Pharmacology Biochemistry & Behavior, Vol. 6, pp. 627–630.

Further Studies on BL-3912A: Effects on Avoidance Behavior of Rats with Low Baselines and on Reaction Thresholds to Electric Footshock

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(Received 27 January 1977)

TILSON, H. A., J. H. CHAMBERLAIN AND J. A. GYLYS. *Further studies on BL–3912A: effects on avoidance behavior of rats with low baselines and on reaction thresholds to electric footshock.* PHARMAC. BIOCHEM. BEHAV. 6(6) 627–630, 1977. – Rats selected for their low performance baselines in an active avoidance shuttle box task were given various doses of R–(–)-2-amino-l-(2,5-dimethoxy-4-methylphenyl) butane (BL–3912A), S-amphetamine or piracetam. BL–3912A at 1 mg/kg IP had no significant behavioral effects, while 5 and 10 mg/kg significantly increased the number of avoidance responses without affecting responses during the intertrial interval (ITI). Statistically reliable effects on behavior were not observed following 20 mg/kg of BL–3912A. S-Amphetamine at 0.5 and 1 mg/kg IP also facilitated avoidance responding, but could be differentiated from BL–3912A in that the S-amphetamine significantly increased shuttles during the ITI. S-Amphetamine at 0.1 mg/kg was not effective, while 2 mg/kg increased ITI activity. Piracetam (50 or 200 mg/kg) had no significant effects on avoidance or shuttles during ITI. Using an electric shock titration procedure, BL–3912A at 10 and 20 mg/kg IP had no significant effect on reaction thresholds. Animals receiving 100 mg/kg of p-chlorophenylalanine po for 3 days and tested 2 days later showed hyperalgesia to the electric shock. In summary, BL–3912A facilitated shuttle box avoidance responding of rats with low performance baselines. Behavioral facilitation occurred without concomitant increases in noncontingent activity or apparent changes in reactivity to electric footshock.

BL–3912A Low baseline responders in shuttle box Reaction threshold to electric footshock

R–(–)–2–AMINO–1–(2,5-dimethoxy-4-methylphenyl) butane (BL–3912A) has been reported to facilitate the performance of naive rats in a massed trial shuttle box task [11]. Behavioral facilitation occurred with doses of BL–3912A (10 and 20 mg/kg) that had little or no effect on unacclimated motor activity or continuous avoidance responding of rats. These results, as well as other work in our laboratory [7], indicate that BL–3912A has a psychopharmacological profile that is different from other phenylalkylamines such as S-amphetamine and R–DOM.

The behavioral facilitation observed with BL–3912A in naive rats prompted us to test this agent in other populations of animals, such as those with low performance baselines. Other investigators have demonstrated that poor performer rats in the shuttle box are sensitive to the effects of various psychoactive drugs [4,5]. It has also been reported that drugs such as p-chlorophenylalanine (p-CPA) may influence behavior by increasing the sensitivity of the test animal to external stimulation, particularly in tasks motivated by electric footshock [8]. Thus, it was decided to study the effects of BL–3912A on pain or reaction threshold to footshock.

METHOD

Animals

Male, hooded rats of the Long-Evans strain, obtained from Blue Spruce Farms (Altamont, NY) and weighing 250–350 g were used in these experiments. The animals were housed either individually (shuttle box experiment) or in groups of 4 (reaction threshold experiment) in facilities described previously [10].

Low Baseline Avoidance Responding

Avoidance behavior was assessed in 2 automated shuttle cages (BRS/LVE, model RSC-044) contained within a light and sound-attenuated cabinet equipped with a ventilation

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fan [11]. Initially, rats were given 120 massed acquisition trials to determine performance in the shuttle box. Each trial was 30 sec in duration and consisted of a 5 sec conditioned stimulus (CS, light) followed by 5 sec of electric footshock (0.8 mA scrambled shock) applied to the same side of the shuttle cage as the rat and CS. Crossing over to the other side of the cage during the CS (avoidance) or after shock onset (escape) terminated the CS and/or shock and initiated an intertrial interval (ITI) having a duration of at least 20 sec. Following the initial 120 trial acquisition session, the rats were selected for low performance baselines during 30 trial tests conducted twice weekly (usually Tuesdays and Thursdays). Only those animals making more than 1, but less than 16 avoidances out of 30 trials for 2 weeks of testing were considered for subsequent testing.

