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Release of endogenous dopamine from rat isolated striatum: effect of clorgyline and (−)-deprenyl

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1 High performance liquid chromatography with electrochemical detection was used to measure the release and content of dopamine and dihydroxyphenylacetic acid (DOPAC) from rat isolated striatum. The effects of the monoamine oxidase (MAO) inhibitors clorgyline and (−)-deprenyl on dopamine and DOPAC release and contents, and the IC_{50} values of these compounds for inhibition of dopamine deamination in rat striatum were determined.

2 Dopamine release was significantly increased by elevated KCl (22 mM) in a Ca^{2+}-dependent manner, and by ouabain (20 μM), whereas the release of DOPAC remained constant. The loss in striatal dopamine content during the incubation period (67% of initial content) was far greater than the amount of dopamine recovered in the incubation fluid (16% of initial content), suggesting that much of the DOPAC, released during incubation originated from the conversion of dopamine to DOPAC within the striatum.

3 A concentration-dependent decrease in DOPAC efflux, both during rest and stimulation periods, was observed in the presence of clorgyline (10^{-8} M–10^{-7} M) and (−)-deprenyl (10^{-5} M–10^{-4} M).

4 Higher concentrations of clorgyline (10^{-7} M) and (−)-deprenyl (10^{-4} M), which inhibited dopamine deamination by 85–90%, enhanced both the resting and KCl-induced release of dopamine.

5 The total amount of dopamine plus DOPAC that was released in the presence of clorgyline or (−)-deprenyl did not differ from control values, suggesting that the increase in dopamine release elicited by MAO inhibitors might result from reduced degradation of dopamine to DOPAC.

6 The IC_{50} values of clorgyline (5×10^{-9} M) and (−)-deprenyl (5×10^{-6} M) for inhibition of dopamine deamination indicate that dopamine is a substrate for type A MAO in rat striatum.

Introduction

It is generally accepted that dopamine released from axon terminal varicosities of the nigrostriatal pathway has an important role in the regulation of striatal neurotransmission (Vizi *et al.*, 1977; Dray, 1979; Lehmann & Langer, 1983). Yet the release of endogenous dopamine as a possible index of dopaminergic neural activity is not commonly measured because of the small amount of dopamine released from brain slice preparations. Drugs affecting the release of dopamine in the striatum have been investigated mainly by indirect methods such as measurement of [^{3}H]-dopamine synthesis from [^{3}H]-tyrosine (Glowinski, 1976) or determination of ^{3}H outflow from striatal slices preloaded with [^{3}H]-dopamine (Starke *et al.*, 1978; Cubeddu & Hoffman, 1982). Studies with the [^{3}H]-dopamine method, however, have often produced conflicting data. For example, when the effects of opioid peptides were studied, it was observed in one study that β-endorphin but not Met enkephalin, inhibited striatal dopamine release (Loh *et al.*, 1976), whereas in other studies, β-endorphin was reported to have no effect (Arbilla & Langer, 1978) and Met enkephalin was found to be inhibitory on dopamine release (Subramanian *et al.*, 1977). Similarly, studies of the effects of neuroleptics on striatal dopamine release have yielded contradictory data (Farnebo & Hamberger, 1971; Seeman & Lee, 1975).

In this paper an attempt has been made to characterize the release of endogenous dopamine *in vitro* from isolated striata of the rat using high performance liquid chromatography with electrochemical detection (h.p.l.c.-e.d.). The high sensitivity of the h.p.l.c.-e.d., technique makes the determination of

dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) possible in the perfusion fluid from brain slices (Bennett *et al.*, 1981; Kapoor, 1982; Kapoor & Arbuthnott, 1982). The effects of the monoamine oxidase (MAO) inhibitors clorgyline, which is selective for type A MAO (Johnston, 1968), and (−)-deprenyl, which is selective for type B (Knoll & Magyar, 1972), on striatal dopaminergic neurotransmission were investigated. It was found that inhibition of dopamine deamination by clorgyline or (−)-deprenyl leads to an increase in dopamine release and a reduction of DOPAC efflux, whereas the total amount of dopamine and DOPAC measured in the striatal perfusate is unchanged.

