Pharmacology Biochemistry & Behavior, Vol. 6, pp. 325–329.

Quipazine-Induced Head-Twitch in Mice[1]

JEFFREY B. MALICK,[2] ELEANOR DOREN AND ALLEN BARNETT

Department of Pharmacology, Biological Research Division, Schering Corporation
Bloomfield, NJ 07003 U.S.A.

(Received 26 January 1976)

MALICK, J. B., E. DOREN AND A. BARNETT. *Quipazine-induced head-twitch in mice.* PHARMAC. BIOCHEM. BEHAV. 6(3) 325–329, 1977. – Quipazine has been reported to be a direct serotonin receptor agonist. In this laboratory, quipazine produced head-twitch in mice similar to that produced by the serotonin precursor, 5-hydroxytryptophan (5-HTP). Three antiserotonergic drugs (methiothepin, methysergide, and cinanserin) antagonized both the 5-HTP and quipazine-induced head-twitch responses. In addition, the quipazine response was significantly potentiated by a monoamine oxidase (MAO) inhibitor, pargyline. Since it is not likely that quipazine itself is metabolized by MAO, these results suggested that quipazine might cause release of endogenous serotonin. Parachlorophenylalanine, a serotonin depletor, significantly antagonized the potentiation of quipazine by the MAO inhibitor but failed to antagonize the head-twitch produced by quipazine itself. The present studies suggest that quipazine influences serotonin receptors in the brain to produce head-twitch by two mechanisms of action: (1) by direct serotonin receptor activation, and (2) indirectly by causing a release of endogenous serotonin.

Quipazine-induced head-twitch Mechanism of action Serotonin-like activity Serotonin receptor antagonists
5-HTP-induced head-twitch

QUIPAZINE has been reported to have activity at sites outside of the central nervous system that are sensitive to serotonin [9], and it has been suggested that its activity was produced by serotonin receptor activation [10].

Quipazine also has been reported to have serotonin-like activity in the central nervous system (CNS) since it produced sham-rage in the cat [7, 9, 10, 23] that was similar to the response produced by 5-hydroxytryptophan (5-HTP), the serotonin precursor [2]. The central serotonin-like actions of quipazine were abolished by the antiserotonergic drugs, cyproheptadine [10] and cinanserin [21]. Grabowska and co-workers [6] found that parachlorophenylalanine (PCPA), a serotonin depletor, failed to significantly inhibit the actions (e.g., stereotyped head movements, sniffing and gnawing) of quipazine in the rat; however, methysergide, a serotonin receptor antagonist, inhibited all of the symptoms observed following quipazine; in addition, quipazine may also interact with dopamine receptors since haloperidol and pimozide inhibited the gnawing and sniffing produced following its administration. Quipazine did not significantly alter total brain levels of serotonin or norepinephrine in the rat [20]; however, it inhibited ^{3}H-serotonin uptake into rat corpus striatum in vitro [16].

In this laboratory, it was observed that quipazine produced head-twitching in mice that appeared to be the same as that which was produced by 5-HTP [3,15]. The present studies were designed to investigate further this serotonin-like activity of quipazine and to determine whether it would be antagonized by serotonin receptor antagonists or by serotonin depletion.

GENERAL METHOD

CF No. 1-S male mice (20–24 g) were used throughout these studies. All drug doses were calculated in terms of mg/kg of free base, and all treatments were administered using aqueous solutions or suspensions of drug in 0.4% methyl-cellulose solution. Volumes used were 20 ml/kg for the oral (PO) route and 10 ml/kg for the intraperitoneal (IP) route. The drugs used in these studies were the maleate salts of methiothepin, methysergide and quipazine and the hydrochloride salts of cinanserin and pargyline. dl-Parachlorophenylalanine was used as the free acid (Nutritional Biochemical Corporation). L-5-hydroxytryptophan was used in these studies. Pilot studies were not performed blind; however, all of the results used in this paper were from studies confirmed under blind conditions (e.g., the technician did not know what drugs were being tested).

