Keyphrases

Chlorpheniramine, dealkylated metabolites—analysis
Urine—chlorpheniramine, metabolites, separation, determination
Ion-pair partition chromatography—separation
GLC—analysis
UV spectrophotometry—analysis

Comparative Effects of Chlorpromazine Hydrochloride and Quaternary Chlorpromazine Hydrochloride on the Central Nervous Systems of Rats and Mice

By N. WATZMAN*, A. A. MANIAN†, H. BARRY, III, and J. P. BUCKLEY

The effects of quaternary chlorpromazine HCl (QCPZ) and the tertiary parent compound chlorpromazine hydrochloride (CPZ) on the central nervous system were investigated in male rats and mice. CPZ was much more potent than QCPZ in inducing depressant effects on spontaneous activity of mice, forced motor activity of rats, continuous shock avoidance of rats, and potentiation of hexobarbital sleeping time in mice. Neither compound significantly altered the threshold of convulsive seizures induced by either pentylenetetrazol or strychnine. Although quaternization of the CPZ molecule at the terminal side chain nitrogen produced a decrease in central nervous system activity, the resulting compound was much more toxic than CPZ.

THE QUATERNIZATION of chlorpromazine (CPZ) by introducing a functional ^{14}C-methyl group into the terminal side chain nitrogen provides a highly sensitive and convenient method for the quantitative analysis of minute amounts of this compound and some of its metabolites in human blood plasma (1). Quaternary ammonium compound formation, in all likelihood, could also be used for determining plasma levels of other psychoactive amines employed in the treatment of the mentally ill.

Scientific literature discloses two references to biological information on this quaternary compound.[1] The first appears in a summary of an investigation by Seeman (2) on *in vitro* membrane stabilization by the phenothiazines, and the other by Fog *et al.* (3) describing the effects of quaternary phenothiazines administered *via* intracerebral injection in rats. Some quaternary phenothiazines such as 10-(α-dimethylamino-propionyl)-phenothiazine methobromide[2] (4, 5) have anticholinergic and ganglion-blocking actions while others such as 1-(10-phenothiazinylmethyl)-ethyl-2-hydroxyethyldimethyl ammonium chloride[3] (6–9) possess antihistaminic activity. Excellent review articles on the chemistry and pharmacologic actions of quaternary ammonium compounds, in general, have been presented by Cavallito and Gray (10) and D'Arcy and Taylor (11, 12).

This current study was undertaken to investigate the comparative pharmacologic activity of the quaternary chlorpromazine, 1-(2-chloro-10-phenothiazinyl)propyl - 3 - trimethylammonium chloride (QCPZ) (I) and the tertiary parent drug chlorpromazine HCl (CPZ),[4] in a battery of biological tests.

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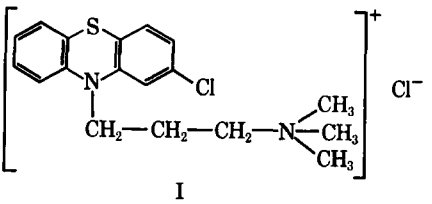
[1] Generously supplied by Dr. Leo E. Gaudette, Biomedical Assay Laboratories, a Division of the New England Nuclear Corp., Worcester, Mass.

[2] Secergan, Aktiebolaget Astra, Sodertalje, Sweden.
[3] Aprobit, Recip Co., Stockholm, Sweden.
[4] Thorazine, Smith, Kline & French, Philadelphia, Pa.



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METHODS

Subjects—Male albino mice (Swiss-Webster) and rats (Wistar descendant) were supplied by Hilltop Laboratory Animals, Inc., Scottdale, Pa.

Drug Administration—Both QCPZ and CPZ were injected intraperitoneally in an isotonic saline solution of 0.1 ml./g. for mice or 1.0 ml./kg. for rats. The same volume of the isotonic saline served as the placebo.

Gross Behavior—QCPZ was evaluated by a gross-observation rating scale which reflects qualitative drug effects on gross behavior. The scale is divided into four major drug actions: (*a*) stimulation, (*b*) depression, (*c*) autonomic activity, (*d*) reflexes and tone. Each of these actions is, in turn, subdivided into component characteristic responses; for example, motor activity and body tremors are two of the components of stimulation. Each trial consisted of the observation of a drug-treated animal simultaneously with a nontreated one. Items on the scale were checked 15, 30, 60, 120, and 240 min. after drug administration, so that the time course of the drug effect might be ascertained. QCPZ was administered intraperitoneally to two to six rats and mice at each dose level, ranging from 6.25 to 100 mg./kg. The effects of CPZ, in comparable doses, were observed in the rats used in the spontaneous activity test and in the mice used in the hexobarbital potentiation test. In addition, the 72-hr. LD_{50} was determined for both compounds, with 12–14 mice at each dose level. Toxic doses of QCPZ were also tested in a small number of rats.