Methods

Release of dopamine and DOPAC from rat isolated striatum

All experiments were performed on rat isolated caudate nucleus. Wistar rats weighing 120–150 g were killed by decapitation and the brain was immediately removed and rinsed in ice-cold saline. The striatum was dissected on ice according to Glowinski & Iversen (1966). Striata, each weighing 30–40 mg, were pooled from 3 rats as the amount of dopamine and DOPAC released from this quantity of tissue was sufficient for reliable determination. Striata were incubated in organ baths at 37°C in 1 ml of Krebs-bicarbonate buffer containing Na_2EDTA (27 μM) and ascorbic acid (0.28 mM) and bubbled with 5% CO_2 in O_2. The Krebs-bicarbonate buffer had the following composition (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, and glucose 11.5. Incubation of striata was carried out in a 5 ml double-walled glass chamber with oxygen supplied through capillary tubing at bottom. No dopamine uptake inhibitor was used in this study since it was reported that nomifensine failed to counteract K^+-depolarization-evoked dopamine release from superfused rat striatum (Kapoor & Arbuthnott, 1982). The tissues were equilibrated under resting conditions for 60 min before collection of samples. Subsequently, the Krebs-bicarbonate buffer was replaced at 20 min intervals and the collected incubation fluid was assayed for released dopamine and DOPAC. During the third 20 min period, the incubation fluid contained either ouabain (20 μM) or high potassium (22 mM KCl) to stimulate the efflux of dopamine and DOPAC. When the concentration of KCl was elevated, the amount of NaCl in the incubation fluid was proportionally reduced to maintain iso-osmolarity. When the effects of clorgyline or (−)-deprenyl on the release of dopamine and DOPAC were measured, these MAO inhibitors were added at the beginning of the 60 min preincubation period to allow sufficient time for complete MAO inhibition to develop.

Striatal tissues were also removed for measurement of dopamine and DOPAC, either from freshly decapitated brains (non-incubated tissue) or at the end of sample collection (incubated tissue). In some experiments striata were incubated for two hours in Krebs-bicarbonate buffer which also contained 0.25 mM L-tyrosine.

Determination of dopamine and DOPAC with h.p.l.c.-e.d.

At the end of each collection period the incubation fluid was removed, frozen on dry ice, lyophilized, then reconstituted in 200 μl of 0.2 mM perchloric acid containing 0.11 mM ascorbic acid and centrifuged. Twenty μl of the clear supernatant was injected directly into the h.p.l.c. without further purification as described by Loullis *et al.* (1980) and Lyness (1982). Striatal tissues were homogenized by ultrasonication in 400 μl of 0.2 M perchloric acid containing 0.11 M asorbic acid and centrifuged. The clear supernatant was transferred to a fresh tube and 20 μl was injected into the h.p.l.c. as described by Lyness (1982). In some experiments catechol compounds were extracted on alumina as described by Mefford (1981). In this case, protocatechuic acid (300 pmol) was added as an internal standard.

The Biotronik h.p.l.c.-e.d. system (Biotronik Wissenschaftliche Geräte GmbH, Frankfurt am Main, West Germany) consisted of: BT 3020 high pressure pump with pulse dampener, Rheodyne 7125 injector with 20 μl loop, 150 × 4.6 mm Nucleosil C_{18} reverse phase column, 5 μm particles (Bischoff Analysentechnik), and ESA (Environmental Sciences Associates Inc., Bedford, MA) electrochemical detector with an ESA Model 5020 guard cell at a voltage of +0.6 V and an ESA Model 5010 dual electrode analytical cell with porous graphite electrodes at voltages of −0.5 V and +0.3 V versus palladium reference electrodes. Signals of the electrochemical detector were monitored on a two-channel chart recorder.

Mobile phase consisted of 0.1 M sodium acetate/citric acid, pH 4, containing 8% methanol and 0.4 mM sodium octyl sulphate. The mobile phase was filtered then degassed in an ultrasonic generator. Flow rate was 0.7 ml min^{-1}.

