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DISCRIMINATIVE STIMULUS PROPERTIES OF QUIPAZINE*

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Summary—Rats were trained to discriminate quipazine (2.5 mg/kg) from saline in a two-lever, drug discrimination task. After high levels of accuracy (i.e. 90% correct choice responding) were attained, dose and time parameters of the quipazine cue were studied during extinction test sessions. Quipazine was highly discriminable at doses of 1.25 and 0.94 mg/kg. With decreasing doses, the percentage of responding on the quipazine lever declined in a dose-related fashion. By varying the normal 30-min time interval between injection and testing, the onset and duration of action of quipazine were determined to be about 5–15 min and 60–90 min respectively.

After injections with novel drugs, animals were tested during extinction sessions. Lysergic acid diethylamide, (LSD), at doses of 0.08 mg/kg and 0.10 mg/kg, transferred completely to the quipazine cue (i.e. above 95% quipazine responding); neither D-amphetamine (1.0 and 2.0 mg/kg) nor apomorphine (0.5 and 1.0 mg/kg) showed any transfer to the quipazine cue.

By administering various neurotransmitter blockers in conjunction with quipazine and testing during extinction sessions, it was found that the putative central 5-HT antagonists cyproheptadine (1.0 mg/kg), methysergide (1.0 mg/kg), and methiothepin (1.0 mg/kg) produced significant decreases in the discriminability of quipazine. The dopamine antagonists haloperidol (0.05 and 0.10 mg/kg) and fluphenazine (1.0 mg/kg) were without effect. It was concluded that LSD and quipazine have a common mechanism of action which may (or may not) involve 5-HT; a role for DA was not indicated.

Quipazine (2-piperazinyl quinoline) has been shown to act peripherally and centrally as a serotonin (5-HT) agonist (Hong, Sancillo, Vargas, and Pardo, 1969; Rodriguez and Pardo, 1971; Rodriguez, 1972; Medon, Leeling and Phillips, 1973; Grabowska, Antkiewicz and Michaluk, 1974b; Costall and Naylor, 1975; Jacoby, Howd, Levin and Wurtman, 1975; Green, Youdim, and Grahame-Smith, 1976). More specifically, there is behavioural (Rodriguez, 1972; Green *et al.*, 1976; Samanin, Bernasconi and Quattrone, 1976), pharmacological (Grabowska *et al.*, 1974b) and physiological (Costall and Naylor, 1975) evidence that the effects of this compound are mediated by direct stimulation of 5-HT receptors although certain indirect mechanisms (e.g. 5-HT uptake inhibition and monoamine oxidase inhibition) cannot be ruled out (Fuller, Snoddy, Perry, Rousch, Molloy, Bymaster and Wong, 1976; Hamon, Bourgoin, Enjalbert, Bockaert, Hery, Ternaux and Glowinski, 1976). Grabowska, Antkiewicz and Michaluk (1974a) and Green *et al.* (1976) have suggested that quipazine may also stimulate dopamine (DA) receptors, but this contention has not been consistently supported (Costall and Naylor, 1975; Green *et al.*, 1976).

Since LSD has also been reported to exert its behavioural (discriminable) effects by interacting with 5-HT receptors (Greenberg, Kuhn and Appel, 1975a; Hirschhorn, Hayes and Rosecrans, 1975), the similarity of these drugs was tested in a drug discrimination task. When quipazine (2.5 mg/kg) was administered to rats trained to discriminate D-LSD (0.08 mg/kg) from saline, responding occurred predominantly (85%) on the lever associated with LSD administration (Kuhn, White, and Appel, 1976b). Since this is the most complete transfer to LSD yet demonstrated, more than is typically seen following known hallucinogens such as mescaline or psilocybin (Cameron and Appel, 1973), it was decided to investigate the discriminative stimulus properties of quipazine and to determine the dose and the temporal parameters of these effects. By subsequently administering LSD, as well as various other drugs during transfer or antagonism tests, it was hoped to further elucidate the mechanism of action of quipazine.

METHODS

Subjects

Twelve experimentally naive rats (Sprague–Dawley), weighing between 275–320 g, were used in all phases of the experiment. They were maintained at

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80% of free-feeding weight by limiting water intake and were housed individually in a room at constant temperature (21–23°C) and humidity (40–50%) on a 12-hr light–dark cycle.

