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Inhibitory effects of type A and type B monoamine oxidase inhibitors on synaptosomal accumulation of [^{3}H]dopamine: a reflection of antidepressant potency

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Inhibitors of monoamine oxidase (MAO) are principally used in medicine in the treatment of depressive illness [1]. While these agents demonstrate quite similar MAO inhibitory potencies, clinical studies have shown differences in their antidepressant efficacy [2, 3]. Tranylcypromine, for example, is a highly effective antidepressant, while nialamide is very weak, with pargyline assuming an intermediate position in the spectrum of antidepressant potency. In search of an explanation, Hendley and Snyder [4] reported that the clinical efficacy of these antidepressants was closely reflected in their potency as inhibitors of brain catecholamine uptake.

More recently, multiple forms of MAO (i.e. type A and type B) have been described in a variety of tissues including brain [5, 6]. Moreover, new selective inhibitors of each form of MAO have been developed [7, 8]. Clorgyline (a type A inhibitor) and deprenyl (a type B inhibitor) were the first of these to be described. Recent clinical studies have shown that these selective MAO inhibitors are also effective as antidepressant drugs [9–13]. Thus, it became of interest to know if these agents demonstrated inhibitory properties on brain catecholamine uptake that were similar in potency to the non-selective but highly effective antidepressant, MAO-inhibitory drugs (e.g. tranylcypromine). Accordingly, we have examined the effects of a number of clinically active selective and non-selective MAO inhibitors on the accumulation of [^{3}H]dopamine (DA) in synaptosomal-rich homogenates of rat forebrain.

Materials and methods

Adult male Sprague–Dawley rats (200 g) were obtained from Hilltop Laboratory Animals, Inc. (Scottsdale, PA) and housed in the university animal facility for 1 week prior to use. These animals were permitted food and water *ad lib.*

Tissue preparation. Rats were decapitated, and their brains were removed and placed in ice-cold saline. Forebrain sections were obtained by making a coronal cut from the rostral border of the superior colliculi to the optic chiasm. The entire dissection was performed in the cold.

Accumulation studies. Rats were pretreated with 2.5 mg/kg (i.p.) reserpine and 100 mg/kg (i.p.) nialamide 2 hr prior to decapitation, to eliminate the storage and metabolism components of the catecholamine neuronal accumulation process [14]. This dose of nialamide caused complete inhibition of brain MAO activity. Forebrain sections from these animals were homogenized in 10 vol. of 0.32 M sucrose, and a crude synaptosomal-rich (P_2) fraction was prepared as described by Whittaker *et al.* [15]. Aliquots (200 μl) of the resuspended P_2 fraction were assayed for inhibitory effects by clinically active MAO-inhibitor drugs on the high-affinity accumulation of [^{3}H]catecholamine, using the method described by Coyle and Snyder [16]. [^{3}H]DA (0.1 μM; 11.4 Ci/mmole) was used as the substrate of the high-affinity transport system. The incubation was carried out for 5 min (37°) in the presence of a wide concentration range (0.1 μM to 1 mM) of each inhibitor drug. Diffusion blanks were obtained at 0°, and total protein was determined by the biuret method [17]. Tissue/medium ratios were obtained and percent inhibition of uptake was calculated as previously described [18]. IC_{50} Values for each inhibitor drug were obtained on logarithmic-probability paper [19].

MAO assay. Forebrain tissue was assayed for type A and type B MAO activity using methods previously described [20]. Aliquots of a crude mitochondrial fraction [15] were preincubated with each MAO-inhibitor drug. [^{14}C]Serotonin (47.2 mCi/mmole; 0.1 mM) and [^{14}C]β-phenylethylamine (41.6 mCi/mmole; 5 μM) were the substrates used to assay type A and type B MAO respectively. Percent inhibition of MAO activity was calculated, and IC_{50} values were obtained as described above.

Statistical analysis. Each difference reported in this manuscript was analyzed for statistical significance using Student's *t*-test. Differences were considered to be statistically significant at the $P < 0.05$ level. Degrees of freedom $= n - 1$.

Results

Six clinically effective MAO-inhibitor drugs were tested for their effects on MAO activity and [^{3}H]catecholamine accumulation. A summary of the effects of each of these agents on type A ([^{14}C]serotonin deamination) and type B ([^{14}C]phenylethylamine deamination) MAO activity in rat forebrain is presented in Table 1. Clorgyline and Lilly 51641 were clearly more effective as inhibitors of [^{14}C]serotonin deamination. In contrast, deprenyl and pargyline were 100 to 1000 times more active as inhibitors of

