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Short communication

Mecamylamine-precipitated nicotine-withdrawal aversion in Lewis and Fischer 344 inbred rat strains

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Abstract

Many studies have indicated that Lewis and Fischer 344 inbred rat strains show marked differences in behavioral responses to abused drugs. In the present study, we sought to determine whether these two strains of rats show different responses in mecamylamine-precipitated nicotine-withdrawal aversion using the conditioned place preference paradigm. Rats were treated subcutaneously with 10 mg/kg/day nicotine for 7 days using an osmotic minipump. After chronic nicotine infusion, the nicotinic receptor antagonist mecamylamine produced a significant place aversion in Lewis, but not in Fischer 344 rats. These results suggest that mecamylamine-precipitated nicotine-withdrawal aversion is strongly regulated by genetic factors.

Keywords: Conditioned place preference paradigm; Mecamylamine; Withdrawal aversion; Physical dependence; Nicotine; Lewis rat; Fischer 344 rat

1. Introduction

In pharmacogenetic studies, inbred or selectively bred strains are usually used to determine whether genetic differences may be involved in different pharmacological effects of drugs. For example, Lewis and Fischer 344 inbred rat strains are useful for investigating genetic differences in the behavioral and biochemical effects of several abused drugs.

Recent findings have suggested that Lewis and Fischer 344 inbred rat strains show marked differences in behavioral responses to several abused drugs. For example, the reinforcing effects of alcohol in Lewis rats are greater than those in Fischer 344 rats (Suzuki et al., 1988). In addition, Fischer 344 rats that were chronically treated with diazepam, ethanol or barbital showed greater withdrawal signs than Lewis rats (Suzuki et al., 1992a,b). These results suggest that the reinforcing effects of and development of physical dependence on abused drugs may be influenced by genetic factors.

The conditioned place preference paradigm has been demonstrated to be an effective method for assessing the rewarding effects of abused drugs, such as cocaine, amphetamine, morphine and nicotine. Moreover, strain differences in the rewarding effects of abused drugs in Lewis and Fischer 344 inbred rats have also been investigated using this procedure. For example, morphine-, cocaine- and nicotine-induced conditioned place preferences in Lewis are greater than those in Fischer 344 rats (Guitart et al., 1992; Kosten et al., 1994; Horan et al., 1997).

Antagonist-precipitated aversive effects can also be estimated using this paradigm. In fact, we have found that mecamylamine-precipitated nicotine-withdrawal aversion, which may reflect physical dependence on nicotine, can be evaluated using the conditioned place preference paradigm (Suzuki et al., 1996). Therefore, in the present study, we examined whether physical dependence on nicotine, as estimated by mecamylamine-precipitated nicotine-withdrawal aversion, is influenced by genetic factors using Lewis and Fischer 344 inbred rat strains.

2. Materials and methods

The present studies were carried out in accordance with the Guide for Care and Use of Laboratory Animals adopted by the Committee on Care and Use of Laboratory Animals of Hoshi University, which is accredited by the Ministry of Education, Science, Sports and Culture, Japan.

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2.1. Animals

Male Lewis and Fischer 344 rats (Charles River Japan, Atsugi, Japan), weighing 300–400 g, were housed in groups of 4 in a temperature-controlled (25 ± 1°C) specific pathogen free room. The animals were maintained on a 12-h light/dark cycle (lights on 8:00 AM to 8:00 PM) with laboratory rat chow and tap water available ad libitum.

2.2. Apparatus

The apparatus consisted of a shuttle box (30 × 60 × 30 cm: $w \times l \times h$) 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

On day 1, an osmotic minipump (Alzet 2001, Alza, CA, USA) with a flow rate of 1.03 μl/h filled with (−)-nicotine hydrogen tartrate in saline was subcutaneously implanted in rats that had been anesthetized with diethylether. Continuous subcutaneous infusion of nicotine hydrogen tartrate was adjusted to a rate of 10 mg/kg/day, which is similar to that in a previous report (Suzuki et al., 1996). Nicotine-naive rats received sham operations: they were subjected to the same anesthesia and surgical procedure as the implanted animals except for implantation of an osmotic minipump.

On days 4 and 5, habituation was 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. Afterwards, rats were allowed to freely move in the shuttle box for 900-s.

On day 6, pre-tests of conditioning were performed. 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 lx) and masking white noise.