EXPERIMENT 1. HEAD-TWITCH PRODUCED BY QUIPAZINE ALONE AND IN COMBINATION WITH PARGYLINE

Method

Head-twitch was defined as distinct side-to-side movement of the head which was independent of body jerking. In pilot studies, quipazine produced head-twitching responses that were evenly distributed over the 30-min observation period following drug administration. In all subsequent studies, head-twitches were counted between 10 and 20 min after quipazine so that the results could be compared with those obtained in 5-HTP-induced head-twitch studies (Experiment 2). Mice were housed 5 per

[1] Paper presented in part at FASEB Meeting, Atlantic City, New Jersey, April, 1975 (Abstract: *Fedn Proc.* 34: 801, 1975).

[2] Requests for reprints should be sent to Dr. Jeffrey B. Malick, Biomedical Research Department, Pharmacology Section, ICI United States Inc., Wilmington, DE 19897 U.S.A.

chamber in a quiet area during testing. The data were expressed as the mean number of head-twitches for the 10-min observation period.

Initially, quipazine was studied by itself to determine its ability to produce head-twitching over a wide range of doses (1–10 mg/kg, IP). Subsequently, the production of head-twitches by quipazine in the presence of a monoamine oxidase inhibitor (MAOI), which would prevent the breakdown of any released monoamines, was studied. In drug combination studies, the MAOI, pargyline (100 mg/kg, IP; this dose was chosen on the basis of previous 5-HTP head-twitch studies [7]), was administered 3 hr prior to quipazine administration and head-twitches were once again counted between 10 and 20 min post-quipazine. Head-twitch responses following quipazine alone were compared to those obtained in combination with pargyline via a Student's *t*-test (one-tailed).

In order to adequately control these experiments, separate groups of mice were treated either with vehicle or pargyline 180 min prior to a second vehicle injection; head-twitches were then counted between 10 and 20 min after the second injection as in the other experiments. This experiment was necessary to control for spontaneous head-twitching and to determine whether pargyline by itself would induce head-twitching.

Results

A few vehicle-injected control mice exhibited a very low level of spontaneous head-twitching during the 10-min observation period (Table 1). Administration of pargyline (100 or 200 mg/kg, IP) failed to significantly ($p>0.10$; Student's *t*-test) increase the low level of head-twitching observed in the vehicle controls (Table 1).

Quipazine produced relatively low levels of head-twitching over a wide range of doses (Table 2); however, quipazine produced significantly ($p<0.05$; Student's *t*-test) greater head-twitches than observed in vehicle treated controls. No dose-response relationship existed when quipazine was given by itself. Pretreatment with pargyline resulted in a significant ($p<0.01$; Student's *t*-test) potentiation of the head-twitch response at all doses of quipazine that were tested (Table 2); in the presence of pargyline, the activity of quipazine was dose related. The quipazine/pargyline combination also produced significantly ($p<0.05$; Student's *t*-test) greater head-twitching than observed in mice only treated with pargyline (Table 1). Thus, quipazine produced head-twitching in mice which appeared to be similar to that produced by the administration of 5-HTP, the serotonin precursor [3,15]. In addition, the head-twitch response produced by quipazine can be significantly potentiated by pretreatment with an MAOI, much the same as has been observed with the 5-HTP-induced head-twitch response [3].

Modigh [17] has observed tremor, limb abduction, and hyperextension in addition to rapid head movements following high doses (400 to 800 mg/kg, IP) of 5-HTP; in the present study, quipazine only produced head-twitching except after the highest dose tested (10 mg/kg, IP) at which a few mice exhibited slight tremoring.

TABLE 1

HEAD-TWITCH FOLLOWING VEHICLE OR PARGYLINE ADMINISTRATION

Treatment*	Dose (mg/kg,ip)	N†	No. of Twitches (Mean ± SEM)
Vehicle Controls	10 ml/kg	10	0.3 ± 0.2
Pargyline	100	20	0.4 ± 0.2
Pargyline	200	10	0.4 ± 0.2

*Mice were treated with either vehicle (0.4% methylcellulose) or pargyline 180 min prior to a second vehicle injection; head-twitches were counted between 10 and 20 min after the second injection.
†Number of mice tested at each dose.