Standards were diluted in 0.2 M perchloric acid with 0.11 mM asorbic acid from stock solutions stored frozen in 1 N HCl. The detector response was linear in a range of 1.5–200 pmol 20 μl^{-1} of dopamine and DOPAC. Dopamine and DOPAC in the samples were identified by retention times and quantified by peak heights, and their contents were calculated from

standard curves determined by linear regression (correlation coefficient $r > 0.99$).

The concentration of dopamine and DOPAC in non-incubated and incubated rat striatum was expressed as nmol g^{-1}. The release of dopamine (DA) and DOPAC was calculated as follows:

DA or DOPAC release (pmol g^{-1} min^{-1}) =

$$\frac{\text{DA or DOPAC in sample (pmol)}}{\text{incubation time (min)} \times \text{tissue weight (g)}}$$

For statistical comparison of the data either the paired *t* test or two-tailed *t* test was used. Differences were considered significant when $P < 0.05$.

Determination of monoamine oxidase activity

Rat striatal mitochondria were prepared by the method of Suzuki *et al.* (1979). In some studies, mitochondria were prepared from striatum which had been incubated for 2 h as described above. Aliquots of the mitochondrial pellet (protein content 0.37 mg) were suspended in a final volume of 1 ml in 0.1 M sodium phosphate buffer (pH 7.4) containing 1 mM Na_2EDTA and 17 mM ascorbic acid and saturated with 95% O_2:5% CO_2. In some experiments clorgyline or (−)-deprenyl was added. After 10 min preincubation at 37°C the reaction was initiated by addition of 0.5 mM [^{3}H]-dopamine as substrate. After 20 min, 0.4 ml N HCl was added to terminate the reaction. Blanks were prepared by adding HCl at 0 time. The ^{3}H metabolites formed were extracted into 5 ml ethylacetate and the radioactivity of the organic phase was measured by liquid scintillation spectrometry. MAO activity was calculated by subtracting the blank value and the results were expressed as % of control. Protein content was measured by the method of Lowry *et al.* (1951).

Drugs

The following drugs and compounds were used in this study: dopamine HCl, 3,4-dihydroxyphenylacetic

