ELSEVIER

Pharmacology Biochemistry and Behavior, Vol. 53, No. 2, pp. 243-247, 1996

0091-3057/96 $15.00 + .00

0091-3057(95)00203-0

Conditioned Taste Aversion is a Confound in Behavioral Studies that Report a Reduction in the Reinforcing Effects of Drugs

WILLIAM J. PIZZI[1] AND DAVID F. COOK

Neuropsychology Laboratory, Northeastern Illinois University, Chicago, IL 60625

Received 19 December 1994; Revised 7 March 1995; Accepted 9 March 1995

PIZZI, W. J. AND D. F. COOK. *Conditioned taste aversion is a confound in behavioral studies that report a reduction in the reinforcing effects of drugs.* PHARMACOL BIOCHEM BEHAV **53**(2) 243-247, 1996. – Pharmacologic agents with a potential to attenuate the reinforcing properties of drugs of abuse may have an important role in the treatment of drug addiction. The reduction of drug self-administration and sweet solution intake are two common animal models employed to screen for promising therapeutic agents. When these agents are effective in suppressing the behavior maintained by drugs of abuse, the cause is usually attributed to a neuronal mechanism such as the modification of neurotransmitters that subserve reinforcement. These experiments present data for an alternate interpretation which suggest that some of these agents produce a conditioned taste aversion (CTA) that acts as a confounding variable in the screening of potential therapeutic agents. Both carbamazepine and isradipine were shown to establish a CTA at doses reported to attenuate the reinforcing properties of drugs of abuse. It is concluded that CTA represents a potential experimental confound in studies of pharmacologic agents that appear to attenuate the reinforcing properties of drugs. These results suggest that screening for a CTA is necessary in any paradigm that measures the suppression of consummatory behavior in response to pharmacologic intervention.

Carbamazepine Isradipine Cocaine Conditioned taste aversion Reinforcement Drugs of abuse Sweet solution consumption

RECENTLY, a number of reports have claimed that certain pharmacologic agents produce a general suppressant effect on behavior maintained by cocaine (2,5,9,10) and morphine (4). The suppression of behavior in these reinforcement paradigms is usually attributed to a modification of brain monoamine neurotransmitters that subserve brain reward mechanisms, especially dopamine (DA). It would appear that agents acting as DA antagonists should effectively reduce the reinforcing properties of cocaine and morphine. In clinical situations, a major concern with this approach is that many DA antagonists are likely to produce unwanted side-effects or serious toxicity. Unwanted side-effects lead to patient noncompliance, and serious toxicity would make the treatment inappropriate. This situation has led to a continuing search for pharmacologic agents that possess little toxicity while effectively reducing the reinforcing properties of drugs of abuse.

In screening for drugs with potential therapeutic value, a reinforcement paradigm is often used in which an experimental animal is required to reduce drug self-administration or reduce its intake of a highly palatable solution. When a pharmacologic agent results in the diminution of the reinforced behavior, its effects are generally attributed to its known or putative interaction with a neurotransmitter system subserving reinforcement. An alternate explanation for the reduction in a particular behavior is that it may be the result of a toxic effect produced by the agent being tested. In this situation, an agent that produced a toxic effect (unknown to the experimenter) would represent an experimental confound.

Recently, two unrelated pharmacologic agents have been reported to attenuate the reinforcing properties of cocaine, morphine, and a highly palatable solution of glucose and saccharin (G + S). Carbamazepine (CBZ), an antiepileptic agent,

[1] Requests for reprints should be addressed to W. J. Pizzi, Neuropsychology Laboratory, Northeastern Illinois University, 5500 N. St. Louis Ave., Chicago, IL 60625.

has been reported to effectively reduce cocaine self-administration and the consumption of G + S (2). Several reports from different laboratories have claimed that the dihydropyridine calcium antagonist isradipine (ISR) effectively reduces the rewarding properties of cocaine (5,7), morphine (4,5), and G + S (1). These studies employed several different behavioral paradigms including the consumption of sweet solutions (1), conditioned place preference (4,7), and drug self-administration (2,5).

