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Buspirone Fails to Affect Cocaine-induced Conditioned Place Preference in the Mouse

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ALI, I. AND M. E. KELLY *Buspirone fails to affect cocaine-induced conditioned place preference in the mouse.* PHARMACOL BIOCHEM BEHAV **58**(2) 311–315, 1997.—The conditioned place preference (CPP) procedure was employed to examine the effects of the 5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$) receptor agonist, buspirone, on cocaine reinforcement. Cocaine (5.0 mg/kg, SC, 30 min) produced a significant place preference whereas buspirone (0.5–2.0 mg/kg, IP, 30 min) per se failed to induce a CPP. Buspirone pretreatment (0.5–2.0 mg/kg, IP) 30 min prior to cocaine (5.0 mg/kg, SC, 30 min) conditioning had no effect on the acquisition of cocaine-induced CPP. Pretreatment with buspirone on the postconditioning test day also failed to affect the expression of an already established place preference response to cocaine. These results demonstrate an inability of buspirone to block both the acquisition and the expression of cocaine reward, as modeled in the CPP para- digm. © 1997 Elsevier Science Inc.

Cocaine Buspirone 5-HT$_{1A}$ receptor Conditioned place preference Mouse Acquisition Expression

PREVIOUS research would suggest that changes in dopaminergic function are the underlying mechanism for the reinforcing effects of cocaine and other psychostimulants, such as amphetamine (18,25,27). However, recent evidence also suggests that 5-hydroxytryptamine (5-HT) may play an important facilitatory role in the mediation of psychomotor stimulant reinforcement. For example, self-administration of amphetamine varies depending on whether brain 5-HT levels are elevated or depressed; enhanced 5-HT levels achieved by manipulation of the 5-hydroxytryptaminergic system through treatment with the 5-HT precursor, L-tryptophan; the 5-HT uptake inhibitor, fluoxetine; or the 5-HT agonist, quipazine, all lead to a decrease in amphetamine self-administration (12). In contrast, destruction of 5-HT containing neurones by intraventricular injections of 5,7-dihydroxytryptamine (5,7-DHT) results in an increase in amphetamine self-administration (14).

Similar results have been achieved with cocaine self-administration. Reduced levels of brain 5-HT achieved through intracerebral injections of 5,7-DHT into the medial forebrain bundle or amygdala increase the breakpoints on a progressive ratio schedule of reinforcement, suggesting that depletion of forebrain 5-HT increases the rewarding effect of cocaine (13). On the other hand, elevated brain 5-HT levels through enhanced dietary L-tryptophan (3) or treatment with fluoxetine (4) or the 5-HT$_{1A}$ agonist, 8-OH-DPAT, leads to a decrease in cocaine self-administration (23).

In discriminative stimulus studies, the 5-HT$_{1A}$ agonist, buspirone, has been shown to block the effects of apomorphine in the rhesus monkey (11), but fails to alter the discriminative stimulus effects of cocaine in the rat (24). To further clarify the role of buspirone on cocaine reinforcement, the present investigation utilized the conditioned place preference (CPP) paradigm to investigate the effects of buspirone on the acquisition of cocaine CPP. In addition, the effect of buspirone on the postconditioning expression of cocaine CPP was also investigated. In most studies, this aspect of the CPP response receives little or no attention, even though it may be more reflective of the self-administration procedure because the drug of interest is not administered until after successful conditioning with the rewarding drug (6). Finally, buspirone was examined in the CPP procedure per se because conflicting data have been generated in previous studies using the CPP paradigm (9,19).

METHOD

Subjects and Apparatus

Male adult (3 months old) BKW (University of Bradford

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bred) mice weighing 28–50g were housed in a colony holding room in groups of 10 under conditions of reversed lighting (lights off between 07:00 and 19:00) and constant temperature (~21°C), and free access to food and water.

