DIFFERENTIAL EFFECTS OF CLORGYLINE ON CATECHOLAMINE AND INDOLEAMINE METABOLITES IN THE CEREBROSPINAL FLUID OF RHESUS MONKEYS

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Abstract—The effects of acute and chronic administration of clorgyline, an irreversible inhibitor of monoamine oxidase type A (MAO-A), on the deaminated metabolites of norepinephrine, dopamine and serotonin were examined in rhesus monkey cerebrospinal fluid (CSF). Acute clorgyline treatment resulted in highly significant, dose-dependent reductions in 3-methoxy-4-hydroxyphenylglycol (MHPG) of 50% (1 mg/kg) and 68% (2 mg/kg) compared to pretreatment values. Chronic clorgyline administration (0.25 to 0.5 mg/kg × 24 days) resulted in a 67% reduction in CSF MHPG. In contrast, the concentrations of 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) were less affected by acute clorgyline administration, being reduced significantly only after the 2 mg/kg dose, which lowered 5-HIAA 27% and HVA 48%. Chronic clorgyline treatment had no significant effect on the CSF concentrations of HVA and 5-HIAA. These data, which suggest that MAO-A inhibition by clorgyline *in vivo* is more closely associated with changes in the noradrenergic than the serotonergic or dopaminergic systems in nonhuman primates, are in general agreement with the effects of clorgyline on CSF and urinary biogenic amine metabolites in man. They differ from several *in vitro* studies which indicate a primary role of MAO-A in the metabolism of serotonin and of MAO-B in norepinephrine degradation in primate brain. The discrepancies may reflect modulating effects of synaptic feedback mechanisms on the actions of clorgyline *in vivo* or perhaps a failure of CSF metabolites to adequately reflect brain amine metabolism changes. The lack of change in platelet MAO-B activity during clorgyline treatment together with the minimal changes in HVA concentrations indicate that the selective inhibitory effects of clorgyline on MAO-A were maintained during chronic administration of low drug doses.

Clorgyline, a highly selective inhibitor of monoamine oxidase type A (MAO-A), is an effective antidepressant with clinical activity equaling [1] or exceeding [2] that of the standard tricyclic antidepressants, imipramine and amitriptyline. Clorgyline has also been found to be a more effective antidepressant than another structurally-related acetylenic MAO inhibitor with relatively selective effects toward MAO type B, pargyline [3, 4].

In studies of human and nonhuman primate brain *in vitro*, clorgyline manifests highly selective effects on certain substrates for MAO, inhibiting the oxidative deamination of serotonin at $<10^{-8}$ M concentrations, while somewhat higher concentrations are required to inhibit *l*-norepinephrine and dopamine deamination; considerably higher clorgyline concentrations ($>10^{-6}$ M) are required to inhibit the degradation of other substrates principally deaminated by MAO-B such as phenylethylamine [5–8]. Although some species differences exist, particularly for dopamine degradation [6, 7, 9, 10], several *in vivo* studies in rodents have demonstrated that clor-

gyline has highly substrate-selective MAO-inhibiting properties similar to those observed *in vitro* [11, 12].

Close correspondence has been found between clorgyline's therapeutic effects and changes in several different measures reflecting noradrenergic system function, including norepinephrine and MHPG concentrations in cerebrospinal fluid and urine as well as blood pressure alterations [4, 13–15]. Of concern in regard to the *in vitro* evidence that clorgyline preferentially affects serotonin rather than norepinephrine is that similar correlations with clinical response like those found for noradrenergic system changes were not obtained for measures reflecting changes in the serotonergic system, including 5-HIAA reductions in cerebrospinal fluid [15] and urine [13, 14]. One prior study in humans found that clorgyline led to reductions in CSF concentrations of MHPG, HVA and 5-HIAA [16]. However, only one time point (3–4 weeks after clorgyline treatment) was examined, and only six patients were available for study in this earlier report. In another clinical study, clorgyline reduced urinary norepinephrine and its metabolite output more than the urinary metabolites of serotonin and dopamine [14]. To investigate in greater detail the relative changes in the metabolism of norepinephrine, dopamine and

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serotonin following acute and chronic clorgyline administration, we have utilized a nonhuman primate model which permitted continuous measurement of CSF amine metabolite concentrations following several acute clorgyline doses as well as following longer-term treatment with clorgyline.

