J. Pharm. Pharmacol. 2000, 52: 977–981
Received January 10, 2000
Accepted March 28, 2000

Tiospirone and the Reinforcing Effects of Cocaine in the Conditioned Place Preference Paradigm in Rats

MARIA PIA AROLFO AND BRIAN A. MCMILLEN

Center for Alcohol and Drug Abuse Studies, Department of Pharmacology, Brody School of Medicine at East Carolina University, Greenville, NC 27858, USA

Abstract

Tiospirone (TSP) is an atypical antipsychotic drug. It has 5HT-2 antagonistic properties as well as affinity for D2, 5HT-1a, 5HT-6 and sigma receptors.

Behavioural studies in our laboratory, which used a 24 h free access to food and fluids paradigm, showed a decreased alcohol and increased food intake after twice-daily administration of TSP; the maximal effect was obtained at a dose of 0·48 mg kg^{-1}. This study used the conditioned place preference paradigm to determine the effect of TSP on the reinforcing properties of cocaine. Intraperitoneal administration of 5·0 mg kg^{-1} cocaine, but not saline, increased the time rats spent in the drug-paired compartment of a three-compartment shuttle box by 104·9%. Two doses of TSP, 0·143 and 0·48 mg kg^{-1}, were tested subcutaneously 60 min before saline or cocaine administration during the conditioning phase only. A dose-response effect was observed with a significant reduction in the time rats spent in the cocaine-paired compartment on the drug-free test day produced by the dose of 0·48 mg kg^{-1} (an increase of only 38·1% when post-conditioned times were compared with preconditioned times).

These findings suggest that TSP reduces the reinforcing properties of cocaine exhibited in the conditioned place preference paradigm.

Tiospirone (8-{4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl]butyl}-8-azaspiro (4·5) decane-7,9-dione hydrochloride, TSP) is an atypical antipsychotic drug and an analogue of buspirone. Clinical studies have shown its effectiveness as an atypical antipsychotic drug in schizophrenic patients (Jain et al 1987; Moore et al 1987). The term 'atypical antipsychotic' is used to denote an agent that is effective in tests predictive of antipsychotic activity, such as inhibition of a conditioned avoidance response, with weak effects in tests for extrapyramidal risk, such as the induction of catalepsy (McMillen 1985; Jain et al 1987). TSP, unlike classical antipsychotic drugs, is six to eight times more potent for inhibition of conditioned avoidance response than for induction of catalepsy (Riblet et al 1982a). It shows anti-dopaminergic properties such as inhibition of the conditioned avoidance response in rats and blockade of the stereotyped effects of dopamine agonists, including apomorphine in rats (Riblet et al 1982a) and amphetamine in dogs (Eison et al 1984). Unlike classical neuroleptics, it does not cause supersensitivity of dopamine receptors on subchronic administration (Riblet et al 1982b; McMillen et al 1983). TSP is a 5HT-2 antagonist with affinity for D2, 5HT-1a, 5HT-6 and sigma receptors (Borison et al 1989; Meltzer et al 1989a,b; Roth et al 1994; Lesage et al 1995; Sumiyoshi et al 1995). Furthermore, TSP is potentially sedative as it potentiates central nervous system depressants and reduces shock-elicited aggression in mice. It also reduces spontaneous motor activity in rats, and at high doses induces muscle weakness and motor incoordination (Eison et al 1985). Behavioural studies in our laboratory showed that rats decreased volitional consumption of alcohol and increased food intake after twice-daily administration of TSP in a 24 h access paradigm. The maximal effect was obtained at a dose of 0·48 mg kg^{-1} (McMillen et al 1996). Recently, we demonstrated that although 0·143 and 0·48 mg kg^{-1} TSP (0·3 and 1·0 μmol kg^{-1}, respectively) significantly decreased the number of food reinforcements obtained by rats in

Correspondence: B. A. McMillen, Department of Pharmacology, Brody School of Medicine, East Carolina University, Greenville, NC 27858, USA.
E-Mail: mcmillen@brody.med.ecu.edu

an FR5 schedule, these doses also induced signs of akinisia and catalepsy (Arolfo & McMillen 1999). These studies support the idea that TSP actions are mediated through its affinity for 5HT-2, 5HT-1a, 5HT-6 and D2 receptors.