Apparatus

Four two-lever operant chambers (Lehigh Valley Electronics Model 143–24), housed in sound- and light-attenuating outer chambers (LVE Model 132-04) were used throughout the experiment. Each chamber contained a dipper, mounted between two levers, which delivered 0.01 ml tap water as reinforcement. The chambers were illuminated by a 28 V house light and were ventilated during testing. Programming and recording were controlled electromechanically in an adjoining room.

Training procedure and acquisition of the quipazine–saline discrimination

The rats were injected with either quipazine (2.5 mg/kg) or saline (in equivalent volume) 30 min prior to testing. They were then placed in experimental chambers and training was begun on a continuous schedule of water reinforcement (FR 1). During this phase of the experiment, only the stimulus (quipazine or saline) appropriate lever was present. For half of the subjects, the right lever was correct for quipazine and the left for saline, while the opposite levers were correct for the other half of the subjects. The number of bar-pressing responses required per reinforcement was gradually increased to 32 (FR 32). The order of quipazine–saline administration was random with the restriction that neither condition was present for more than three consecutive days. When responding stabilized at FR 32, both levers were presented simultaneously and discrimination training was begun. The animals were reinforced only for responding on the stimulus-appropriate lever; there was no programmed consequence for incorrect responding. The sessions were 30 min long and were conducted five days per week (Mon–Fri).

Dose parameters of the quipazine cue

When discrimination was consistently above 85%, tests with different doses of quipazine were begun. The rats were injected (in random order) with 1.25, 0.94, 0.63, 0.47, and 0.31 mg/kg and were tested for lever choice 30 min later. All generalization tests were conducted during extinction (i.e. the animals were not reinforced) to avoid possible confounding with additional learning. The extinction session ended for each rat when a total of 32 responses had been made on one of the levers. All extinction sessions were conducted on the day following a saline session so that there could be no residual drug effect. Thus, during any week, extinction tests occurred on Tuesdays and Fridays, saline sessions occurred on Mondays and Thursdays, and a quipazine re-training session was held on Wednesdays.

Temporal parameters of the quipazine cue

The time interval between injection of quipazine and initiation of testing was varied and lever choice was observed during extinction. Animals were injected at 5, 15, 30, 60, 90, 120, 180 and 240 min prior to testing. Since the sessions lasted for 5 min (or until the subjects made 32 responses) each time interval represented is actually a 5-min interval. The order of these tests was random over a five-week period of testing.

Transfer of the quipazine cue to other drugs

The animals were injected with other drugs and tested for lever choice 30 min afterward. The drugs tested were D-amphetamine (1.0 and 2.0 mg/kg) and apomorphine (0.5 and 1.0 mg/kg), both of which stimulate dopamine receptors and D-LSD (0.02–0.10 mg/kg) a drug thought to stimulate 5-HT receptors.* All transfer tests were conducted during extinction sessions and were randomly administered throughout the testing period to avoid order effects.

Antagonism of the quipazine cue

The putative serotonin antagonists, methysergide (1.0 mg/kg), cyproheptadine (1.0 mg/kg) and methiothepin (1.0 mg/kg), and the dopamine antagonists, fluphenazine (0.5 and 1.0 mg/kg) and haloperidol (0.05 and 0.10 mg/kg), were administered in combination with quipazine (either 30 or 60 min prior to quipazine injection) and the animals were tested for lever choice during extinction.

Pharmacological procedures

Haloperidol was dissolved in a stoichiometric volume of lactic acid and diluted with distilled water containing methyl and propyl parabens. Apomorphine was dissolved in 0.2% ascorbic acid. Cyproheptadine was dissolved in 15% aqueous propylene glycol. All other drugs were dissolved in 0.09% NaCl. Injections were given intraperitoneally.

RESULTS

The acquisition of the quipazine–saline discrimination is shown in Figure 1. As can be seen, the rats acquired the discrimination rapidly. Only on sessions 3 and 4 did accuracy fall below 70%. By session 12, accuracy reached 80% correct and was never again less than 85% correct.

Response rates on days during which quipazine was administered were significantly lower than on days when saline was given during the first 30 days of discrimination training ($P < 0.001$; t-tests for correlated measures).

