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Chronic Clorgyline and Pargyline Increase Apomorphine-Induced Stereotypy in the Rat

IAIN C. CAMPBELL,* MICHAEL J. DURCAN,* ROBERT M. COHEN,† DAVID PICKAR,† DIANE CHUGANI† AND DENNIS L. MURPHY†

**Institute of Psychiatry, DeCrespigny Park, London SE5 8AF, UK*
†Clinical Neuropharmacology Branch, NIMH, Bethesda, MD 20205

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CAMPBELL, I. C., M. J. DURCAN, R. M. COHEN, D. PICKAR, D. CHUGANI AND D. L. MURPHY. *Chronic clorgyline and pargyline increase apomorphine-induced stereotypy in the rat.* PHARMACOL BIOCHEM BEHAV **23**(6) 921–925, 1985.—The effects of monoamine oxidase inhibiting antidepressant drugs on behavioral and biochemical measures of dopamine receptor status were measured in the rat. Male Wistar rats received clorgyline (1 mg/kg/day for 21–28 days), pargyline (1 mg/kg/day for 21–28 days) or a combination of these regimens. They were then either tested for stereotypy induced by 1 mg/kg SC injection of apomorphine or were sacrificed and their striata used to measure specific [^{3}H]spiroperidol binding. All three chronic treatment regimens produced statistically significant increases in apomorphine induced stereotypy: there was, however, no significant difference between the three drug regimens. None of the antidepressant drug treatments significantly affected [^{3}H]spiroperidol binding in the corpus striatum. This study demonstrates that behavioral and biochemical measures of dopamine function may not always be closely correlated. It is proposed that the behavioral changes may be related to alterations in other monoaminergic systems, which are known to have fibres running into the nigrostriatal pathway.

Antidepressants Clorgyline Pargyline Stereotypy Apomorphine [^{3}H]spiroperidol Rats

BIOCHEMICAL studies of depression and antidepressant treatments have concentrated primarily on noradrenergic and serotonergic systems [8,26], though a partial role for dopamine has been proposed [2]. Within this context, we have examined the effects of two monoamine oxidase inhibitor (MAOI) antidepressants, clorgyline and pargyline, on behavioral and pharmacological measures of the dopamine receptor. These drugs are reported to differ in their antidepressant response, clorgyline being more potent than pargyline [27]. *In vitro*, dopamine is a substrate for both MAO A and MAO B [35] but *in vivo* in the rat, deamination is reported to occur primarily via MAO A [30], whereas in primates, dopamine deamination is principally by MAO B [19]. Thus, in this study, it would be expected that clorgyline, which is a relatively MAO A specific inhibitor, might produce more changes than pargyline, which is a relatively specific MAO B inhibitor. In previous studies we have shown that these chronic drug regimens cause change in α and β adrenoceptors, whereas when administered acutely they have no apparent effect [4,9]. This small study on the effects of the chronic drug regimens on central dopaminergic receptors was undertaken within the context that the therapeutic effects of the drugs are only seen after repeated administration.

METHOD

Animals and Treatment Regimens

Male Wistar rats (~200 g) received the clorgyline, pargyline or clorgyline + pargyline (1 mg/kg per day for 21–28 days). The drugs were administered intrapertioneally (controls received saline, 0.2 ml). These dose regimens are similar to those which have been used clinically [27]. Measurements of stereotypy were made four hours after the last dose of drug.

Measurement of Stereotypy

Measurements were made by two observers. In the initial study, the animals (6/group) received apomorphine [16] intraperitoneally at a series of concentrations. In the second experiment (using different animals, 12/group) the rats were injected at 1 min intervals with apomorphine HCl (1 mg/kg, SC in the flank, dissolved in 0.1% ascorbate isotonic saline) and placed in individual 30×30×30 cm observation chambers, which had wire grid (1.5 cm) bottoms and clear Perspex sides. Ten minutes after receiving the injection of apomorphine, the first animal was observed for 60 sec and a stereotypy rating score noted: this procedure was repeated until each animal had a rating score (taken 10 min after its injection of apomorphine). The animals were rated again in the same way, the process continuing until each animal had five stereotypy ratings. The scale used [14] rated absence of stereotyped behavior (0), discontinuous sniffing and exploratory behavior (1), continuous sniffing and exploratory behavior (2), continuous sniffing and discontinuous licking or mouthing with or without exploratory activity (3), continuous sniffing and licking or mouthing with discontinuous

