.9 (5)	74.1 ± 6.2 (5)
Lower brain stem		
Area postrema	25.9 ± 1.1 (5)	

* Results are mean values [μmoles products·(g dry wt)$^{-1}$·hr^{-1}] ± S.E.M. with the number of determination given in parentheses. The final concentrations of PEA and 5-HT were 10 μM and 1 mM respectively. PEA–MAO activities represent apparent units of the enzyme activity, since the PEA concentration used was equal to the K_m value (10.1 μM).

† From our previous data [7, 8].

time and tissue used under the experimental conditions. In the present assay system, slight substrate inhibition was observed at 20 μM PEA and it became stronger at higher concentrations, as described by others [11, 19]. In addition, the present results represent apparent units of type B MAO activities, since the apparent K_m value for PEA was 10.1 μM and the PEA concentration used in the present study was equal to the K_m value. Using this assay, the reaction products were within 12 per cent of the total cpm added, and the experimental values ranged from three to eight times the blank value (120 cpm).

Recently, Kunimoto *et al.* [20] showed the distribution of MAO activities, using PEA as substrate, in individual brain nuclei of the rat. Their results, however, may reflect not type B MAO but activities of both types of MAO, since they assayed MAO activities using a high concentration of PEA (1 mM), and since it was recently indicated by Suzuki *et al.* [10, 11] that PEA at high concentration (1 mM) was oxidized by both types of MAO, based on inhibition studies with clorgyline and deprenyl.

The regional distribution of MAO, using 10 μM PEA as substrate (type B MAO), in discrete hypothalamic and circumventricular nuclei is summarized in Table 1. Of the hypothalamic nuclei and circumventricular regions examined, PEA–MAO activities varied about 5-fold between the region with the highest and the lowest values. The highest activities were found in the posterior part of the nucleus arcuatus. The medial part of the nucleus arcuatus, the nucleus periventricularis, the nucleus preopticus suprachiasmaticus, the organon vasculosum laminae terminalis (OVLT), the area postrema, and the median eminence, which are all close to the ventricle, had high specific activities. In contrast, the nucleus hypothalamicus anterior, the nucleus hypothalamicus posterior, and the medial part of the lateral hypothalamic area had low PEA–MAO activities. As suggested previously [7–9], the present results indicate directly that the circumventricular regions or nuclei are generally rich in type B MAO and that an active metabolism of PEA, or of other endogenous monoamines specific for type B MAO, may occur in those regions. The circumventricular regions are in contact with the ventricular cerebrospinal fluid, and some circumventricular structures are thought to be involved in the process of neurohumoral regulation [21, 22]. Indeed, we found previously differential effects of thyroidectomy on the two types of MAO in some circumventricular nuclei (the nucleus arcuatus and the nucleus periventricularis) 4 days [23] and 8 days (unpublished observation) after the operation.

Comparing the regional distribution of PEA–MAO with that of 5-HT–MAO [7, 8], PEA–MAO activities in discrete hypothalamic nuclei are distributed differently from those of 5-HT–MAO. PEA–MAO activities, as indicated above, are highly concentrated in the circumventricular regions, while the highest 5-HT–MAO activities are found in the hypothalamic nuclei of ventromedialis, premammillaris ventralis, paraventricularis, and dorsomedialis. It may be concluded, therefore, that the regional distributions of type A and type B MAO are different from one another in individual hypothalamic and circumventricular nuclei of the rat.

In summary, we have demonstrated type B MAO distribution, using 10 μM PEA as substrate, in discrete hypothalamic and some circumventricular nuclei of the rat. The present study indicates directly that the circumventricular nuclei or organs are generally rich in type B MAO.

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