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Comparison of Routes of Administration and Time Course Effects of Zacopride and Buspirone in Mice Using an Automated Light/Dark Test

RICHARD YOUNG[1] AND DAVID N. JOHNSON[2]

Department of Pharmacology, A.H. Robins Co., Inc., 1211 Sherwood Avenue, Richmond, VA 23261-6609

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YOUNG, R. AND D. N. JOHNSON. *Comparison of routes of administration and time course effects of zacopride and buspirone in mice using an automated light/dark test.* PHARMACOL BIOCHEM BEHAV **40**(4) 733–737, 1991.—The behavioral effects of zacopride and buspirone were assessed in mice in a fully automated 2-compartment light/dark test. A significant increase in time mice spent in the lit area was used as an indication of anxiolytic-like action. Doses of zacopride from 0.0001 to 17.8 mg/kg, IP, and buspirone from 3.16 to 17.8 mg/kg, IP, produced significant increases in time mice spent in the lit area of the chamber. In addition, zacopride and buspirone were compared for oral potency and for duration of action after IP and PO administration. Zacopride and buspirone produced anxiolytic-like activity between doses of 0.001 to 100.0 mg/kg, PO, and 10.0 to 56.2 mg/kg, PO, respectively. The duration of effect of buspirone was 2 to 4 h after IP or PO administration, while that for zacopride was ≥16 h by either route of administration. Thus, when compared for anxiolytic-like effects in this test, zacopride is a more potent and longer acting agent than buspirone.

Anxiolytic Light/dark test Serotonin Buspirone Zacopride

IN the previous investigation (8) a fully automated and computer-integrated 2-compartment light/dark apparatus for mice was described and characterized for its pharmacological selectivity to anxiolytic agents. The measurement found most useful for assessing anxiolytic-like action was time mice spent in the lit area. The administration of an anxiolytic that is thought to act by a benzodiazepine-receptor related mechanism (diazepam) and anxiolytics that are thought to act by serotonin mechanisms (ipsapirone, ondansetron) significantly increased the time mice spent in the lit area.

In the present study a more extensive evaluation of the test system was undertaken by assessing some pharmacological parameters of the selective 5-HT_3 receptor antagonist zacopride (6). A previous study has shown it to be potently active in another version of the light/dark test (1). In fact, if zacopride's potency is confirmed clinically, it will be among the most potent psychoactive compounds known. Thus the present effort was undertaken to determine if the automated light/dark apparatus would be useful for gathering data concerning zacopride's relative potency via different routes of administration (i.e., IP and PO) and time course (i.e., onset to action and duration of effect) effects. For comparative purposes the effects of buspirone, a known anxiolytic drug that has also been shown to be active in other light/dark exploratory situations (2–5), was also evaluated.

METHOD

Animals

Female ICR-DUB albino mice, 17 to 35 g, obtained from Dominion Labs (Dublin, VA) were used. Thirty mice were normally housed in each cage and given free access to food and water. The mice were kept in the Robins vivarium on a 12-h light and 12-h dark cycle with lights off at 1800 h and on at 0600 h. On test days, 60 to 63 mice were used. The mice were naive to the test apparatus.

Apparatus

Experiments were performed in a sound-attenuated room illuminated with a 25-watt red bulb. Behavioral testing was conducted with three 2-compartment automated test chambers (Digiscan, Model RXYZCM16, Omnitech Electronics Inc., Columbus, OH). Access between the lit and dark areas was provided by a 7.5×7.5 cm passageway. A 90-watt light bulb located 30 cm above the box was used to provide light to 1 compartment (hereafter called the lit area). The intensity of light was constant at 260 lux. Interruptions of the infrared beams in the chamber were automatically recorded by the digiscan analyzer and then transmitted to a "VAX cluster" which consisted of a VAX 11/785 computer and a VAX 85-30 computer (Digital

[1]Present address: Department of Medicinal Chemistry, Box 540, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0540.

[2]Present address: National Institute on Drug Abuse, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857.

