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Withdrawal From Chronic Nicotine Fails to Produce a Conditioned Taste Aversion to Saccharin in Rats

HEIDI F. VILLANUEVA, SHAHWALI AREZO, JOHN R. JAMES AND JOHN A. ROSECRANS[1]

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298

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VILLANUEVA, H. F., S. AREZO, J. R. JAMES AND J. A. ROSECRANS. *Withdrawal from chronic nicotine fails to produce a conditioned taste aversion to saccharin in rats.* PHARMACOL BIOCHEM BEHAV **37**(1) 59–61, 1990.—Sprague-Dawley rats were maintained on a daily regimen of nicotine, morphine or saline administration for 28 days. Following the discontinuation of the daily drug regimen, rats were given a choice of tap water or a saccharin-water solution. The rats previously receiving morphine drank significantly less saccharin-water solution than did the rats receiving nicotine or saline injections. The failure of the nicotine rats to display a conditioned aversion to the novel saccharin flavor suggests that nicotine did not produce a physiological withdrawal syndrome analogous to morphine withdrawal in this paradigm.

Conditioned taste aversion Morphine Nicotine Dependence Withdrawal

BOTH clinical and animal data indicate that nicotine meets the criteria for the potential to produce dependence (4, 9, 10), and that repeated administration of nicotine can produce a state of physiological dependence which will produce a nicotine abstinence syndrome when the drug is withdrawn (5–7, 11, 12). However, despite the clinical reports that humans attempting to abstain from cigarette smoking experience intense cravings for nicotine, there exists no widely accepted animal model of nicotine withdrawal. It has been suggested that conditioned aversion may be an appropriate technique to assess the otherwise immeasurable effects of abstinence from certain dependence-producing compounds. For example, Parker and Radow (17) demonstrated that if a saccharin solution is introduced at the onset of morphine withdrawal, an animal will avoid the saccharin, thus producing a conditioned aversion to the saccharin. Mucha, Walker and Fassos (14) have recently replicated this effect, demonstrating that withdrawal from the opiate sufentanil also produces a conditioned aversion to saccharin. This methodology, therefore, presents an ideal opportunity to investigate the dependence-producing and withdrawal effects of other compounds. The present investigation attempts to replicate the results presented by Parker and Radow (17) in morphine-dependent rats, and to determine if similar results can be achieved during withdrawal from chronically administered nicotine.

METHOD

Subjects

Twenty-four male Sprague-Dawley rats were individually housed in a temperature-controlled environment with ad lib food and water. The animals were approximately 60 days old and weighed 250–275 g at the start of the experiment.

Initial Preference Test

All animals were given a 5-day preference test between tap water and water sweetened with 0.23% sodium saccharin. Affixed to each cage were two 50 ml fluid bottles, one containing tap water and the other containing the saccharin solution. Daily consumption from each bottle was recorded and the left-right positions of the bottles were switched to control for position preferences. Following the preference test, both bottles were filled with tap water and placed on the cages. The animals were matched according to their preference for saccharin and randomly assigned to one of three drug groups: saline, morphine or nicotine (N = 8/group). Drug treatments were initiated approximately ten days following the initial preference test.

[1]Requests for reprints should be addressed to John A. Rosecrans, Department of Pharmacology and Toxicology, Virginia Commonwealth University, MCV Box 613, Richmond, VA 23298-0613.

Drug Treatments

Using the "staircase" regimen employed by Parker and Radow (17) and designed after Nichols *et al.* (16), the morphine group was assigned a maintenance dose of 160 mg/kg and the nicotine group was assigned a maintenance dose of 2.4 mg/kg. The morphine group received daily intraperitoneal (IP) injections of morphine sulfate (dissolved in 0.9% saline) at a volume of 4 ml/kg. The daily regimen began at a dose of 20 mg/kg and was followed by daily dosage increases of 7 mg/kg for 20 consecutive days until the maintenance dose of 160 mg/kg was attained on day 21. This group then received 160 mg/kg for an additional 7 days. Four of the vehicle control animals received daily injections of 4 ml/kg saline IP on each of the 28 days of drug treatment. The nicotine group received daily subcutaneous (SC) injections of (−)-nicotine bi-tartrate (concentrations expressed as the free base) at a volume of 1 ml/kg. The daily regimen began at a dose of 0.3 mg/kg and was followed by daily dosage increases of 0.1 mg/kg for 20 consecutive days until the maintenance dose of 2.4 mg/kg was achieved on day 21. This group then received 2.4 mg/kg nicotine for an additional 7 days. The other four vehicle control animals received daily injections of 1 ml/kg saline SC on each of the 28 days of drug treatment. All injections were administered between 11 a.m. and 2 p.m. daily.

Postdrug Preference Test

Twenty-four hours after the last injection on day 28, one water bottle was again replaced with the saccharin solution and a postdrug preference test was carried out for 21 days. Every 24 hours, consumption of saccharin and water was recorded and the left-right positions of the bottles were switched.

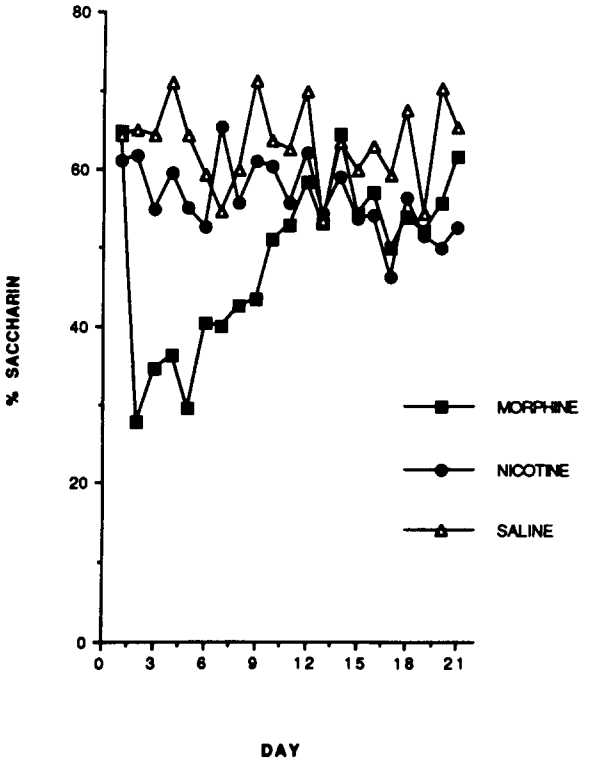

FIG. 1. Percent of fluid consumed as saccharin during drug withdrawal.