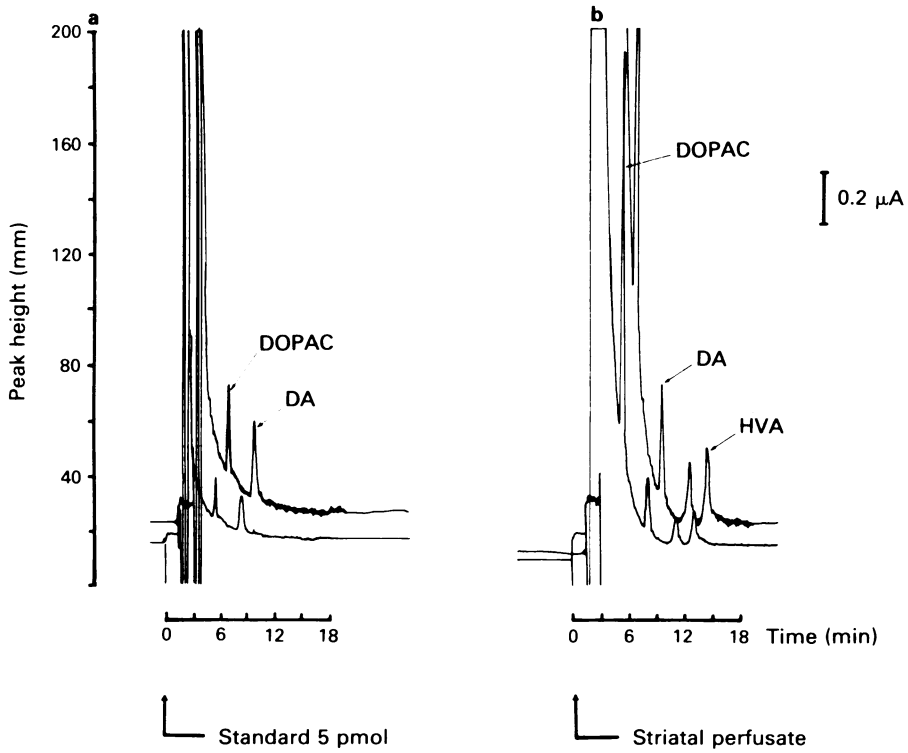

Figure 1 (a) H.p.l.c.-e.d. elution profile for mixture of external standard containing 5 pmol dihydroxyphenylacetic acid (DOPAC) and dopamine (DA). (b) Typical h.p.l.c.-e.d. chromatogram for dopamine and DOPAC released from incubated striata of the rat. The sample was collected in resting conditions, collection time 20 min. An unknown peak was observed between dopamine and homovanillic acid (HVA) and appeared to be sufficiently different in retention time from either dopamine or DOPAC. For registration a two-channel recorder was used with different sensitivities. For sample preparation and chromatographic conditions see Methods.

acid (DOPAC), protocatechuic acid, uric acid, homovanillic acid (HVA), Sigma; L-tyrosine, Serva; ouabain, Calbiochem; ascorbic acid, octyl sodium sulphate, Merck; (−)-deprenyl HCl, Chinoin; clorgyline HCl, May and Baker Ltd; Na_2EDTA, Reanal; [2,5,6-^{3}H]-dopamine (specific activity 500 GB mmol^{-1}), Amersham.

Results

Identification of dopamine and DOPAC in biological samples

Figure 1a shows a typical chromatogram for standards containing 5 pmol of dopamine and DOPAC. Retention times for dopamine and DOPAC were 5.3 and 8.1 min, respectively. Peaks identified as dopamine and DOPAC by retention times were obtained in striatal incubation fluid (Figure 1b) as well as in tissue extracts. No difference was found between dopamine content in tissue extracts measured by direct injection of unpurified supernatant (45.1 ± 1.5 nmol g^{-1}) or determined after alumina absorption (42.5 ± 7.7 nmol g^{-1}; $n = 4$; $P > 0.70$), indicating that the peaks were not contaminated by unknown materials.

Release of dopamine and DOPAC from rat isolated striatum

The resting release of dopamine and DOPAC from incubated striatum gradually decreased during the initial 60 min preincubation period (Figure 2a). The release of dopamine varied between 53.6 and 38.8 pmol g^{-1} min^{-1} under resting conditions. The rate of DOPAC output declined from 203.5 to 87.6 pmol g^{-1} min^{-1} and reached an equilibrium in about 60 min. Incubation of striata with Krebs-bicarbonate buffer containing 22 mM KCl resulted in a 2 fold increase of dopamine release (Figure 2b and Table 1). The efflux of dopamine min^{-1} was enhanced from 0.33 to 0.70% of total dopamine content. Omission of Ca^{2+} from the incubation fluid abolished the increase in dopamine efflux elicited by high KCl (Table 1). Ouabain, an inhibitor of Na^+-K^+-activated adenosinetriphosphatase (Vizi, 1978), added in a concentration of 20 μM to the incubation medium evoked a 3 fold increase in the amount of dopamine released. In contrast, DOPAC release was not increased by depolarization with either elevated KCl or ouabain (Table 1).

The dopamine content of striata which were not incubated was 31.8 ± 1.4 nmol g^{-1} ($n = 4$). After 120 min of incubation, the dopamine content of the

