Pharmacology Biochemistry & Behavior, Vol. 35, pp. 841–845. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

Benzodiazepines Impair a Behavioral Effect Induced by Stimulation of 5-HT1B Receptors

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Received 15 August 1989

FRANCÈS, H., F. KHIDICHIAN AND C. MONIER. *Benzodiazepines impair a behavioral effect induced by stimulation of 5-HT1B receptors*. PHARMACOL BIOCHEM BEHAV **35**(4) 841–845, 1990. — Seven days of isolation induce in mice a social behavioral deficit (decrease in escape attempts) reversed by TFMPP acting through activation of 5-HT1B receptors. The present experiments were performed to investigate the interaction between tranquillizing drugs and one aspect of the serotonergic functioning through the TFMPP-induced increase in escape attempts. The benzodiazepines diazepam, alprazolam, triazolam and chlordiazepoxide impaired significantly TFMPP-induced increase in escape attempts at behaviorally inactive doses. Buspirone opposed TFMPP effect, but the active doses 4 and 16 mg/kg alone decreased the number of escape attempts. ICS 205-930 in a large dose range (0.001–1 mg/kg) modified neither the number of escape attempts nor the increase induced by TFMPP. Chronic (11 days) treatment with buspirone (16 mg/kg) or ICS 205-930 (1 mg/kg) modified neither the number of escape attempts of isolated mice nor the increase induced by TFMPP. These results suggest that tranquillizing drugs of the benzodiazepines group, but not of other groups, interact with the 5-HT1B receptors; they add to the knowledge of relations between benzodiazepines and serotonin by specifying the involvement of 5-HT1B receptors.

Benzodiazepines Nonbenzodiazepine tranquillizing drugs 5-HT1B stimulation Isolated mice

A link between serotonin and anxiety constitutes an increasing field of research. It led to the development of serotonergic anxiolytic drugs such as buspirone (18) and to the elaboration of hypotheses such as the 5-HT hypersensitivity hypothesis of anxiety proposed by Kahn *et al.* (24).

Several recent reports (16, 21, 24, 35, 38) have reviewed these proposals. Serotonin acts in the brain through various receptor subtypes: 5-HT1A, 5-HT1B, 5-HT1C, 5-HT1D, 5-HT2, 5-HT3; several of them have been involved in animal models of anxiety. The present work is restricted to the study of the 5-HT1B subtype of serotonin receptors.

Drugs acting on 5-HT1B receptors exert some behavioral effects in man and in animals. M-CPP, a serotonergic stimulant binding to 5-HT1B, but also to numerous other receptors in brain (19), has been reported to induce anxiety and/or panic attacks in healthy volunteers or patients (5, 23, 41). In animals, RU 24969, a serotonergic stimulant binding to 5-HT1A and 5-HT1B receptors (20), depressed responding in a licking conflict, but evoked a slight increase in punished responding in a food-motivated conflict (17) and reduced punished and unpunished responding in a test of conditioned suppression of drinking (4). These anxiogenic and anxiolytic-like activities of the drug have been ascribed by the authors to changes in motor behavior rather than to an action on anxiety. The focal neurostimulation of periaqueductal grey matter provoked in rats unpleasant or aversive effects which are attenuated by classical anxiolytics (2): on this model, m-CPP exerted an antiaversive effect (22). In the anxiety model "defensive burying," the 5-HT1B receptors are reported to play a role (3). The sensory motor reactivity as measured in the acoustic startle reflex is decreased by m-CPP (8) and also by quipazine acting probably through the stimulation of 5-HT1B receptors (26).

Francès (10) developed an animal model in which mice isolated for 7 days were observed in pairs with grouped mice (one isolated + one grouped mice) under an inverted beaker for 2 minutes. The mice attempted to escape; however, the number of escape attempts of isolated mice was half that of grouped mice. This decrease in escape attempts of isolated mice relative to grouped mice was named the "isolation-induced social behavioral deficit." In a further study (13), it was demonstrated that this deficit was dependent upon circumstances. For example, isolated mice tested alone under the inverted beaker attempted to escape more often than grouped mice tested alone. The conclusion was that isolation induced in mice a state of hyperreactivity and that testing in pairs induced, in addition, a behavioral inhibition.

