ELSEVIER

European Journal of Pharmacology 314 (1996) 281–284

ejp

Short communication

Mecamylamine-precipitated nicotine-withdrawal aversion in rats

Tsutomu Suzuki *, Yuya Ise, Makoto Tsuda, Jun Maeda, Miwa Misawa

Department of Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142, Japan

Received 18 July 1996; accepted 6 September 1996

Abstract

The present study examined a rapid and convenient model for evaluating nicotine dependence using the conditioned place preference paradigm. Rats were chronically infused subcutaneously with 9 mg/kg per day nicotine using an osmotic minipump. After nicotine infusion for 7 days, the nicotinic receptor antagonist mecamylamine produced a place aversion in nicotine-dependent rats, but not in acute nicotine-treated rats or sham-operated rats. These results suggest that the mecamylamine-precipitated withdrawal aversion in rats chronically treated with nicotine may result from physical dependence on nicotine, and may be useful for studying the physical dependence on nicotine.

Keywords: Nicotine; Mecamylamine; Conditioned place preference paradigm; Place aversion; Physical dependence

1. Introduction

In pharmacological research, the conditioned place preference paradigm has been demonstrated to be an effective method for assessing the rewarding effects of several abused drugs, such as cocaine, amphetamine, morphine and nicotine (Hoffman, 1989). The aversive effects of drugs can also be estimated using this paradigm. For example, the κ-opioid receptor agonist U50,488H produces conditioned place aversion (Mucha and Herz, 1985). Moreover, naloxone-precipitated withdrawal aversion in morphine-dependent rats can also be assessed using the conditioned place preference paradigm (Mucha, 1987). In fact, when the morphine-withdrawal aversive response was paired with specific environmental cues, the animal subsequently avoided the environment. Thus, the conditioned place preference paradigm may also be useful for assessing antagonist-precipitated withdrawal aversion.

Nicotine addiction, which has been likened to the addiction produced by cocaine or heroin, has become widely accepted as the mechanism which supports chronic tobacco use (US Department of Health and Human Services); nicotine withdrawal produces a withdrawal syndrome, which includes irritability, anxiety, inhibition of concentration, insomnia and craving in nicotine-dependent subjects (Hughes et al., 1991). Although animal models of physical dependence on nicotine are potentially useful for investigating nicotine dependence, there are currently few such models.

There have been several reports that the chronic administration of nicotine can produce physical dependence on nicotine in animal studies. For example, Costall et al. (1990) reported that nicotine-withdrawal aversive responses could be estimated by the light/dark exploration test in mice treated with nicotine for 14 days. Moreover, Malin et al. (1994) reported that rats that had been chronically treated with nicotine for 7 days showed several withdrawal signs, such as gasping/writhing, teeth chatter/chewing, shakes/tremors and ptosis after mecamylamine injection.

In the present study, we examined a convenient and sensitive model for evaluating nicotine dependence using the conditioned place preference paradigm.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Tokyo Experimental Animals, Tokyo, Japan), weighing 270–320 g, were housed in

* Corresponding author. Tel.: (81-3) 5498-5787; Fax: (81-3) 5498-5787.

PII S0014-2999(96)00723-6

groups of 2–3 in a temperature-controlled room (22 ± 1°C). The animals were maintained on a 12-h light/dark cycle (lights on 8:00 a.m. to 8:00 p.m.) with laboratory rat chow and tap water available ad libitum.

2.2. Apparatus

Place conditioning was conducted according to the method of Suzuki et al. (1990). The apparatus consisted of a shuttlebox (30 × 60 × 30 cm: w × l × h) which was divided into two compartments of equal size. One compartment was white with a textured floor and the other was black with a smooth floor.