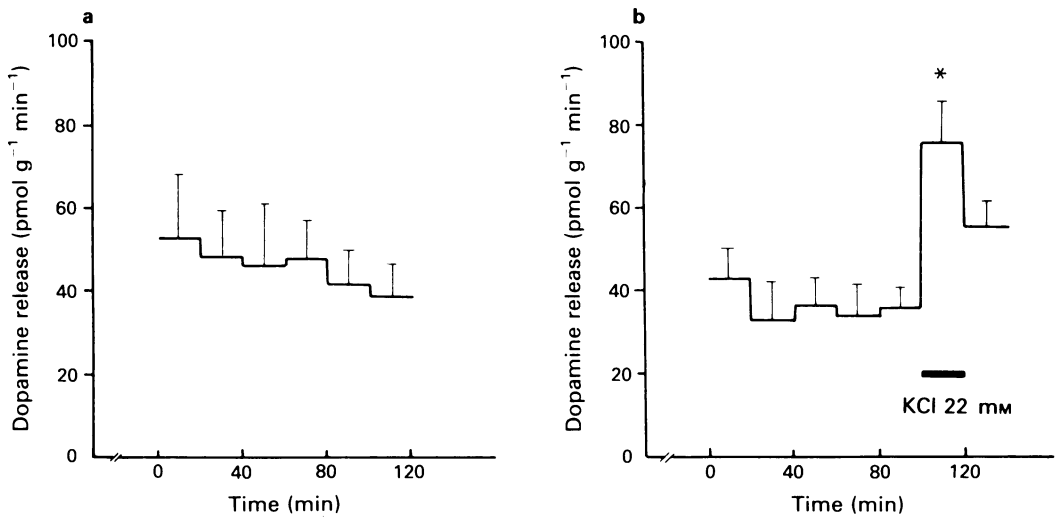

Figure 2 (a) Spontaneous dopamine release from rat isolated striatum. Rat striata, prepared as described in Methods, were placed in a thermostated (37°) glass chamber and incubated in Krebs-bicarbonate buffer containing asorbic acid (0.28 mM) and Na_2EDTA (27 μM) and were bubbled with 5% CO_2 in O_2. Dopamine released into the bathing solution was determined in serial fractions (20 min each) by h.p.l.c.-e.d. Mean of 5 experiments, vertical lines represent s.e.mean. (b) Increase by KCl (22 mM) of dopamine release from rat isolated striatum. Mean of 3 experiments, vertical lines indicate s.e.mean. KCl (22 mM) evoked a significant increase of dopamine release (*$P < 0.05$, paired t statistic).

Table 1 Release of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) from isolated striata of rat

Condition	*DA release* (pmol g^{-1} min^{-1})		*DOPAC release* (pmol g^{-1} min^{-1})	
Resting	42.5 ± 7.1	(13)	75.4 ± 10.0	(13)
KCl 22 μM	81.2 ± 11.2	(13)*	70.2 ± 10.1	(13)
Resting				
Ca^{2+} omitted	46.1 ± 9.0	(4)	122.7 ± 11.2	(4)
KCl 22 mM				
Ca^{2+} omitted	51.4 ± 8.4	(4)	84.4 ± 10.9	(4)*
Resting	32.7 ± 2.2	(4)	99.0 ± 16.0	(4)
Ouabain 20 μM	113.9 ± 16.1	(4)*	95.1 ± 17.7	(4)

Mean ± s.e.mean, number of experiments in parentheses, *$P < 0.05$, paired t test.

tissue was reduced to 10.5 ± 0.2 nmol g^{-1} ($n = 4$, $P < 0.01$), indicating that 67% of the initial dopamine content was lost during incubation. This decline of dopamine content could not be prevented by addition of L-tyrosine (0.25 mM) to the incubation fluid: dopamine contents measured at the end of incubation were 11.0 ± 2.5 and 12.0 ± 0.6 nmol g^{-1} in the absence and presence of L-tyrosine, respectively ($n = 4$, $P > 0.70$). About 16% of the original dopamine content was released and recovered in the perfusate (5.2 nmol g^{-1} in 120 min of incubation) whereas 51% of the dopamine which was present in the striata before incubation could not be accounted for. No significant change was found in the DOPAC content of incubated and non-incubated striatum (2.9 ± 0.1 and 3.2 ± 0.1 nmol g^{-1}, respectively, $n = 4$, $P > 0.05$). A high amount of DOPAC (13.8 ± 1,9 nmol g^{-1} 120 min^{-1}) was, however, released from striata and recovered from the perfusion fluid.