EXPERIMENT 2. ANTAGONISM OF QUIPAZINE- AND 5-HTP-INDUCED HEAD-TWITCH

Since quipazine produced head-twitching in mice that appeared to be indistinguishable from that produced by the serotonin precursor, 5-HTP, studies were designed to determine whether the quipazine-induced response could be pharmacologically antagonized by serotonin receptor antagonists. In addition, for comparative purposes, the pharmacological antagonism of the 5-HTP-induced head-twitch response was studied at the same time.

Method

Antagonism of quipazine-induced head twitch. In drug antagonism studies, pargyline (100 mg/kg, IP) was given

TABLE 2

HEAD-TWITCH INDUCED BY QUIPAZINE ALONE AND IN COMBINATION WITH PARGYLINE

	Induction of head-twitch in mice				
	Quipazine alone		Quipazine + pargyline‡		
Dose (mg/kg, ip)	No. of Twitches (Mean ± SEM)*	N†	No. of Twitches (Mean ± SEM)*	N†	P Value§
1.0	4.8 ± 0.8	10	20.9 ± 4.1	10	<0.01
3.0	1.6 ± 0.5	5	26.8 ± 4.9	5	<0.01
10.0	4.4 ± 1.1	10	61.0 ± 16.2	5	<0.01

*Mean number of head-twitches 10–20 min after quipazine administration ± standard error of the mean.
†Number of mice tested at each dose.
‡Mice pretreated with pargyline (100 mg/kg, ip) 3 hr prior to administration of quipazine.
§Student's *t*-test comparing quipazine-pargyline combination to quipazine alone.

2 1/2 hr prior to the oral administration of test drug (serotonin receptor antagonist); 30 min after test drug, the mice were challenged with quipazine (1.0 mg/kg, IP) and head-twitches were counted between 10 and 20 min as before. This dose of quipazine was chosen because it produced a frequency of head-twitching in combination with pargyline that was approximately the same as that observed in the 5-HTP-induced head-twitch procedure.

The antiserotonergic drugs (i.e., drugs which inhibit the pharmacological effects of serotonin at specific receptor sites) used in these studies (i.e., methiothepin [18,22], methysergide [4], and cinanserin [5]) were selected because they were previously demonstrated to be antiserotonergic drugs that were devoid of anticholinergic activity in mice, in contrast to other antiserotonergic drugs (e.g., cyproheptadine, pizotyline, and xylamidine) that also exhibited potent anticholinergic activity [15]. Five mice were used to evaluate each dose of the serotonin receptor antagonists, and the dose intervals were kept constant (0.5 $\log_{10}$ units) for each drug. The mean number of head-twitches per mouse was determined for each group, and this value was compared to the same day control value. The MED50, the minimal dose of test drug that produced a reduction in the mean number of head-twitches of 50% or more compared to the appropriate control group receiving saline and tested on the same day, was obtained for each of the antiserotonergic drugs. The dose-response curves generated in this procedure did not permit ED50 determinations because the slopes of the curves were too steep.

Antagonism of 5-HTP-induced head-twitch. 5-HTP, the serotonin precursor, produces head-twitching in mice which has been shown to be blocked by serotonin antagonists [3]. Pretreatment with pargyline potentiates the response presumably by preventing the catabolism of the serotonin formed from the 5-HTP. Pargyline (100 mg/kg, IP) was given 2 1/2 hr prior to the oral administration of each antiserotonergic drug; 30 min later, the mice were challenged with 5-HTP (25 mg/kg, IP). Head-twitches were counted between 10 and 20 min after 5-HTP. The antiserotonergic drugs were tested at the same doses as in the quipazine test, and the data were analyzed in the same way to obtain MED50's.

Results

All three serotonin receptor antagonists (methiothepin, methysergide, and cinanserin) inhibited both the 5-HTP-induced and the quipazine-induced head-twitch responses (Table 3); the order of potency was the same in both procedures, and the MED50 values for antagonism of head-twitch were also quite similar (Table 3).