Spontaneous Motor Activity—The effect of both compounds on the spontaneous motor activity of rats was measured in four photocell activity cages (Actophotometer, Metro Industries, Inc., New York, N. Y.). A single animal was placed in each photocell cage 1 hr. after drug administration and counts were recorded every 15 min. for a total of 1 hr. The doses were selected from the data obtained in the gross-observation studies. Animals tested simultaneously were administered saline, 1, 2, and 4 mg./kg. in one experiment, and saline, 6.25, 12.5, and 25.0 mg./kg. in the other experiment. Each dose was tested in a factorial design in each of the four activity cages in order to negate the differences in sensitivity among the units. This design, requiring 16 animals, was replicated once so that a total of 32 animals was tested for each compound (eight animals per dose level, including saline). The ED_{50} of each compound, defined as the dose which decreased the level of performance to 50% of the control scores, was calculated according to the method of Miller and Tainter (13).

Forced Motor Activity—The effects of the two compounds on forced motor activity of mice were compared on a rotarod, 5.08 cm. (2 in.) in diameter. The procedure was similar to that described by Watzman and Barry (14): the wooden rod rotated at 4 r.p.m. during the first 30 sec. of each trial, at 6 r.p.m. during the next 30 sec., and at progressively increasing speeds thereafter at 30-sec. intervals until the animals fell off. Six animals, tested simultaneously, were given 10 trials, with two spaced several hours apart on each of 5 consecutive days. The tenth trial was preceded at an interval of 1 hr. by the administration of saline or one of three doses of either compound (1, 2, and 4 mg./kg.). The use of eight groups of six animals provided a total of 48 test subjects with 12 animals per dose level, including the saline group. The animals tested simultaneously included each of the four dosage conditions; each dose was tested an equal number of times in each of the six rod positions in a factorial design. The drug or placebo effects for each animal were computed as the ratio of performance time on the tenth trial divided by performance time on the ninth trial. A second experiment was performed with QCPZ only, using 6.25, 12.5, and 25 mg./kg.

Continuous Shock Avoidance—Standard operant-conditioning chambers (Lehigh Valley Electronics, Inc., Fogelsville, Pa.), each with two levers on one wall, were used for a two-lever continuous shock-avoidance task with a separate shock-escape response. Painful electric shocks (250 v. a.c. through a series resistance of 150,000 ohms) were programmed to be delivered on the grid floor for a duration of 2 sec. with a 5-sec. interval between shocks. A press on one lever (Escape) terminated the shock, initiating the 5-sec. S-S interval. Each press on the other lever (Avoidance), provided the shock was not currently on, initiated a longer, 20-sec. R-S interval until the next shock. Effects of the compounds were tested in 17 rats which had acquired a stable and proficient level of avoidance performance in more than 20 previous 3-hr. sessions. The test sessions were preceded at an interval of 1 hr. by injection of CPZ, QCPZ, or saline. Drug sessions alternated with saline sessions. The first two drug treatments were 2 mg./kg. CPZ and 2 mg./kg. QCPZ. Each of these treatments was given first to half the animals in order to counterbalance the sequence. Subsequent drug sessions consisted of progressively higher doses of QCPZ (4, 8, and 16 mg./kg.). Eight of the animals were selected to receive additional doses of QCPZ (24, 32, and 48 mg./kg.) without intervening saline sessions.

Hexobarbital Sleeping-Time Potentiation—A total of 40 mice was divided into four groups of 10 animals each; three groups received 4, 8, or 16 mg./kg. QCPZ 1 hr. prior to the intraperitoneal administration of 100 mg./kg. of hexobarbital; the last group received 100 mg./kg. hexobarbital only. The same drug regimen was used for CPZ, except that only the two lowest doses (4 and 8 mg./kg.) were administered. Sleeping times for the animals of all groups were recorded as the interval between the loss and return of righting reflex. The criteria for the loss and return of the reflex are defined as the inability and ability, respectively, of the animal to right itself within 5 sec. in three successive trials when placed on its back. The trials were arbitrarily terminated 3 hr. after the loss of righting reflex if the animals continued to sleep.

