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Anticholinergic activity of antipsychotic drugs in relation to their extrapyramidal effects

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Antipsychotic drugs were evaluated with two indices of anticholinergic activity, mydriasis in mice *in vivo* and antagonism of carbamylcholine-induced contractions of guinea-pig tracheal strips *in vitro*. The drugs from most to least potent as oral mydriatic agents were mepazine, clozapine, thioridazine, promazine and chlorpromazine. Trifluoperazine, pimozide and haloperidol were inactive. These results were consistent with the hypothesis that anticholinergic activity of antipsychotic drugs is inversely related to their propensity to produce extrapyramidal effects in man. *In vitro* results appeared to predict the incidence of extrapyramidal effects less accurately than *in vivo* results.

An indicant of anticholinergic activity of drugs is their ability to displace muscarinic receptor binding agents in rat brain homogenates (Miller & Hiley, 1974; Snyder, Greenberg & Yamamura, 1974). Antipsychotic drugs with potent anticholinergic activity in this test appear to produce fewer extrapyramidal effects in man than antipsychotic drugs with less anticholinergic activity. The purpose of the present study was to determine whether results similar to those previously reported would be obtained *in vivo* using mydriatic activity in mice and *in vitro* using guinea-pig trachea.

MATERIALS AND METHODS

Pupil diameters in mice

Pupil diameter was measured with a Bausch and Lomb, Model BKT-5, stereomicroscope with a 100 unit scale in the eyepiece (see Pulewka, 1932; Ing, Dawes & Wajda, 1945, for prior descriptions of method). The scope was set full tilt at 6·6 × magnification, the mirror illumination was not used and the illumination on the stage of the scope was 24 ft candles. Illumination for the scope was provided with two fluorescent 15 w lights directed 45° toward the stage at a distance of about 30 cm. Illumination for the room was provided by overhead fluorescent lights.

Male Swiss-Webster mice, 18–22 g, (Taconic Farms, N.Y.) were taken in their home cages to the test room 30 min before measurement of pupil diameter. The size of the left pupil was measured to the nearest scale unit, 100 μm, and immediately after the mice were dosed orally then each was placed in a 18 × 8 × 4 cm mesh cage. Pupil measurements were repeated 1 and 4 h after medication. Because peak mydriatic activity of the drugs at their minimum effective doses was noted 1 h after medication, 1 h measurements were reported. The mean change in pupil diameter was the average difference in pupil diameter in groups of 10 mice immediately before and 1 h after medication.

Guinea pig tracheae

Hartley guinea-pigs of either sex, 180–280 g, were anaesthetized with urethane, 1·5–3·0 g kg^{-1} intraperitoneally. The tracheae were removed, freed from connective tissue, placed on wooden sticks and cut 45° to form a strip. The strips under 2 g tension were placed in 50 ml muscle baths, filled with Krebs-Henseleit solution at 35°, and gassed with carbon dioxide in oxygen. During the 1 h equilibration period the warmed physiological solution was changed several times. Isometric tension was recorded with a Grass force displacement transducer, Model FTO3C, on a Grass polygraph, Model 7.

Carbamylcholine, 3 × 10^{-7}M, added to the bath to constrict each tracheal strip to about 80% of the maximum response, remained in contact with each tissue for 15 min, after which the test drug was cumulatively added in log increments, 10^{-9}–10^{-4}M. Isoprenaline, 10^{-6}M, was added to the final concentration of the test drug, 10^{-4}M, to determine the maximal extent of relaxation of the tracheal strips. The tracheal strips were allowed to stabilize at each new concentration of test agent. Each tracheal strip was exposed to only one test drug.

* Correspondence

Data from each tissue were subjected to an arcsin transformation before determining the slope of the dose-response relation and the concentration that was effective in eliciting 50% of the total response (EC50). Results from eight to ten tissues were averaged and the s.e. calculated.

Drugs. Clozapine (Sandoz Pharmaceuticals), haloperidol (Janssen Pharmaceutica), mepazine hydrochloride (Warner Lambert Research Institute), pimozide (Janssen Pharmaceutica), promazine hydrochloride (Wyeth Institute), thioridazine hydrochloride (Sandoz Pharmaceuticals), and trifluoperazine dihydrochloride (Smith, Kline and French Laboratories) were all kindly donated. Atropine sulphate (Merck and Co., Inc.) was used as an antiacetylcholine reference agent.

Drugs were made up in a 1% gum tragacanth suspension for oral medication and administered at a volume of 0·1 ml per 10 g body weight. The suspension alone served as the control vehicle. Doses were reported in mg kg^{-1} of the free base.

RESULTS

Mydriasis in mice

Three groups of 10 mice each were medicated with gum tragacanth. One h later five of the 30 mice exhibited a decrease of 100 μm in pupil diameter. The remaining 25 mice showed no change (Table 1).

The drugs from most to least potent in producing a statistically significant ($P = 0{\cdot}05$ level) amount of mydriasis were atropine, mepazine, clozapine, thioridazine, promazine and chlorpromazine. Trifluoperazine, pimozide and haloperidol were inactive. The highest dose of haloperidol tested was 64 mg kg^{-1} because 128 mg kg^{-1} caused clonic convulsions in all 10 mice within 1 h and 256 mg kg^{-1} killed four of the 10 mice.

Guinea-pig tracheae

Table 2 shows the antagonistic effect of atropine and antipsychotic agents on carbamylcholine-induced constriction of guinea-pig tracheal strips. The drugs from most to least potent were atropine, clozapine, thioridazine, promazine, mepazine, chlorpromazine, haloperidol and trifluoperazine. The differences in activity between thioridazine, promazine, mepazine and chlorpromazine were at best slight.