The laboratory was illuminated with red light at all times and subjects were allowed to habituate for at least 1 h before testing commenced. All experiments were conducted between 09:30 and 17:00. Four automated place conditioning boxes (76 × 30 × 30 cm), each with three distinctive interconnected Plexiglass chambers, were used (see Fig. 1). The two outer chambers measured 30 × 30 × 30 cm; one consisted of striped wood walls with a striped textured glass floor, and the other of metal walls with a striped wood floor. The smaller central chamber (16 × 30 × 30 cm) connected the two outer chambers and consisted of a black painted floor with clear Plexiglass walls. All three chambers were connected by black Plexiglass guillotine doors which were staggered to prevent visual communication between the three chambers.

Experimental Procedure

The CPP protocol consisted of three phases. In the preconditioning phase, the initial preferences of mice were determined by allowing them free access to the entire apparatus. The time spent in each of the two outer chambers during the 15-min period was recorded automatically using a photocell system linked to a timer. The time spent in the central chamber was calculated by subtraction (15 min minus the sum of the time spent in the outer two chambers). This was repeated for three consecutive days and the average time for the three days was taken as the preconditioning (baseline) preference. From this, the preferred and nonpreferred chamber for each subject was determined. The conditioning phase consisted of an eight-day period in which each subject received vehicle (0.9% saline) or drug and was confined individually to one of the outer two chambers for 30 min. On alternate days, the subjects received the other treatment and were placed in the opposing outer chamber, such that each subject received four drug and four vehicle pairings. Drug pairing was counterbalanced to both the preferred and nonpreferred chambers. Finally, the effect of drug conditioning was determined by the postconditioning test, in which each subject was allowed free access to the entire apparatus. The time spent in the outer two chambers was again recorded automatically over a period of 15 min (time spent in the central chamber determined by subtraction), as in the preconditioning phase. At the end of each phase of the experiment, mice were held in recovery boxes in their original groups, and returned to the respective home cage at the end of the experimental period.

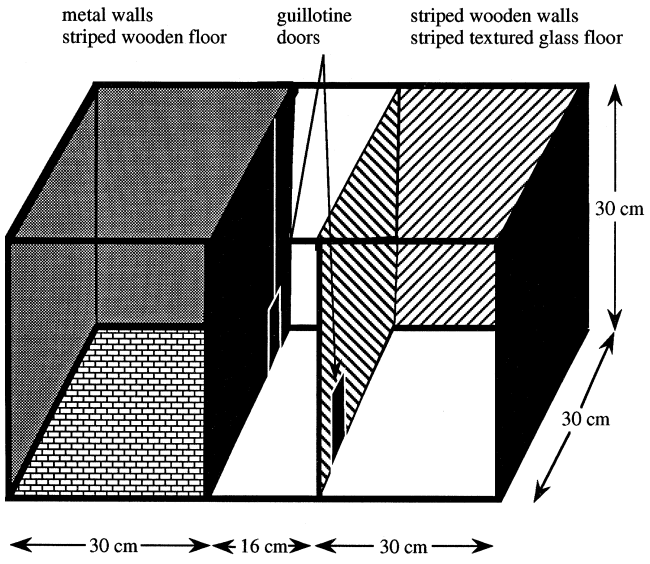

FIG. 1. A diagrammatic representation (not drawn to scale) of the CPP apparatus used in the present study. The box measured 76 × 30 × 30 cm. Dimensions of the three distinctive chambers are given.

Buspirone CPP

The above protocol was followed. During the conditioning phase, vehicle (0.9% saline, $n = 9$) administration was paired with one of the two outer chambers and buspirone (0.5, 1.0, or 2.0 mg/kg, IP, $n = 10$ per dose group) with the other for 30 min.

Acquisition Experiments

For the acquisition experiments, the initial preferences of groups of mice ($n = 10$ per group) were determined as above. During the conditioning phase, each group was pretreated with vehicle (0.9% saline) or buspirone (0.5, 1.0, or 2.0 mg/kg) in the home cage for 30 min followed by the administration of vehicle (0.9% saline) or cocaine (5.0 mg/kg, SC) and confinement to one of the outer two chambers for 30 min. On alternate days, the subjects were given the other treatment and confined to the opposing chamber so that each subject received four cocaine and four saline pairings. Drug pairing was counterbalanced as before, so that subjects receiving buspirone pretreatment were always conditioned with cocaine and confined to one chamber and those receiving vehicle pretreatment were always conditioned with cocaine vehicle (except the vehicle control group) in the alternative chamber. Finally, the effect of drug conditioning was determined by the postconditioning test as above.