In this article, we present the results of several experiments using a conditioned taste aversion (CTA) paradigm to determine whether CBZ or ISR is capable of producing a toxic effect. Following a standard test to determine whether a CTA was established, we tested the animals on a reinforcing glucose and saccharin solution to determine whether the CTA would generalize to this highly palatable solution. If CBZ and ISR lead to the establishment of a CTA, we will have demonstrated an important experimental confound that calls into question the mechanism by which these agents produce changes in consummatory behavior.

METHODS

Subjects

The subjects in these experiments were female Sprague-Dawley rats obtained from Holtzman Farms (Madison, WI). Each treatment group consisted of six rats with body weights between 250 and 300 g. Animals were individually housed in polycarbonate cages with ad lib access to food and tapwater before the onset of the experiments. The colony was maintained on a 12 L : 12 D cycle throughout the experiment.

Drugs

CBZ was purchased from Sigma Chemical Co. (St. Louis, MO) and mixed in corn oil with a Brinkman homogenizer (Westbury, NY). ISR was a gift from Sandoz Pharmaceuticals (East Hanover, NJ). ISR was dissolved in dimethyl sulfoxide (DMSO) or mixed in corn oil.

Procedure

Animals were adapted to the laboratory for a minimum of 7 days with ad lib access to food and tapwater. At the beginning of each experiment, the animals were randomized into treatment or vehicle control groups. The rats were deprived of water for 23 h and 50 min for a 4-day period to establish a baseline for water intake in the deprivation condition, which was in force throughout the experiment. The 10-min access to all solutions was carried out in separate test cages without food available during the test periods. On conditioning days (saccharin + agent) the animals were returned to their home cages without pelleted food for the 1st h. All solutions were presented in the test cages from a standard drinking bottle. Fluid intake during these test sessions was measured by weighing bottles before and after the test presentations. Water consumption on test day 4 was used as the animals' baseline consumption of tapwater. On test day 5, the animals were given a 0.125% saccharin solution during the 10-min test period followed by the administration of the test agent (CBZ or ISR) within 30 min. On days 6 and 7, the animals were given tapwater during their drinking sessions to allow recovery from any residual sickness. On test day 8, the animals were again presented with the 0.125% saccharin solution to determine whether a conditioned taste aversion had been established. The animals were again presented with tapwater during their drinking session on day 9. On test day 10, the animals were presented with a glucose (3%) + saccharin (0.125%) solution to determine whether the CTA would generalize to this highly palatable solution.

Each experiment used six animals per treatment level. CBZ was administered via oral gavage at doses of 0, 10, 40, and 160 mg/kg. In an initial experiment, ISR was dissolved in corn oil and administered via oral gavage at doses of 0 and 30 mg/kg or dissolved in DMSO and administered via intraperitoneal (IP) injection at doses of 0 and 30 mg/kg. A second experiment tested for the presence of a CTA following the administration ISR at 1, 3, and 10 mg/kg dissolved in DMSO and delivered by IP injections. All drugs were administered at a constant volume of 1 ml/kg body weight.

Data Analysis

In experiments using two groups in each treatment condition, a Student's *t*-test for independent groups was used. Experiments with three or more groups were analyzed using a one-way analysis of variance and post hoc testing employed the Bonferroni *t*-test. All significance levels are two-tailed. The InStat program was used for all calculations (Intuitive Software for Science, San Diego, CA).