2.3. Procedure

2.3.1. Motivational effect of mecamylamine after chronic treatment with nicotine

On day 1, an osmotic minipump (Alzet 2001, Alza Corporation, CA, USA) with a flow rate of 1.03 μl/h filled with (−)-nicotine tartrate in saline was subcutaneously implanted in rats that had been anesthetized with diethylether. The concentration of nicotine was adjusted for differences in body weight, but was approximately 116 mg/ml, resulting in continuous subcutaneous infusion of nicotine tartrate at the rate of 9 mg/kg per day according to the method of Malin et al. (1993, 1994). Nicotine-naive rats received sham operations: they were subjected to the same anesthesia and surgical procedure as the implanted animals except for the implantation of an osmotic minipump.

In the morning (9:00 a.m.) on day 7 of nicotine infusion, rats were subcutaneously injected with mecamylamine (0.1–1.0 mg/kg) or saline (1.0 ml/kg), and immediately confined to one compartment of the test apparatus for 60 min. In the evening (7:00 p.m.) on the same day, rats were then treated with saline or mecamylamine, respectively, and confined to the other compartment for 60 min. The pairings of injection (mecamylamine or saline) and compartment (white or black) were counterbalanced across all of the subjects. The control rats in the sham-operated and nicotine-infused groups were injected with saline (1.0 ml/kg, s.c.) instead of mecamylamine in the conditioning session. After the saline injections, the rats were confined to one compartment in the morning and to the other compartment in the evening. Before the start of the experiments, either saline control (black- or white-floored compartment place) was randomly chosen to be the substitute for the mecamylamine-associated place.

In the morning on day 8, tests of conditioning were performed as follows: the partition which separated the two compartments was raised to 12 cm above the floor, and a neutral platform was inserted along the seam separating the compartments. The time spent in each compartment during a 900-s session was measured automatically in a blind fashion by an infrared beam sensor (KN-80, Natsume Seisakusho, Tokyo, Japan). The position of the rat was defined by the position of its body. All sessions were conducted under conditions of dim illumination (40 lux) and masking white noise.

2.3.2. Motivational effect of mecamylamine after acute nicotine injection

In the morning (9:00 a.m.) on day 1, rats were treated with nicotine (0.2–0.8 mg/kg, s.c.). Five minutes after nicotine treatment, rats were subcutaneously injected with mecamylamine (1.0 mg/kg) or saline (1.0 ml/kg), and immediately confined to one compartment for 60 min. In the evening (7:00 p.m.) on the same day, rats were again treated with nicotine and saline or mecamylamine, respectively, and confined to the other compartment for 60 min. The control rats were injected with saline (1.0 ml/kg, s.c.) instead of nicotine 5 min before mecamylamine injection. The pairings of injection (mecamylamine or saline) and compartment (white or black) were counterbalanced across all of the subjects. In the morning on day 2, conditioning was tested in the same manner as in the chronic nicotine-treated group.

2.4. Drugs

The drugs used in the present study were (−)-nicotine hydrogen tartrate (Sigma, St. Louis, MO, USA) and mecamylamine hydrochloride (Sigma). All drugs were dissolved in saline and injected in a volume of 1.0 ml/kg.

2.5. Data analysis

Conditioning scores represent the time spent in the drug-paired place minus the time spent in the vehicle-paired place and are expressed as the mean ± S.E.M. Behavioral data were statistically evaluated with a one-way ANOVA followed by a Dunnett's multiple comparison test, which was used to determine whether an individual dose produced a significant place conditioning, and with a two-way ANOVA, which was used to determine the effects of treatment on mecamylamine-induced place conditioning.

3. Results

3.1. Motivational effect of mecamylamine after chronic treatment with nicotine

As shown in Fig. 1, the saline-control rats exhibited no preference for either compartment. The mean conditioning scores in sham-operated and chronic nicotine-infused rats were 7.3 ± 67.2 s ($n = 8$) and −12.3 ± 63.4 s ($n = 8$),