TABLE 1

MONOAMINE OXIDASE ACTIVITY IN RAT CORTEX FOLLOWING ADMINISTRATION OF DRUGS FOR 28 DAYS

	Control	Clorgyline	Pargyline	Clorgyline + Pargyline
5 HT Deamination	100.0± 5.4	9.1±1.1	37.3±10.0	5.0±1.0
PEA Deamination	100.0±10.8	73.0±2.2	10.0± 2.0	4.9±0.9

Values are expresssed as % of corresponding controls and are the mean ± SD of 3 assays derived from cortices of 6 animals/group. The specific activity for 5HT is 118 nmoles deaminated/mg protein/hr and the corresponding value for PEA is 41 nmoles deaminated/mg protein/hr.

gnawing with or without exploratory activity (4) and continuous gnawing biting and licking with no exploratory activity (5). The reliability of the raters was very high with few disagreements; if disagreement occurred (this was never greater than between adjacent scale points) the lower score was taken.

Measurement of Dopamine (D2) Receptors

Four hours after the last dose of MAOI, striata were removed from groups of rats which did not receive apomorphine. Briefly, the tissues (~50 mg/per assay) were homogenised in Tris buffer (50 mM, pH=7.7) containing NaCl (120 mM), KCl (5 mM), $MgCl_2$ (1 mM) and $CaCl_2$ (2 mM), using a Brinkman polytron (setting 7), and after two centrifugations (50,000 g × 10 min) the final tissue suspension was 1.2 mg/1.5 ml. Tissues were pre-incubated at 37°C for 15 min and then with [^{3}H]spiroperidol (21 Ci/nmol) at six concentrations (0.05–1.25 nM). Non specific binding was defined by sulpiride (2×10^{-5} M). After incubation at room temperature for 30 min, samples were filtered (Whatman GF/B), washed and counted by scintillation spectrometry [32]. B_{max} and K_D were calculated using a direct linear plot [10].

Measurement of Monoamine Oxidase Activity in Rat Cortex

MAO activity was determined by methods previously described using [^{3}H]phenylethylamine (PEA, for MAO B) and [^{3}H]5-hydroxytryptamine (5HT, for MAO A) as substrates [5]. The assay, on crude sonicated tissue homogenates (in 80 mM phosphate buffer, pH=7.2), involved incubating the sample with appropriate [^{3}H]monoamine at 37°C, followed by separation of the substrate and product using an amberlite CG50 ion exchange resin [15]. The products formed were counted by liquid scintillation spectrometry.

RESULTS

The various MAOI regimens produced no obvious changes in the animals. In the first study, (containing 6 animals/group), the mean weights at the end of 28 days of treatment were: control, 282±37 g; pargyline, 289±16 g; clorgyline, 292±23 g; and pargyline + clorgyline, 291±9 g.

Monoamine Oxidase Activity

Table 1 shows the effects of the various drug treatments on MAO A (5HT deamination) and MAO B (PEA deamination) activities in cerebral cortex after 28 days. Clorgyline reduces MAO A activity by approximately 90% and MAO B activity by 27%. Pargyline reduced MAO B activity by 90% but also had a substantial effect on MAO A, reducing its activity by 63%. The combined treatment with clorgyline + pargyline resulted in 95% inhibition of both MAO A and MAO B.