Pentylenetetrazol Antagonism—The protection of mice from tonic convulsions and death produced by pentylenetetrazol was studied in the following manner. A total of 77 male albino mice was divided into

four treatment groups: the first five of these (10 mice each) received 4 mg./kg. CPZ or QCPZ, 8 mg./kg. CPZ or QCPZ and saline, respectively, 1 hr. prior to the subcutaneous administration of 100 mg./kg. pentylenetetrazol; the last three groups (nine animals) received 16 mg./kg. CPZ or QCPZ and saline, respectively, 1 hr. prior to the administration of pentylenetetrazol. Protection from convulsions was indicated if tonic extension of the hind limbs for 5 sec. or longer did not occur. Time of convulsions and onset of death were recorded for each animal.

Strychnine Antagonism—The same procedure and same number of animals as for pentylenetetrazol antagonism were used except that strychnine (2 mg./kg., i.p.) was the convulsant employed. The highest dose level (16 mg./kg.) of both CPZ and QCPZ was tested simultaneously on a different day than the two lower dose levels, thus requiring a separate control group (saline).

RESULTS

Gross Behavior—The onset of action of QCPZ usually occurred within 15 min. in mice and 30 min. in rats. Minimal depression was produced in rats by doses of 6.25, 12.5, and 25.0 mg./kg. and consisted of decreased motor activity, decreased irritability, and decreased startle response with no signs of autonomic effects or effects on reflexes and tone. Mice, however, did not display central nervous system depression, but autonomic activity was indicated by exophthalmos, mydriasis, and increased urination. Both mice and rats treated with the same doses of CPZ were so profoundly depressed that they did not move even when prodded.

Toxic effects of QCPZ were noted in doses ranging from 30–100 mg./kg. These effects usually consisted of ataxia and labored respiration. Death usually occurred within 5 min. in mice, which was much quicker than in rats. The 72-hr. LD_{50} in mice was 170 ± 69 mg./kg. for CPZ and 35.5 ± 2.1 mg./kg. for QCPZ. A lethal effect of QCPZ occurred in one of two rats after 30 mg./kg. and in three of four rats after 100 mg./kg. The LD_{50} for CPZ injected intraperitoneally was 115 mg./kg. for mice and 85 mg./kg. for rats in one prior study (15) and 215 mg./kg. for mice and 150 mg./kg. for rats in another prior study (16). The LD_{50} values of the present study are similar for CPZ but much lower for QCPZ.

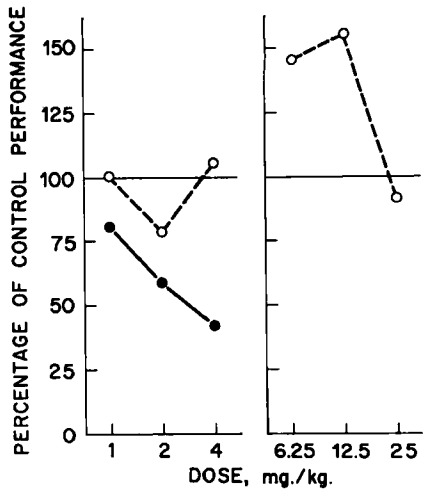

Fig. 2—Comparative effects of chlorpromazine hydrochloride and quaternary chlorpromazine hydrochloride on rotarod performance of mice. Key: O--O, QCPZ; ●—●, CPZ.

Spontaneous Motor Activity—Figure 1 shows the comparative effects of CPZ and QCPZ on spontaneous activity of rats in two separate experiments. In both the lower and higher dose ranges, CPZ decreased activity to a greater degree. The ED_{50} values were 12.0 mg./kg. for QCPZ and 4.3 mg./kg. for CPZ. Thus QCPZ appears to have much less potency than CPZ.

Forced Motor Activity—Figure 2 shows the comparative effects of CPZ and QCPZ on the rotarod performance times of mice. In doses of 1, 2, and 4 mg./kg., CPZ clearly decreased motor coordination, whereas QCPZ had no reliable inhibiting effect. In the second experiment, higher doses of 6.25, 12.5, and 25 mg./kg. QCPZ likewise had no reliable inhibitory effect. In this test of forced motor activity, the ED_{50} for CPZ was 2.8 ± 1.1 mg./kg., whereas the

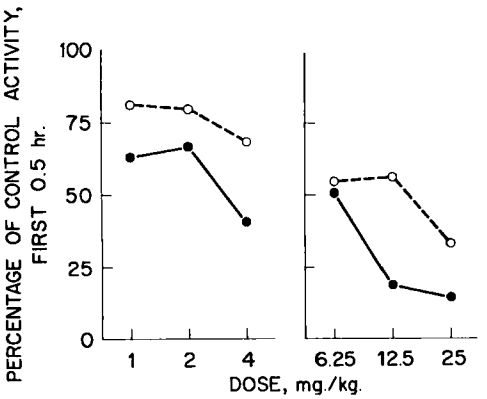

Fig. 1—Comparative effects of chlorpromazine hydrochloride and quaternary chlorpromazine hydrochloride on the spontaneous activity of rats. Key: O- -O, QCPZ; ●—●, CPZ.