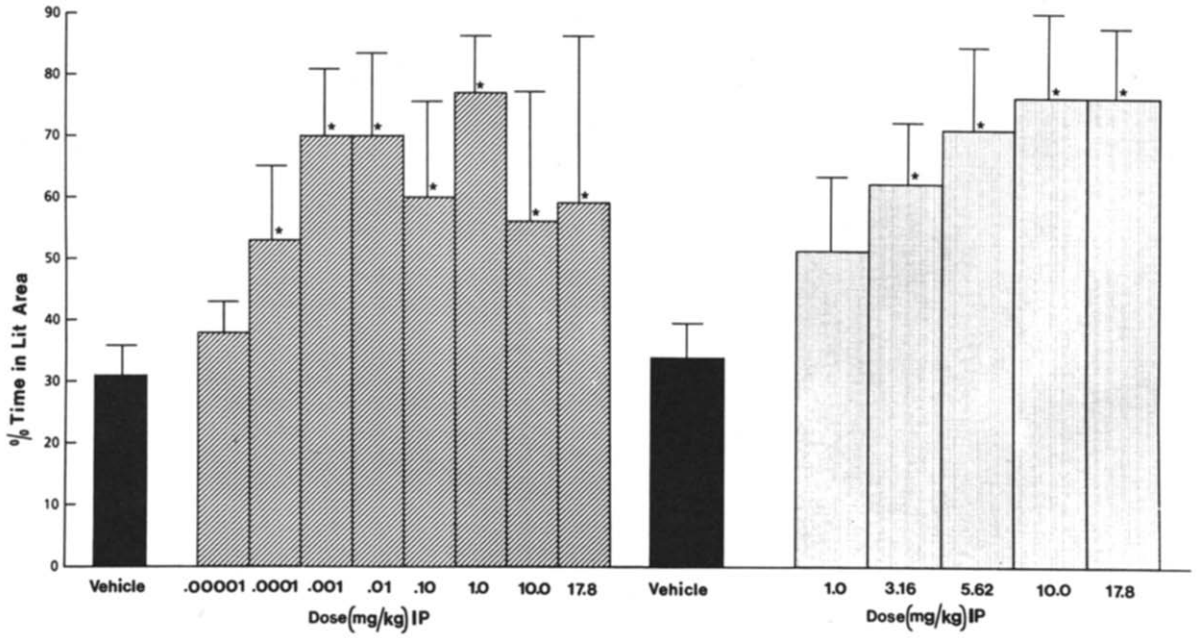

FIG. 1. The effect of IP administration of zacopride (left) and buspirone (right) on the time mice spent in the lit area (±S.E.M.). The * indicates values that are significantly different from vehicle; $p<0.05$, n = 7/dose, Dunnett's t-test.

Equipment Corp., DEC, Maynard, MA). Computer software programs were written for summarizing (mean, standard deviation) data and performing statistical analyses (see below).

Procedure

At approximately 0900 h on the day of an experiment, the mice were taken from a vivarium holding room to the sound-attenuated, darkened room. The mice were then randomized into dose groups according to a table of random numbers (7) and placed into individual holding cages. The mice stayed in the dark room for a subsequent 4-h period.

Starting at approximately 1230 h, each mouse received either an IP or PO dose of either saline, buspirone, or zacopride. Thirty min later the animal was placed at the center of the lit area and behavioral activity was tallied over a 5-min period by