RESULTS

These experiments were conducted in a fixed order for the two agents under consideration with the high dose of each

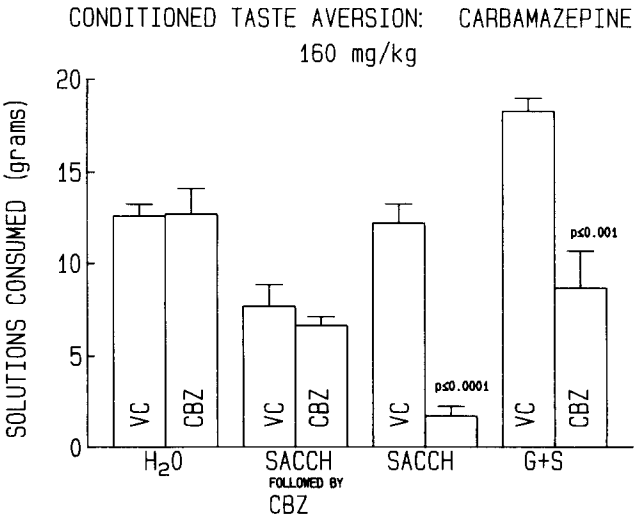

FIG. 1. Mean (SEM) fluid intake during a 10-min test session to test for the establishment of a CTA to carbamazepine (160 mg/kg). The first pair of bars represents baseline water intake on day 4 of the test protocol. The second pair of bars represents the intake of saccharin upon initial presentation as a novel taste stimulus. Immediately following the 10-min drinking session, the experimental animals were orally gavaged with 160 mg/kg CBZ (day 5). The third pair of bars represents the test day to determine whether a CTA was established to the saccharin solution (day 8). The final pair of bars represents a test of generalization of the CTA to a highly palatable glucose plus saccharin (G + S) solution (day 10). All statistical comparisons are with control groups using the same route of administration (Figs. 1–5).

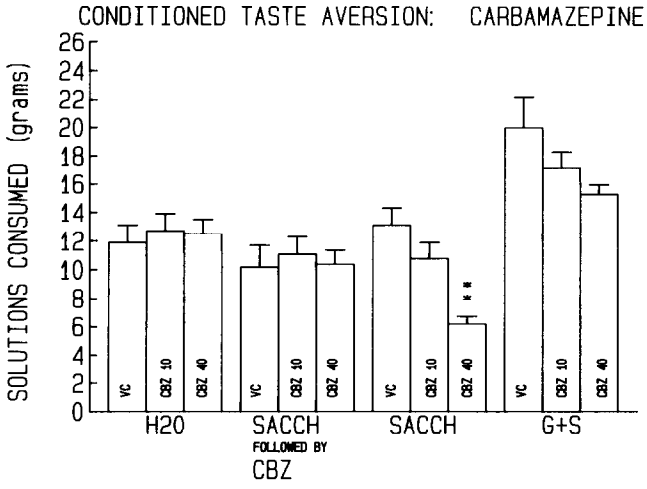

FIG. 2. Mean (SEM) fluid intake during a 10-min test session to test for the establishment of a CTA to carbamazepine (10 and 40 mg/kg, orally). The testing sequence and drug administration were the same as in Fig. 1. **Statistically significant difference in saccharin solution consumed ($p < 0.01$).

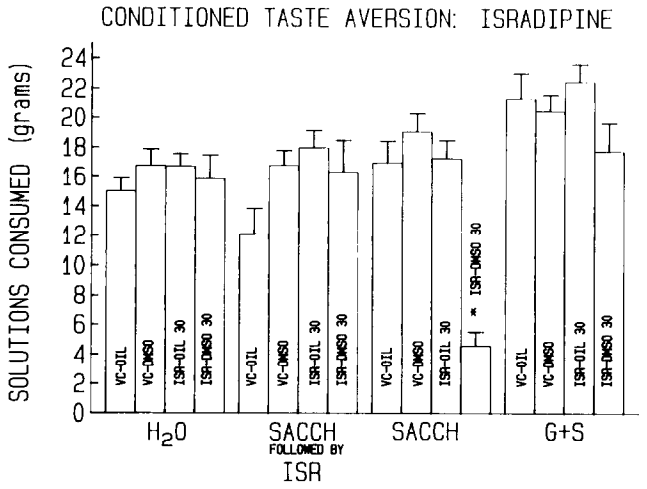

FIG. 3. Mean (SEM) fluid intake during a 10-min test session to test for the establishment of a CTA to isradipine (30 mg/kg). Testing sequence was the same as Fig. 1. Each test session was composed of two control groups (VC-Oil, orally, and VC-DMSO, IP) and the corresponding 30-mg/kg isradipine groups.

drug tested first and separately from a follow-up experiment. The second experiment with each agent was a range-finding experiment aimed at determining the dose which was ineffective at producing the CTA.