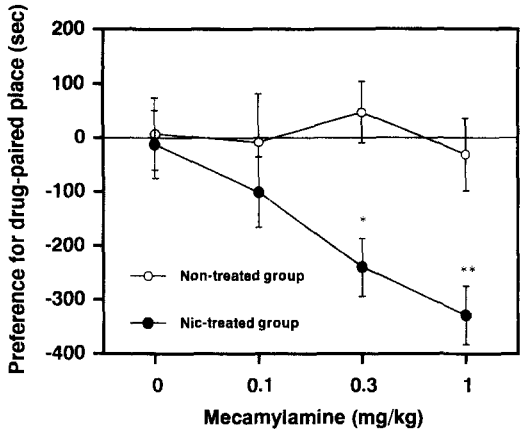

Fig. 1. Place conditioning produced by mecamylamine in rats that were chronically treated with nicotine (Nic) using an osmotic minipump. Rats were injected with saline (1.0 ml/kg, s.c.) or mecamylamine (0.1–1.0 mg/kg, s.c.). Each point represents the mean conditioning score with S.E.M. of 8–12 rats. * $P < 0.05$, ** $P < 0.01$ vs. saline-treated control.

respectively. Mecamylamine (0.1, 0.3 and 1.0 mg/kg) did not produce either significant place preference or place aversion in sham-operated rats. The mean conditioning scores associated with 0.1, 0.3 and 1.0 mg/kg mecamylamine were -8.4 ± 90.2 s ($n = 8$), 47.8 ± 57.1 s ($n = 8$) and -31.0 ± 67.4 s ($n = 8$), respectively. On the other hand, mecamylamine produced place aversion ($F(1,60) = 14.18$, $P < 0.01$) in chronic nicotine-infused rats. The lowest dose of mecamylamine (0.1 mg/kg) induced a slight place aversion, but this effect was not significant, with a mean conditioning score of -101.1 ± 65.3 s ($n = 8$). Significant place aversion was observed at 0.3 and 1.0 mg/kg mecamylamine, with mean conditioning scores of -240.0 ± 53.6 s ($n = 12$, $P < 0.05$) and -329.0 ± 53.8 s ($n = 8$, $P < 0.01$), respectively. There was no significant

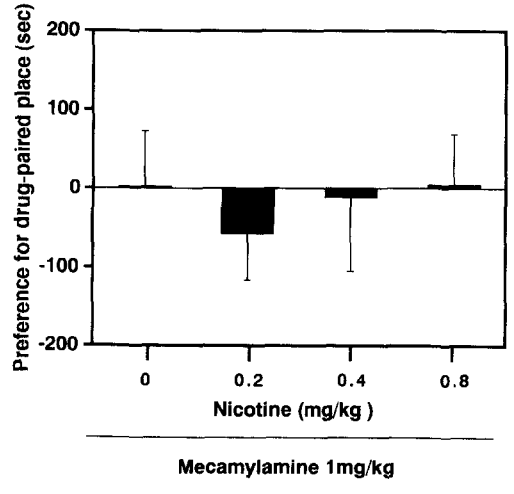

Fig. 2. Place conditioning produced by mecamylamine in rats that were acutely injected with nicotine twice a day. Rats were injected with saline (1.0 ml/kg, s.c.) or mecamylamine (1.0 mg/kg, s.c.) 5 min after treatment with nicotine (0.2–0.8 mg/kg, s.c.) in the morning and the evening. Each point represents the mean conditioning score with S.E.M. of 8 rats.

effect of dose ($F(3,60) = 2.62$, $P > 0.05$) or treatment × dose interaction ($F(3,60) = 2.28$, $P > 0.05$).

3.2. Motivational effect of mecamylamine after acute nicotine injection

As shown in Fig. 2, mecamylamine (1.0 mg/kg) did not produce place aversion in acute nicotine-treated rats. The mean conditioning scores associated with saline (control), 0.2, 0.4 and 0.8 mg/kg nicotine were 2.5 ± 70.5 s ($n = 8$), -56.1 ± 60.1 s ($n = 8$), -10.9 ± 94.9 s ($n = 8$), and 4.1 ± 65.0 s ($n = 8$), respectively.