Those animals meeting the low performance criteria continued to be tested twice weekly. The first session of the week consisted of an IP injection of the vehicle 15 min prior to the 30 trial sessions. If the performance during the first session was appropriate, the rats were given IP injections of BL−3912A (1, 5, 10 or 20 mg/kg), S-amphetamine (0.1, 0.5, 1 or 2 mg/kg) or piracetam (50 or 200 mg/kg) dissolved in a water vehicle on the second test session. Drugs were studied in the above order, while dosages within each experiment were investigated in a random fashion. Those animals not maintaining a baseline consistent with the established criteria for 4 consecutive control sessions were dropped from further study. A total of 42 animals were selected for study during various stages of the study. In the latter stages of testing for 20 of the animals, it was decided to inject the vehicle on both days of testing. This was done in order to measure any conditioned drug effects that may have resulted from the testing procedure.

The number of avoidance responses in 30 trials and shuttles during the ITI after drug were compared to data acquired during matching control sessions of the same week. Statistical differences between means were tested by a Wilcoxson matched pair, signed-rank test [6] with $p<0.05$ (two-tailed) set as the accepted level of significance.

Reaction Threshold-Apparatus and Procedure

The test apparatus was a commercially available modular unit (Lafayette Instrument Co., Model 85020) equipped with a one-way viewing mirror and internal light source. Scrambled, electric shock was delivered to the grid floor by means of a constant voltage shock source with a manual control to regulate shock intensity. Following a one min acclimation period, shocks 0.5 msec in duration were delivered automatically every 30 sec. Shock levels were raised gradually from 0.1 to 1.0 mA in 0.05 mA steps until the appropriate behavioral response was elicited. The threshold reaction was defined as a rapidly occurring oscillation of 3 or more paws usually associated with vocalization.

Preinjection thresholds were always obtained prior to drug testing and served as matched control readings for subsequent measurements obtained after vehicle or drug injections. Differences between pre- and postinjection thresholds were evaluated by a matched pair t-test [2] with $p<0.05$ as the accepted level of significance.

Twenty-four hr after measurement of the first preinjection thresholds, 12 rats were randomly divided into 2 equal groups and given IP injections of solutions from coded vials containing either 10 mg/cc of BL−3912A or distilled water (1 ml/kg body weight). Reaction thresholds were measured 30 min later, which corresponds to the period of time performance enhancement was observed in the previous experiment. Seventy-two hr later, the entire procedure was repeated for 20 mg/kg of BL−3912A.

At least 72 hr following the experiments with BL−3912A, preinjection thresholds were measured and the rats were again randomized into 2 equal groups. On the following day, the rats were given 6 mg/kg of morphine sulfate or vehicle from coded vials and thresholds measured 1 hr later. This corresponds to the time of peak analgesia, as measured by this technique [9].

Seventy-two hrs after the morphine experiment, reaction thresholds were measured again. Immediately afterward, 6 randomly selected rats received 100 mg/kg po of p-chlorophenylalanine (p-CPA, Aldrich Chemical Co., Milwaukee, WI) suspended in 1% carboxymethylcellulose (CMC) saline solution for 3 successive days. The remaining 6 animals received the CMC vehicle. Ninety-six hr after initiation of p-CPA or vehicle treatment, reaction thresholds were measured in a blind fashion.

RESULTS

Low Avoidance Baseline

Twenty randomly selected animals having received drugs on at least 2−4 test sessions showed no significant differences in avoidances or shuttles during the ITI when the vehicle was given on both sessions of the week (Table 1). These data argue against the possibility that behavioral facilitation seen on some drug sessions was somehow related to a conditioned drug effect or some other artifact of the sequential testing procedure.

The IP administration of 1 mg/kg of BL−3912A had no significant effect on the number of avoidances, while 5 and 10 mg/kg of BL−3912A significantly increased the number of avoidances made during the 30 trial test procedure (Table 1). The highest dose of BL−3912A tested (20 mg/kg) tended to elevate avoidance responding, but the effect was not statistically reliable. None of the doses of BL−3912A that were tested produced significant effects on the number of shuttles occurring during the ITI. These data indicate that BL−3912A facilitates the shuttle box avoidance responding of rats without influencing a measure of noncontingent shuttle activity.