Figure 1 shows the results from the high-dose CBZ experiment. In this experiment, a CTA was established on the saccharin test day ($p < 0.0001$). This figure also shows an example of generalization of the CTA to the highly palatable G + S solution ($p < 0.001$).

The second experiment employed doses of 10 and 40 mg/kg of CBZ. These doses were chosen because 10 mg/kg is the approximate dose that protects 50% of rats from seizures in a maximal electroshock test. Figure 2 shows that the 10-mg/kg dose failed to produce a CTA, whereas 40 mg/kg did produce a CTA ($p < 0.01$). Neither of these doses of CBZ resulted in a generalization of the CTA to G + S consumption.

Figure 3 shows the results obtained with the highest dose of ISR used in our experiments (30 mg/kg). This experiment employed two control groups: one for the oil vehicle used in the oral gavage administration, and a second control group to determine whether the DMSO vehicle would produce a CTA. Only the ISR dissolved in DMSO and administered IP was able to produce a CTA. This CTA did not generalize to the G + S test, although there was a slight reduction in G + S consumption.

In the second ISR experiment, doses of 1, 3, and 10 mg/kg were used because the highest of these doses has been reported to be effective in reducing the reinforcing effects of cocaine and morphine, whereas the low dose was reported to be ineffective. Figure 4 shows that the two higher doses (3 and 10 mg/kg, IP, of ISR dissolved in DMSO) resulted in the establishment of a CTA. As with the 30-mg/kg dose of ISR, the CTA did not generalize to the G + S solution.

Figure 5a shows the dose-response relationship that existed between various doses of CBZ and the establishment of the CTA. Figure 5b shows a similar dose-response curve for the various ISR doses.

DISCUSSION

These experiments demonstrate that both CBZ and ISR are capable of producing a conditioned taste aversion at doses that other reports claim to be effective in reducing the reinforcing effects of cocaine and morphine. Because the establishment of a CTA has been proposed as a reliable indicator of a drug's toxicity (8), these findings suggest that the suppression of behavior seen in other reports may actually be the result of the toxic effects of these agents on animals. This conclusion is supported by the fact that the doses reported to

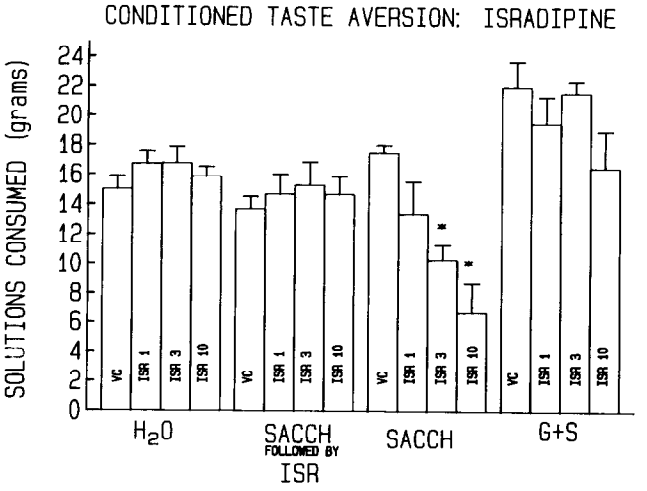

FIG. 4. Mean (SEM) fluid intake during a 10-min test session to test for the establishment of a CTA to isradipine (1, 3, and 10 mg/kg). The testing sequence was the same as Fig. 1. Isradipine was dissolved in DMSO and administered IP. Significant difference in saccharin solution consumed: *$p < 0.05$; **$p < 0.01$.