* LSD was obtained from the National Institute on Drug Abuse. Expressed concentrations refer to the tartrate salt.

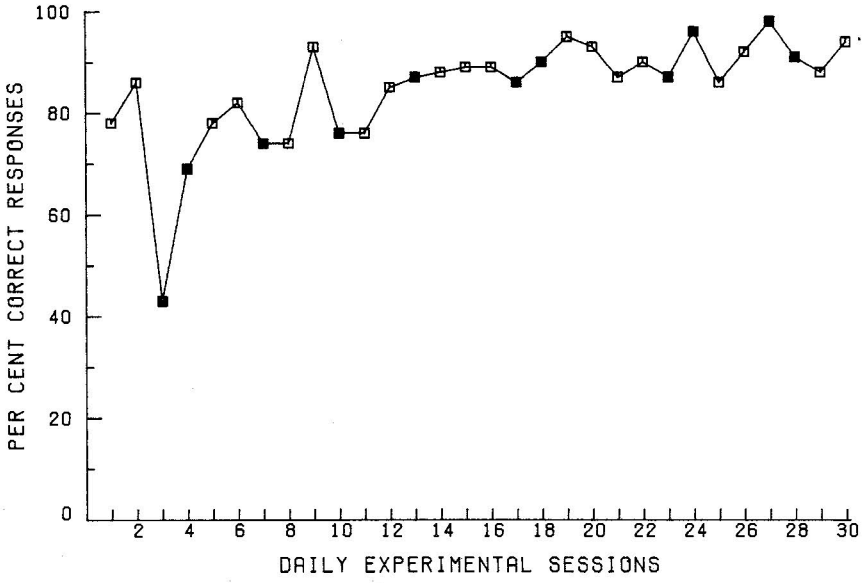

Fig. 1. Acquisition of quipazine–saline discrimination. All points are the mean of 12 *Ss* and are expressed as the percentage of responses on the correct lever before the first reinforcement. Solid squares represent quipazine sessions; open squares represent saline sessions.

Dose parameters of the quipazine discrimination

The dose–response curve of the quipazine discrimination is shown in Figure 2. When half of the training dose (1.25 mg/kg) of quipazine was administered, the average percentage responding on the quipazine lever was 90%. Thereafter, the percentage declined with decreasing doses of quipazine.

Temporal parameters of the quipazine cue

Figure 3 shows the percent of quipazine responding as a function of time between injection and testing. The discriminability of quipazine was 82% at 5 min after injection. The percentage of appropriate responses to the drug was above 90% at 15 min, 98% at 30 min (the training interval), 85% at 45 min and declined to 75% at 60 min after injection. Drug-related responding gradually decreased and, by 240 min (4 hr after injection), was no longer evident (i.e. there was 94% saline responding).

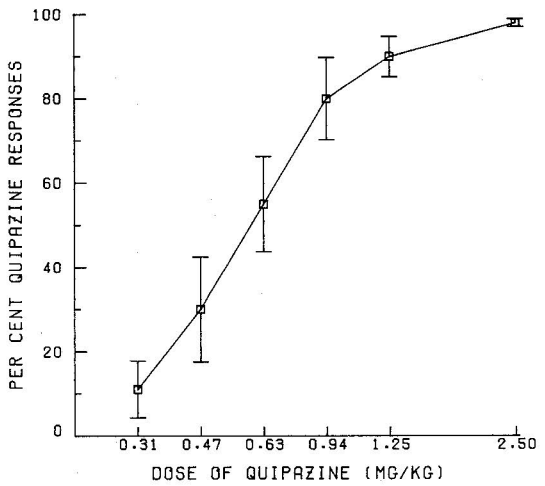

Fig. 2. Generalization of the quipazine-cue to other doses of quipazine (log scale). Data points are the mean of 12 *Ss* (±S.E.) and are expressed as the percentage of responses on the quipazine lever during extinction.