TABLE 1

EFFECTS OF ZACOPRIDE AND BUSPIRONE ON TOTAL LOCOMOTOR ACTIVITY IN THE LIGHT/DARK TEST

Drug[a]	Dose mg/kg	Mean Total Locomotor Counts	Drug	Dose mg/kg	Mean Total Locomotor Counts
Vehicle (IP)	10 ml/kg	1205.3	Vehicle (PO)	10 ml/kg	1357.7
Zacopride (IP)	0.00001	1103.3	Zacopride (PO)	0.0001	1066.0
	0.0001	1107.4		0.001	1463.1
	0.001	929.9		0.01	1370.4
	0.01	906.6		0.10	1241.5
	0.10	1116.6		1.0	1527.9
	1.0	934.6		10.0	1095.0
	10.0	1035.5		100.0	907.2
	17.8	1212.5			
	100.0	312.6*			
Vehicle (IP)	10 ml/kg	1164.0	Vehicle (PO)	10 ml/kg	1357.9
Buspirone (IP)	1.0	1313.5	Buspirone (PO)	1.0	1347.8
	3.16	1343.1		3.16	1425.4
	5.62	1152.6		10.0	1341.6
	10.0	1157.4		17.8	1313.0
	17.8	953.2		31.6	1129.3
	31.6	534.2*		56.2	747.5*

*Indicates values that are significantly different from the appropriate vehicle value.
[a]For each vehicle treatment and each dose of each drug n = 7.

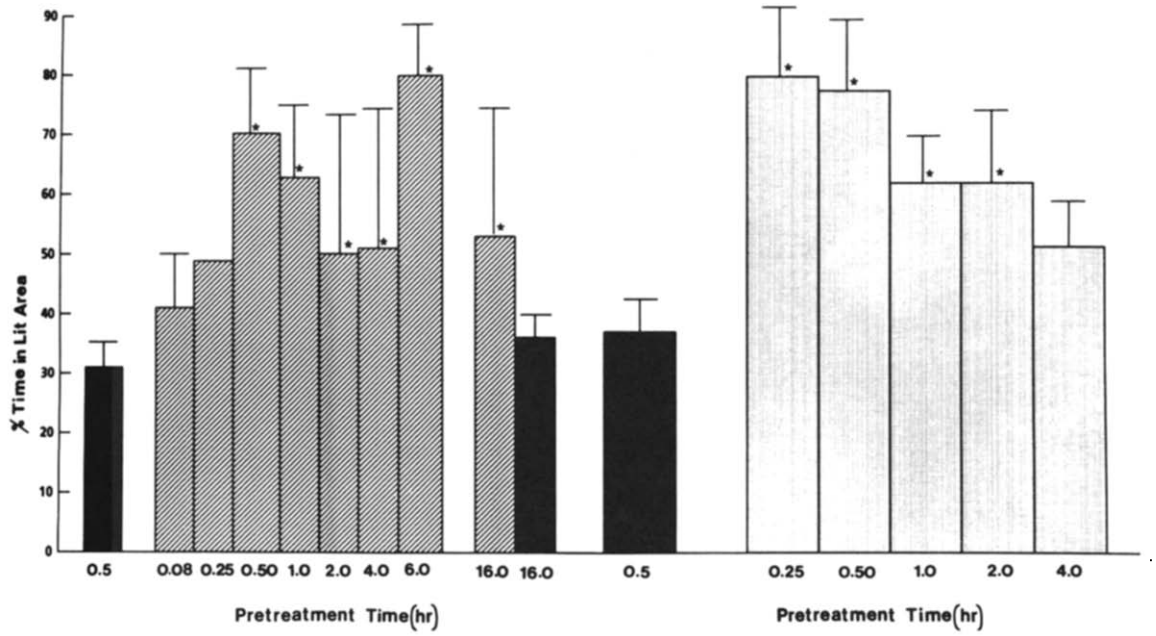

FIG. 2. The time course effect (±S.E.M.) of 0.001 mg/kg, IP, of zacopride (left) and 5.62 mg/kg, IP, of buspirone (right). Mice were injected with either vehicle (shaded areas), zacopride, or buspirone at various pretreatment intervals prior to being placed at the center of the lit area. The * indicates values that are significantly different from appropriate vehicle test; $p<0.05$, n = 7/ pretreatment time, Dunnett's t-test.