The isolation-induced social behavioral deficit may not be seen as a model of anxiety or depression since neither tranquillizing drugs (11) nor tricyclic antidepressant drugs (12) are able to impair it. The decrease in escape attempts of isolated mice tested in pairs with grouped mice is only reversed by serotonergic drugs (10) acting through the 5-HT1B receptors (14,15). The interpretation of such data is not unambiguous. It may be postulated that isolated mice are abnormal relative to grouped mice and that agonists of 5-HT1B receptors "normalize" their behavior. However, it may also be postulated that the normal behavior of isolated mice tested

in pairs is to observe the other grouped mouse rather than attempting to escape, so that, in this context, the agonists of 5-HT1B receptors would act in disturbing the normal attentive behavior of isolated mice by increasing "impulsivity" or inducing "panic."

Whatever the interpretation, this model, until now, appears specific for 5-HT1B agonists. So, given the effects of m-CPP and RU 24969 on anxiety in man and in animal and the hypothesized interpretation of the effects of 5-HT1B agonists in this model as possibly inducing "panic" or "impulsivity," we undertook to study the possible interaction between tranquillizing drugs and the effects of TFMPP in this model.

METHOD

Animals

Male Swiss NMRI mice (20–24 g), from CERJ, Genest St. Isle, 53940 France, were used in all experiments. Mice were either housed in groups of 6 in home cages of 30 × 20 × 10 cm or isolated in home cages of 24 × 10 × 8 cm. Mice were 4 weeks old at the beginning of isolation. The room was thermostatically maintained at 21 ± 1°C with a 12-hour light/dark schedule. Food and water were freely available. The duration of isolation was 7 days.

Experimental Procedure

Mice were tested in pairs (one grouped mouse + one isolated mouse) under a transparent beaker (height: 14 cm; diameter: 10 cm) inverted on a rough surface glass plate. The number of escape attempts was counted for 2 minutes. An escape attempt was defined as any one of the following: 1) the two forepaws were leaned against the beaker wall, 2) the mouse was sniffing, its nose into the spout of the beaker, 3) the mouse was scratching the glass floor.

There was no minimal duration for one attempt. When an attempt lasted a long time, a new attempt was counted for each period of 3 seconds. However, the escape attempts were very rapid movements and the longest duration observed lasted between 3 and 6 seconds (counted as 2 attempts). Behavioral observations were taped by an observer blind to the treatments received by the mice.

Drugs

Drugs used were: 1-(3-trifluoromethylphenyl) piperazine (TFMPP—Aldrich Chemical Co.: Strasbourg, France); diazepam and chlordiazepoxide (Hoffmann-La Roche & Co.: Basel, Switzerland); triazolam, alprazolam (Upjohn: Kalamazoo, MI); buspirone (Bristol-Myers: Paris, France); ICS 205-930 (Sandoz: Basel, Switzerland).

Drugs were either dissolved in demineralized water or suspended in arabic gum. Tranquillizing drugs were administered 45 minutes and TFMPP 30 minutes before testing. All drugs were administered by IP route under a volume of 0.25 ml/20 g body weight.

In chronic experiments, mice received the drug twice daily (9 a.m., 5 p.m.): they were grouped by 6 in home cages for the first 4 days of treatment and then isolated for the 7 following days of treatment. They were tested the day after the last treatment.

Treatments were administered only to isolated mice, the controls being isolated mice receiving water. The grouped mice were only partners without any treatment.

Statistics

Results were analyzed using the one-way analysis of variance followed by Dunnett's *t*-tests.

TABLE 1

EFFECT OF BENZODIAZEPINES ON THE TFMPP-INDUCED INCREASE IN ESCAPE ATTEMPTS OF ISOLATED MICE

Drugs mg/kg −45 min		−30 min	n	Escape Attempts mean ± S.E.M.		p
Diazepam		TFMPP				
0	+	0	27	10.8 ± 1.6		
0	+	2	28	23.2 ± 2.8	c	F(5,91) =
2	+	0	10	11.0 ± 2.6	NS	6.43
2	+	2	10	19.9 ± 3.9	NS	$p<0.001$
4	+	0	9	10.0 ± 1.7	NS	
4	+	2	10	11.7 ± 1.7	a'	
Alprazolam		TFMPP				
0	+	0	27	11.7 ± 1.8		
0	+	2	28	23.6 ± 2.2	c	F(6,101) =
0.25	+	2	10	29.6 ± 2.6	NS	20.65
0.5	+	0	10	8.1 ± 2.0	NS	$p<0.001$
0.5	+	2	9	7.4 ± 2.3	c'	
1	+	0	10	9.1 ± 1.7	NS	
1	+	2	20	4.1 ± 0.9	c'	
Triazolam		TFMPP				
0	+	0	30	9.8 ± 1.2		
0	+	2	30	25.1 ± 2.5	c	F(6,136) =
0.008	+	2	9	20.0 ± 5.0	NS	9.76
0.016	+	0	18	8.0 ± 1.5	NS	$p<0.001$
0.016	+	2	20	18.4 ± 3.1	NS	
0.03	+	0	17	8.5 ± 1.8	NS	
0.03	+	2	19	12.1 ± 1.8	c'	
Chlordiazepoxide		TFMPP				
0	+	0	27	14.3 ± 1.4		
0	+	2	28	28.3 ± 2.1	c	F(3,108) =
16	+	0	27	15.7 ± 1.5	NS	14.68
16	+	2	30	13.1 ± 2.1	c'	$p<0.001$

