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Correlation of changes in α_2-adrenoceptor number and locomotor responses to clonidine following clorgyline discontinuation

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[^{3}H]-clonidine binding *in vitro* and the locomotor response to clonidine *in vivo* were studied over an eight week period following four weeks of treatment with the monoamine oxidase-inhibiting antidepressant, clorgyline (1 mg kg^{-1} day^{-1}). Long-term clorgyline administration caused decreases in responsiveness to clonidine and in the number of α_2-adrenoceptors; these changes reverted towards pretreatment values very gradually over an eight week period following discontinuation of the drug. This study provides some of the first detailed evidence regarding the slow return of adaptional changes following discontinuation of an antidepressant drug in animals and has implications for understanding some delayed drug interactions associated with MAO-inhibiting antidepressants in man.

Introduction Clorgyline, a selective inhibitor of monoamine oxidase (MAO) type A, has been shown to possess antidepressant effects in quite severely depressed patients (Lipper, Murphy, Slater & Buchsbaum, 1979). The clinical antidepressant effects of MAO inhibitors, like those of tricyclic antidepressants, are observed only after two or more weeks of their administration. Animal studies of molecular mechanisms, pertinent to antidepressant efficacy, have concentrated on adaptational changes that occur during this same time period.

Several investigators have reported delayed drug interactions following discontinuation of MAO-inhibiting antidepressants (Cousins & Maltby, 1971; Feinberg, De Vigne, Kronfol & Young, 1981; Insel, Roy, Cohen & Murphy, 1982). One obvious explanation for these delayed drug interactions may be the delayed recovery of the enzyme (MAO) activity (Felner & Waldmeier, 1979). However, besides enzyme activity alterations, other changes that could contribute to a drug interaction may also persist. Previously, we have reported that 21-day but not 3-day treatment with clorgyline decreases the number and sensitivity of α_2-adrenoceptors (Cohen, Aulakh & Murphy, 1982a). In this study, we examine the time course of the return of these changes to normal by measuring the binding of [^{3}H]-clonidine *in vitro* and the locomotor response to clonidine administration *in vivo* over an eight week period following four weeks of clorgyline (1 mg kg^{-1} day^{-1}) treatment.

Methods In this study, 36 male Wistar rats weighing approximately 180 g at the beginning of the study were used. All the animals were housed individually and had free access to food and water. They were divided into three main groups, with 12 rats in each group. Each group was further divided into 6 control and 6 clorgyline treatment subgroups. All three groups were used for receptor binding studies; one group was used for the behavioural studies. The first group was killed on the 7th day, the second group on the 21st day, and the third group (used for the behavioural studies) on the 56th day after clorgyline discontinuation. Clorgyline (1 mg kg^{-1} day^{-1}) was subcutaneously administered continuously for 28 days by means of Alzet minipumps (Alza Corporation) which were reimplanted at two weeks (Cohen, Campbell, Dauphin, Tallman & Murphy, 1982b).

Locomotor behaviour Locomotor activity of individual rats was recorded daily for a period of 40 min with Selective Activity Meters at the same time of day, six days a week. After 5–7 days of adaptation to the experimental situation, 6 animals were implanted with clorgyline-containing minipumps and the other 6 were sham-implanted under light anesthesia. Sham-implanted controls as well as clorgyline-treated animals were challenged with 50 μg kg^{-1} clonidine given intraperitoneally at the end of the first and third weeks, as well as at the end of each week for a period of seven weeks after discontinuation of clorgyline. The effect of clonidine in each animal at various times was calculated as the percentage difference from the baseline value (mean of previous 4–5 days results before clonidine injection). Selection of the clonidine dose (50 μg kg^{-1}) was based on our previous work (Cohen, *et al.*, 1982a).

Receptor studies The α_2-adrenoceptor binding was studied in cortical membranes using [^{3}H]-clonidine as a ligand and the method was essentially the same as described earlier from this laboratory (Cohen, *et al.*, 1982b).

Statistics For the purpose of statistical analysis, two-way analysis of variance, one-way repeated measures analysis of variance and Student's *t*-test were used wherever appropriate.

Results

Locomotor activity Clorgyline did not significantly alter locomotor behaviour during the period of its administration or following its discontinuation [$f(1,10) = 0.20$, $P > 0.05$). Clonidine decreased [$f(1,10) = 45.9$, $P < 0.01$] motor activity in both the sham-implanted control and clorgyline-treated animals, with no significant difference ($P > 0.05$) between the two groups during the first week of drug treatment. As previously reported, by the third week of treatment, the suppressant effect of clonidine on motor activity was attenuated in clorgyline-treated animals with a significant ($P < 0.01$) difference between the two groups (Figure 1). After discontinuation of clorgyline, the pretreated group was found to be less sensitive to the motor suppressant effect of clonidine than the control group for up to seven weeks or as long as the animals were tested (Figure 1), with these differences remaining statistically significant ($P < 0.05$) up to the end of four weeks, following drug discontinuation.