4. Discussion

There are several reports that chronic administration of nicotine can produce physical dependence in animals, which show various withdrawal signs when nicotine is withdrawn. For example, Fung et al. (1996) reported that nicotine-dependent rats showed a decrease in locomotor activity after nicotine withdrawal. In addition, the administration of mecamylamine to rats that had been chronically treated with nicotine using an osmotic minipump induced various withdrawal signs, such as gasping/writhing, teeth chatter/chewing, shakes/tremors and ptosis (Malin et al., 1994). In the present study, nicotine was subcutaneously infused with an osmotic minipump according to the method of Malin et al. (1993, 1994). However, we did not observe mecamylamine-precipitated withdrawal signs, except for ptosis. Takada et al. (1988) reported that the chronic administration of nicotine using an osmotic minipump can produce behavioral signs, such as penis erection, grooming and an increase in water consumption after removal of the osmotic minipump, but it is unclear whether these behaviors are signs of withdrawal from nicotine. Signs of nicotine withdrawal are weaker than those with opioids, barbiturates and alcohol. Thus, it is difficult to observe marked nicotine withdrawal signs. In the present study, we focused on mecamylamine-precipitated withdrawal aversion as a sign of nicotine withdrawal.

The conditioned place preference paradigm can be used to evaluate not only the rewarding effects but also the aversive effects of drugs. Using the conditioned place preference paradigm, naloxone-precipitated withdrawal aversion was observed in morphine-dependent rats, suggesting that place aversion may be a sign of morphine withdrawal (Mucha, 1987). Naloxone-precipitated withdrawal aversion was produced in morphine-dependent rats by a low dose of naloxone which does not induce marked withdrawal signs. Therefore, the appearance of naloxone-precipitated withdrawal aversion may be a very sensitive sign of morphine withdrawal. Since the conditioned place preference paradigm possesses these properties, antagonist-precipitated withdrawal aversion may be useful for investigating dependence on nicotine as well as opioids.

In the present study, we found that mecamylamine produced place aversion in chronically nicotine-treated rats, but not in sham-operated rats. Therefore, mecamylamine-precipitated withdrawal aversion may be a sign of nicotine withdrawal, similar to naloxone-precipitated withdrawal aversion in morphine-dependent rats. There is a possibility that mecamylamine produces place aversion due to its antagonistic effects after acute nicotine injection. However, we found that mecamylamine did not produce place aversion when mecamylamine-precipitated place aversion was examined after the acute injection of nicotine (0.2–0.8 mg/kg). The highest dose (0.8 mg/kg) of nicotine is the maximal dose without a sedative effect. Therefore, the mecamylamine-precipitated withdrawal aversion resulting from chronic treatment with nicotine may reflect physical dependence on nicotine, i.e. a withdrawal sign. These results suggest that mecamylamine-precipitated withdrawal aversion in the conditioned place preference paradigm may be useful for investigating physical dependence on nicotine.

Previous studies have indicated that several mechanisms contribute to the appearance of nicotine withdrawal signs. For example, Harris et al. (1986) reported that nicotine withdrawal partially generalizes to the discriminative stimulus effects of pentylenetetrazole, an anxiogenic drug. In addition, Costall et al. (1990) reported that an aversive response caused by nicotine withdrawal was detected by the light/dark exploration test; this aversive behavior is related to an anxiogenic response which is mediated by overstimulation of central serotonergic neurons. Moreover, the opioid system may play a role in physical dependence on nicotine, since naloxone precipitates withdrawal signs in nicotine-dependent rats and morphine inhibits withdrawal signs after the termination of nicotine (Malin et al., 1993). Since multiple neurotransmitter systems are modulated by nicotine, these systems may contribute to nicotine-withdrawal aversion. Nicotine produces a conditioned place preference (Fudala et al., 1985; Risinger and Oakes, 1995) and an increase in dopamine release from the nucleus accumbens (Di Chiara and Imperato, 1988), as does morphine. On the other hand, naloxone-precipitated withdrawal in morphine-dependent animals produced a conditioned place aversion (Mucha, 1987) and a decrease in dopamine turnover in the limbic forebrain (Suzuki et al., 1995). Moreover, dopamine contents in the nucleus accumbens decreased after the termination of nicotine treatment in nicotine-dependent rats (Fung et al., 1996). These results suggest that mecamylamine-precipitated withdrawal aversion may be related to a decrease in the activity of the mesolimbic dopaminergic system.