The lowest dose of S-amphetamine tested (0.1 mg/kg) did not alter shuttle box responding, while 0.5 and 1 mg/kg significantly increased the number of avoidances. The IP injection of 2 mg/kg of S-amphetamine also increased avoidance responding, but the effect was not statistically significant. S-amphetamine at 1 and 2 mg/kg produced marked increases in the number of shuttles during the ITI. Thus, the increase in avoidance responding following 1 mg/kg of S-amphetamine was associated with an increase in noncontingent activity during the ITI.

Piracetam at 50 or 200 mg/kg IP had no significant effect on shuttle box responding of rats with low baselines (Table 1). The higher dose of piracetam tended to increase shuttles during the ITI, but the effect was not statistically reliable.

Reaction Threshold

The IP administration of 10 mg/kg of BL−3912A had

TABLE 1

EFFECTS OF BL-3912A, S-AMPHETAMINE AND PIRACETAM ON SHUTTLE BOX RESPONDING OF POORLY PERFORMING RATS

Treatment* (mg/kg, IP)	N	Average Avoidances per 30 Trials ± 1 SE	Average Shuttles During the ITI ± 1 SE
Vehicle (session 1)	20	8.2 ± 1.1	11.3 ± 2.4
Vehicle (session 2, sequential control)		8.4 ± 1.2	11.2 ± 3.0
Vehicle	6	7.7 ± 1.1	9.2 ± 3.1
BL-3912-A, 1.0		6.5 ± 2.1	7.0 ± 4.5
Vehicle	9	7.4 ± 1.4	8.8 ± 3.5
BL-3912-A, 5.0		14.7 ± 2.3†	11.1 ± 3.8
Vehicle	12	6.4 ± 1.2	10.3 ± 2.5
BL-3912-A, 10.0		10.8 ± 2.3†	11.0 ± 2.8
Vehicle	11	7.3 ± 1.0	6.1 ± 2.1
BL-3912-A, 20.0		10.0 ± 1.7	6.6 ± 3.4
Vehicle	7	7.6 ± 0.8	12.6 ± 5.1
S-amphetamine, 0.1		7.1 ± 1.7	10.1 ± 5.0
Vehicle	7	7.6 ± 1.9	8.2 ± 0.7
S-amphetamine, 0.5		13.4 ± 3.4†	9.6 ± 3.5
Vehicle	17	7.9 ± 1.0	7.8 ± 2.1
S-amphetamine, 1.0		11.2 ± 1.7†	13.9 ± 4.0†
Vehicle	8	8.9 ± 1.3	10.9 ± 3.6
S-amphetamine, 2.0		10.8 ± 1.5	20.6 ± 7.6†
Vehicle	6	7.7 ± 1.2	8.8 ± 2.5
Piracetam, 50		7.7 ± 1.4	8.1 ± 2.4
Vehicle	11	6.7 ± 1.4	9.3 ± 2.6
Piracetam, 200		9.7 ± 2.1	15.5 ± 4.4

*Data are average ± S.E. avoidances or ITI shuttles during 30 trial sessions. Vehicle was given on the first session of each week, while drug was given on the second day. In the sequential control, vehicle was given on both the first and second test days.

†Statistically different from matched vehicle control (Wilcoxson Matched-Pairs Signed-Ranks Test, $p<0.05$).

no significant effect on the shock required to elicit the designated response (Table 2). Although 20 mg/kg of BL–3912A tended to decrease the threshold in some of the rats (89.3% of preinjection control), the effect was not statistically reliable. As reported previously using a similar procedure [9], the IP administration of 6 mg/kg of morphine sulfate produced a significant increase in the reaction threshold. These data indicate that the technique was sensitive to changes in threshold produced by a standard analgesic agent.

The oral administration of the CMC vehicle for 3 days had no effect on the reaction threshold measured 2 days later, while those rats receiving p-CPA suspended in CMC had significantly decreased thresholds (82.8% of preinjection thresholds). Thus, at a time when brain serotonin is depleted [3], p-CPA produced a significant hyperalgesia.

DISCUSSION

The main finding of these experiments is that BL–3912A facilitated the shuttle box performance of rats selected on the basis of low performance criteria. This observation supports and extends our previous work in which it was observed that BL–3912A facilitated shuttle box avoidance responding of naive retired breeder rats [11]. It is also noteworthy that the facilitation of avoidance responding in the shuttle box by BL–3912A was not correlated with increases in the frequency of other types of behaviors, such as shuttle activity during the ITI. It has been reported elsewhere that doses of BL–3912A which facilitate shuttle box responding (5–20 mg/kg) have no effect on or decrease rates of negatively reinforced operant responding and horizontally-directed motor activity of rats measured under novel environmental conditions [11].