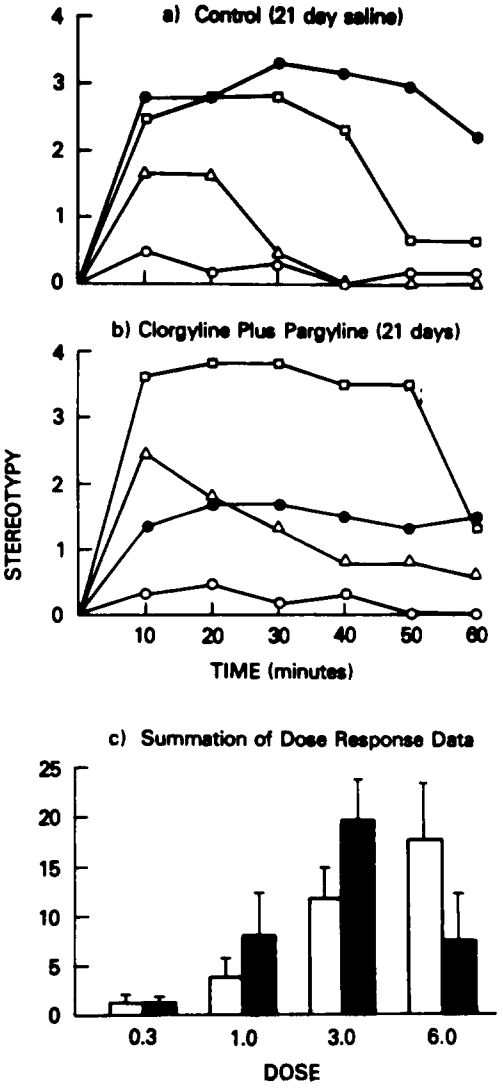

FIG. 1. Dose response curve for the effects of intraperitoneal apomorphine (APO) in control (saline treated) animals, (a), and those which had received clorgyline + pargyline (both at 1 mg/kg/day) for 21 days (b). 0.3 mg/kg APO (○—○); 1.0 mg/kg APO (△—△); 3.0 mg/kg APO (□—□); 6.0 mg/kg APO (●—●). The summated dose response data is presented where the solid bars are the MAOI-treated group and the open bars are controls. Values are mean±SEM (6 animals) and testing was 4 hr after the last dose of MAOI.

Behavioral Responses to Apomorphine

In the first experiment there were two groups of 6 animals: control and those which received clorgyline + pargyline (1 mg/kg/day for 21 days). This was a pilot investiga-

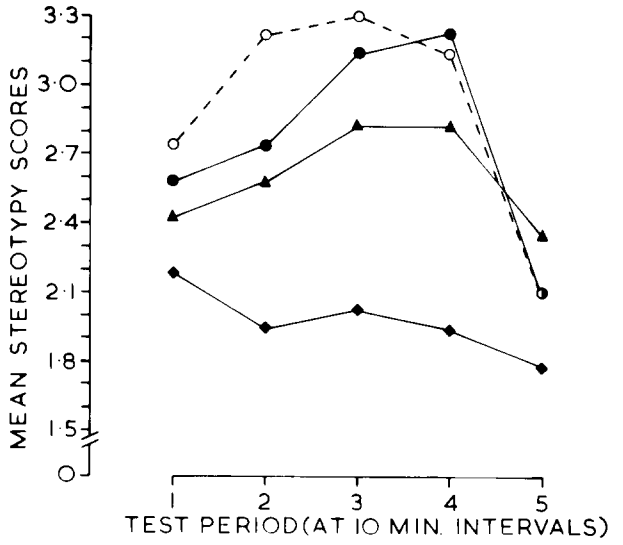

FIG. 2. Mean stereotypy scores in control (◆—◆), clorgyline (1 mg/kg/day; ●—●), pargyline, (1 mg/kg/day; ○—○) and clorgyline + pargyline (both at 1 mg/kg/day; ▲—▲) treatment groups, after the drugs had been administered for 21–28 days. Apomorphine was administered at a dose of 1 mg/kg/SC: there were 12 animals in each treatment group.

tion and compared the effects of complete MAO inhibition against corresponding controls. Although no significant differences were observed, the changes suggested that the MAOI regimen was altering the behavioral sensitivity to apomorphine.

Figure 1 shows the stereotypy scores in the control and clorgyline + pargyline groups in response to several concentrations of apomorphine. In control animals, there is an increase in stereotypy with increasing doses of apomorphine (Fig. 1a). The same pattern is followed in the MAOI-treatment group except that at 6.0 mg/kg there appears to be a decreased response to the challenge (Fig. 1b). The summated data (Fig. 1c) shows that a dose of 0.3 mg/kg of apomorphine does not allow a distinction between the control and MAOI treatment groups while 1.0 mg/kg and 3.0 mg/kg elicit a marked increase in stereotypy in the clorgyline + pargyline group relative to the corresponding controls.

In the second experiment, there were 12 animals/group and the data was subjected to a more complete statistical analysis. The animals received apomorphine as 1 mg/kg (SC) in their flank. The results are shown in Fig. 2. A significant elevation of stereotypy in the drug treated animals against controls was noted, $F(3,44)=5.39$, $p<0.005$. A curvilinear change in stereotypy over time was found (a Quadratic trend over trials, $F(1,44)=55.50$, $p<0.0001$, which is particularly marked in the drug treated animals as compared to the saline controls (Trial × Drug × Quadratic interaction, $F(3,44)=9.00$, $p<0.0001$) and can be seen in Fig. 2. A significant cubic trend over trials was also detected, $F(1,44)=16.01$, $p<0.0005$. Paired comparisons of the groups revealed that while all the treated groups differed from controls for stereotypy, they did not differ from one another.