Effect of clorgyline and (−)-deprenyl on dopamine and DOPAC release from rat striata

In another series of experiments, striata were incubated for 120 min in the presence of clorgyline or (−)-deprenyl. As is shown in Table 2, clorgyline and (−)-deprenyl added in concentrations of 10^{-8}–10^{-7} and 10^{-5}–10^{-4} M, respectively, reduced both the resting and KCl-induced release of DOPAC. Higher concentrations of clorgyline (10^{-7} M) and (−)-deprenyl (10^{-4} M) were needed to evoke elevation of resting and KCl-elicited release of dopamine from rat isolated striatum (Table 2).

Inhibition by clorgyline and (−)-deprenyl of dopamine deamination is shown in Table 3. The calculated IC_{50} values of clorgyline and (−)-deprenyl for MAO inhibition using dopamine as substrate were 5×10^{-9} and 5×10^{-6} M, respectively, in striatal mitochondria prepared from non-incubated tissue or from striatal tissue which had been incubated for 2 h. Inhibition of DOPAC efflux by MAO inhibitors was associated with a 67–76% inhibition of dopamine deamination whereas a greater inhibition (85–96%) was required for elevation of dopamine release (Tables 2 and 3). When the total amount of dopamine plus DOPAC released was calculated, no significant differences were found between the control values and those measured in the presence of clorgyline and (−)-deprenyl (Table 4).

Table 2 Effect of clorgyline and (−)-deprenyl on the release of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) from rat isolated striata

Treatment	Concentration (M)	*DA release* Resting	*DA release* KCl-induced	*DOPAC release* Resting	*DOPAC release* KCl-induced	(pmol g^{-1} min^{-1})	
Control	—	31.1 ± 2.6	77.3 ± 2.9	81.1 ± 7.2	76.7 ± 10.3		(5)
Clorgyline	10^{-9}	26.2 ± 3.2	71.5 ± 16.3	73.0 ± 4.4	60.3 ± 7.1		(3)
Clorgyline	10^{-8}	22.6 ± 5.7	89.2 ± 17.0	47.6 ± 1.8*	35.3 ± 2.2*		(3)
Clorgyline	10^{-7}	51.3 ± 0.9**	108.1 ± 12.5*	36.2 ± 8.5**	27.2 ± 7.1*		(3)
Control	—	39.4 ± 9.8	64.0 ± 7.8	74.5 ± 1.29	78.5 ± 13.6		(6)
(−)-Deprenyl	10^{-6}	38.8 ± 4.8	77.6 ± 3.8	28.0 ± 11.6	28.5 ± 11.7		(3)
(−)-Deprenyl	10^{-5}	29.1 ± 6.2	71.4 ± 12.0	18.7 ± 2.8*	24.7 ± 6.9*		(3)
(−)-Deprenyl	10^{-4}	87.3 ± 15.0*	141.9 ± 6.4***	7.0 ± 2.2**	6.7 ± 2.0**		(5)

Rat isolated striata were incubated in the presence of clorgyline or (−)-deprenyl for 2 h. The release was stimulated by 22 mM KCl.
Mean ± s.e.mean, number of experiments in parentheses, *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; two-tailed t test in comparison with controls.

Table 3 Effect of clorgyline and (−)-deprenyl on dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) content and [^{3}H]-dopamine deamination in rat isolated striatum

Treatment	Concentration (M)	Content of DA (nmol g^{-1})	DOPAC (nmol g^{-1})		Ratio of DA to DOPAC	Inhibition of [^{3}H]-DA deamination (%)
Control	—	12.1 ± 1.0	2.6 ± 0.2	(5)	4.6	—
Clorgyline	10^{-9}	13.1 ± 2.4	2.2 ± 0.03	(5)	5.9	14
Clorgyline	10^{-8}	19.1 ± 2.8*	1.5 ± 0.05**	(3)	12.7	67
Clorgyline	10^{-7}	19.8 ± 0.9**	0.8 ± 0.1***	(3)	24.7	85
Control	—	13.7 ± 1.8	2.5 ± 0.1	(6)	5.4	—
(−)-Deprenyl	10^{-6}	17.1 ± 2.5	1.8 ± 0.3*	(3)	9.5	30
(−)-Deprenyl	10^{-5}	24.5 ± 2.4**	1.8 ± 0.1**	(3)	13.6	76
(−)-Deprenyl	10^{-4}	19.0 ± 1.2*	0.5 ± 0.09***	(5)	38.0	96