On day 7, conditioning sessions were performed as follows; in the morning (9:00 AM), rats were subcutaneously injected with saline (1.0 ml/kg), and immediately confined to the non-preferred compartment of the shuttle box for 60 min. In the evening (7:00 PM) on the same day, rats were then treated with mecamylamine (0.3–3.0 mg/kg, s.c.) and confined to the preferred compartment for 60 min. 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.

On day 8, post-tests of conditioning were performed, similar to the pre-tests of conditioning. The time spent in each compartment during a 900-s session was again measured. The degree of nicotine-withdrawal place aversion was estimated by subtracting the time spent in the preferred compartment during the pre-conditioning session from the time spent in the mecamylamine-injected compartment during the post-conditioning session.

2.4. Drugs

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

2.5. Data analysis

Conditioning scores represent the time spent in the mecamylamine-injected compartment in the post-conditioning test minus the time spent in the preferred compartment in the pre-conditioning test and are expressed as the mean (sec) ± S.E.M. Behavioral data were statistically evaluated with a one-way repeated analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, which was used to determine whether an individual dose produced significant place conditioning, and with a two-way ANOVA, which was used to determine the effect of strain differences on mecamylamine-induced place conditioning.

3. Results

As shown in Fig. 1, the saline-control rats exhibited no preference for either compartment. The mean conditioning scores in Lewis and Fischer 344 rats were −11.6 ± 51.9 s ($n = 8$) and −1.8 ± 74.5 s ($n = 8$), respectively. Mecamylamine (0.3, 1.0 and 3.0 mg/kg) produced neither significant place preference nor place aversion in nicotine-treated

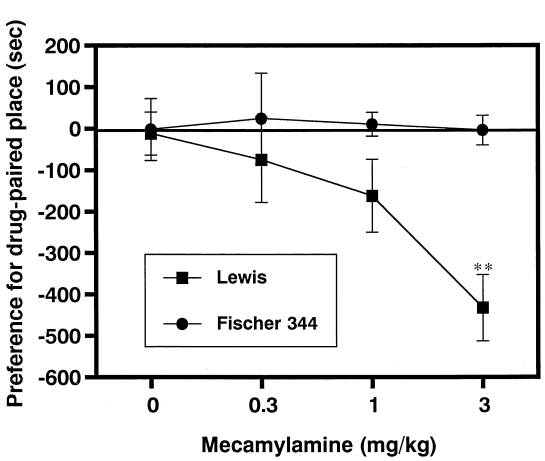

Fig. 1. Place conditioning produced by mecamylamine in Lewis (closed squares) and Fischer 344 (closed circles) rats that were chronically treated with nicotine using an osmotic minipump (10 mg/kg/day). Rats were injected with saline (1.0 ml/kg, s.c.) or mecamylamine (0.3–3.0 mg/kg, s.c.). Each point represents the mean conditioning score with S.E.M. of 6–8 rats. $^{**}P < 0.01$ vs. saline-treated control.

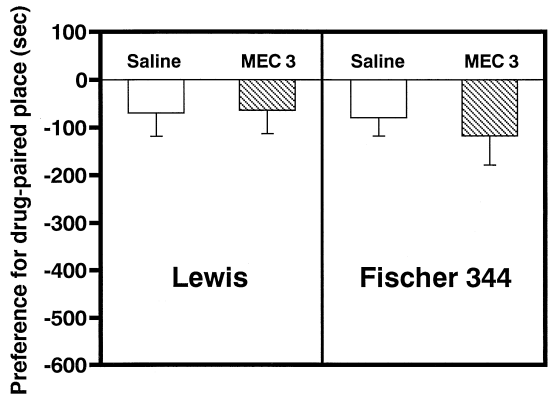

Fig. 2. Place conditioning produced by mecamylamine (MEC) in Lewis and Fischer 344 rats that were not treated with nicotine. Rats were injected with saline (1.0 ml/kg, s.c.) or mecamylamine (3.0 mg/kg, s.c.). Each column represents the mean conditioning score with S.E.M. of 7–8 rats.