MATERIALS AND METHODS

Sample collection. Nine adult male rhesus monkeys (*Macaca mulatta*) were adapted to chair restraint with the lights on from 7:00 a.m. to 7:00 p.m. and off from 7:00 p.m. to 7:00 a.m. Water was available *ad lib.* and food (Ralston Purina Monkey Chow No. 5038) was provided at 9:00 a.m. and 4:00 p.m. and not withdrawn.

A polyethylene cannula was inserted between the lumbar vertebrae of the animal and advanced to the high cervical subarachnoid space during ketamine HCl (7 mg/kg) and xylazine (0.6 mg/kg) anesthesia. The cannula was encased in a water jacket cooled to 10° which fed into a fraction collector housed in a −40° freezer. CSF was collected continuously in 90-min aliquots at a flow rate of approximately 1.5 ml/hr. Within 18 hr after collection, the samples were subdivided and stored at −80° until analyzed. To provide a volume necessary to measure the metabolites in addition to several neuroendocrine measures for other studies, the aliquots were pooled according to the following time points: 3:00 p.m. to 7:30 p.m., 7:30 p.m. to 1:30 a.m., 1:30 a.m. to 7:30 a.m., and 7:30 a.m. to 3:00 p.m.

Blood samples for the determination of platelet MAO activity, plasma amine oxidase activity, and platelet counts were drawn by femoral venipuncture before and on day 24 of chronic clorgyline treatment. Blood samples were processed, stored at −80° and assayed for platelet MAO activity, using [^{14}C]benzylamine as the substrate, as described previously [17].

Acute and chronic clorgyline administration. CSF collection began at least 24 hr following the lumbar puncture procedure to provide a post-anesthesia recovery time. Baseline CSF was collected for 2–3 days, with intramuscular (i.m.) saline injections given daily at 3:00 p.m. Clorgyline (1 mg/kg) was administered i.m. to seven animals at 3:00 p.m. followed by a 2 mg/kg dose of the drug at 3:00 p.m. the second day, with continuous CSF collection. Two additional animals received 0.1 mg/kg and 0.5 mg/kg of clorgyline following the same protocol.

Immediately following acute clorgyline administration, four animals were returned to their individual cages and treated chronically with clorgyline as follows: two monkeys received 0.5 mg/kg/day i.m. and two received 0.25 mg/kg/day i.m. at 3:00 p.m. for 24 days. These doses were chosen on the basis of human [18] and rodent [11] studies as most likely to yield selective MAO-A inhibition during longer-term administration. On day 24 the animals were reanesthetized, lumbar punctures were performed and cannulae were inserted and advanced to the cervical subarachnoid space, and the animals were chaired for CSF collection beginning at least 24 hr after surgery. Daily clorgyline administration at the same dose was continued at 3:00 p.m. each day during this period. CSF was collected for 2–3 days and processed as described above.

Liquid chromatography of CSF MHPG, 5-HIAA and HVA. 3-Methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) were quantitated with high performance liquid chromatography with electrochemical detection using 5-fluoro-HVA as an internal standard as described previously [19]. The detection limits of the procedure, using a 100 μl injection volume, were 8 nM for MHPG, 10 nM for 5-HIAA, and 20 nM for HVA. Intra- and interassay coefficients of variation were 2–6% and <10% respectively.

RESULTS

The effects of acute and chronic clorgyline administration on the monoamine metabolites in CSF of rhesus monkeys are presented in Fig. 1. Clorgyline administration (1 mg/kg) resulted in a 50% reduction in CSF MHPG from baseline (P < 0.01). Lesser, non-significant reductions were found in CSF HVA (22%) and 5-HIAA (7%).

Increasing the dose of clorgyline to 2 mg/kg for 1 day resulted in a further decrease in CSF MHPG (68% from baseline, P < 0.01) and HVA (48% from baseline, P < 0.01). At this clorgyline dose, 5-HIAA was reduced significantly (27% from baseline, P < 0.05).