Significations were expressed: c vs. water + water; a',c' vs. water + TFMPP; a' $p<0.05$; c c' $p<0.001$.

RESULTS

When a pair of mice (one isolated mouse + one grouped mouse) was observed under the reversed beaker for 2 minutes, the number of escape attempts of the isolated mice were half those of grouped mice (isolated mice: 9.5 ± 1.1, n = 20; grouped mice: 20.7 ± 1.8, n = 20; $p<0.001$). After administration of TFMPP (2 mg/kg), the number of escape attempts of isolated mice was about twice that of isolated control mice receiving water: this may be seen in any of the tables.

Diazepam, devoid of effect by itself, antagonized completely (4 mg/kg) the TFMPP-induced increase in escape attempts (Table 1). The dose of 2 mg/kg was inactive. Alprazolam without significant effect per se at 0.5 and 1 mg/kg impaired completely TFMPP effect at 0.5 mg/kg and even reversed it at 1 mg/kg (Table 1). Triazolam impaired significantly and in a dose-dependent manner the effect of TFMPP with the doses of 0.016 and 0.03 mg/kg which were without effect by themselves (Table 1). Chlordiazepoxide 16 mg/kg antagonized the effect of TFMPP and was inactive alone (Table 1). Buspirone reduced dose-dependently

TABLE 2

EFFECT OF NONBENZODIAZEPINE ANXIOLYTIC DRUGS ON THE TFMPP-INDUCED INCREASE IN ESCAPE ATTEMPTS OF ISOLATED MICE

Drugs mg/kg				Escape Attempts		
−45 min		−30 min	n	mean ± S.E.M.		p
Buspirone		TFMPP				
0	+	0	30	10.5 ± 1.3		
0	+	2	30	24.1 ± 2.2	c	$F(7,150) =$
0.5	+	2	10	26.6 ± 4.1	NS	16.13
1	+	2	9	22.4 ± 4.1	NS	$p<0.001$
4	+	0	20	5.8 ± 1.0	b	
4	+	2	20	16.7 ± 1.8	a'	
16	+	0	20	6.5 ± 1.1	a	
16	+	2	19	10.4 ± 1.8	c'	
ICS		TFMPP				
205-930						
0	+	0	21	11.3 ± 1.5		
0	+	2	18	28.1 ± 3.2	c	$F(8,114) =$
0.001	+	0	12	7.8 ± 0.9	NS	9.34
0.01	+	0	19	15.0 ± 1.7	NS	$p<0.001$
0.01	+	2	10	31.8 ± 3.6	NS	
0.1	+	0	10	13.9 ± 1.6	NS	
0.1	+	2	11	26.6 ± 4.6	NS	
1	+	0	11	14.1 ± 2.8	NS	
1	+	2	11	21.8 ± 3.8	NS	

Significations were expressed: a,b,c vs. water + water; a',c' vs. water + TFMPP; a a' $p<0.05$; b $p<0.01$; c c' $p<0.001$.

TABLE 3

EFFECT OF CHRONIC NONBENZODIAZEPINE ANXIOLYTIC DRUGS ON THE TFMPP-INDUCED INCREASE IN ESCAPE ATTEMPTS OF ISOLATED MICE

Drugs mg/kg			Escape Attempts		
Chronic	−30 min	n	mean ± S.E.M.	p	
Water	Water	15	8.9 ± 1.1		
(×22)	TFMPP 2 mg/kg	14	19.8 ± 2.3	<0.001	(a)
Buspirone 16 mg/kg (×22)	Water	15	12.1 ± 2.2	NS	(a)
	TFMPP 2 mg/kg	15	22.7 ± 2.2	NS	(b)
ICS 205-930 1 mg/kg (×22)	Water	14	10.1 ± 1.3	NS	(a)
	TFMPP 2 mg/kg	16	17.6 ± 2.2	NS	(b)
	$F(5,83) = 8.20$, $p<0.001$				

Significations were expressed: (a) vs. water + water, (b) vs. water + TFMPP.

the effect of TFMPP; however, the active doses 4 and 16 mg/kg alone reduced the number of escape attempts (Table 2). ICS 205-930 studied in a large dose range did not modify the TFMPP-induced increase in escape attempts.