Fischer 344 rats. The mean conditioning scores associated with 0.3, 1.0 and 3.0 mg/kg mecamylamine were 23.9 ± 109.5 s ($n = 7$), 9.9 ± 28.6 s ($n = 7$) and −3.9 ± 35.6 s ($n = 8$), respectively. On the other hand, mecamylamine produced a dose-dependent place aversion ($F(1,50) = 11.39$, $P < 0.01$) in nicotine-treated Lewis rats. Low doses (0.1 mg/kg and 0.3 mg/kg) of mecamylamine did not induce a significant place aversion, with mean conditioning scores of −74.8 ± 102.7 s ($n = 6$) and −162.0 ± 88.1 s ($n = 7$), respectively. Significant place aversion was observed at 3.0 mg/kg mecamylamine, with a mean conditioning score of −432.7 ± 80.3 s ($n = 7$, $P < 0.01$). Both dose ($F(3,50) = 3.32$, $P < 0.05$) and strain differences × dose interaction ($F(3,50) = 2.94$, $P < 0.05$) had significant effects.

As shown in Fig. 2, mecamylamine (3.0 mg/kg) did not produce place aversion in sham Lewis or Fischer 344 rats that were not treated with nicotine. The mean conditioning scores associated with saline and 3.0 mg/kg mecamylamine in Lewis rats were −70.3 ± 49.3 s ($n = 7$) and −64.4 ± 49.8 s ($n = 8$), respectively, and those in Fischer 344 rats were −80.3 ± 38.0 s ($n = 8$), and −118.8 ± 61.3 s ($n = 8$), respectively.

4. Discussion

In the present study, a significant mecamylamine-precipitated nicotine-withdrawal aversion was observed in Lewis, but not in Fischer 344 inbred rats that had been chronically treated with nicotine. However, mecamylamine did not produce a significant place aversion in either strain of rat that was not treated with nicotine. These results suggest that mecamylamine-precipitated nicotine-withdrawal aversion is strongly influenced by genetic factors, and Lewis rats may be sensitive to the development of physical dependence on nicotine compared to Fischer 344 rats.

On the other hand, we previously reported that withdrawal signs of diazepam, ethanol and barbital are greater in Fischer 344 rats than in Lewis rats (Suzuki et al., 1992a,b). The action sites of these drugs differ from that of nicotine; diazepam, ethanol and barbital act on $GABA_A$ receptors, while nicotine acts on nicotinic receptors. Therefore, the relative rank for the development of physical dependence on nicotine (Lewis > Fischer 344) might be reversed in Lewis and Fischer 344 rats, compared to that with diazepam, ethanol and barbital (Lewis < Fischer 344).

Our recent studies have shown that 1.0 mg/kg of mecamylamine produces a significant place aversion (Suzuki et al., 1996). In the present study, the same dose of mecamylamine induced a slight place aversion in Lewis rats, but this effect was not significant. The behavioral effects of drugs may be influenced by various factors such as species, gender, strain, and the environment in which the rats were housed before drug administration (Milton et al., 1995). Our previous research (Suzuki et al., 1996) was performed using Sprague–Dawley outbred rats that were housed in a clean room. On the other hand, the present study was performed using inbred strains of rats that were housed in a specific pathogen free room. Thus, the difference in nicotine-withdrawal aversion produced by the same dose of mecamylamine may be explained by the differences in the strains and the environment in which the rats were housed.

In the present study, we found that the mecamylamine (3 mg/kg)-precipitated nicotine-withdrawal aversion in Lewis rats was greater than that in Fischer 344 rats. This strain difference does not seem to be due to genetic differences in nicotine metabolism, since there are no differences between the nicotine levels in the two strains when chronically treated with nicotine (Horan et al., 1997). Thus, nicotine metabolism in Lewis and Fischer 344 inbred rats may not be involved in the strain difference in the mecamylamine-precipitated nicotine-withdrawal aversion.

It is unclear why mecamylamine-precipitated nicotine-withdrawal aversion was observed in Lewis, but not in Fischer 344 rats. However, previous studies have indicated that Lewis and Fischer 344 rat strains show differences in biochemical and neurochemical parameters in the mesolimbic dopaminergic system. For example, Lewis and Fischer 344 rat strains differ with regard to tyrosine hydroxylase and neurofilament protein levels in the ventral tegmental area, and adenylate cyclase, cyclic AMP and G-protein levels in the nucleus accumbens (Guitart et al., 1992). Moreover, different levels of basal dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the nucleus accumbens have been shown between Lewis and Fischer 344 inbred rat strains (Strecker et al., 1995). Recent studies have indicated that nicotine-withdrawal may reduce the function of the mesolimbic dopaminergic system. For example, Fung et al. (1996) reported that nicotine-withdrawal hypolocomotion, which may reflect physi-