Transfer of the quipazine cue to other drugs

The results of transfer tests with D-LSD, amphetamine, and apomorphine are presented in Table 1. As can be seen, the transfer of D-LSD to quipazine was virtually complete (above 95%) at both 0.08 and 0.10 mg/kg. Following 0.06 mg/kg and 0.04 mg/kg of D-LSD, responding was slightly below chance (35–45%) and after 0.02 mg/kg of D-LSD, responding was primarily on the saline lever. There was no transfer (12–39%) to the quipazine discrimination with D-amphetamine (1.0 mg/kg) or apomorphine (0.50 and 1.0 mg/kg). Tests with larger doses of these drugs resulted in behavioural disruption, (i.e. no responding) and therefore could not be interpreted.

Antagonism of the quipazine cue

Table 2 shows the results of antagonism studies with the 5-HT antagonists cyproheptadine, methysergide and methiothepin and the dopamine antagonists fluphenazine and haloperidol. All the 5-HT antagonists, administered in conjunction with quipazine, produced a significant block of the quipazine discrimination (Table 2). Methysergide (1.0 mg/kg) reduced the amount of quipazine responding to 71%. Cyproheptadine (1.0 mg/kg) and methiothepin (1.0 mg/kg) blocked the discrimination to 28% and 25% respectively. The dopamine antagonists, fluphenazine and

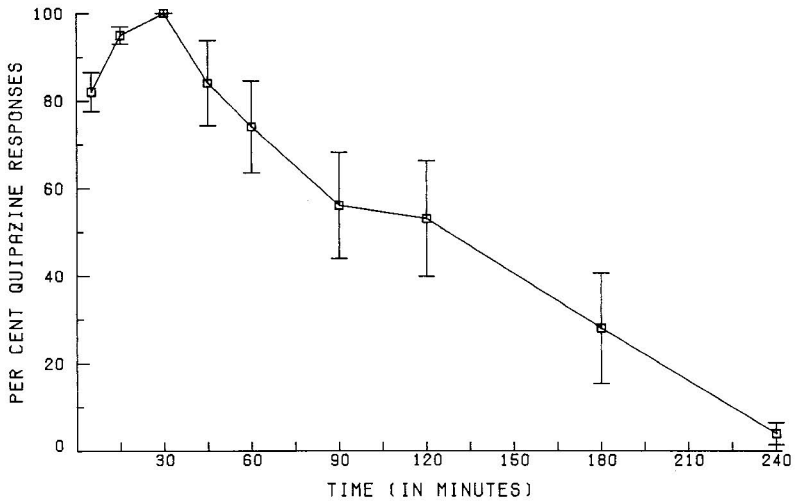

Fig. 3. Temporal parameters of the quipazine cue. Percentage responding on the quipazine lever as a function of the interval between injection and initiation of testing is shown. Data points are the mean of 12 *S*s (±S.E.) and are expressed as the percentage of responses on the quipazine lever during extinction.

Table 1. Mean percentage responding on the quipazine lever after injection of novel drugs

Drug	Dose	*n*	Percentage*
D-LSD	0.02	12	12 ± 8[a]
	0.04	12	39 ± 13[b]
	0.06	12	44 ± 13[b]
	0.08	11	95 ± 2[c]
	0.10	12	96 ± 1[c]
Apomorphine	0.5	8	39 ± 12[a]
	1.0	9	0 ± 0[b]
D-amphetamine	1.0	8	12 ± 12[a]

* Each value refers to the mean percentage (±S.E.) responding on the quipazine lever.
[a] Not significantly different from saline control.
[b] Not significantly different from chance responding.
[c] Not significantly different from quipazine control.

haloperidol, had no effect on the quipazine discrimination.

DISCUSSION

The results of this experiment indicate that quipazine can acquire and maintain strong control of choice responding in a two-lever drug discrimination task. The acquisition was relatively rapid (12 sessions) and was maintained at high levels (above 85%) throughout the experiment. Thus, these results are comparable to those seen in this laboratory with other drugs, e.g. D-amphetamine (Kuhn, Appel and Greenberg, 1974), D-LSD (Kuhn *et al.*, 1976b), pentazocine (Kuhn, Greenberg and Appel, 1976a), Δ^9-THC and psilocybin (Greenberg, Kuhn and Appel, 1975b), using the same apparatus and similar procedures.