Rat isolated striata were incubated in the presence of clorgyline or (−)-deprenyl for 2 h. Inhibition of [^{3}H]-DA deamination was measured in striatal mitochondrial preparation. Control MAO activity: 94 nmol mg^{-1} protein 20 min^{-1}.
Mean ± s.e.mean, number of experiments in parentheses, *$P<0.05$; **$P<0.01$; ***$P<0.001$; two-tailed t test in comparison with controls.

Table 4 The total amount of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) released from rat isolated striata in the presence of monoamine oxidase (MAO) inhibitors

Treatment	Concentration (M)	DA plus DOPAC released: resting condition (pmol g^{-1} min^{-1})		KCl stimulated (pmol g^{-1} min^{-1})		
Control	—	112.3 ± 6.8		154.1 ± 10.5	(5)	
Clorgyline	10^{-6}	93.4 ± 3.4	$P>0.05$	135.3 ± 14.2	(3)	$P>0.30$
Control	—	114.3 ± 16.6		143.0 ± 9.2	(6)	
(−)-Deprenyl	10^{-4}	94.4 ± 16.3	$P>0.40$	148.7 ± 6.4	(5)	$P>0.60$

The release was stimulated by 22 mM KCl.
Mean ± s.e.mean, number of experiments in parentheses, two-tailed t test in comparison with controls.

Reduction of DOPAC output in response to MAO inhibition by either clorgyline or (−)-deprenyl was accompanied by a gradual decrease in the DOPAC content of striatal tissue when measured at the end of 120 min incubation (Table 3). A significant increase in dopamine content was also observed in the presence of 10^{-8} M clorgyline and 10^{-5} M (−)-deprenyl (Table 3). The ratio of dopamine to tissue DOPAC showed a concentration-dependent increase in response to MAO inhibitors (Table 3).

Discussion

We have used h.p.l.c. in combination with electrochemical detection for investigation of dopaminergic neurotransmission in rat isolated striata. Application of an analytical cell with dual electrodes in the redox mode provides selective detection of dopamine and DOPAC, compounds capable of undergoing reversible oxidation/reduction reactions. The redox mode of electrochemical detection greatly reduces the possibility of interference from unknown compounds when testing substances in biological samples. While some unidentified peaks appeared in the chromatograms of incubation fluid and tissue extracts, they did not interfere with either dopamine or DOPAC. Thus, we concluded that interference from unknown compounds can be ruled out under our h.p.l.c. conditions.

The levels of dopamine and DOPAC measured in non-incubated striatum were comparable with those reported by others who used h.p.l.c.-e.d. for determination (Westerink & Mulder, 1981; Wagner *et al.*, 1982). In addition the rate of dopamine and DOPAC efflux determined in our experiments proved to be in

the same range as found by Kapoor & Arbuthnott (1982). The efflux of both dopamine and DOPAC decreased as a function of incubation time and reached an equilibrium after about 60 min. As has been found with other putative neurotransmitters (Vizi, 1978), the release of dopamine from striatum was stimulated by ouabain and by elevated KCl; the enhancement of dopamine by KCl was Ca^{2+} dependent. Kapoor (1982) and Kapoor & Arbuthnott (1982) reported that DOPAC release was increased by KCl or electrical stimulation. However, in our experimental conditions the efflux of DOPAC was not affected by either ouabain or by elevated KCl. This difference between dopamine and DOPAC release might suggest differences in the mechanisms by which the two compounds are released; whereas dopamine reaches the synaptic cleft through a carrier-mediated transport system, DOPAC is not retained by neural structures of the axon terminals (Arbilla & Langer, 1980).

