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BRIEF COMMUNICATION

Minipump Clorgyline Administration and CSF Amine Metabolites in Unrestrained Monkeys[1]

M. COX, N. GARRICK, M. REITE[2] AND M. GENNARO

Department of Psychiatry, University of Colorado Health Sciences Center, Denver, CO 80262 and National Institute of Mental Health, Bethesda, MD 20892

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COX, M., N. GARRICK, M. REITE AND M. GENNARO. *Minipump clorgyline administration and CSF amine metabolites in unrestrained monkeys*. PHARMACOL BIOCHEM BEHAV **38**(3) 677–679, 1991.—The irreversible MAO-A inhibitor clorgyline was administered in doses of 0.5 mg/kg (N = 1), 1 mg/kg (N = 3), and 2 mg/kg (N = 1) to 5 young (age 5.5 to 23.9 months) pigtail (*M. nemestrina*) monkeys using a 28-day (Alza 2ML4) osmotic minipump. CSF MHPG, 5-HIAA, HVA, and plasma MHPG were measured before and at approximately weekly intervals after pump implantation. Implants were well tolerated. CSF MHPG decreased about 75%, 5-HIAA 30%, and HVA from 30–50% with a tendency to plateau by the second week. Plasma MHPG decreased to undetectable levels. The findings demonstrate that long-term inhibition of MAO-A can be produced in unrestrained monkeys by minipump administered clorgyline. There is an apparently greater effect on the norepinephrine system relative to the serotonin and dopamine systems.

Clorgyline MAOI Osmotic minipump *M. nemestrina* Monkeys MHPG 5-HIAA HVA Catecholamine Nonhuman primates Cerebrospinal fluid CSF

THIS study was undertaken to determine if the antidepressant monoamine oxidase type A (MAO-A) inhibitor clorgyline could be chronically administered to unrestrained social group living monkeys by means of osmotic minipump, and to determine what dose would effectively alter brain monoamine metabolism as determined by cerebrospinal fluid (CSF) amine metabolite levels.

Social separation paradigms in monkeys are useful animal models of separation and loss-related depression in humans. A useful strategy in such paradigms is treatment of the separated animal with agents known to be effective in human depression to determine the effect on the separation response (9). We preferred not to have to repeatedly capture the group living animals to administer oral or parenteral drugs. Furthermore, we found that relatively high dose agents, such as tricyclics, could not be effectively administered by minipump due to adverse tissue reaction (7). Accordingly, we determined to try minipump administration of a relatively more potent MAOI agent. Clorgyline is a MAO-A inhibitor that has been shown to be an effective antidepressant in man (4,6), and whose effects on CSF amine metabolites in rhesus monkey are known (3). Inhibition of MAO-A by clorgyline would be expected to result in decreases in these products of MAO activity.

METHOD

Subjects were five young (age 5.5 to 23.9 months) pigtail (*M. nemestrina*) monkeys. All were laboratory born, two were housed individually at the time of the study, and three were socially group housed. For all subjects, the light-dark cycle was 13/11 (lights off 2000–0700 h). Feeding (monkey chow and fruit) occurred at approximately 0900 daily, while water was available ad lib.

We administered the irreversible MAO-A inhibitor clorgyline in doses of 0.5 mg/kg/day (N = 1), 1 mg/kg/day (N = 3), and 2 mg/kg/day (N = 1) using a 28-day (Alza 2ML4) constant infusion rate osmotic minipump implanted under the skin of the back. Implantations were performed under ketamine anesthesia. Incisions were closed with interrupted stainless steel sutures that were removed approximately ten days after implantation. Subjects received antibiotics (30 mg/kg Kefzol and 10 mg/kg Unipen or Polycillin per day) for from five to twelve days postimplant (sub-

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[2]Requests for reprints should be addressed to Martin Reite, M.D., UCHSC, Box C268, 4200 East Ninth Avenue, Denver, CO 80262.

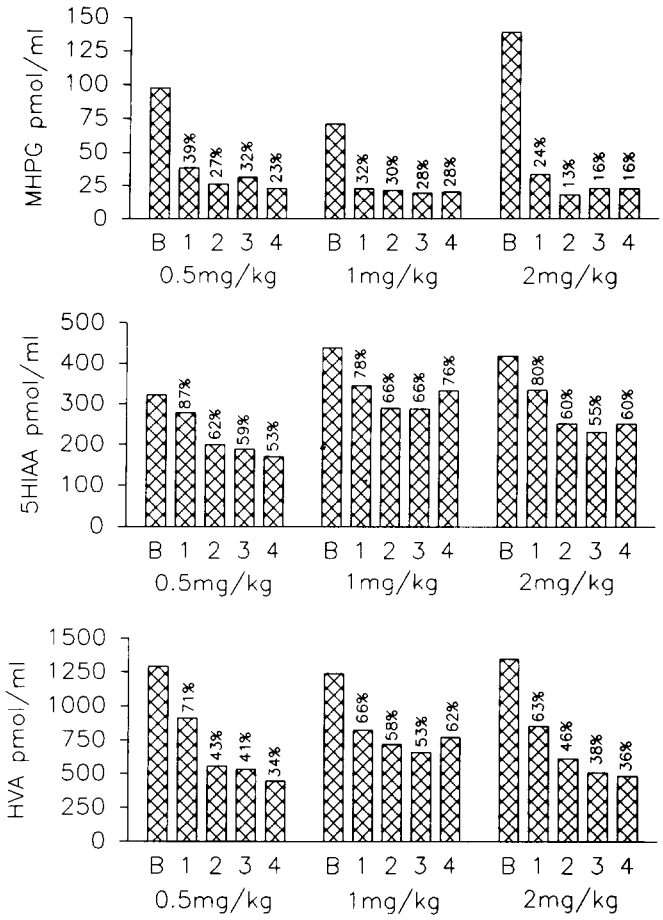

FIG. 1. Bar graphs of CSF MHPG (top), 5-HIAA (center) and HVA (bottom) in pmol/ml during predrug baseline (B), and 1, 2, 3, and 4 weeks (ordinate) after minipump implantation, for 3 dose levels, 0.5 (N = 1), 1.0 (N = 3), and 2.0 (N = 1) mg/kg. Percent of baseline values are on top of bar graphs for weeks 1, 2, 3 and 4.

sequent work indicates this period of antibiotic treatment may not be necessary). Clorgyline was dissolved in sterile saline to make a final volume of 2 cc, then filtered through a 0.2 μm syringe filter for pump loading. Osmotic minipumps reach their constant infusion rate within four hours after implantation.

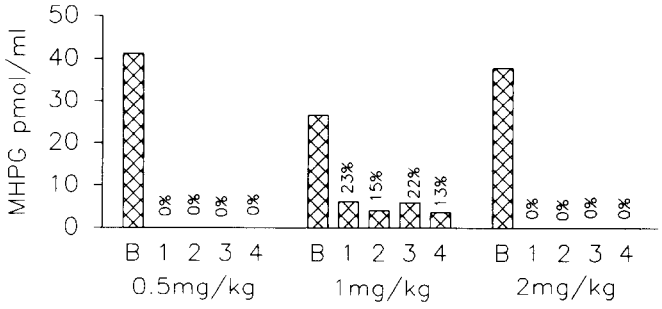

FIG. 2. Plasma MHPG in pmol/ml during predrug baseline (B), and at 1, 2, 3, and 4 weeks (ordinate) following minipump implantation, for 3 dose levels, 0.5 (N = 1), 1.0 (N = 3), and 2.0 (N = 1) mg/kg. Percent of baseline values are above each bar graph for weeks 1, 2, 3, and 4.

CSF (cisternal tap under ketamine anesthesia) and blood (peripheral limb venipuncture) were sampled once immediately before, and at approximately weekly intervals after pump implantation for four weeks. Ketamine does not affect CSF norepinephrine levels in baboons (2), or affect levels of HVA or 5-HIAA in rodent brain (1). CSF was collected immediately after blood collection and was frozen in a 1.0 ml cryotube at −70°C. Blood was collected in a heparinized tube, plasma was separated by centrifugation within 15 minutes, then frozen at −70°C in a 1.0 ml cryotube.