In conclusion, we found using the conditioned place preference paradigm that mecamylamine produces a place aversion in rats that have been chronically treated with nicotine. However, mecamylamine-precipitated withdrawal aversion was not observed after the acute injection of nicotine. Therefore, mecamylamine-precipitated withdrawal aversion resulting from chronic treatment with nicotine may reflect physical dependence on nicotine, i.e. withdrawal, and this method may be useful for investigating physical dependence on nicotine.

Acknowledgements

This work was supported in part by a Research Grant (5A-7) for Nervous and Mental Disorders and a Research Grant from the Ministry of Health and Welfare to T.S. We wish to thank Ms. Seiko Katsuike and Mr. Tsuyoshi Yoshida for their expert technical assistance.

References

Costall, B., B.J. Jones, M.E. Kelly, R.J. Naylor, E.S. Onaivi and M.B. Tyers, 1990, Sites of action of ondansetron to inhibit withdrawal from drugs of abuse, Pharmacol. Biochem. Behav. 36, 97.

Di Chiara, G. and A. Imperato, 1988, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats, Proc. Natl. Acad. Sci. USA 85, 5274.

Fudala, P.J., K.W. Teoh and E.T. Iwamoto, 1985, Pharmacologic characterization of nicotine-induced conditioned place preference, Pharmacol. Biochem. Behav. 22, 237.

Fung, Y.K., M.J. Schmid, T.M. Anderson and Y.-S. Lau, 1996, Effects of nicotine withdrawal on central dopaminergic systems, Pharmacol. Biochem. Behav. 53, 635.

Harris, C.M., M.W. Emmett-Oglesby, N.G. Robinson and H. Lal, 1986, Withdrawal from chronic nicotine substitutes partially for the interoceptive stimulus produced by pentylenetetrazol (PTZ), Psychopharmacology 90, 85.

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

Hughes, J.R., S.W. Gust, K. Skoog, R.M. Keenan and J.W. Fenwick, 1991, Symptoms of tobacco withdrawal: a replication and extension, Arch. Gen. Psychiatry 48, 52.

Malin, D.H., J.R. Lake, V.A. Carter, J.S. Cunningham and O.B. Wilson, 1993, Naloxone precipitates nicotine abstinence syndrome in the rat, Psychopharmacology 112, 339.

Malin, D.H., J.R. Lake, V.A. Carter, J.S. Cunningham, K.M. Hebert, D.L. Conrad and O.B. Wilson, 1994, The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat, Psychopharmacology 115, 180.

Mucha, R.F., 1987, Is the motivational effect of opiate withdrawal reflected by common somatic indices of precipitated withdrawal? A place conditioning study in the rat, Brain Res. 418, 214.

Mucha, R.F. and A. Herz, 1985, Motivational properties of kappa- and mu-opioid receptor agonists studied with place and taste preference conditioning procedures, Psychopharmacology 86, 274.

Risinger, F.O. and R.A. Oakes, 1995, Nicotine-induced conditioned place preference and conditioned place aversion in mice, Pharmacol. Biochem. Behav. 51, 457.

Suzuki, T., Y. Masukawa and M. Misawa, 1990, Drug interactions in the reinforcing effects of over-the-counter cough syrups, Psychopharmacology 102, 438.

Suzuki, T., M. Tsuji, T. Mori, M. Misawa and H. Nagase, 1995, Effect of naltrindole on the development of physical dependence on morphine in mice: a behavioral and biochemical study, Life Sci. 57, PL247.

Takada, K., S. Katoh and T. Yanagita, 1988, Nicotine dependence in rats, Jpn. J. Psychopharmacol. 8, 295.