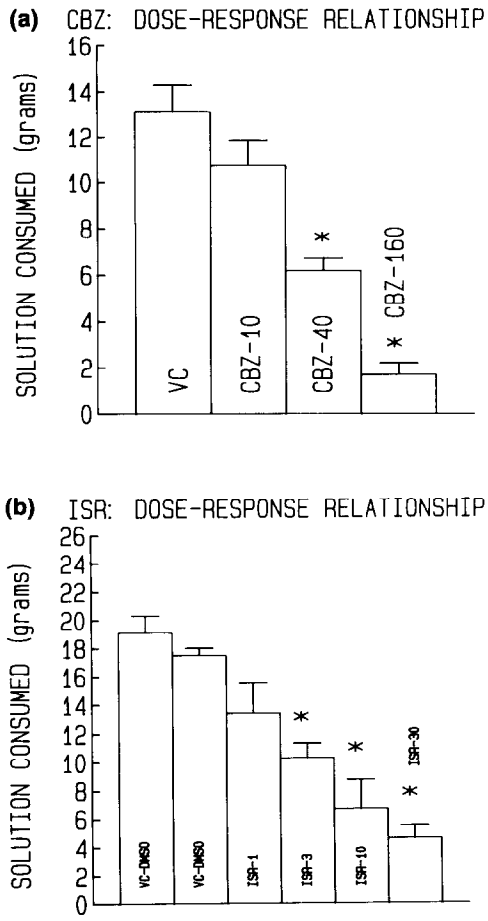

FIG. 5. (a and b) Mean (SEM) saccharin solution consumed on the CTA test day (8) showing a dose-response relationship between drug dose and reduction of saccharin intake (a = CBZ; b = ISR). In (a), the CBZ was administered by oral gavage. (b) The results of two separate experiments in which ISR was administered IP, Both DMSO control groups are represented; however, all statistical comparisons used their respective control groups. *Statistically significant decrease in saccharin solution consumed.

be effective in reducing the reinforcing effects of drugs of abuse overlap those that produce a CTA. ISR has been reported to be effective in blocking the reinforcing effects of cocaine at 3, 10, and 30 mg/kg, IP (1), whereas it blocks the reinforcing effects of morphine at 2.5 mg/kg, subcutaneously, in a place preference test (4). Likewise, the dose that has been reported to be ineffective (1 mg/kg ISR) does not establish a CTA.

In these experiments with ISR, the route of administration was critical. ISR dissolved in DMSO and administered by IP injection produced a CTA, whereas the highest dose of ISR (30 mg/kg), dissolved in corn oil and administered by oral gavage, did not produce a CTA. The studies reporting a reduction in drug self-administration or sweet solution intake following ISR all used the IP route of administration (1).

Although these data call into question the existing studies of ISR and its ability to reduce the reinforcing effects of drugs, they do not necessarily contradict the reported pharmacologic effects on various brain neurotransmitters. To demonstrate that any of these pharmacologic changes affect the reinforcing properties of drugs of abuse, it will be necessary to disentangle the confounding variable of CTA from any specific pharmacologic effects of ISR. Keeping in mind that ISR was developed as a compound to be administered orally, and that the 30-mg/kg oral dose used in this study did not produce a CTA, a replication of the drug self-administration paradigm with oral doses of ISR would support a pharmacologic interpretation of its effects on reinforcement. A second method that could in theory disentangle these two variables would be intraventricular (IT) administration of the drug. However, even if IT administration were technically feasible for a given agent, it would be necessary to screen this preparation to rule out the production of a CTA.