DISCUSSION

Table 3 shows the rank-order potencies of antipsychotic drugs as measured by mydriasis in mice and by reversal of carbamylcholine-induced constric-

Table 1. *Effect of atropine and antipsychotic drugs on pupil diameter in mice.* The mean change was the difference in pupil diameters of groups of 10 mice measured immediately before and 1 h after oral medication. The average pupil diameter in mice before medication was 199 μm.

Drug	Dose mg kg^{-1}	Mean change ± s.e. (μm)	
Gum tragacanth	nil	− 10	7
	nil	− 10	7
	nil	− 30	11
Atropine	0·25	+ 20	9
	0·5	+130	15***
	1·0	+700	26***
Mepazine	4·0	0	15
	8·0	+ 80	14**
	16·0	+140	28**
	32·0	+460	19***
Clozapine	8·0	+ 10	13
	8·0	+ 30	11
	16·0	+ 70	15**
	32·0	+300	24***
Thioridazine	32·0	0	11
	32·0	+ 30	24
	64·0	+150	16***
	128·0	+180	14***
Promazine	32·0	− 10	13
	64·0	+ 40	16
	64·0	+ 70	21*
	128·0	+120	18***
Chlorpromazine	128·0	0	0
	256·0	+ 70	18*
	256·0	+120	25**
Haloperidol	32·0	− 20	9
	64·0	0	11
Pimozide	64·0	− 30	11
	128·0	+ 20	17
	256·0	− 10	7
Trifluoperazine	64·0	+ 10	7
	128·0	− 20	9
	256·0	+ 20	9

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, according to the two-tailed paired *t*-test.

Table 2. *Antagonistic effect of atropine and antipsychotic agents on carbamylcholine-induced constriction of guinea-pig tracheal strips.*

Drug	−Log EC50 ± s.e. M†	Slope ± s.e. radians
Atropine	8·88 ± 0·08	0·76 ± 0·11
Clozapine	5·71 ± 0·15	0·56 ± 0·06
Thioridazine	5·41 ± 0·08	0·43 ± 0·04
Promazine	5·39 ± 0·19	0·43 ± 0·06
Mepazine	5·38 ± 0·12	0·59 ± 0·06
Chlorpromazine	5·35 ± 0·18	0·49 ± 0·02
Haloperidol	4·95 ± 0·08	0·72 ± 0·05
Trifluoperazine	Inactive‡	—

† The EC50 equals the concentration of test drug that was effective in eliciting 50% of the total effect in eight to ten tissues.

‡ Relaxation was 0·04 ± 0·02 g at 10^{-4} M.

Table 3. *Rank-order potency of drugs in anticholinergic tests in A, mouse mydriasis, B, guinea-pig trachea, C, rat brain homogenate and D, in producing extra-pyramidal effects in man*†.

	A	B	C[1]	C[2]	D
Mepazine	1	4	—	—	‡
Clozapine	2	1	1	2	6
Thioridazine	3	2	2	1	5
Promazine	4	3	3	—	4
Chlorpromazine	5	5	4	3	3
Pimozide	7	—	—	4	‡
Trifluoperazine	7	7	5	5	2
Haloperidol	7	6	6	—	1

† The numeral 1 indicates the most potent drug for each column. Note that the differences in potency between mepazine, thioridazine, promazine and chlorpromazine on the guinea-pig trachea were at best slight as indicated on the results section.

‡ See text for discussion of extrapyramidal effects. C[1] Snyder & others, 1974 C[2] Miller & Hiley, 1974.

tion in guinea-pig tracheae. Included in Table 3 are previous studies with rat brain homogenates and estimates of the incidence of extrapyramidal effects in man (Miller & Hiley, 1974; Snyder & others, 1974). Pimozide and mepazine were not ranked for extrapyramidal effects. Pimozide, however, produces extrapyramidal effects (Huber, Serafetinides & others, 1971), probably with an incidence less than that for fluphenazine (Morris, MacKenzie & Masheter, 1970). Mepazine, which has poor antipsychotic efficacy, rarely produces extrapyramidal effects (Bruckman, Kitchener & others, 1957; Feldman, 1957a; Lomas, 1957; Mitchell, Sykes & King, 1957; Denber, 1958; Ayd, 1961). The incidence of extrapyramidal effects for mepazine appears to be lower than that for chlorpromazine and promazine (Feldman, 1957b; Freyhan, 1959; Hollister, Caffey & Klett, 1960), and mepazine may even reduce extrapyramidal effects produced by chlorpromazine (Bowes, 1956). Mepazine frequently produces atropine-like effects in man (Bowes, 1956; Feldman, 1957a; Lomas, 1957; Hollister & others, 1960).

Data of Table 3 for each of the four tests show that the antiacetylcholine activity of antipsychotic drugs in animals was inversely correlated with the incidence of extrapyramidal effects in man. In Table 3 the lowest Spearman rank-order correlation coefficient was between the *in vivo* mydriatic test in mice and the *in vitro* test in guinea-pigs (0·78, $P < 0·05$ one tailed test). This was primarily due to the discrepancy in the potency estimates for mepazine. The differences in potency between mepazine, thioridazine, promazine and chlorpromazine were at best slight in the guinea-pig trachea.

Whether the *in vitro* results in guinea-pig trachea accurately predict the incidence of extrapyramidal effects in man is questionable. *In vitro* haloperidol was more potent than trifluoperazine, and chlorpromazine was about as potent as thioridazine and mepazine, whereas in man haloperidol produced a greater incidence of extrapyramidal effects than trifluoperazine, and chlorpromazine produced a greater incidence of extrapyramidal effects than thioridazine and mepazine.

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