The dose–response curve showed that at doses of

Table 2. Mean percentage responding on quipazine lever following administration of neurotransmitter antagonists

Drug	Dose of antagonist (mg/kg)	Time‡ interval (min)	*n*	Percentage*
Methysergide + quipazine†	1.0	30	12	71 ± 12′
Methysergide + saline	1.0	30	10	4 ± 1[b]
Cyproheptadine + quipazine	1.0	60	11	28 ± 12[a]
Cyproheptadine + saline	1.0	60	5	0 ± 6[b]
Methiothepin + quipazine	1.0	60	9	25 ± 11[a]
Methiothepin ± saline	1.0	60	6	0 ± 0[b]
Haloperidol + quipazine	0.05	30	11	98 ± 2[c]
	0.10	60	8	100 ± 0[c]
Haloperidol + saline	0.10	60	6	0 ± 0[b]
Fluphenazine + quipazine	0.05	30	8	96 ± 2[c]
	0.10	60	2	100 ± 0[c]
Fluphenazine + saline	0.10	60	5	0 ± 0[b]

* Each value refers to the mean percentage (±S.E.) responding on the quipazine lever.
† Quipazine (2.5 mg/kg) was always injected 30 min prior to testing.
‡ Time interval refers to the interval between the injection of antagonist and the injection of quipazine or saline.
[a] Significantly different from quipazine control.
[b] Not significantly different from saline control.
[c] Not significantly different from quipazine control.

1.25 mg/kg and 0.94 mg/kg, quipazine was still highly discriminable (i.e. above 80%). Coupled with the fact that the training dose of 2.5 mg/kg produced slight disruption of bar-pressing (decreased rate), the dose–response data suggest that an equally effective discrimination might be established with lower doses of quipazine without the behavioural depression seen at 2.5 mg/kg. However, it has been shown that the shape of generalization gradients such as that in Figure 2 are, to a great extent, dependent on the training dose used in establishment of a drug discrimination (Overton, 1974).

Determination of the temporal parameters of quipazine discriminability showed that the onset of the discriminative effect is rapid (82% at 5 min after injection) and remains at high levels for between 60–90 min. (By 180 min responding was predominantly on the saline lever.) Although these temporal parameters are also largely determined by the dose and time interval used in training, it is interesting that the quipazine-induced hypermotility (Green *et al.*, 1976) antinociception (Samanin *et al.*, 1976), and neurochemical alterations (Grabowska *et al.*, 1974) have similar times of onset (5–15 min) and durations of action (60–90 min).

The most interesting finding of this experiment is the dramatic transfer of LSD to the quipazine cue. Although such transfer of drug-stimulus properties in rats does not necessarily imply subjective similarity in man (Kuhn *et al.*, 1976b), the strength of this transfer (95%) and the fact that it is symmetrical (Kuhn *et al.*, 1976b) suggests that these two compounds have similar mechanisms of actions. Although it is reasonable to assume this common mechanism involves the direct stimulation of 5-HT receptors, it is possible that it involves some other structural or pharmacological similarity such as the quinoline moiety which these drugs have in common.

The putative 5-HT antagonists methiothepin, methysergide, and cyproheptadine were found to be effective antagonists of the quipazine discriminative stimulus, confirming the results of other, different behavioural tests with quipazine. However, since the ability of methiothepin, methysergide and cyproheptadine to serve as central 5-HT antagonists has been seriously questioned (Haigler and Aghajanian, 1974), it cannot be concluded that quipazine is exerting its discriminable effects by activating 5-HT receptors, *per se*. However, interpretation of the results with the DA antagonists is far more certain. Since fluphenazine and haloperidol, at doses far larger than those needed to completely block the stimulus properties of apomorphine (unpublished observations), were without effect on the quipazine cue, it is clear that DA receptors are not involved in the quipazine discriminative stimulus. This is further substantiated by the finding that DA agonists do not transfer to quipazine (Table 1). Additional studies on the neural mechanisms underlying the behavioural effects of quipazine are currently underway in this laboratory.

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REFERENCES

Cameron, O. G. and Appel, J. B. (1973). A behavioral and pharmacological analysis of some discriminable properties of D-LSD in rats. *Psychopharmacologia* **33:** 117–134.

Costall, B. and Naylor, R. J. (1975). The role of the raphe and extrapyramidal nuclei in the stereotyped and circling response to quipazine. *J. Pharm. Pharmac.* **27:** 368–371.