Table 1. Inhibition of type A and type B MAO activities in rat forebrain

MAO inhibitor	IC_{50} Values* (moles/l) [^{14}C]Serotonin deamination	[^{14}C]Phenylethylamine deamination
Clorgyline	$11.7 (\pm 1.1) \times 10^{-9}$	$89.3 (\pm 3.8) \times 10^{-6}$
Lilly 51641	$40.5 (\pm 2.3) \times 10^{-9}$	$5.3 (\pm 0.2) \times 10^{-6}$
Deprenyl	$49.3 (\pm 2.7) \times 10^{-6}$	$49.7 (\pm 6.3) \times 10^{-9}$
Pargyline	$5.0 (\pm 1.1) \times 10^{-6}$	$46.7 (\pm 5.4) \times 10^{-9}$
Tranylcypromine	$1.6 (\pm 0.2) \times 10^{-6}$	$0.6 (\pm 0.1) \times 10^{-6}$
Nialamide	$51.0 (\pm 9.0) \times 10^{-6}$	$70.1 (\pm 10.2) \times 10^{-6}$

* Means ± S.E.M. of four experiments run in triplicate. Each value was obtained by probit plot analysis.

Table 2. Inhibition of synaptosomal accumulation of [^{3}H]dopamine in rat forebrain

MAO inhibitor	ID_{50} for [^{3}H]Dopamine accumulation* (μmoles/l)
Tranylcypromine	4.36 ± 1.30
Lilly 51641	5.53 ± 1.08
Deprenyl	23.30 ± 1.59
Clorgyline	34.03 ± 9.76
Pargyline	378.10 ± 30.32
Nialamide	>1000.00

* Means ± S.E.M. of four experiments performed in duplicate. Each value was obtained by probit plot analysis.

[^{14}C]phenylethylamine deamination. The final two agents, tranylcypromine and nialamide, were non-selective type A or type B MAO inhibitors, showing similar inhibitory potencies on [^{14}C]serotonin or [^{14}C]phenylethylamine deamination.

Table 2 shows the effects of each of these agents on synaptosomal accumulation of [^{3}H]DA in rat forebrain. The drugs are listed in decreasing order, according to their inhibitory potencies. With the exclusion of nialamide, all agents were effective inhibitors of synaptosomal [^{3}H]DA accumulation. Tranylcypromine and Lilly 51641 demonstrated inhibitory potencies that were 4–6 times greater than deprenyl or clorgyline and nearly 100 times greater than pargyline. No clear relationship between selectivity with regard to MAO inhibition (i.e. type A or type B inhibition) and the inhibitory potency on [^{3}H]DA accumulation was observed.

Discussion

In the present study, a number of clinically active MAO-inhibitor drugs were examined for their effects on [^{3}H]catecholamine accumulation in brain tissue. From our experiments, it is clear that classic agents such as tranylcypromine, pargyline and nialamide demonstrate inhibitory potencies on [^{3}H]DA accumulation that are consistent with their clinical efficacy as antidepressants [2]. Thus, based upon this relationship and the data presented here for the newer agents, one might predict that Lilly 51641, deprenyl, and clorgyline would fall into a class of MAO-inhibitor antidepressants that Davis [2] has described as highly effective and certainly more effective than pargyline. Each of these agents was 10 to 70 times more potent as inhibitors of [^{3}H]DA accumulation than was pargyline. A recent study by Lipper *et al.* [9] would indeed support this suggestion. In a crossover study between clorgyline and pargyline, in a total of twenty-four depressed patients, clorgyline demonstrated greater antidepressant properties. If this finding remains consistent in the hands of others, then similar comparative studies should be performed between pargyline and deprenyl or Lilly 51641. The latter agent was six times more potent as an inhibitor of [^{3}H]DA accumulation than clorgyline.

The finding that clorgyline and deprenyl were essentially equal as inhibitors of [^{3}H]DA accumulation is not consistent with previous findings in rat brain tissue. In a study on the effects of these two drugs on a variety of monoamine uptake systems, it was demonstrated that clorgyline (IC_{50} = 56 μM) was approximately ten times more potent than deprenyl (IC_{50} = 530 μM) in inhibiting [^{3}H]DA uptake [21]. The reason for this discrepancy between the two studies is not readily evident. However, it should be noted that Lai *et al.* [21] neglected to pretreat their animals with an MAO inhibitor or reserpine in order to eliminate the catabolism and storage components of the accumulation process [14]. Since clorgyline and deprenyl are selective MAO inhibitors (type A and type B, respectively), the localization and activity of each form of MAO within the tissue might influence the amount of unmetabolized [^{3}H]DA retained by the tissue under these experimental conditions. Through *in vivo* pretreatment with nialamide and reserpine, we have minimized these variables and can assume that the effects observed were primarily through interactions of these drugs with the high-affinity neuronal membrane transport system for [^{3}H]DA. In support of our findings, Knoll [22] has demonstrated that deprenyl is a highly potent inhibitor of the catecholamine neuronal uptake system in peripheral adrenergic neurons.