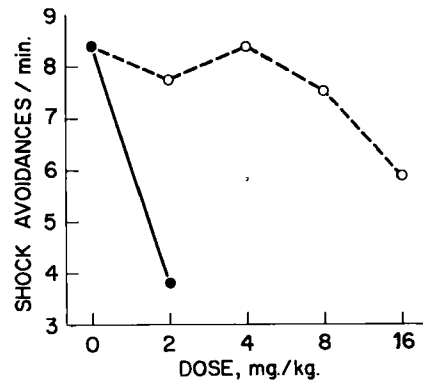

Fig. 3—Comparative effects of chlorpromazine hydrochloride and quaternary chlorpromazine hydrochloride on continuous shock avoidance by rats. Key: O-O, QCPZ; ●—●, CPZ.

TABLE I—COMPARATIVE EFFECTS OF QUATERNARY CHLORPROMAZINE HCl AND CHLORPROMAZINE HCl ON THE HEXOBARBITAL SLEEPING TIME OF MICE IN MINUTES

Mouse No.	QCPZ				CPZ		
	Control[a]	4[b]	8[b]	16[b]	Control[a]	4[b]	8[b]
1	50	100	32	77	17	101	172
2	41	94	135	82	21	95	180+
3	34	155	23	101	—	89	180+
4	46	151	65	41	33	117	180+
5	41	109	70	84	40	119	180+
6	56	90	76	82	54	161	127
7	48	155	60	33	25	116	167
8	56	126	67	66	45	97	126
9	56	93	66	77	23	111	140
10	78	143	40	90	35	157	180+
$\bar{X}$	50.6	121.6	63.4	73.3	32.6	116.3	163.2
± *SE*	3.8	8.7	9.7	6.7	4.1	7.8	7.3
% (incr.)	—	142.0	25.3	45.0	—	256.7	400.6

[a] = hexobarbital (100 mg./kg. i.p.) only. [b] = mg./kg.

ED_{50} for QCPZ could not be determined but was definitely above 25 mg./kg.

Continuous Shock Avoidance—Figure 3 shows the rate of shock avoidances for the entire 3-hr. session in four placebo sessions averaged together and after CPZ (2 mg./kg.) and QCPZ (2, 4, 8, and 16 mg./kg.). Performance was decreased to 46% of the control rate by 2 mg./kg. CPZ, whereas a reliable effect of QCPZ was found only at 16 mg./kg., with performance at 70% of the control rate. A precise ED_{50} could not be determined for CPZ because of the single dose used, but appears to be less than 2 mg./kg., in agreement with previous reports that shock avoidance is impaired by low doses of this compound (17). The ED_{50} for QCPZ could not be determined but appears to be greater than 16 mg./kg. Evidence for severe toxicity of QCPZ at 16 mg./kg. was found in that five of the 17 animals died within 24 hr. after this dose. Among the eight animals tested with higher doses of QCPZ, two died within 4 hr. after 32 mg./kg. and three within 4 hr. after 48 mg./kg. In spite of these lethal effects in the majority of animals, no consistent performance decrement was found in the survivors, even at 48 mg./kg.

Hexobarbital Sleeping-Time Potentiation—Table I shows the comparative effects of CPZ and QCPZ on the hexobarbital sleeping times of mice. CPZ, 8 mg./kg., prolonged average sleeping time almost to the maximum of 180 min., whereas QCPZ was much less effective and the responses were not dose related.

Pentylenetetrazol Antagonism—Pentylenetetrazol produced both clonic and tonic convulsions, the former occurring approximately 3–5 min. after injection, whereas tonic extension of the hind limbs and arching of the back began approximately 10 min. after administration. Death usually occurred immediately following the tonic seizures. Neither CPZ nor QCPZ had any statistically significant effects, but only seven of the ten animals convulsed after 8 mg./kg. of QCPZ, and there was a slight increase in the time before death after 4 and 8 mg./kg. of CPZ.