The antagonism of quipazine by the serotonin receptor antagonists was surmountable since, when a higher dose of quipazine (10 mg/kg, IP) was used to induce the head-twitch, the methysergide MED50 for antagonism of the response was 10 mg/kg, PO as compared to 3 mg/kg, PO versus the lower quipazine dose (1 mg/kg, IP).

Thus, the head-twitch produced by quipazine in combination with pargyline appeared to be the result of activity at serotonin receptors since it was pharmacologically antagonized by antiserotonergic drugs that were also antagonists of the 5-HTP-induced head-twitch response.

EXPERIMENT 3. EFFECTS OF PCPA ON QUIPAZINE-INDUCED HEAD-TWITCH

This experiment was designed to determine whether serotonin depletion with PCPA would diminish the head-twitch produced either by quipazine by itself or by quipazine plus pargyline. PCPA has been shown to be a relatively selective depletor of brain serotonin in mice [12,13] although higher doses are required in mice than in rats. The dose of PCPA used in this study was chosen since Kilian and Frey [12] observed that brain levels of serotonin were significantly reduced (58% decrease) in mice 24 hr following a high dose (900 mg/kg) of PCPA whereas no significant alterations were observed in the brain levels of the catecholamines (norepinephrine or dopamine).

Method

Two groups of 20 mice each were administered either placebo (vehicle control) or PCPA (1000 mg/kg, IP) 24 hr prior to the administration of quipazine (1 mg/kg, IP). In addition, both of these groups were divided in half, and 10 mice from each group received placebo, and the remaining 10 mice from each group received pargyline (100 mg/kg, IP) treatments 3 hr prior to quipazine. Once again, head-twitches were counted between 10 and 20 min post quipazine, and the groups were compared utilizing a Student's *t*-test.

Results

PCPA significantly antagonized ($p<0.01$; Student's *t*-test) the potentiation of the quipazine response produced by pretreatment with pargyline but failed to alter significantly the head-twitch response produced by quipazine by itself (Table 4).

TABLE 3

ANTAGONISM OF 5-HTP- AND QUIPAZINE-INDUCED HEAD-TWITCH IN MICE BY SEROTONIN RECEPTOR ANTAGONISTS

Treatment	Inhibition of 5-HTP-induced head-twitch MED50 (mg/kg p.o.)*	N†	Inhibition of quipazine-induced head-twitch MED50 (mg/kg, p.o.)*	N†
Methiothepin	1.0	50	3.0	35
Methysergide	1.0	40	3.0	25
Cinanserin	10.0	20	10.0	20

*Minimum effective dose producing a 50% or greater decrease in head-twitch as compared to control.

†Number of mice used for each drug.

TABLE 4

EFFECT OF PCPA ON QUIPAZINE-INDUCED HEAD-TWITCH IN MICE

Treatment*	Induction of head-twitch in mice: Quipazine alone No. of Twitches (Mean ± SEM)†	Quipazine + pargyline‡ No. of Twitches (Mean ± SEM)†	p Value§
Placebo Control	4.5 ± 0.8	11.6 ± 1.1	<0.01
PCPA//	6.1 ± 0.6	5.6 ± 0.8	NS
p Value**	NS	<0.01	

*N=10 mice/treatment group.
†Mean number of head-twitches 10–20 min after quipazine administration ± standard error of the mean.
‡Mice pretreated with pargyline (100 mg/kg, IP) 3 hr prior to quipazine administration.
§Student's t-test comparing quipazine-pargyline combination to quipazine alone.
//Parachlorophenylalanine (1000 mg/kg, IP) administered 24 hr prior to quipazine.
**Student's t-test comparing placebo to PCPA-treated group for quipazine alone and quipazine-pargyline groups.