TABLE 2

TIME COURSE EFFECTS OF ZACOPRIDE (0.001 mg/kg, IP, 0.01 mg/kg, PO) AND BUSPIRONE (5.62 mg/kg, IP, 17.8 mg/kg, PO) ON TOTAL LOCOMOTOR ACTIVITY IN THE LIGHT/DARK TEST

Drug and Dose[a]	Pretreatment Time (h)	Mean Total Locomotor Counts	Drug and Dose	Pretreatment Time (h)	Mean Total Locomotor Counts
Vehicle (10 ml/kg, IP)	0.5	1164.6	Vehicle (10 ml/kg, IP)	0.5	1202.7
Zacopride (0.001 mg/kg, IP)	0.08	1282.0	Buspirone (5.62 mg/kg, IP)	0.08	259.5*
	0.25	1265.0		0.25	870.4
	0.50	929.9		0.50	1152.6
	1.0	859.7		1.0	1244.1
	2.0	1038.7		2.0	1243.4
	4.0	1008.5		4.0	1309.2
	6.0	917.3			
Vehicle (10 ml/kg, IP)	16.0	1340.6			
Zacopride (0.001 mg/kg, IP)	16.0	1525.7			
Vehicle (10 ml/kg, PO)	0.5	1357.7	Vehicle (10 ml/kg, PO)	0.5	1388.1
Zacopride (0.01 mg/kg, PO)	0.08	1143.0	Buspirone (17.8 mg/kg, PO)	0.08	848.9*
	0.25	1391.2		0.25	1091.7
	0.50	1370.4		0.50	1313.0
	1.0	1053.3		1.0	1362.0
	2.0	1121.8		2.0	1083.7
	4.0	1441.7		4.0	1251.8
				6.0	1301.3
Vehicle (10 ml/kg, PO)	8.0	1323.5			
Zacopride (0.01 mg/kg, PO)	8.0	1090.2			
Vehicle (10 ml/kg, PO)	16.0	1453.7			
Zacopride (0.01 mg/kg, PO)	16.0	1249.9			

*Indicates values that are significantly different from the appropriate vehicle value.
[a]For each vehicle treatment and each dose of each drug at each pretreatment time n = 7.