Fuller, R. W., Snoddy, H. D., Perry, K. W., Rousch, B. W., Molloy, B. B., Bymaster, F. P. and Wong, D. T. (1976). The effects of quipazine on serotonin metabolism in rat brain. *Life Sci.* **18:** 925–134.

Grabowska, M., Antkiewicz, L. and Michaluk, J. (1974a). A possible interaction with central dopamine structures. *J. Pharm. Pharmac.* **26:** 74–76.

Grabowska, M., Antkiewicz, L. and Michaluk, J. (1974b). The influence of quipazine on the turnover rate of serotonin. *Biochemical Pharmac.* **23:** 3212.

Greenberg, I., Kuhn, D. M. and Appel, J. B. (1975a). Behaviorally induced sensitivity to the discriminable properties of LSD. *Psychopharmacologia* **43:** 229–232.

Greenberg, I., Kuhn, D. M. and Appel, J. B. (1975b). Comparison of the discriminative stimulus properties of Δ^9-THC and psilocybin in rats. *Pharm. Biochem. Behav.* **3:** 931–934.

Green, A. R., Youdim, M. B. H. and Grahame-Smith, D. G. (1976). Quipazine: Its effects on rat brain 5-hydroxytryptamine metabolism, monoamine oxidase activity and behaviour. *Neuropharmacology* **15:** 173–179.

Haigler, H. J. and Aghajanian, G. K. (1974). Peripheral serotonin antagonists: Failure to antagonize serotonin in brain areas receiving a prominent serotonergic input. *J. Neural Trans.* **35:** 257–273.

Hamon, M., Bourgoin, S., Enjalbert, A., Bockaert, J., Hery, F., Ternaux, J. and Glowinski, J. (1976). The effects of quipazine on 5-HT metabolism in the rat brain. *Naunyn-Schmiedebergs Arch. exp. Path. Pharmak.* **294:** 99–108.

Hirschhorn, I. D., Hayes, R. L. and Rosecrans, J. A. (1975). Discriminative control of behavior by electrical stimulation of the dorsal raphe nucleus: generalization to LSD. *Brain Res.* **86:** 134–138.

Hong, E., Sancillo, L. F., Vargas, R. and Pardo, E. G. (1969). Similarities between the pharmacological actions of quipazine and serotonin. *Eur. J. Pharmac.* **6:** 274–280.

Jacoby, J. H., Howd, R. A., Levin, M. S. and Wurtman, R. J. (1975). Mechanisms by which quipazine alters brain 5-hydroxyindole metabolism. *Soc. Neurosci.* **250:** 86.

Kuhn, D. M., Appel, J. B. and Greenberg, I. (1974). An analysis of some discriminable properties of D-amphetamine. *Psychopharmacologia* **39:** 57–66.

Kuhn, D. M., Greenberg, I. and Appel, J. B. (1976a). Stimulus properties of the narcotic antagonist pentazocine: similarity to morphine and antagonism by naloxone. *J. Pharmac. exp. Ther.* **196:** 121–127.

Kuhn, D. M., White, F. J. and Appel, J. B. (1976b). Discriminable stimuli produced by hallucinogens. *Psychopharm. Commun.* **2:** 345–348.

Medon, P. J., Leeling, J. L. and Phillips, B. M. (1973). Influence of quipazine, a potential anti-parkinsonian agent on the uptake of 3H-dopamine and 3H-serotonin into rat striatal tissue *in vitro*. *Life Sci.* **13:** 685–691.

Overton, D. A. (1974). Experimental methods for the study of state-dependent learning. *Fedn Proc. Fedn Am. Socs exp. Biol.* **33:** 1800–1813.

Rodriguez, R. (1972). Antagonism of tremorine-induced tremor by serotonergic agents in mice: interactions with levodopa. *Life Sci.* **13:** 685–691.

Rodriguez, R. and Pardo, E. G. (1971). Quipazine, a new type of anti-depressant agent. *Psychopharmacologia* **21:** 89–100.

Samanin, R., Bernasconi, S. and Quattrone A. (1976). Antinociceptive action of quipazine: relation to central serotonergic receptor stimulation. *Psychopharmacologia* **46:** 219–222.