As experiments in our laboratory have shown that TSP decreased alcohol consumption in rats, this study was designed to investigate the ability of this drug to inhibit the rewarding effects of cocaine, another drug of abuse. The rewarding effects of cocaine are widely believed to be mediated by dopaminergic activity in the mesolimbic system (Koob 1992). In this experiment, the conditioned place preference paradigm was used. This model is an effective method for assessing the rewarding effects of different drugs or stimuli (Hoffman 1989) and testing the potential treatments for drugs of abuse (Nomikos & Spyraki 1987; Kosten & Nestler 1994; Jones & McMillen 1995).

Materials and Methods

Drugs

Cocaine–HCl was purchased from Sigma Chemical Co. (St Louis, MO). Cocaine was dissolved in 90% H_2O, 10% 0·1 M sodium citrate, pH 6·3. TSP–HCl (TSP) was supplied by Bristol-Myers Squibb (Wallingford, CT). TSP was dissolved in distilled water and acetic acid.

Rats

Forty male Sprague-Dawley rats (Harlan Sprague-Dawley, Frederick, MD), initially weighing 200–250 g, were group-housed (two to four per cage) in a noise-free, temperature (21°C)-controlled environment with a 14 : 10 h light–dark cycle. The rats remained in this room for the duration of the experiment. Food (Purina rat chow) and water were freely available.

Conditioned place preference

The behavioural training and testing apparatus was a painted, rectangular wooden box consisting of three compartments. The two large outer compartments (34 × 25 cm) were separated by guillotine doors from a small central area (11 × 25 cm) that served as a neutral or choice point. Each of the two large compartments was distinctive in wall and floor covering. One compartment consisted of a grid floor and a gray wall with a black line on it, the other compartment consisted of a mesh floor and a black wall with a white circle on it.

Protocol to establish a cocaine-conditioned place preference

For three consecutive days (preconditioning phase) individual rats were placed in the neutral compartment before the guillotine doors were opened, and then the doors were removed to allow free access to each compartment of the box for 15 min (phase 1). On the third day the time spent in each compartment of the shuttle box was recorded (using the BEHAVIOR program written by L. W. Means, East Carolina University, NC). Phase 1 determined the preconditioned place preference for each rat. During phase 2 (the conditioning phase) the rats were alternately administered i.p. injections of saline or cocaine–HCl (5 mg kg^{-1}) for 8 days. Previous reports from our laboratory showed that rats develop cocaine-conditioned place preference for doses of 2·5, 5·0 and 10·0 mg kg^{-1} (Jones & McMillen, 1995). We used a dose of 5·0 mg kg^{-1} cocaine–HCl in this study. On training days 1, 3, 5 and 7, an intra peritoneal injection of cocaine was administered immediately before the rat was confined to the least preferred compartment for 15 min. On days 2, 4, 6, and 8, saline was administered before the rat was placed in the initially preferred side of the shuttle box for 15 min. One group received saline each day during the training phase in order to test the effect of handling on behaviour and to serve as the control. On the test day (phase 3, the post-conditioning phase) no drugs were administered. The drug-free rat was placed in the neutral compartment before opening the guillotine doors to allow free access to the three chambers. The time each rat spent in each chamber was recorded in order to establish the post-conditioned place preference.

Protocol to investigate the effects of TSP on the conditioned place preference

Three groups of rats were trained in the conditioned place preference paradigm as outlined above. However, TSP (0·143 or 0·48 mg kg^{-1}) or saline was administered subcutaneously to each rat 60 min before injection of cocaine or saline each day during the 8-day conditioning phase. On the test day, post-conditioning phase, the drug-free rats were placed in the neutral chamber before opening the guillotine doors to allow free access to the three chambers. The time spent by each rat in each chamber was recorded to establish the post-conditioned place preference.

Statistical analysis

The data for the behavioural test comparing preconditioned place preference to post-conditioned

place preference for saline- and saline/cocaine-treated rats were compared with a paired-sample t-test. The data for conditioned place preference after saline/cocaine and TSP/cocaine-treated rats were analysed with a two-way repeated measure analysis of variance and a Newman-Keuls post-hoc test (Zar 1984). The accepted level of significance for all tests was $P < 0.05$, two-tailed. All statistical analyses were run on GB-STAT software (Dynamic Microsystems, Silver Spring, MD).