Chronic treatment of four monkeys with clorgyline at 0.25–0.5 mg/kg/days × 24 days resulted in an equivalent reduction from baseline MHPG (67%) to that seen with acute clorgyline at 2 mg/kg (68%). As indicated in Fig. 2, essentially identical MHPG reductions were found in the two monkeys receiving 0.25 mg/kg/day × 24 days (65% from baseline) compared to two receiving 0.5 mg/kg/day (71%). A very low (0.1 mg/kg/day) dose of clorgyline given acutely to two monkeys had minimal effects on CSF MHPG (Fig. 2b), 5-HIAA and HVA (data not shown).

Changes in CSF HVA following chronic clorgyline administration were less (13%) than those resulting from acute clorgyline at 2 mg/kg (48%). CSF 5-HIAA values were not reduced by chronic clorgyline treatment (Fig. 1).

Clorgyline given chronically did not alter platelet MAO-B activity measured with benzylamine as the substrate. Mean pretreatment platelet MAO activity was 2.40 ± 0.31 nmoles/10⁸ platelets/hr; on day 24 of clorgyline treatment, it was 2.41 ± 0.51 nmoles/10⁸ platelets/hr (P = NS).

DISCUSSION

This study in rhesus monkeys is in general agreement with the one previous investigation in humans of the effects of clorgyline on CSF monoamine metabolites in demonstrating that low concentrations of the drug given chronically had apparently preferential effects on norepinephrine deamination [16]. Similar clorgyline doses in the previous study in man of 0.37 ± 0.04 mg/kg × 21–28 days, compared to those in the present study (0.25 to 0.5 mg/kg × 24 days), led to marked reductions in CSF MHPG averaging 91%; lesser reductions in HVA (34%) and 5-HIAA (45%) than in MHPG were observed [16]. In the present study, substantial CSF MHPG reductions were found, with minimal changes in HVA (13%) or 5-HIAA (0%), during chronic clorgyline treatment.
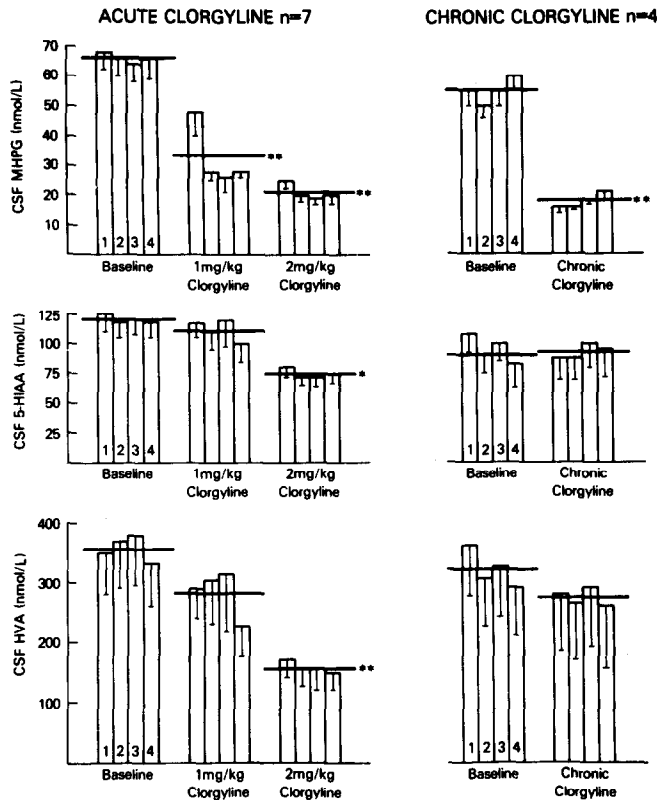
ACUTE CLORGYLINE n=7 **CHRONIC CLORGYLINE n=4**

Fig. 1. Effects of acute (1 and 2 mg/kg, N = 7) and chronic (0.25 to 0.5 mg/kg/day × 24 days, N = 4) clorgyline administration on 24 hr rhesus monkey CSF metabolites. Numbers in the baseline bars represent CSF collected according to the following time periods: (1) 3:00 p.m. to 7:30 p.m.; (2) 7:30 p.m. to 1:30 a.m.; (3) 1:30 a.m. to 7:30 a.m.; and (4) 7:30 a.m. to 3:00 p.m. Lights were on from 7:00 a.m. to 7:00 p.m. Saline or clorgyline injections were given at 3:00 p.m. Error bars on individual time points represent S.E.M. Horizontal lines indicate mean 24 hr values. See Materials and Methods for further detail. Key: (*) P < 0.05 and (**) P < 0.01, paired t-test, two-tailed, compared to baseline.