A great deal of the dopamine originally present in striatum was lost during incubation. Only a small fraction of the dopamine lost from striatal stores was recovered in the incubation fluid; the rest was probably metabolized within the tissue. The decline of dopamine content in incubated striatum might be due to a rapid breakdown of dopamine to DOPAC which is subsequently released from the striatal preparation. We found that the decrease in the dopamine concentration of incubated striatum could not be prevented by addition of L-tyrosine, a precursor of dopamine. Since it has been shown that dopamine can be synthesized from tyrosine under *in vitro* conditions (Glowinski, 1976) we speculate that the rate of dopamine synthesis is much less that the rate of metabolism in the incubated striatal tissue.

Evidence has been obtained that type A MAO is present in nigrostriatal neurones: lesioning of the substantia nigra pars compacta with 6-hydroxydopamine treatment led to selective reduction in type A MAO in the ipsilateral striatum (Demarest *et al.*, 1980). Other observations have also indicated that dopamine might be a substrate for type A MAO in rat striatum (Braestrup *et al.*, 1975; Waldmeier *et al.*, 1976; Hársing *et al.*, 1979; Demarest *et al.*, 1980) and the present findings are consistent with the previous observations. It is highly probable that in our experimental conditions clorgyline affected dopaminergic transmission by selective inhibition of type A MAO, whereas (−)-deprenyl exerted its action at concentrations known to inhibit type B as well as type A MAO. The IC_{50} value of (−)-deprenyl for selective inhibition of MAO B using *m*-iodobenzylamine or phenylethylamine (both are MAO B substrates) was reported to be 10^{-7}–10^{-8} M (Knoll & Magyar, 1972; Yang & Neff, 1973). Thus, it seems very likely that (−)-deprenyl exerts its action on dopaminergic neurotransmission in the rat by non-selective MAO inhibition.

Both the efflux and content of DOPAC in the striatum were reduced by doses of clorgyline which are selective for type A MAO and which caused greater than 50% inhibition of dopamine deamination. This suggests that the pharmacological effects of MAO inhibition may be seen only when MAO is inhibited by more than 50%. Whereas one-half of the type A MAO activity in the striatum is associated with dopaminergic neurones, type B MAO activity appears to be restricted to nondopaminergic neural structures (Demarest *et al.*, 1980). This might explain why (−)-deprenyl only reduced DOPAC efflux and content in concentrations that inhibited type A MAO. Whereas the efflux of DOPAC was reduced by MAO inhibitors, the release of dopamine under both resting and stimulated conditions was enhanced. These effects of clorgyline and (−)-deprenyl are probably due to protection of dopamine from enzymatic degradation to DOPAC, which would lead to changes in the proportion of dopamine to its metabolite within the nerve terminals. This is supported by the observation that, although the proportion was changed, the total amount of dopamine plus DOPAC that was released in control conditions or in the presence of clorgyline or (−)-deprenyl did not differ significantly.

From the present data we conclude that selective doses of clorgyline, which inhibit type A MAO, and non-selective doses of (−)-deprenyl, which inhibit both type A and B MAO, affect nigrostriatal dopaminergic neural transmission. This suggests that dopamine might be a substrate for type A MAO in rat striatum. Clorgyline and (−)-deprenyl do not alter the amount of dopamine plus DOPAC released whereas the proportion of dopamine and DOPAC released is changed, with an increase in dopamine and a reduction in DOPAC release. Changes in the proportion of dopamine to DOPAC in response to MAO inhibitors, however, might lead to enhanced dopaminergic tone and as a consequence, a reduction in cholinergic transmission in the striatum (Hársing *et al.*, 1979). In addition, it has been shown that (−)-deprenyl inhibits dopamine uptake in the rat striatum and forebrain (Hársing *et al.*, 1979; Lai *et al.*, 1980; Azzaro & Demarest, 1982) which might also result in enhancement of dopaminergic tone. In human striatum, where dopamine is a substrate for MAO-B (Glover *et al.*, 1977), (−)-deprenyl might exert its therapeutic action in Parkinson's disease through these mechanisms.

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