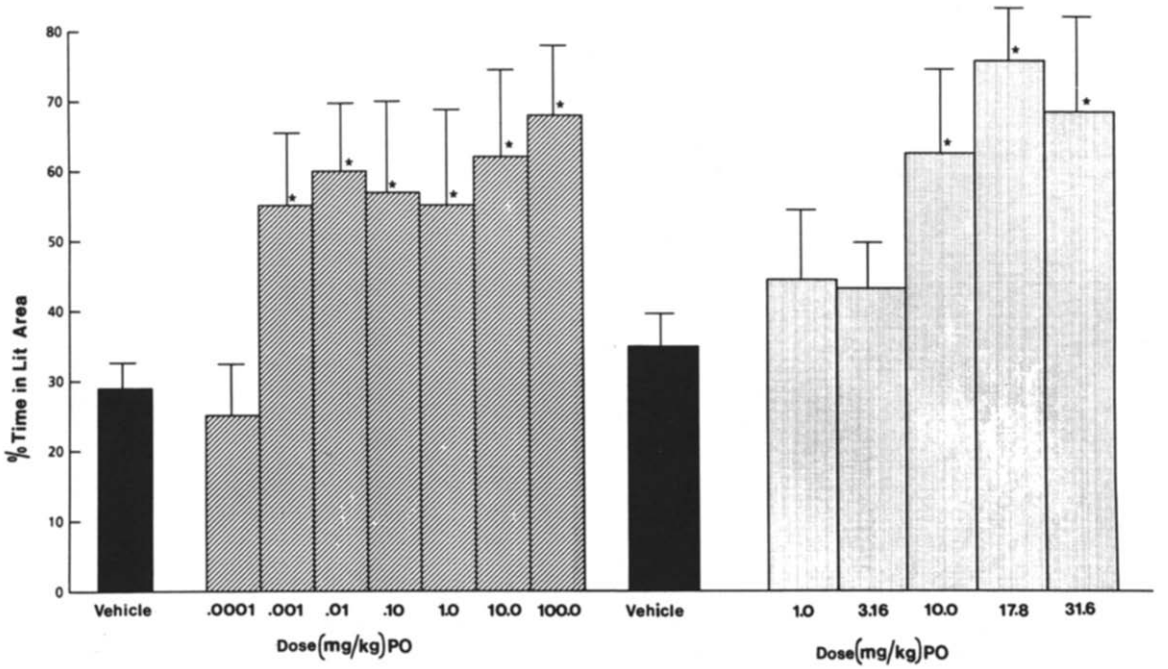

FIG. 3. The effect of PO administration of zacopride (left) and buspirone (right) on the time mice spent in the lit area (±S.E.M.). The * indicates values that are significantly different from vehicle; $p<0.05$, n=7/dose, Dunnett's *t*-test.

the Digiscan analyzer. Eight behavioral measures were recorded: the time spent in the lit and dark areas, locomotor activity counts in each area, number of rearings in each area, number of transitions between the 2 areas, and latency to make the first transition from the lit area to the dark area. Statistical analyses were performed on the percent of time spent in the lit area and on total locomotor activity counts by using Dunnett's *t*-test. The previous study (8), using reference anxiolytics, suggested that

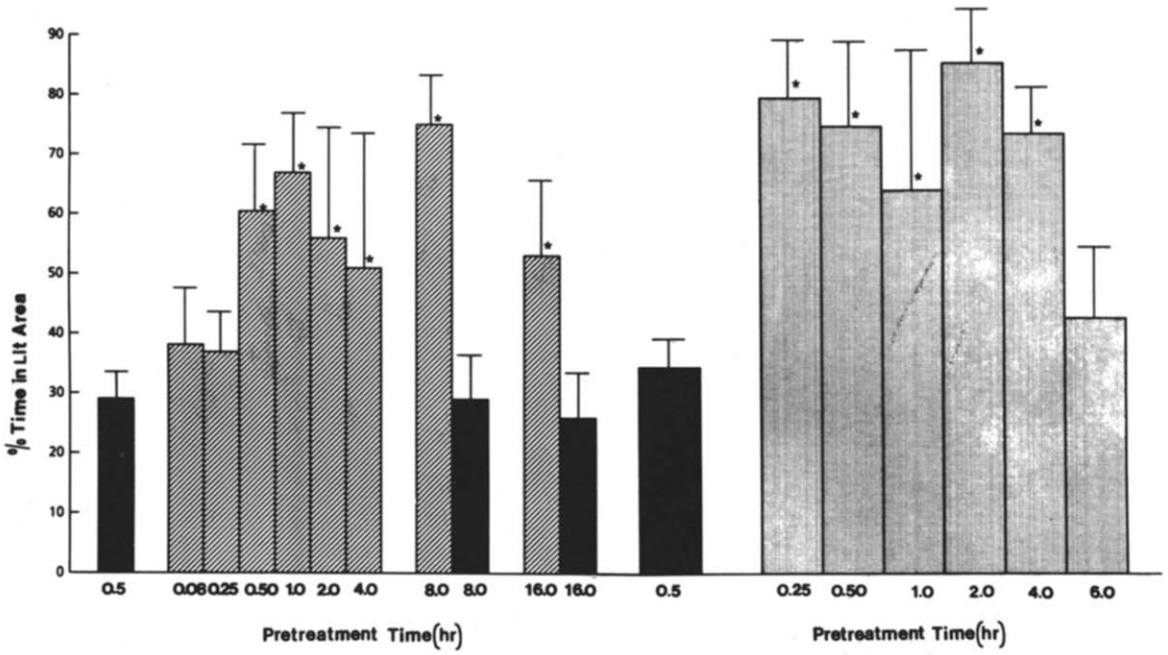

FIG. 4. The time course effect (±S.E.M.) of 0.01 mg/kg, PO, of zacopride (left) and 17.8 mg/kg, PO, of buspirone (right). Mice were injected with either vehicle (shaded areas), zacopride, or buspirone at various pretreatment intervals prior to being placed at the center of the lit area. The * indicates values that are significantly different from appropriate vehicle test; $p<0.05$, n=7/pretreatment time, Dunnett's *t*-test.

the former measure may be the best indicator of anxiolytic-like activity, while the latter measure was used as an indicator of behavioral disruption (i.e., sedation or ataxia). All significant differences were determined by using a p value of ≤ 0.05.