Strychnine Antagonism—Strychnine produced seizures in the form of tonic extension of the hind legs and arching of the back about 3–7 min. after administration, usually followed by death. CPZ and QCPZ had almost exactly the same effects as in the pentylenetetrazol test; only seven of the nine animals convulsed after 16 mg./kg. QCPZ, whereas all nine control animals convulsed, and CPZ showed slight evidence of increasing the time of onset before convulsions or before death. None of these effects of QCPZ or CPZ was statistically reliable.

DISCUSSION AND CONCLUSION

QCPZ and CPZ were studied in a battery of tests designed to compare their effects on the central nervous system. The tests were: gross behavior, spontaneous motor activity, forced motor activity, continuous shock avoidance, hexobarbital sleeping-time potentiation, and pentylenetetrazol and strychnine antagonism. In each of the first five tests, CPZ clearly appeared to be the more potent compound, although the LD_{50} was much higher for CPZ than for QCPZ. In the gross-observation experiments, both compounds produced a sedative profile in doses ranging from 1–25 mg./kg. CPZ produced generalized depression to a large degree, whereas QCPZ-induced depression was minimal. From the standpoint of the central nervous system activity of QCPZ, the ED_{50} and doses producing minimal depression are close to the lethal dose range, so that the theoretical therapeutic index is much smaller than that of CPZ. Both compounds potentiated hexobarbital sleeping times with CPZ producing the greater effect. However, neither compound protected the test animals from death due to strychnine or pentylenetetrazol. The data suggest that QCPZ, in common with 1-(10-phenothiazinylmethyl)-ethyl-2-hydroxyethyldimethyl ammonium chloride (9), fails to penetrate the blood-brain barrier to an appreciable degree. Therefore, the effect of low doses of CPZ on various measures of performance, especially shock avoidance (17), can be attributed to the central rather than peripheral actions of CPZ.

In conclusion, quaternization of the CPZ molecule at the terminal side chain nitrogen produces a marked increase in toxicity and a decrease in central nervous system activity.

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Keyphrases

Chlorpromazine HCl—quaternary, tertiary forms
Quaternary, tertiary chlorpromazine HCl—activity compared
Motor activity, spontaneous, forced—chlorpromazines
Shock avoidance—chlorpromazines
Hexobarbital sleep-time—chlorpromazines

Interaction of Methyl and Propyl Parabens with Selected Sucrose Esters

By CARLOS VALDEZ, EUGENE I. ISAACSON, and FRANK P. COSGROVE*

The interaction of methyl and propyl *p*-hydroxybenzoates with two sucrose esters, sucrose monotallowate and sucrose monococoate, was studied at different temperatures and concentrations of the sucrose ester by the solubility method. Aspects of the qualitative and quantitative nature of the interaction are reported and a possible mechanism of interaction based on hydrogen and hydrophobic bonding is proposed. Predictive effects on preservative activity due to intermolecular association were tested by microbiologic studies.

NUMEROUS REPORTS in recent years have noted the inactivation of various preservatives in the presence of surface-active agents commonly employed in pharmaceutical preparations (1–11). Examples which have been frequently cited are the inhibition of preservative activity of phenolic compounds in the presence of polyether derivatives of fatty acid esters.

The introduction, in 1956, of a novel series of nonionic surfactants, the fatty acid esters of sucrose, and their proposed use in forming emulsion bases and as dispersing agents in pharmaceutical suspensions warrants a consideration of their possible interaction with preservatives commonly used in such systems. A previous investigation by Blaug and Ebersman (12) using the dialysis method revealed evidence of interaction between fatty acid mono and diesters of sucrose and fatty acid monoesters of propoxylated sucrose and derivatives of benzoic acid. The present study employs the solubility method to investigate the interaction between methyl and propyl *p*-hydroxybenzoates with sucrose monotallowate and sucrose monococoate. The effect of elevation of temperature and change of concentration of surfactant on the association is considered. Microbiologic studies have been designed so as to permit a direct correlation of preservative activity and the degree of binding of the preservative.

EXPERIMENTAL

Materials—Recrystallized methyl *p*-hydroxybenzoate, m.p. 124–126°; recrystallized propyl *p*-hydroxybenzoate, m.p. 95–96°, Eastman Organic Chemicals; sucrose monotallowate; sucrose monococoate, Sucro-Chemical Division, Colonial Sugar Co., Gramercy, La.

Solubility Method—The interaction of methyl and propyl parabens with sucrose monotallowate and sucrose monococoate was studied at different concentrations and temperatures according to the Higuchi and Zuck (13–15) solubility method.

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