Results

The dose of 5·0 mg kg^{-1} cocaine–HCl significantly increased the amount of time the rats spent in the initially least preferred (cocaine-paired) compartment by 104·9%, $P < 0.01$ (Figure 1). Furthermore, the cocaine-treated rats increased their activity in the conditioned place preference apparatus as their number of chamber entries increased from 42.0 ± 5.4 to 67.8 ± 6.9 ($P < 0.01$). Saline-treated rats did not develop a place preference for the initially least preferred compartment and did not change the frequency of chamber entries. This result shows that the handling of the rats alone did not change their place preference.

For the experimental groups which received either saline or TSP pretreatments, analysis of variance revealed a significant change between pre- and post-conditioning (F1, 17 = 17·966, $P < 0.001$). Figure 2 shows that rats pretreated with saline or 0·143 mg kg^{-1} TSP 60 min before treatment with 5·0 mg kg^{-1} cocaine–HCl and conditioned to the least preferred compartment, significantly increased the time they spent in the cocaine-paired compartment (75%, $P < 0.01$, and 126·3%, $P < 0.05$, respectively). Rats pretreated with 0·48 mg kg^{-1} TSP did not significantly increase the time they spent in the least preferred cocaine-paired compartment (38·1% increase, not significant). The difference between preconditioned and post-conditioned time in the least preferred compartment decreased with the increase in the TSP dose (Figure 3). A dose–response effect was observed in that the higher dose of TSP produced a result that was not significantly different from either the preconditioned time or the increased time observed after conditioning in the saline/cocaine group.

Finally, there were no statistical differences in the frequency of chamber entries for any

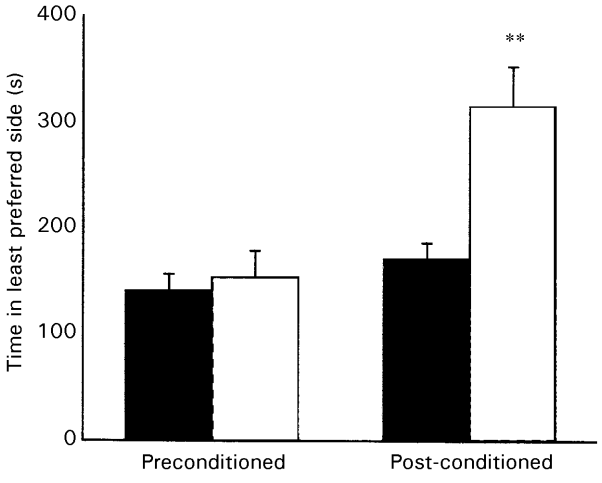

Figure 1. Time spent by rats in the initially least preferred compartment before and after conditioning with saline only or 5 mg kg^{-1} cocaine. Saline only ■, cocaine/saline alternating □. Data represent mean ± s.e.m. ** $P < 0.01$ compared with preconditioned values, paired t-test.

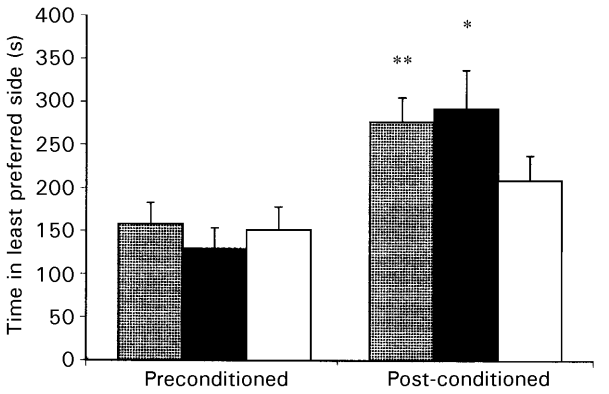

Figure 2. Time spent by rats in the initially least preferred compartment before and after pretreatment with saline (▩), 0·143 mg kg^{-1} TSP (■) or 0·48 mg kg^{-1} TSP (□) and conditioned with 5 mg kg^{-1} cocaine. Data represent mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$ compared with preconditioned values, Newman-Keuls test.