Statistical Analyses

The mean total volume of liquid consumed, the mean volume of saccharin and the mean percentage of saccharin consumed were calculated for each of the three groups. Dunnett's tests (3) were used to compare each drug group to the vehicle group.

RESULTS

Two animals died during the course of the experiment. One animal died on the first day of morphine administration (20 mg/kg) and one vehicle animal (IP) died on day 27 of injection. Analysis of variance revealed no significant differences between the IP and SC vehicle groups, $F(1,5)=0.374$, $p>0.05$, therefore the results for these two groups were combined and used as a single control group in further analyses.

Dunnett's tests revealed that forty-eight hours following the last injection (day 2, Fig. 1), the morphine group was consuming significantly less saccharin than the vehicle group ($p<0.05$). This difference was significant through day 10, after which time the consumption of saccharin by the morphine group returned to vehicle levels. Total fluid consumption was also significantly less than vehicle ($p<0.05$) during the first 72 hours of morphine abstinence (Fig. 2).

The nicotine group did not display a consistent reduction in saccharin consumption, although there were significant differences from vehicle on days 4, 17 and 20 ($p<0.05$). These differences, however, appear to be attributable to increases in consumption by the vehicle group, and not to decreases on the part of the nicotine group (Fig. 1).

DISCUSSION

As expected, the rats receiving morphine displayed a reliable aversion to the saccharin solution during morphine abstinence. Those animals receiving nicotine, however, displayed no aversion to the saccharin solution during nicotine abstinence, suggesting that a morphine-like dependence does not develop to the effect of nicotine in rats. The results of this study are in agreement with those reported by Mucha, Walker and Fassos (14). Mucha and colleagues found that withdrawal from the opiate sufentanil produced an aversion to the saccharin solution, while withdrawal from the psychostimulant amphetamine did not produce an aversion to saccharin, but produced an increase in saccharin consumption. The lack of increase in saccharin consumption after nicotine withdrawal in this study may be associated with the method of drug administration; Mucha and colleagues (14) administered drug via osmotic minipumps, while the present study employed daily injections of gradually increasing concentrations of drug.

While the present study constitutes only a preliminary investigation of conditioned aversion to nicotine, the negative results presented are supported by a majority of the literature involving animals models of nicotine withdrawal. Nicotine abstinence does not generally produce changes in behavior or locomotion (13,18), although it has been suggested that rats will display an abstinence syndrome in response to nicotine if stressed (15). The degree to which rats exhibit a nicotine abstinence effect may be related to the physiological state of the animal during drug administration and abstinence (1). Only a few studies have reported the presence of a nicotine abstinence syndrome in the absence of aversive environmental stimuli. For example, Corrigal, Herling and Coen (2) have demonstrated in the rat that withdrawal from 50 days of 2 mg/kg daily nicotine produces performance deficits on a fixed interval operant schedule, and that these deficits disappear within three days of nicotine withdrawal. Hendry and Rosecrans (8) also found evidence of withdrawal in the mouse during a fixed ratio operant

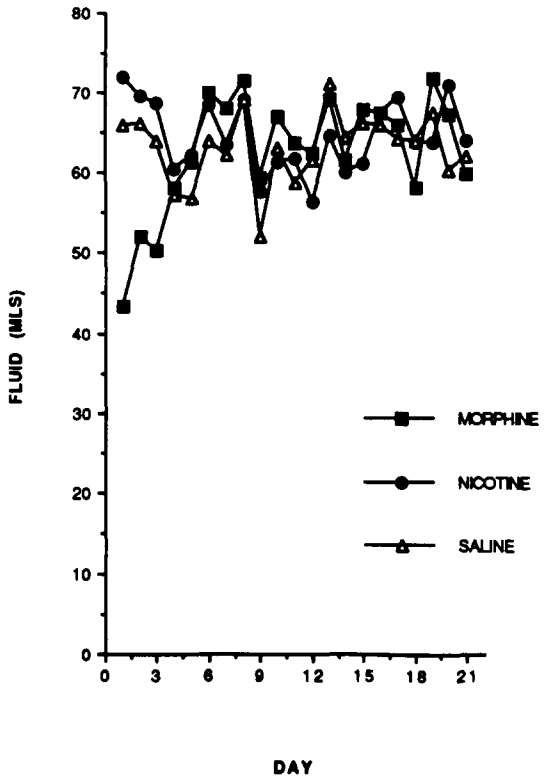

FIG. 2. Total fluid consumed each day during drug withdrawal.

schedule following 30 days of 1.2 mg/kg nicotine administration. While neither of these studies represent a clear model of physiological withdrawal from nicotine, these experiments suggest that behavior may play a significant role in the development of a nicotine withdrawal syndrome.

Previous research and this preliminary study provide support for the hypothesis that nicotine does not produce physical dependence in rats. However, further studies investigating the effects of different doses and routes of administration of nicotine are necessary to determine whether withdrawal from chronic administration of nicotine can produce a conditioned taste aversion in rats.

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