Two recent reports of urinary monoamine metabolite changes following clorgyline administration for 3–4 weeks in depressed patients also document greater reductions in MHPG excretion (70–85%) than in 5-HIAA (30–45%), HVA (40%), or phenylethylamine (18%) excretion [13, 14]. A post-mortem study of brain amine concentrations following clorgyline administration to depressed patients at a dose of 20 mg/day for an average of 33 days also found greater increases in brain norepinephrine (3.12-fold) than in dopamine (1.44- to 1.61-fold) or serotonin (2.15-fold) concentrations [20].

Acute clorgyline treatment in the present study led to more immediate reductions in MHPG concentrations (42% at 4 hr post-drug) which also occurred at lower dose levels than those needed to affect HVA and 5-HIAA concentrations. While there appear to be no prior studies similar to the present one on monoamine metabolism following acute clorgyline administration to either human or nonhuman primates, clorgyline has been shown to elevate rat brain norepinephrine concentrations acutely and to suppress locus ceruleus firing rates within an hour following drug administration [21]. In rodents, however, both dopamine and serotonin deamination are affected by clorgyline to an equal or greater extent than norepinephrine deamination; these dif-

ferences have been attributed to the greater proportion of MAO-A than MAO-B in rodent brain compared to human and nonhuman primate brain as studied in vitro [7–12, 22].

In most investigations comparing MAO-A and MAO-B in brain, clorgyline has been used to discriminate these two enzyme forms based upon its >1000-fold greater potency in inhibiting the deamination of the prototypical MAO-A substrate, serotonin, than the selective MAO-B substrate, phenylethylamine [23]. These in vitro comparisons generally have proved to be valid in vivo, as clorgyline as well as other selective MAO-A inhibitors such as Lilly 51641 have greater effects on serotonin metabolism, while MAO-B inhibitors such as deprenyl and pargyline much more markedly alter the degradation of MAO-B substrates such as phenylethylamine, phenylethanolamine and tele-methyl histamine in both rodents and man [11–13, 23–26].

Dopamine has been found to be selectively deaminated by MAO-A in rodents [9, 10] but predominantly by MAO-B in human and nonhuman primate brain in vitro [6, 7]. Studies of dopamine metabolite formation following selective MAO-A or MAO-B inhibition by clorgyline, pargyline or deprenyl, and non-selective MAO inhibition by phenelzine, have clearly supported this distinction [12–15, 27, 28].
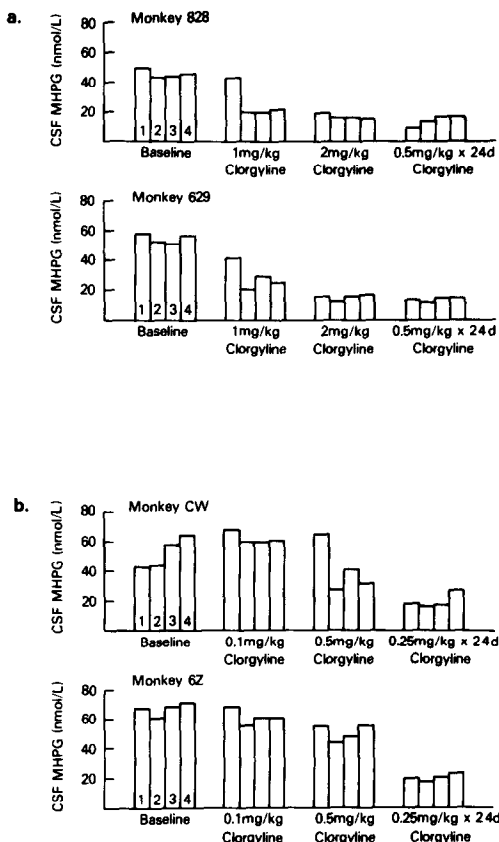
Fig. 2. Effects of various acute and chronic doses of clorgyline on rhesus monkey CSF MHPG concentrations. CSF collection and drug administration are described in the legend of Fig. 1 and Materials and Methods.