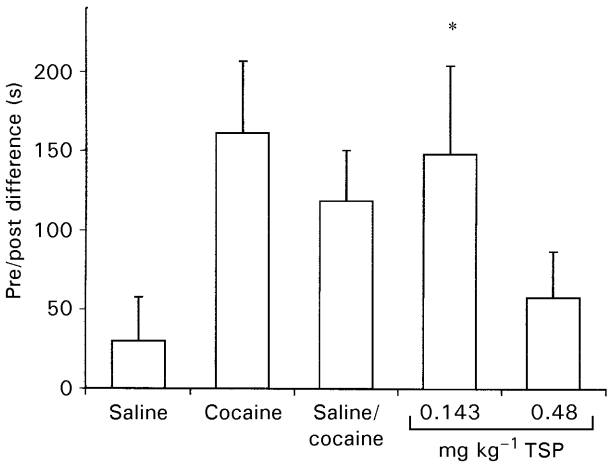

Figure 3. Difference between preconditioned and post-conditioned time (s) spent in the least preferred compartment for each group (saline, cocaine, saline/cocaine, 0·143 mg kg^{-1} TSP and 0·48 mg kg^{-1} TSP). Data represent mean ± s.e.m. * $P < 0.05$ compared with saline.

group, either between preconditioning and post-conditioning (F1, $17 = 4{\cdot}474$, not significant) or as the result of drug treatment (F2, $36 = 0{\cdot}727$, not significant). All of the groups had a small increase, 8–13, in chamber entries during the post-conditioning test, but the variance in this measure prevented these data from reaching a significant level.

Discussion

Our results suggest that pretreatment with TSP attenuates the development of a cocaine-conditioned place preference in a dose-dependent fashion. When increasing the pretreatment dose of TSP, a decrease in the difference between time spent in the least preferred compartment before and after treatment with $5\,mg\,kg^{-1}$ cocaine–HCl was observed. In this study a conditioned place preference was achieved with a dose of $5\,mg\,kg^{-1}$ of cocaine–HCl. The group treated with saline instead of cocaine showed no significant difference in the time spent in the least preferred compartment before and after the conditioning phase. This indicates that the increased time spent in the initially least preferred compartment was not due to handling or reduced aversion to the least preferred compartment.

The doses of TSP used in this study were previously tested in a food reinforcement paradigm. In that study we demonstrated that $0{\cdot}143$ and $0{\cdot}48\,mg\,kg^{-1}$ TSP significantly decreased the number of food reinforcements obtained by food-deprived rats in an FR5 schedule. These doses also induced akinisia and catalepsy (Arolfo & McMillen 1999). One could argue that the akinetic and cataleptic effects of the doses of TSP used were responsible for the decrease in time spent in the least preferred compartment after conditioning with $5\,mg\,kg^{-1}$ cocaine–HCl. This is unlikely, however, as although rats may have shown decreased locomotor activity during the conditioning phase, the test sessions were conducted in a drug-free state. If anything, the rats were more active in the chambers during the post-conditioning test. In our interpretation of the results, TSP reduces the rewarding effects of cocaine in a conditioned place preference paradigm. This interpretation supports the conclusions drawn for the consumption of alcohol, another drug of abuse, in previous experiments in our laboratory. In those studies it was demonstrated that TSP decreased alcohol intake and increased food intake after twice-daily administration of the drug and measurement of consumption in periods of 24 h in rats, with the maximal effect obtained at a dose of $0{\cdot}48\,mg\,kg^{-1}$ (McMillen et al 1996).

The hypothesis that the dopaminergic mesolimbic pathway mediates the rewarding effect of drugs is supported by extensive evidence (Koob 1992). Although the exact mechanism of action of TSP in the mesolimbic pathway is not clearly known, it may indirectly influence the mesolimbic system through serotonergic connections. This idea is supported by studies that demonstrated that clozapine attenuates the cocaine-conditioned place preference (Kosten & Nestler 1994) and ritanserin inhibits a place preference induced by (+)-amphetamine (Nomikos & Spyraki 1987).

In general, our conclusions seem to be in accordance with the view of other authors that there must be multiple reward systems that function independently and are organized parallel with one another. The fact that TSP decreases alcohol consumption (McMillen et al 1996) as well as reducing the rewarding effects of cocaine in a conditioned place preference paradigm makes this drug a possible candidate in the psychotherapy of drug abuse. This is especially important since the abuse of cocaine is often accompanied by abuse of alcohol and other depressants. An agent that diminishes the reward components of both abused drugs has a great advantage as an adjunct to psychotherapy.

Acknowledgements

We thank L. W. Means for the use of his BEHAVIOR program and H. L. Williams for her technical assistance. This work was supported by a grant from the NC Governor's Institute of Alcohol and Drug Abuse, Inc. awarded to M. P. Arolfo.