While each of the MAO-inhibitor drugs was effective in inhibiting [^{3}H]DA accumulation, it was clearly evident that their potencies as selective MAO inhibitors were much greater (see Table 1). The IC_{50} values for selective MAO inhibition were two to three orders of magnitude less than those obtained for inhibition of [^{3}H]DA uptake (see Table 2). Thus, it is clear that each of these agents represents a substantial improvement over previous MAO-inhibitor drugs (e.g. tranylcypromine) with which it is difficult to separate an inhibitory effect on monoamine uptake from that seen on MAO. These selective properties of the newer MAO-inhibitor agents should prove to be of value in their use as experimental tools.

In summary, we have shown that the newer selective MAO-inhibitor drugs, which demonstrate clinical efficacy as antidepressants, also inhibit the accumulation of [^{3}H]catecholamines by brain tissue. These results are consistent with the previous suggestion that clinical efficacy of antidepressant MAO inhibitors is reflected in the inhibitory potency of these drugs on catecholamine neuronal uptake.

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Potassium release by α_2-adrenergic receptor stimulation of rat parotid acinar cells

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A classification of α-adrenergic receptors [1–3] has been developed based on the abilities of certain selective agents to preferentially elicit or block α-adrenergic responses. The agonists clonidine and α-methyl norepinephrine and the antagonist yohimbine are more potent at α_2-adrenergic receptors, while the agonist phenylephrine and the antagonists WB-4101 and prazosin are more potent at α_1 receptors. Fain and Garcia-Sainz [4] noted that known α_1-adrenergic receptors appear to mediate responses by facilitating the entry of extracellular calcium into target cells, while α_2-adrenergic receptors probably mediate responses by inhibiting cAMP* formation.

Parotid acinar cells release K^+ when exposed to α-adrenergic agonists. K^+ release appears to result from Ca^{2+} entry since the response is Ca^{2+} dependent and can be elicited by Ca^{2+} ionophores [5]. Radioligand binding studies of rat submaxillary glands have shown the presence of receptor binding sites with both α_1 [6] and α_2[6, 7] characteristics, while the binding sites in parotid have not been classified. Accordingly, we undertook a characterization of the α-adrenergic response in dispersed parotid cells with selective antagonists in order to test the hypothesis that Ca^{2+} entry is mediated by α_1-adrenergic receptors.

Materials and methods

Cell preparation. Dispersed parotid cells were prepared as described previously [8, 9]. Freshly excised parotid glands were minced and subsequently incubated in collagenase and hyaluronidase for 1 hr with gentle mechanical disruption. After filtration through nylon mesh, the cells were washed twice and resuspended in Hanks–Hepes* buffer [8] (143.3 mM NaCl, 5.4 mM KCl, 0.81 mM $MgSO_4 \cdot 7H_2O$, 2.4 mM $CaCl_2$, 20.0 mM Hepes, and 5.6 mM dextrose) gassed with 95% O_2–5% CO_2. Approximately 1–2 $\times 10^8$ cells were routinely obtained from the parotid glands of five or six male Sprague–Dawley rats (Zivic—Miller Laboratories, Allison Park, PA).

Potassium release. K^+ release was measured in 50 μl of cells (approximately 5×10^6 cells) suspended in gassed Hanks–Hepes buffer at 37°. In most experiments, epinephrine alone or in combination with an antagonist in Hanks–Hepes buffer was added directly to the cell preparation. After 30 sec of incubation, the suspension was centrifuged (Beckman microfuge) for an additional 30 sec and 25 μl of the supernatant fraction was diluted with 1 ml of an Acationox (Scientific Products, Charlotte, NC) solution. K^+ was measured with an atomic absorption spectrometer (Perkin–Elmer 303). In some experiments, the cells were preincubated for 30 min in buffer containing one of the α-adrenergic antagonists before being exposed to epinephrine for 30 sec.

High concentrations of prazosin were insoluble in buffer alone. A stock solution of prazosin (10 μM) was routinely prepared in buffer with 10% ethanol. This stock solution was diluted with buffer alone. Control experiments with 10% ethanol showed that neither this concentration nor more dilute solutions of ethanol had any effect on the K^+ release elicited by epinephrine from intact cells.

Materials. WB-4101 was donated by WB Pharmaceuticals, Ltd., Berkshire, U.K.; prazosin HCl was supplied by Pfizer, Inc., Brooklyn, NY, U.S.A.; and yohimbine HCl

* Abbreviations: cAMP, cyclic 3′,5′-adenosine monophosphate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethyleneglycolbis(aminoethylether)tetra-acetate; and WB-4101, (2-[2-(2′,6′-dimethoxyphenoxy)ethylaminomethyl]-1,4-benzodioxane HCl.