α_2-Adrenoceptors In the control animals, [^{3}H]-clonidine binding varied between 29.72 ± 2.23 and 31.95 ± 3.15 fmol mg^{-1} protein, and none of the differences between the groups was statistically significant. Compared to their respective controls, [^{3}H]-clinidine binding was found to be significantly reduced ($P < 0.01$) in all three clorgyline pretreated groups. However, the decrease was less in the 8th week (18%) as compared to the first (37%) and third (31%) weeks following clorgyline discontinuation. The Scatchard analysis of binding data confirmed that the binding changes observed were the result of a change in the numbers of α_2-receptors (B_{max}), and not an affinity change.

Discussion The present study demonstrates that the decreased sensitivity and number of α_2-adrenoceptors which develop during chronic treatment with the MAO-A-inhibiting antidepressant, clorgyline (Cohen, *et al.*, 1982a), revert towards pretreatment values very gradually over an eight week period following discontinuation of the drug. There are several clinical reports which indicate that some effects of MAO-inhibiting antidepressants persist for a long time after discontinuation of the drug. Cohen, Pickar, Garnett, Lipper, Gillin & Murphy (1982c) observed that four weeks of clorgyline treatment caused near total suppression of REM sleep time and caused an increase in awake time which did not return to baseline three weeks after discontinuation of the drug. In another study, unusual adverse reactions (similar to 5-hydroxytryptamine (5-HT) syndrome reactions observed in rodents) occurred in

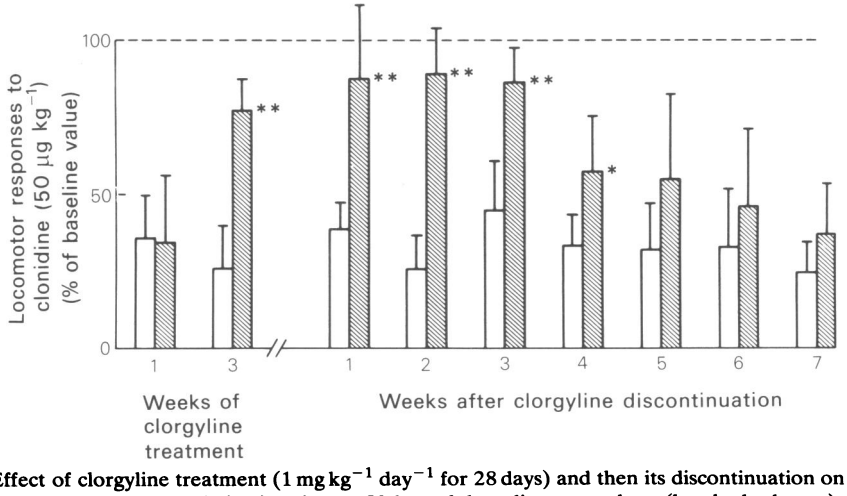

Figure 1 Effect of clorgyline treatment (1 mg kg^{-1} day^{-1} for 28 days) and then its discontinuation on clonidine-induced suppression of locomotor behaviour in rats. Values of clorgyline-treated rats (harched columns) significantly different from controls (open columns) are represented by $^*P < 0.05$; $^{**}P < 0.01$.

two patients given single doses of the tricyclic antidepressant, clomipramine (a potent 5-HT uptake inhibitor) four to five weeks after discontinuation of clorgyline (Insel, *et al.*, 1982). Feinberg *et al.*, (1981) reported hypertensive responses to amphetamine challenges in two patients following phenelzine discontinuation for periods of 16 and 19 days respectively. Similarly, three weeks after pargyline discontinuation, methamphetamine has been found to cause hypertensive responses (Cousins & Maltby, 1971).

Platelet MAO activity (which is MAO type B) has been reported to return to baseline after 10 to 14 days following discontinuation of the non-selective MAO inhibitor, phenelzine (Murphy, Brand, Goldman, Baker, Wright, Van Kammn & Gordon, 1977). The half-life for recovery of MAO-A in the rat brain homogenates after administration of clorgyline (10 mg kg^{-1} day^{-1} for 14 days) has been reported to be 12 days (Felner & Waldmeier, 1979) and 11 days after a single dose (10 mg kg^{-1}) of pargyline (Goridis & Neff, 1971). Clorgyline, pargyline and phenelzine are all irreversible inhibitors of MAO; recovery of enzyme activity is thought to depend upon new enzyme synthesis. Thus, in addition to the slow return of the MAO activity, the decreased sensitivity and number of α_2-adrenoceptors observed in the present investigation may also be partially responsible for delayed drug interactions associated with MAO-inhibiting antidepressants. Furthermore, the present study also indicates that in psychobiological research, the commonly used two-week drug wash-out period may be an insufficient time to assume a return to baseline function.

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