Norepinephrine generally has been considered to be preferentially deaminated by MAO-A [29], although two recent studies indicated that it is less selectively deaminated by MAO-A than is serotonin, particularly in human and nonhuman primates [5, 8]. Specifically, serotonin was found to have a 2- to 4-fold smaller K_m than norepinephrine for MAO-A, and to be deaminated 2- to 5-fold more actively than norepinephrine across a number of rodent, monkey and human tissues [8]. Additionally, serotonin has been reported to have a higher affinity than norepinephrine for the MAO-A active site according to data demonstrating that 16-fold lower concentrations of serotonin ($K_i = 40 \mu M$) than norepinephrine ($K_i = 640 \mu M$) inhibit the binding of a reversible selective MAO-A inhibitor, radiolabeled harmaline [30]. Moreover, serotonin deamination has been shown to be inhibited 80–100% by $10^{-7} M$ clorgyline in human, nonhuman primate and rodent brain *in vitro*, while norepinephrine deamination is inhibited to a lesser extent by the same concentration of clorgyline in rodent brain (75–85%), human brain (58%) or rhesus monkey brain (37%) [8].

The present data and the aggregate of limited but nonetheless highly consistent human CSF, urinary and post-mortem brain data are not in full agreement with the *in vitro* data reviewed above in regard to the selective actions of clorgyline on serotonin versus

norepinephrine deamination. While several bases for this discrepancy exist, one likely possibility relates to the presence *in vivo* of a number of adaptive mechanisms responsive to acute and chronic changes in synaptic neurotransmitter concentrations such as those known to follow MAO-inhibitor treatment. Specifically, relatively greater MHPG reductions could result from more sensitive synaptic feedback mechanisms in the noradrenergic than serotonergic neurotransmitter system. In one pertinent experiment, Lin et al. [31] found that short-term treatment with high, non-selective doses of pargyline leads to significant elevations of both brain serotonin and norepinephrine in rats; however, serotonin synthesis rates remain unchanged while norepinephrine synthesis from tyrosine is reduced to less than 50% of the baseline control values. In addition to this feedback modulation of amine synthesis, direct inhibition of locus ceruleus firing rates, which also would be expected to reduce norepinephrine release and MHPG formation, follows acute clorgyline treatment [21], while with chronically administered clorgyline, multiple adaptational changes in α_1-, α_2- and β-adrenergic receptors occur and apparently lead to a reduction in central synaptic outflow accompanied by reduced plasma norepinephrine and MHPG concentrations [32, 33]. This hypothesis of a greater sensitivity to feedback inhibition in noradrenergic neurons than in serotonergic neurons seems most compatible with all of the available data, but other pharmacological consequences of MAO inhibition could also be contributory to the MHPG vs 5-HIAA differences observed [13, 34]. Furthermore, there is continuing debate about the extent to which MHPG, 5-HIAA and other CSF metabolites adequately reflect brain amine metabolism, particularly in regard to contributions from the spinal cord and the periphery when lumbar CSF is sampled. However, in the present study, lumbar cannulae were inserted and advanced to the high cervical subarachnoid space, just below the cisterna magna, and the CSF measurements are more likely to reflect predominantly brain rather than cord metabolism, with some contributions from the periphery [35]. While direct measurement of amine metabolites in brain tissue following clorgyline administration would aid in evaluating whether CSF metabolite concentrations adequately reflect inhibition of MAO-A activity in brain, these data are not readily attainable in primates.

The present study also demonstrated that clorgyline can produce marked changes in norepinephrine metabolism without affecting platelet MAO activity, which, in rhesus monkeys as in man, is totally of the MAO-B type [36]. As platelet MAO is essentially completely inhibited by clinically used doses of the MAO-B inhibitors deprenyl and pargyline [37], the present data offer further support for the evidence from studies in man and rodents that chronic administration of low doses of clorgyline can lead to the maintenance of a highly selective inhibition of MAO-A without appreciable effects on MAO-B. Studies which have suggested otherwise generally have used higher drug doses [11, 38]. The lack of change in HVA concentrations offers further validation of the platelet MAO-B data, as dopamine is preferentially

deaminated by MAO-B in the nonhuman primate [7]. These data, then, indicate that the CSF MHPG changes reported in this paper were principally, if not nearly exclusively, the result of selective MAO-A inhibition by clorgyline. Overall, our results offer further support for the proposition that selective noradrenergic system effects may be most closely associated with the therapeutic effects of MAO-A inhibition produced by clorgyline in man.

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