Expression Experiments

For the expression experiments, the initial preferences of four groups of mice ($n = 10$ per group) were determined followed by conditioning with cocaine (5.0 mg/kg, SC) for 30 min over a period of eight days, as above. On the postconditioning test day, the subjects were pretreated with buspirone (0.5, 1.0, or 2.0 mg/kg, IP) or vehicle for 30 min in the home cage prior to being tested for changes in preference behavior as above; 24 h later, the subjects were tested again, but in a drug-free state to ensure that buspirone did not have an adverse effect on cocaine CPP.

Drugs

Cocaine hydrochloride (Sigma Chemical Co., UK) and buspirone hydrochloride (Sigma Chemical Co., UK) were dissolved in normal (0.9%) saline and administered as the base in a volume of 1 ml/100 g body weight. Cocaine was administered via the SC route and buspirone via the IP route.

Statistics

The data were analyzed by two factorial ANOVA with condition (preconditioning/postconditioning) as a within sub-

jects factor and position within the apparatus (drug-paired chamber/center/vehicle-paired chamber) as the other factor followed by post hoc Dunnett's *t*-tests to determine shifts in preference behavior following conditioning.

RESULTS

Effects of Buspirone on CPP

Buspirone had no apparent effect on preference behavior (Fig. 2). Neither vehicle/vehicle nor buspirone (0.5, 1.0, or 2.0 mg/kg) conditioning caused significant changes in preference behavior during the postconditioning test when compared with the preconditioning preferences [$F(2,16) = 0.674$, $F(2,18) = 0.066$, $F(2,18) = 4.228$), and $F(2,18) = 2.654$, $p > 0.05$ in all cases, respectively]. Preconditioning and postconditioning times for every dose of buspirone were comparable to those for the vehicle control (e.g., 306 ± 18 s and 306 ± 25 s for vehicle; 291 ± 19 s and 274 ± 22 s for 2.0 mg/kg buspirone, respectively).

Effects of Buspirone on the Acquisition of Cocaine CPP

The results from the acquisition experiment are presented in Fig. 3. Vehicle pretreatment followed by cocaine (5.0 mg/kg) conditioning produced a significant [$F(2,18) = 11.005$, $p < 0.01$] increase in time spent in the cocaine paired chamber from 273 ± 22 s to 407 ± 29 s primarily at the expense of time spent in the vehicle paired chamber and to a lesser extent time spent in the central chamber (data not shown). Buspirone (0.5, 1.0, or 2.0 mg/kg) pretreatment 30 min prior to cocaine conditioning had no significant effect on the acquisition of cocaine CPP because cocaine conditioning induced a significant increase in time spent in the cocaine-conditioned chamber irrespective of the dose of buspirone administered [$F(2,18) = 16.582$, $F(2,18) = 15.169$, and $F(2,18) = 12.348$, $p < 0.01$ for 0.5, 1.0, and 2.0 mg/kg buspirone, respectively]. The preference behavior for the buspirone-pretreated mice was consistent with the behavior of the vehicle-pretreated controls (e.g., 407 ± 29 s for vehicle pretreatment and 386 ± 26 s, for 2.0 mg/kg buspirone).