References

Arolfo, M. P., McMillen, B. A. (1999) Effects of amperozide and tiospirone, atypical antipsychotic 5HT-2 drugs, on food reinforced behavior in rats. Physiol. Behav. 68: 93–98

Borison, R. L., Sinha, D., Haverstock, S., McLernon, M. C., Diamond, B. I. (1989) Efficacy and safety of tiospirone vs haloperidol and thioridazine in a double-blind, placebo-controlled trial. Psychopharm. Bull. 25: 190–193

Eison, M. S., Taylor, D. P., Riblet, L. A., Temple, D. L., Jr (1984) Determination of functionally-relevant serum levels of MJ 13859-1 in the dog: relationship to blockade of amphetamine stereotypy. Meth. Find. Exp. Clin. Pharmacol. 6: 255–259

Eison, M. S., Minielli, J. L., New, J. S., Taylor, D. P. (1985) BMY 13859. Drugs Future 10: 731–732

Hoffman, D. C. (1989) The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Res. Bull. 23: 373–387

Jain, A. K., Kelwala, S., Moore, N., Gershon, S. (1987) A controlled clinical trial of tiospirone in schizophrenia. Int. Clin. Psychopharm. 2: 129–133

Jones, E. A., McMillen, B. A. (1995) Amperozide attenuates the reinforcing effects of cocaine in the conditioned place preference paradigm in rats. Pharmacol. Sci. 1: 471–474

Koob, G. F. (1992) Neural mechanisms of drug reinforcement. Ann. N.Y. Acad. Sci. 654: 171–191

Kosten, T. A., Nestler, E. J. (1994) Clozapine attenuates cocaine conditioned place preference. Life Sci. 55: 9–14

Lesage, A. S., De Loore, K. L., Peeters, L., Leysen, J. E. (1995) Neuroprotective sigma ligands interfere with the glutamate-activated NOS pathway in hippocampal cell culture. Synapse 20: 156–164

McMillen, B. A. (1985) Acute and subchronic effects of MJ-13859, a potential antipsychotic drug, on rat brain dopaminergic function. J. Pharmacol. Exp. Ther. 233: 369–375

McMillen, B. A., Matthews, R. T., Sanghera, M. K., Shepard, P. D., German, D. C. (1983) Dopamine receptor antagonism by the novel anti-anxiety drug, buspirone. J. Neurosci. 3: 733–738

McMillen, B. A., Williams, H. L., Myers, R. D. (1996) Effect of clozapine, BMY 14802 and tiospirone on the volitional consumption of alcohol by rats. Soc. Neurosci. Abstr. 22: 1927

Meltzer, H. Y., Matsubara, S., Lee, J. C. (1989a) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D1, D2 and serotonin2 pKi values. J. Pharmacol. Exp. Ther. 251: 238–246

Meltzer, H. Y., Matsubara, S., Lee, J. C. (1989b) The ratios of serotonin2 and dopamine2 affinities differentiate atypical and typical antipsychotic drugs. Psychopharmacol. Bull. 25: 390–392

Moore, N. C., Meyendorf, E., Yeragani, V., LeWitt, P. A., Gershon, S. (1987) Tiospirone in schizophrenia. J. Clin. Psychopharmacol. 7: 98–101

Nomikos, G., Spyraki, C. (1987) Effect of ritanserin on the rewarding properties of d-amphetamine, morphine and diazepam revealed by conditioned place preference in rats. Pharmacol. Biochem. Behav. 30: 853–858

Riblet, L. A., Eison, M. S., Taylor, D. P., Temple, D. L., Yevich, J. P. (1982a) Pharmacological profile of a potential antipsychotic agent: MJ-13859-1. Soc. Neurosci. Abstr. 8: 470

Riblet, L. A., Taylor, D. P., Eison, M. S., Stanton, H. C. (1982b) Pharmacology and neurochemistry of buspirone. J. Clin. Psychiat. 43: 11–16

Roth, B. L., Craigo, S. C., Choudhary, M. S., Uluer, A., Mosma, F. J. Jr, Shen, Y., Meltzer, H. Y., Sibley, D. R. (1994) Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptors. J. Pharmacol. Exp. Ther. 268: 1403–1410

Sumiyoshi, T., Suzuki, K., Sakamoto, H., Yamaguchi, N., Mori, H., Shiba, K., Yokogawa, K. (1995) Atypicality of several antipsychotics on the basis of in-vivo dopamine-D2 and serotonin-5HT2 receptor occupancy. Neuropsychopharmacology 12: 57–64

Zar, J. H. (1984) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs