Chlordiazepoxide as an Anticonvulsant in Monkeys.* (27263)

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Experimental studies of potential anticonvulsive agents in the past have depended almost exclusively on the normal or intact animal, usually the mouse, rat or cat. The present method using chronic epileptic monkeys summarizes illustrative data obtained from a study of chlordiazepoxide (Librium).‡ The use of epileptic primates with clinical features resembling those of some human epileptics has the theoretical advantage of more direct application for man. Although this report deals with the results of acute experiments, our method, based on the capacity of a potential anticonvulsive agent to prevent or alter analeptic-induced clinical seizures in epileptic monkeys, might also be readily adapted for chronic experiments.

Method. Chlordiazepoxide was tested in a group of 11 *Macaca mulatta* (2.4-4.8 kg). Five of these had been made chronically epileptic by application of alumina cream to cerebral cortex(1), and 6 remained unoperated (normal). Chlordiazepoxide was injected rapidly intravenously (freshly prepared solutions of 0.5-5% in physiologic saline), and was followed one-half hour later by an intramuscular injection of pentamethylenetetrazole (24 mg/kg for epileptic and 64 mg/kg for normal monkeys). Dosages of chlordiazepoxide tested ranged from 35 mg/kg to 0.55 mg/kg intravenously. At least 2 weeks elapsed between repeated tests of an individual monkey. The minimal effective anticonvulsant dose was considered to be the smallest dose which prevented onset of clinical convulsions within one hour after the intramuscular challenge dose of pentamethylenetetrazole. Daily electroencephalographic and clinical observations were made for periods of 4-7 days following each injection of chlordiazepoxide. Effects of chlordiazepoxide on the EEG were graded quantitatively on a scale of 4+ to 0 (4+ = pronounced effects over 4 days, 3+ = less marked, over 3-4 days, 2+ = definite for 1-2 days, 1+ = slight for 1 day or less, 0 = none). Effects of intramuscular pentamethylenetetrazole on clinical status and EEG were also graded 4-+ to 0 (4+ = generalized tonic-clonic clinical and electrical seizure, 3+ = focal electrical seizure, 2-+ = transient spike and slow waves, 1-+ = minimal changes, 0 = no change). A total of 63 test injections of chlordiazepoxide was made; a maximal dose of 35 mg/kg intravenously was tested 18 times in 5 epileptic and 3 normal monkeys.

Results. Epileptic monkeys. Following injections of the larger doses of chlordiazepoxide, monkeys showed profound ataxia, weakness, emesis and stupor. With smallest doses tested, animals exhibited diminished activity and aggressiveness. Challenge doses of pentamethylenetetrazole (24 mg/kg), which were capable of inducing clinical seizures in these epileptic monkeys, failed to do so following intravenous injection of the larger doses (Table I). The minimal effective doses for the 5 epileptic monkeys were 1.1, 1.1, 2.2, 2.2, and 4.4 mg/kg, respectively. Replacement of high amplitude sharp, slow and spike waves in the EEG by lower amplitude fast activity (25/second) lasting for 3 or more days was noted with the largest doses of chlordiazepoxide, and proportionately reduced effects with the smaller doses (Fig. 1, 2).

Normal monkeys. Clinical and electroencephalographic effects were similar to those produced in the epileptic monkeys. Challenge doses of pentamethylenetetrazole (64 mg/kg i.m.) which were usually capable of inducing clinical seizures failed to do so after injection of the larger doses of chlordiazepoxide. Minimal effective anticonvulsant doses determined in 4 of these monkeys were 1.1, 1.1, 2.2 and 2.2 mg/kg.

Miscellaneous. In 2 monkeys (1 epileptic

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TABLE I. Protocol of Representative Epileptic Monkey (#65) Summarizing Effects of Intravenous Chlordiazepoxide Followed 30 Min. Later by Intramuscular Pentamethylenetetrazole.

Chlordiazepoxide			Pentamethylenetetrazole		
I.V., mg/kg	EEG*	Clinical features	I.M., mg/kg	EEG†	Clinical seizures
35	+++	ataxia +++ emesis	16	0	0
35	+++	*idem* +++	32	±	0
35	++±	ataxia +	48	+	0
17.5	++	ataxia + sl. stupor	24	+	0
4.4	+	quiet	24	++	0
2.2	+		24	++±	0
1.1	±		24	+++	0
.55	0		24	++++	++++
0			24	++++	++++

* Degree of low amplitude fast activity.
† Degree of high amplitude sharp, slow and spike waves.
Tests run at intervals of 2 wk.

and 1 normal), transient gross hematuria followed injections of the laregst doses of chlordiazepoxide used (35 mg/kg i.v.) on 3 occasions, approximately 1½-2 hours after injection. Similar reactions were not noted following 15 other injections of the same dosage.