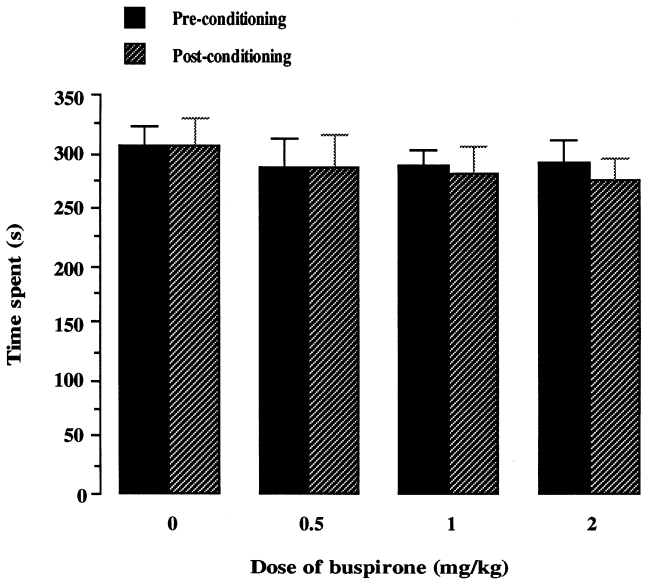

FIG. 2. The effect of buspirone (0.5—2.0 mg/kg, IP) on CPP. The results are expressed as the time spent (s) on the buspirone-paired side pre- and postconditioning and are presented as mean ± SEM ($n = 9$–10). Data were analyzed by two factorial ANOVA at each dose, followed by post hoc Dunnett's *t*-test for significant change in the time spent on the buspirone-conditioned side.

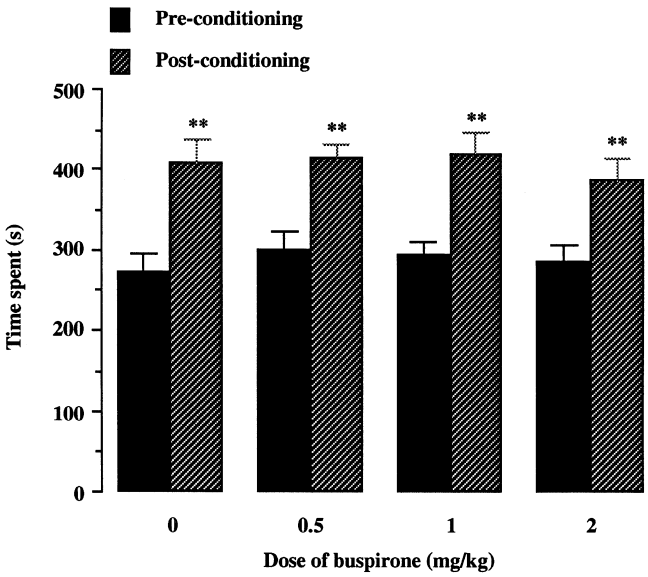

FIG. 3. The effect of buspirone (0.5–2.0 mg/kg, IP) pretreatment on the acquisition of cocaine (5.0 mg/kg, SC) CPP. The results are expressed as the time spent (s) on the cocaine-paired side pre- and postconditioning and are presented as mean ± SEM ($n = 10$). Data were analyzed by two-factorial ANOVA at each dose, followed by post hoc Dunnett's *t*-test for significant change in the time spent on the cocaine-conditioned side. $^{**}p < 0.01$.

Effect of Buspirone on the Expression of Cocaine CPP

Figure 4 shows the results from the expression experiment. Following conditioning with cocaine (5.0 mg/kg), animals pretreated (for 30 min) with the buspirone vehicle prior to the postconditioning test demonstrated a significant [$F(2,18) = 22.317$, $p < 0.01$] shift in preference behavior to the cocaine-associated side from 288 ± 13 s (preconditioning) to 417 ± 28 s (postconditioning). The shift in preference behavior was also significant [$F(2,18) = 7.823$, $p < 0.01$] 24 h following the postconditioning test, when the subjects were tested without buspirone pretreatment (i.e., to 374 ± 19 s).