CSF was subsequently analyzed for 3-methoxy-4-hydroxyphenylglycol (MHPG), 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) using high performance liquid chromatography with electrochemical detection (HPLC-EC) using 5-fluoro-HVA as an internal standard (3,8). MHPG was also analyzed by a slightly modified HPLC-EC assay using deproteinized plasma.

RESULTS

Implants were well tolerated, and incisions healed rapidly without complications. Overt behavioral changes after pump implantation were limited to scratching at the suture line for a short time after suture removal. In the socially housed animals, other animals were observed to groom the sutures before removal and the suture line after removal, but healing was not impaired.

CSF Amine Metabolites

MHPG. CSF MHPG values are illustrated schematically in Fig. 1 (top), in terms of both absolute values and percent of baseline values. Levels decreased to about 25% of baseline (75% reduction) at the time of the second cisternal tap (about two weeks after pump implantation). Relatively little difference was noted between the three dose levels, although only one animal's data was available for the 0.5 and 2 mg/kg dose levels.

5-HIAA. CSF MHPG values are illustrated in Fig. 1 (center). Values decreased by about 30%, by week two, and remained stable. Again, relatively little difference was noted for the three dose levels.

HVA. CSF HVA are illustrated in Fig. 1 (bottom). Values decreased by about 30–50% with a tendency to plateau by the second week. The three dose levels did not differ in terms of percent reduction, again limited by the small N.

Plasma MHPG

Plasma MHPG measurements for the three dose levels as a function of time are schematically illustrated in Fig. 2. Marked reductions were noted beginning with the first week after pump implantation. MHPG levels were undetectable after two weeks in three animals.

These changes in amine metabolites were not the result of the minipump implantation itself, as animals involved in other experiments (to be reported) and implanted with placebo containing minipumps demonstrated no such changes.

DISCUSSION

Our findings demonstrate the feasibility of osmotic minipump administration of MAO inhibitors in young macaque monkeys. Implants are well tolerated, and the animals can be studied in social groups.

The data further suggest long-term inhibition of central norepinephrine, serotonin, and dopamine amine systems is produced by minipump administered clorgyline, with a suggestively greater impact on norepinephrine systems. Overall, reductions in plasma MHPG were greater than in CSF. Relatively little difference was

noted between the three dose levels of clorgyline in terms of effects on amine metabolites, although our N is too small to be certain about this.

Garrick et al. (3) found acute administration of clorgyline (1–2 mg/day for one day) resulted in dose-dependent reductions of 50–68% in CSF (obtained from high cervical subarachnoid space by indwelling cannula) MHPG, 7–28% reductions in 5-HIAA, and 22–48% reductions in HVA. Chronic administration at doses of 0.25–0.5 mg/kg/day IM for 24 days resulted in MHPG reductions of 68 ± 3%, but lower decreases in HVA (14 ± 2%) and 5-HIAA (0 ± 4%). Our findings suggest a greater reduction in 5-HIAA and HVA with chronic minipump administration, with 5-HIAA reduced by about 47% at four weeks, and HVA by about 66% at four weeks for the 0.5 mg/kg dose, and comparable reductions (84% for MHPG, 40% for 5-HIAA, and 64% for HVA) at the 2 mg/kg dose level.

Murphy and co-workers (6) found that clorgyline in orally administered doses of 10–40 mg/day (average 28 mg/day) for a period of four weeks resulted in an 86% reduction in plasma MHPG, and a 91% reduction in CSF MHPG, in patients with depression who, as a group, manifested a significant antidepressant response to clorgyline treatment. Our findings are generally consistent in demonstrating a marked reduction in plasma MHPG, with values being undetectable after week one in several animals. Murphy and co-workers (5,6) also suggest the antidepressant effect of clorgyline in man is highly correlated with effects on the noradrenergic system as manifested by marked reductions in CSF MHPG (91%), which were more substantially decreased by oral clorgyline than were either CSF 5-HIAA (45%) or HVA (34%). The present findings indicate a similar selectivity for NE metabolites in the pigtail monkey, suggesting that similar effective antidepressant dose levels can be obtained in this species by minipump administration.

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