Striatal [^{3}H]Spiroperidol Binding

The results of the binding studies are shown in Table 2.

TABLE 2

[^{3}H]SPIROPERIDOL BINDING TO STRIATAL MEMBRANES

	Control (7)	Clorgyline (7)	Pargyline (3)	Clorgyline + Pargyline (2)
B_{max}	26.8 ± 11.3	23.4 ± 3.6	32.9 ± 8.6	21.3 ± 1.9

Values are expressed as pmoles bound/g tissue (wet weight). In each experiment, striata from 3 control or treated rats were pooled and used for one 6 point saturation curve and the data was analysed using a direct linear plot. Seven separate plots were done from the control clorgyline groups, three in the case of the pargyline group and two in the case of the clorgyline and pargyline group. K_D values in the 0.2–0.6 nM range.

There is no significant difference between any of the groups, control values being 26.8±4.3 pmoles/g; clorgyline 23.4±3.6 pmoles/g; pargyline 32.9±8.6 pmoles/g; and clorgyline + pargyline being 21.3±1.9 pmoles/g.

DISCUSSION

We have observed the development of increased stereotyped responses to apomorphine following chronic doses of MAOI antidepressant drugs. The apparent decrease in stereotypy which is seen in the clorgyline + pargyline group following a 6.0 mg/kg dose of apomorphine (Experiment 1, Fig. 2b) is due to an abnormal response to this dose; the animals remained in one position with their jaws closed on the wire mesh floors of the test arena, and thus did not achieve a 'high' stereotypy score. There was no measurable difference between the relatively MAO A specific (clorgyline) and MAO B specific (pargyline) drugs: however, after 21–28 days of the drug regimens, pargyline has also caused considerable inhibition of MAO A. At the doses of apomorphine used, the behavioral effects are generally believed to be post synaptic [12] and thus the response might be expected to result from supersensitivity of these DA receptors. However, the binding data shows that there is no supersensitivity at the D2 receptor site.

There are numerous reports of drug induced changes in dopamine receptor sensitivity, the most common being the supersensitivity of domapine receptors which develops following chronic administration of neuroleptics [7]. Similar reports of supersensitivity have been made following destruction of dopaminergic pathways with 6-hydroxydopamine [33]. Thus, supersensitivity is generally associated with understimulation of the post synaptic receptor. However, it has also been reported that dopamine administration *in vitro* [22] or prolonged administration of L-dopa *in vivo*, may lead to behavioral supersensitivity of the dopamine receptor [20]. In another study of the effects of MAOI administration on central dopaminergic systems, no effects of clorgyline have been seen on either [^{3}H]spiroperidol binding or stereotypy responses [23] although it is interesting that deprenyl is an antiParkinsonian drug in part by virtue of its MAO B inhibiting effects [3].

The observed behavioral changes may not be simple direct agonist effects as chronic apomorphine administration does not alter dopaminergic sensitivity [17], although it has been reported that a single injection of apomorphine can lead to an enhanced response to a subsequent dose [13]. MAOI's,

being inhibitors of norepinephrine and serotonin metabolism, may cause alterations in these systems in the CNS, but whether such changes influence dopaminergic responses is unclear. Certainly, adrenergic innervation and a high density of β-adrenoceptors are reported to be present in the corpus striatum [18,21] and that these receptors are present on DA neurones has also been shown [28]. Thus, it could be argued that chronic MAOI administration with resultant decreases in β-adrenoceptor numbers [9] might alter DA receptor mediated responses. Alternatively, the changes could be related to MAOI induced changes in the serotonergic system: apomorphine induced stereotypy is respectively increased or decreased by impairment or enhancement of serotonergic function [24]. At present, involvement of changes in other monoaminergic systems seems to be the most reasonable explanation of the MAOI induced behavioral supersensitivity to apomorphine.

In conclusion, the changes which are responsible for the MAOI induced behavioral supersensitivity are unclear. It is of interest, however, that electroconvulsive shock potentiates the effects of dopamine agonists [25,34] and that behavioral [31] and electrophysiological [6] studies suggest that tricyclic antidepressant drugs induce dopaminergic subsensitivity at presynaptic autoreceptor sites [11]. Whether any of these apparent changes in the dopaminergic system are related to the mode of action of antidepressant drugs or are epiphenomena is unknown. Interestingly, however, two relatively new antidepressant drugs, nomifensine and amineptine, also modulate dopaminergic systems [1,29].

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