In line with the acquisition data, buspirone failed to affect the expression of cocaine CPP. Irrespective of buspirone pretreatment (0.5, 1.0, or 2.0 mg/kg), on the postconditioning test day, cocaine conditioning produced a significant [$F(2,18) = 4.936$, $F(2,18) = 5.005$, and $F(2,18) = 19.642$, $p < 0.05$ for 0.5 and 1.0 mg/kg buspirone and $p < 0.01$ for 2.0 mg/kg buspirone, respectively] shift in preference behavior for the cocaine-paired chamber [285 ± 16 s (preconditioning) to 360 ± 32 s (postconditioning), 295 ± 17 s to 360 ± 24 s, and 274 ± 14 s to 394 ± 26 s, respectively, for 0.5, 1.0, and 2.0 mg/kg buspirone]. This preference for the cocaine-paired chamber was also significant [$F(2,18) = 13.290$, $F(2,18) = 8.829$, and $F(2,18) = 10.586$, $p < 0.01$ for 0.5, 1.0, and 2.0 mg/kg buspirone, respectively] when the animals were tested in a buspirone-free state

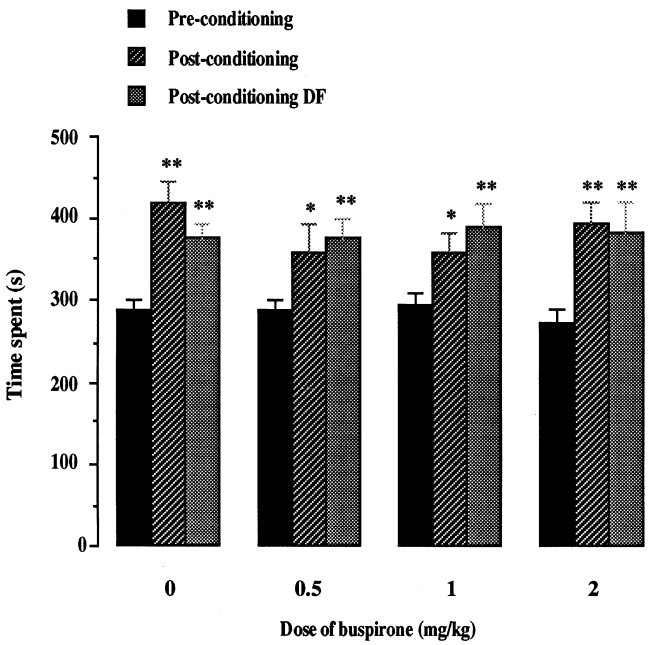

FIG. 4. The effect of buspirone (0.5 –2.0 mg/kg, IP) pretreatment on the expression of cocaine (5.0 mg/kg, SC) CPP. The results are expressed as the time spent (s) on the cocaine-paired side pre- and postconditioning and are presented as mean ± SEM (n = 10). Data were analyzed by two-factorial ANOVA at each dose, followed by post hoc Dunnett's t-test for significant change in the time spent on the cocaine-conditioned side. Postconditioning DF (drug free) represents the response 24 h after buspirone pretreatment. *$p < 0.05$. **$p < 0.01$.

24 h later (377 ± 24 s, 388 ± 28 s, and 384 ± 35 s, respectively, for 0.5, 1.0, and 2.0 mg/kg buspirone).

DISCUSSION

The cocaine-treated mice consistently maintained contact with the environment in which it was administered, thus demonstrating, as in previous studies (18,20), that cocaine is able to produce a robust reinforcing effect following subcutaneous administration (10) demonstrated by an increase in approach to the cocaine-paired chamber at the expense of the vehicle-paired chamber. At the dose of cocaine used, there was no evidence for the development of stereotyped behaviour.

The present data suggest that the 5-HT_{1A} receptor agonist, buspirone, lacks motivational properties. When administered alone, it failed to induce any statistically significant changes in the conditioned place preference paradigm at doses that have been shown previously to have an agonist effect at the 5-HT_{1A} receptor (15). This supports the findings of File (9) who failed to demonstrate a place preference with buspirone in the rat. Montgomery et al. (17) also failed to demonstrate the rewarding properties of buspirone using the intracranial self-stimulation technique, where 8-OH-DPAT produced an enhancement in responding for variable interval, threshold-current self-stimulation of the rat lateral hypothalamus. Similarly, in self-administration studies where rhesus monkeys were trained to self-administer cocaine, buspirone did not reinforce self-administration when substituted for cocaine (2). Additionally, Eison (8) examined the physical dependence properties of buspirone, using rats chronically treated with buspirone or diazepam and measuring weight loss as an indication of the withdrawal syndrome. Animals withdrawn from daily treatment with buspirone demonstrated an enhancement in body weight, whereas diazapam-treated subjects lost weight 24 h following withdrawal, suggesting that buspirone did not produce diazepam-like physical dependence.