The IP administration of 0.5 mg/kg of S-amphetamine to rats with low avoidance baselines significantly increased avoidances, while 1 mg/kg increased both avoidance and shuttles during the ITI. A similar observation has been made for S-amphetamine given to naive retired breeder rats [11]. Thus, the behavioral facilitation produced by at least one dose S-amphetamine is associated with statistically significant increases in behaviors not necessarily dependent upon the avoidance contingency, while BL–3912A facilitated avoidances only.

It is also noteworthy that the rats selected for study in the present investigation resemble closely the Type II poor performers described by Rech [5]. Rech found that these rats seldom, if ever, achieve greater than 50% or better

TABLE 2

EFFECTS OF BL−3912A, MORPHINE SULFATE AND P-CHLOROPHENYLALANINE (P-CPA) ON REACTION THRESHOLDS OF RATS

Treatment (Dose, mg/kg)	Reaction Threshold (mA ± SE)* Preinjection	Postinjection	% of Preinjection
Vehicle Control	0.58 ± 0.05	0.57 ± 0.05	98.3%
BL-3912A (10, IP)	0.56 ± 0.06	0.55 ± 0.04	98.3%
Vehicle Control	0.58 ± 0.05	0.58 ± 0.03	100.0%
BL-3912A (20, IP)	0.56 ± 0.06	0.50 ± 0.05	89.3%
Vehicle Control	0.56 ± 0.06	0.59 ± 0.04	105.4%
Morphine Sulfate (6, IP)	0.58 ± 0.04	0.77 ± 0.02†	132.8%
Vehicle Control	0.61 ± 0.03	0.60 ± 0.03	98.4%
p-CPA (100X3 days, p.o.)	0.58 ± 0.05	0.48 ± 0.04†	82.8%

*Data are means of 6 rats ± S.E. for mA of electrofootshock required to elicit an appropriate behavioral reaction. BL-3912A and morphine sulfate were injected IP 15-20 min and 60 min prior to testing, respectively. p-CPA was given p.o. on 3 successive days (100 mg/kg/day) and reaction thresholds tested two days later. Preinjection thresholds were obtained 24 hr prior to drug treatments.

†Statistically different from matching preinjection threshold (matched pair t-test, $p<0.05$).

correct avoidance, even after prolonged training. In addition, it was observed that 0.5 and 2 mg/kg, but not 0.25 mg/kg, of S-amphetamine, significantly improved the performance of the Type II poor performers. This agrees with the dose response relationship observed in the present study in which 0.10 mg/kg of S-amphetamine was not effective, while 0.5 and 1 mg/kg significantly improved avoidance behavior. In our hands, 2 mg/kg of S-amphetamine increased avoidance responding, but the effect was not statistically significant.

Piracetam (2-oxi-pyrrolidinone-l-acetamide) was included in this study because it is a nonstimulant agent that reportedly facilitates behavior, particularly under hypoxic conditions [1]. Under the conditions of the present study, piracetam (50 or 200 mg/kg) had no significant effect upon avoidance responding or shuttles during the ITI. Thus, the psychopharmacological profile of BL−3912A appears to be different than that observed with piracetam.

The data from this and other investigations [11] indicate that BL−3912A facilitates the performance of rats responding under shock-motivated contingencies. The mechanism by which this behavioral facilitation occurs has yet to be elucidated. However, the results of the reaction threshold experiment argue against the possibility that BL−3912A enhances behavior by lowering the threshold required to elicit a response to electric footshock. Such a mechanism has been proposed to account for the facilitation produced by p-CPA on active avoidance responding of rats [8].

In summary, BL−3912A facilitated the avoidance behavior of rats with low performance baselines. BL 3912A could be differentiated from the psychostimulant S-amphetamine, in that BL−3912A did not increase noncontingent activity. In addition, piracetam, a nonstimulant performance enhancer, was ineffective in these tests. Thus, BL−3912A may prove beneficial in the treatment of individuals having low or deteriorated levels of certain types of associative and psychomotor abilities.

ACKNOWLEDGEMENT

The assistance of Mr. L. L. Gaede in the analysis of the data is gratefully acknowledged.

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