Discussion. Our previous studies(2-5) have shown epileptic monkeys to have lower thresholds than normals for the clinical convulsive effects of pentamethylenetetrazole, picrotoxin, semicarbazide and glutarimide. The ability of a potential anticonvulsive agent to alter this response could depend on a) an effect on the analeptic agent or its mode of action, b) an effect on responsive nervous

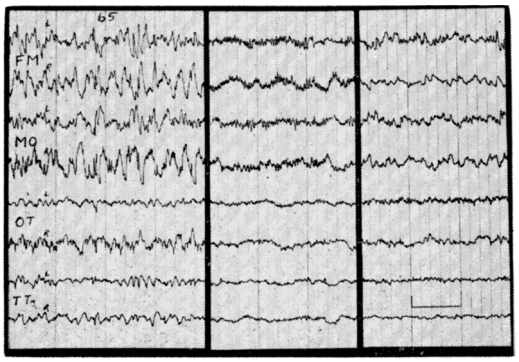

FIG. 1. EEG of epileptic monkey (#65) before, 5 min. after and 48 hr after maximum dosage tested of chlordiazepoxide (35 mg/kg i.v.). F = frontal, M = motor, O = occipital, T = temporal, AT = anterior temporal, R = right, L = left. Calibration: horizontal, 1 sec.; vertical, 50 microvolts.

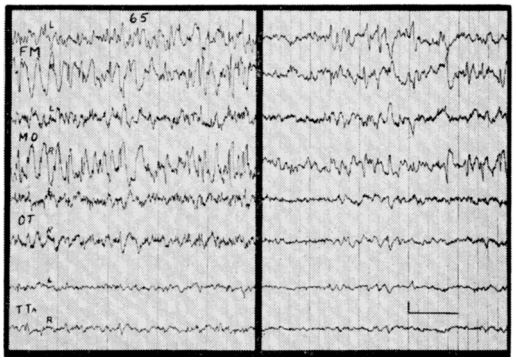

FIG. 2. EEG of same epileptic monkey (#65) before and 14 min. after minimal effective dosage of chlordiazepoxide (1.1 mg/kg i.v.).

and other tissues, or c) both. A potential anticonvulsive drug might therefore yield different results with different test analeptic agents, routes of administration, dosages, etc. Since in these tests intramuscular pentamethylenetetrazole was the analeptic agent employed, the results could be related to the mode of action of this agent.

These studies demonstrated that large doses of intravenous chlordiazepoxide prevented the clinical and EEG convulsant effects of challenge doses of intramuscular pentamethylenetetrazole in normal and epileptic monkeys. The intravenous doses of chlordiazepoxide which were effective exceeded comparatively the oral dosages usually employed in man. It should also be noted that the epilepsy of the monkeys more closely resembles

that of human focal epilepsy of cortical origin than any other of the numerous clinical types.

Although transient hematuria was noted on 3 occasions in 2 monkeys when the highest dosage (35 mg/kg i.v.) of chlordiazepoxide was used this did not occur in 15 other trials of this dosage, or in 45 additional tests of smaller dosages. To our knowledge, hematuria has apparently not been reported in other experimental studies of chlordiazepoxide.

Tower(6) among others has noted the clinical limitations in the predictive value of laboratory tests for anticonvulsant drugs. Extensive batteries of tests have been required since no single test has proved suitable for all drugs. Our method based on ability of a potential anticonvulsant to prevent or alter analeptic-induced seizures in epileptic monkeys should prove a useful additional technic. Theoretically, the best clinical results might be expected when the potential anticonvulsant drug is similarly administered to patients with epilepsy resembling that of the experimental monkey.

Summary. 1) A method using chronic epileptic monkeys is described for testing potential anticonvulsant drugs based on their capacity to prevent or alter clinical seizures induced by analeptic agents. 2) Intravenous chlordiazepoxide in large doses prevented clinical and EEG convulsant effects of challenge doses of intramuscular pentamethylenetetrazole in epileptic and normal monkeys.

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Metabolism of Chlorpromazine. III. Isolation and Identification of Chlorpromazine-N-Oxide.* (27264)

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There is now substantial evidence that chlorpromazine is transformed *in vivo* into an extensive series of metabolites(1-4) whose pattern is species-dependent(4). The observed alterations in the phenothiazine nucleus of chlorpromazine include hydroxylation and sulfoxidation, but there are no indications of sulfone formation(3). The dimethylaminopropyl side chain is subject to either partial or complete demethylation(3,4). This latter phenomenon is especially apparent in humans, who excrete nor$_2$chlorpromazine sulfoxide as the major nonpolar metabolite.

We wish to report here the identification of a new chlorpromazine metabolite (chlorpromazine-N-oxide) which is isomeric with chlorpromazine sulfoxide but represents oxidation of the side-chain nitrogen, rather than the nuclear sulfur atom. Reference is made to Fig. 1 for the structure of this compound (CPNO)† as well as of the other products that have been identified to date.

Experimental. Urine from patients on oral chlorpromazine medication was brought to pH 11:5 and extracted with ethylene dichloride (Eastman) to obtain the desired nonpolar metabolite fraction(4). The extract was reduced in volume, spotted on Whatman #1 paper and chromatographed

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† The following abbreviations are used: CP = chlorpromazine, CPNO = chlorpromazine-N-oxide, CPSO = chlorpromazine sulfoxide, nor$_1$ = desmonomethyl, nor$_2$ = desdimethyl. Also see Table II, footnote (*).