Once the IP and PO dose-effect functions were established with a 30-min pretreatment interval, additional pretreatment periods were investigated. This was accomplished by using 0.001 mg/kg IP and 0.01 mg/kg PO of zacopride, and 5.62 mg/kg IP and 17.8 mg/kg PO of buspirone, doses that produced a significant effect in the time mice spent in the lit area measurement. Pretreatment intervals of 0.08, 0.75, 0.50, 1, 2, 4, 6, 8, and/or 16 h were evaluated. Statistical analyses were performed by using Dunnett's t-test. All significant differences were determined by using a p value of ≤ 0.05.

Drugs

Buspirone HCl (Bristol-Myers, Evansville, IN) and zacopride HCl (synthesized by Chemical Research Group, A.H. Robins, Co., Richmond, VA) were dissolved in 0.9% saline. Doses were expressed as mg of base per kg of body weight. Injections were given IP or PO in a constant volume of 10 ml/kg.

RESULTS AND DISCUSSION

During the course of the study, vehicle-treated mice spent 27–35% of the 5-min test period in the lit area (Figures 1–4). The administration of zacopride and buspirone produced significant increases in time mice spent in the lit area and increases in behavioral activities (see the Procedure section) in this area with concomitant decreases of these activities in the dark area (behavioral activity data not presented). In agreement with the previous report (8), time mice spent in the lit area provided the most consistent dose-effect results with the drugs.

Following IP administration of zacopride and buspirone, mice significantly increased their time spent in the lit area between doses of 0.0001 to 17.8 mg/kg and 3.16 to 17.8 mg/kg respectively (Fig. 1). These increases in time spent in the lit area occurred without a significant change in total locomotor activity. The administration of 100.0 mg/kg (IP) of zacopride or 31.6 mg/kg (IP) of buspirone significantly reduced total locomotor activity (Table 1). The time course effects of zacopride (0.001 mg/kg, IP) and buspirone (5.62 mg/kg, IP) are shown in Fig. 2. The onset to action of zacopride was 0.5 h and the duration of effect was ≥ 16 h. In comparison, the onset to effect of buspirone was 0.25 h and the duration of action was between 2 and 4 h. Interestingly, when mice were administered buspirone at a pretreatment time of 0.08 h a significant impairment of locomotor activity was observed (Table 2).

Oral administration of zacopride and buspirone also produced significant increases in time mice spent in the lit area between doses of 0.001 to 100.0 mg/kg and 10.0 to 31.6 mg/kg respectively (Fig. 3). At those doses, total locomotor activity counts were not significantly altered. The administration of 56.2 mg/kg of buspirone, however, significantly reduced locomotor activity (Table 1). The time course effects of PO administered zacopride (0.01 mg/kg) and buspirone (17.8 mg/kg) are almost identical to those following IP injection (Fig. 4). That is, the onset to action of zacopride was 0.5 h and the duration of effect was ≥ 16 h. Figure 4 also shows that the onset to effect of buspirone was 0.25 h and the duration of effect was 4 h. Again, however, when mice were administered buspirone at a pretreatment time of 0.08 h a significant impairment of locomotor activity was observed (Table 2). The reason(s) for these disruptions of activity by buspirone following a very short pretreatment interval is not clear.

In summary, the present data confirm and extend the results of previous investigations concerning the anxiolytic-like effects of zacopride (1) and buspirone (2, 4, 5) in the light/dark test. Specifically, when compared for anxiolytic-like effects in this test, zacopride is a more potent and longer acting agent than buspirone. Moreover, if the data generated in the present automated test system are paralleled by similar results in the clinic, zacopride will be among the most potent and long acting psychoactive compounds known.

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