In contrast to the present findings, Neisewander et al. (19) were able to demonstrate a place preference with buspirone in the rat. This discrepancy was attributed to procedural variations because Neisewander et al. (19) used a greater number of conditioning trials and a higher concentration of buspirone than File (9) (1.0 and 3.0 mg/kg, compared with 0.25—1.0 mg/kg). Such differences cannot account for the disparity of the present results because the procedures employed and the doses of buspirone, which have been shown previously to be effective in the BKW strain of mouse (5), were very similar. However, the present study utilized mice and hence a species difference, as has been shown clearly in place preference studies utilizing ethanol, cannot be excluded (7).

In addition to an affinity for the 5-HT_{1A} receptor, buspirone has been shown to have affinity for dopamine receptors and appears to block presynaptic dopamine receptors (16), although recent evidence also indicates that it acts as a dopamine D_2 receptor antagonist because it blocks the dopamine D_2 receptor-mediated discriminative stimulus effects of apomorphine in the rhesus monkey (11). However, in the rat, buspirone did not affect the discriminative stimulus properties of cocaine, suggesting that it lacks sufficient anti-dopaminergic activity at the doses tested (24). This may also explain the failure of buspirone to block cocaine CPP in the present study, although it may be argued that, unlike apomorphine, the reinforcing properties of cocaine may not be mediated via the D_2 dopamine receptor (28), hence any blockade of these receptors by buspirone may not necessarily be expected to block the CPP response.

The prototypic 5-HT_{1A} receptor agonist, 8-OH-DPAT, has been shown to possess motivational properties (17,21,26). Low doses of 8-OH-DPAT produce place preference, which is blocked by the preferential 5-HT_{1A} antagonist, spiperone, but not by the D_2 dopamine receptor antagonist sulpiride, suggesting that 8-OH-DPAT-induced CPP involves the 5-HT_{1A} receptor. However, Papp and Willner (21) demonstrated that 8-OH-DPAT-induced CPP was blocked by pimozide and sulpiride, implicating the dopaminergic system in 8-OH-DPAT-induced reward, and suggesting that, at low doses, 8-OH-DPAT acts through 5-HT neurones to disinhibit dopaminergic activity (17).

Buspirone may influence the 5-HT system through two distinct mechanisms; first, it interacts with the presynaptic 5-HT_{1A} receptor (autoreceptor), and second, it acts as a partial agonist at postsynaptic 5-HT_{1A} receptors to reduce 5-hydroxytryptaminergic activity (22). Although there is evidence to suggest that the 5-HT (3,4,13) and, more specifically, the 5-HT_{1A} (23) receptor may be involved in the facilitation of cocaine reinforcement, this remains speculative, because the results of the present investigation do not substantiate this suggestion, given that buspirone failed to modify either the acquisition or the expression of cocaine-induced CPP.

Although it was suggested (6) that the postconditioning expression of the CPP response may be reflective of the self-administration procedure, this does not appear to be the case, because the results obtained in the present investigation do not support the self-administration findings with cocaine and 8-OH-DPAT (23).

CONCLUSIONS

The present series of experiments, in line with behavioral (2,8,9,17) and clinical (1) data, have demonstrated a failure of buspirone to produce reinforcement, which suggest that buspirone lacks abuse potential. Exposure to cocaine produced a robust conditioned place preference response. However, neither the acquisition nor the expression of the response was affected by buspirone pretreatment. Overall, these findings suggest that the acquisition and expression of cocaine CPP in the mouse may be mediated through neural mechanisms that are not affected by buspirone.

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