The tranquillizing drugs inactive after a unique injection were administered according to chronic schedule. Animals receiving chronic buspirone or ICS 205-930 did not differ from control animals receiving water (Table 3). In addition, a chronic treatment with either drug did not impair the TFMPP-induced increase in escape attempts (Table 3).

DISCUSSION

The present results indicate that acutely administered benzodiazepines (diazepam, alprazolam, chlordiazepoxide, triazolam), but not acutely or chronically administered nonbenzodiazepines (ICS 205-930, buspirone) are able to oppose the effect of the stimulation of 5-HT1B receptors in a specific way (without exerting an effect by themselves). The activity of benzodiazepines in this model suggests that it may be seen as a model of anxiety.

The involvement of serotonin in the action of benzodiazepines has been extensively investigated. A benzodiazepine-induced decrease in 5-HT turnover demonstrated by Chase *et al.* (6) and Stein *et al.* (36) suggested that anxiolytic effects of drugs such as benzodiazepines resulted from a reduction in the activity of serotonin systems. However, more recent pharmacological studies have yielded results inconsistent with this hypothesis (34,37). If a reduction in the activity of serotonin neurons was the mechanism responsible for the benzodiazepine effect in our test, so the site of action of 5-HT1B agonists would be post- and not presynaptic. Indeed, the effect of stimulation of 5-HT1B autoreceptors (a decrease in 5-HT release) should have the same result as a reduction by benzodiazepines of 5-HT turnover: this is not the case since benzodiazepines did not increase the number of escape attempts and, in addition, opposed the effect of TFMPP. It may be hypothesized that TFMPP acts by stimulating postsynaptic receptors. If so, the activity of the neurons stimulated by TFMPP may be reduced by benzodiazepines.

Buspirone is a clinically active anxiolytic drug (18) with high affinity for brain 5-HT1A receptors (31). Buspirone is not consistently active in animal models of anxiety; its anxiolytic activity is observed in several tests among which conflict tests (1,9), but not in the elevated plus-maze (30). It may be noted that the doses of 4 and 16 mg/kg reduced the number of escape attempts in acute experiments although 16 mg/kg given during 11 days did not. A tachyphylaxis does not seem to be involved since Wettstein (40) and O'Connor *et al.* (27) did not find any disparities in the effect they observed after durations of treatment of 12 and 14 days, respectively. However, these authors used lower doses (0.01–3 mg/kg) than those used in the present study, and this may also account for the difference observed. Alternatively, this difference may be accounted for by pharmacokinetic reasons since, in chronic experiments, the last injection was performed 17 to 22 hours before the test, but this is only an hypothesis since the half-life of buspirone in mice is unknown. The lack of effect of buspirone vs. TFMPP-induced increase in escape attempts may result from a too brief duration of chronic treatment since Schefke *et al.* (33) increased highly the anticonflict effect of buspirone in rats after 12 weeks of treatment.

ICS 205-930 is antagonist at 5-HT3 receptors (32); these receptors are present in rat brain (25). ICS 205-930 has shown some activity in several animal models of anxiety: the place-aversion conditioning (29) and the two compartments black and white box (7,28), but not in others: conflict tests (39), defensive burying (3). It may not be excluded that the lack of effect after acute and chronic administrations in our model may result either

from a wrong choice in the dose or the schedule of treatments. However, the possibility remains that some form of anxiety is unresponsive to ICS 205-930.

The effect of benzodiazepines and the lack of effect of ICS 205-930 and buspirone in this test call again the multiplicity of animal models for anxiety and the fact that all anxiolytics are not active in all models. In the same way, in man, anxiety is multiple and some forms (panic disorders) are reported to be more responsive to antidepressant drugs than to tranquillizing drugs.

These results suggest that the TFMPP-induced increase in escape attempts of isolated mice may be a model of some form of anxiety. Given the anxiogenic and panic inducing effect of the serotonergic agonist m-CPP and the therapeutic efficacy of antidepressant drugs in panic disorders, these results prompt us to study the possible effect of antidepressant drugs in this model.

ACKNOWLEDGEMENT

This work was supported by the Institut National de la Santé et de la Recherche Médicale.

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