CBZ also produced a CTA at doses of ≥ 40 mg/kg in this study. It is important to recognize that the effective dose of CBZ that blocks 50% of seizures (ED_{50}) in the maximal electroshock paradigm (MES) for inducing seizures in rats is approximately 10 mg/kg. Thus, the doses used in the study by Carroll et al. (2) starting at 80 mg/kg and going as high as 160 mg/kg are outside of the normal therapeutic range for this drug. This makes any attempt to determine the neurochemical mechanism by which CBZ might be effecting the reinforcing properties of drugs an uncertain endeavor. Carroll et al. (2) were aware of the possibilities that extraneous variables might be involved in their experiments, and went so far as to suggest that a conditioned taste aversion may be one such variable. Furthermore, they pointed out that there is a slow return to baseline responding for cocaine or the sweet solution following CBZ treatment, which in turn suggests an aversive effect of the agent. The CBZ doses administered in this study also produced a decrease in locomotor activity, but did not reduce normal eating or drinking. This is consistent with what is known about the CTA paradigm in that the CTA is specific to a novel stimulus and does not generalize to other consummatory behaviors. In testing for the effects of CBZ on the reinforcing properties of drugs of abuse, it would seem that the 10-mg/kg dose representing the ED_{50} for MES should be the reference dose.

Finally, our study used female rats and involved a water deprivation procedure. This paradigm differs from the majority of drug self-administration studies that use male subjects and do not include water deprivation. These differences are not likely to represent a confound in our experiments, because males are more sensitive to the CTA procedure and water deprivation actually reduces the strength of a CTA [see (3) for review].

The experiments reported here underscore the importance of disentangling a potential experimental confound in experiments attempting to determine whether an agent can affect the reinforcing properties of drugs of abuse. This problem is likely to extend beyond the two agents tested for their ability to produce a CTA in our experiments. Our results suggest that a screen for the ability of an agent to produce a CTA is necessary in any paradigm that measures the suppression of consummatory behavior.

ACKNOWLEDGEMENTS

This research was supported in part by Grant no. 1 R15 NS26131 from the National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, and a grant from the Committee on Organized Research, Northeastern Illinois University.

REFERENCES

1. Calcagnetti, D. J.; Schechter, M. D. Attenuation of drinking sweetened water following calcium channel blockade. Brain Res. Bull. 28:967–973; 1992.
2. Carroll, M. E.; Lac, S. T.; Asencio, M.; Halikas, J. A.; Kragh, R. Effects of carbamazepine on self-administration of intravenously delivered cocaine in rats. Pharmacol. Biochem. Behav. 37: 551–556; 1990.
3. Chambers, K. C. Sexual dimorphisms as an index of hormonal influences on conditioned food aversions. In: Braverman, N. S.; Bronstein, P., eds. Experimental assessments and clinical Applications of conditioned food aversions, vol. 443, Annals of the New York Academy of Sciences. New York: New York Academy of Sciences; 1985:110–125.
4. Kuzmin, A.; Patkina, N.; Pchelintsev, M.; Zvartau, E. Isradipine is able to separate morphine-induced analgesia and place conditioning. Brain Res. 593:221–225; 1992.
5. Kuzmin, A.; Zvartau, E.; Gessa, G. L.; Martellotta, M. C.; Fratta, W. Calcium antagonists isradipine and nimodipine suppress cocaine and morphine intravenous self-administration in drug-naive mice. Pharmacol. Biochem. Behav. 41:497–500; 1992.
6. Nachman, M. Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. J. Comp. Physiol. Psychol. 73:22–30; 1970.
7. Pani, L.; Kuzmin, A.; Martellotta, M. C.; Gessa, G. L.; Fratta, W. The calcium antagonists PN 200–110 inhibits the reinforcing properties of cocaine. Brain Res. Bull. 26:445–447; 1991.
8. Riley, A. L.; Tuck, D. L. Conditioned taste aversions: A behavioral index of toxicity. In: Braveman, N. S.; Bronstein, P., eds. Experimental assessments and clinical applications of conditioned food aversions, vol. 443, Annals of the New York Academy of Sciences. New York: New York Academy of Sciences; 1985:272–292.
9. Schecter, M. D. Cocaine discrimination is attenuated by isradipine and CGS 10746B. Pharmacol. Biochem. Behav. 44:661–664; 1993.
10. Sharpe, L. G.; Jaffe, J. H.; Katz, J. L. Carbamazepine produces nonspecific effects on cocaine self-administration in rats. Life Sci. 51:PL13–PL18; 1992.