GENERAL DISCUSSION

Quipazine produced head-twitch by itself in mice which was overtly similar to that produced by 5-HTP, the serotonin precursor [3]. This finding confirmed previous studies that suggested that quipazine had a serotonin-like activity [7, 9, 10, 23] that may be the result of direct serotonin receptor activation [10].

In addition, pretreatment with an MAO inhibitor significantly potentiated the activity of quipazine. Since it is unlikely that quipazine itself is inactivated by MAO [10], one possibility that this finding suggested was that quipazine may have caused a substantial release of endogenous serotonin, which may have been at least partially responsible for its serotonin-like activity. The observation that quipazine by itself does not induce head-twitching in a dose-related manner may, in part, be due to the fact that the effects of the released serotonin may not be apparent in the absence of a monoamine oxidase inhibitor (e.g., pargyline) since it would be catabolized very rapidly by MAO. Jacobs [11] has reported that p-chloroamphetamine, a drug which releases endogenous serotonin, produced lateral head weaving in the rat as part of a syndrome characterized by tremor, rigidity, forepaw treading, Straub tail and hindlimb abduction; this syndrome was blocked by pretreatment with an inhibitor of serotonin biosynthesis (PCPA).

Since the quipazine-induced head-twitch response was antagonized by antiserotonergic drugs that were also antagonists of 5-HTP-induced head-twitch response, it was likely that the activity of the quipazine/pargyline combination was at serotonin receptors.

A controversy exists as to whether the existing serotonin receptor antagonists (e.g., methysergide, methiothepin, cinanserin) block serotonin receptors in the CNS. When serotonin is applied via microiontophoresis to individual neurons in the CNS it either produces inhibition (depression) or excitation of neuronal activity [8]. Segal and Bloom [24] have antagonized the 5-HT inhibitory effects in the hippocampus of rats following the administration of methysergide and cyproheptadine and methiothepin has been shown to inhibit the depressant effects of 5-HT in the lateral geniculate of cats [25]. However, methysergide did not block the depressant effects of 5-HT in the reticular formation [1] or the lateral geniculate [14]. Haigler and Aghajanian [8] failed to antagonize the depressant effects of 5-HT in the ventral lateral geniculate, optic tectum and amygdala of the rat following the administration of several serotonin receptor antagonists (e.g., methysergide, methiothepin, cinanserin); however, the excitatory effects of 5-HT in the reticular formation could be blocked by these antagonists [8]. The excitatory effects of 5-HT also have been blocked by methysergide in the reticular formation [1] and cortex [19] and by cinanserin in the cortex [19].

Thus, although the serotonin receptor antagonists do not consistently block the depressant effects of 5-HT in all brain areas, it is generally accepted that they significantly antagonize the excitatory effects of 5-HT in the CNS. Thus, there may be more than one type of serotonin receptor in the CNS. Since head-twitching appears to be a net excitatory response, the serotonin receptor antagonists probably block this motor response by virtue of their ability to antagonize the excitatory effects of 5-HT in the CNS [1, 8, 19].

Since depletion of brain serotonin with PCPA failed to alter significantly the action of quipazine by itself, quipazine appeared to produce head-twitch by direct receptor stimulation. However, since serotonin depletion significantly reduced the head-twitch produced by the quipazine/pargyline combination, pargyline probably potentiated the action of quipazine by preventing the catabolism of newly released serotonin. Therefore, because of the differential effects of PCPA on head-twitch induced by quipazine itself or in combination with pargyline, it is proposed that quipazine produced head-twitch by two mechanisms: (1) by direct serotonin receptor stimulation, and (2) by a release of endogenous serotonin which can only be demonstrated in the presence of an MAO inhibitor.

Quipazine previously has been reported to be capable of direct receptor activation [10]. The present studies confirm this mechanism of action, but, in addition, they suggest that there may be a second, indirect mechanism of action (i.e., release of endogenous serotonin) for quipazine, both

of which influence the activity of serotonin receptors in the brain.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Jackie Dempsey for valuable technical assistance. The authors are also grateful to Miles Laboratories, Inc. for generously supplying us with quipazine and to the many other pharmaceutical companies who sent us samples of their drugs.

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