cal dependence on nicotine, may be related to the reduction of dopamine turnover in the nucleus accumbens. Moreover, dopamine release in the nucleus accumbens was reduced when rats showed mecamylamine-precipitated nicotine-withdrawal signs such as teeth chatter (Hildebrand et al., 1998). The inhibition of dopamine release in the nucleus accumbens may lead to conditioned place aversion (Schechter and Meehan, 1994). From the previous and present results, Lewis rats may be more sensitive to the decrease in dopamine release in the nucleus accumbens after nicotine withdrawal than Fischer 344 rats. Therefore, mecamylamine-precipitated nicotine-withdrawal aversion was observed in Lewis, but not in Fischer 344 rats.

In conclusion, we observed mecamylamine-precipitated nicotine-withdrawal aversion in Lewis, but not in Fischer 344 rats. Thus, physical dependence on nicotine may be strongly regulated by genetic factors, and Lewis rats may be highly susceptible to the development of physical dependence on nicotine compared to Fischer 344 rats.

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References

Fung, Y.K., Schmid, M.J., Anderson, T.M., Lau, Y.-S., 1996. Effects of nicotine withdrawal on central dopaminergic systems. Pharmacol. Biochem. Behav. 53, 635–640.

Guitart, X., Beitner-Johnson, D., Marby, D.W., Kosten, T.A., Nestler, E.J., 1992. Fischer and Lewis rat strains differ in basal levels of neurofilament proteins and their regulation by chronic morphine in the mesolimbic dopamine system. Synapse 12, 242–253.

Hildebrand, B.E., Nomikos, G.G., Hertel, P., Schilström, B., Svensson, T.H., 1998. Reduced dopamine output in the nucleus accumbens but not in the medial prefrontal cortex in rats displaying a mecamylamine-precipitated nicotine withdrawal syndrome. Brain Res. 779, 214–225.

Horan, B., Smith, M., Gardner, E.L., Lepore, M., Ashby, C.R. Jr., 1997. (−)-Nicotine produces conditioned place preference in Lewis, but not Fischer 344 rats. Synapse 26, 93–94.

Kosten, T.A., Miserendino, M.J.D., Chi, S., Nestler, E.J., 1994. Fischer and Lewis rat strains show differential cocaine effects in conditioned place preference and behavioral sensitization but not in locomotor activity or conditioned taste aversion. J. Pharmacol. Exp. Ther. 269, 137–144.

Milton, G.V., Randall, P.K., Erickson, C.K., 1995. Low-dose effect of ethanol on locomotor activity induced by activation of the mesolimbic system. Alcohol. Clin. Exp. Res. 19, 768–776.

Schechter, M.D., Meehan, S.M., 1994. Conditioned place aversion produced by dopamine release inhibition. Eur. J. Pharmacol. 260, 133–137.

Strecker, R.E., Eberle, W.F., Ashby, C.R. Jr., 1995. Extracellular dopamine and its metabolites in the nucleus accumbens of Fischer and Lewis rats: basal levels and cocaine-induced changes. Life Sci. 56, PL135–141.

Suzuki, T., George, F.R., Meisch, R.A., 1988. Differential establishment and maintenance of oral ethanol reinforced behavior in Lewis and Fischer 344 inbred rat strains. J. Pharmacol. Exp. Ther. 245, 164–170.

Suzuki, T., Lu, M.-S., Motegi, H., Yoshii, T., Misawa, M., 1992a. Genetic differences in the development of physical dependence upon diazepam in Lewis and Fischer 344 inbred rat strains. Pharmacol. Biochem. Behav. 43, 387–393.

Suzuki, T., Motegi, H., Otani, K., Koike, Y., Misawa, M., 1992b. Susceptibility to, tolerance to, and physical dependence on ethanol and barbital in two inbred strains of rats. Gen. Pharmacol. 23, 11–17.

Suzuki, T., Ise, Y., Tsuda, M., Maeda, J., Misawa, M., 1996. Mecamylamine-precipitated nicotine-withdrawal aversion in rats. Eur. J. Pharmacol. 314, 281–284.