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18-Methoxycoronaridine, a potential anti-obesity agent, does not produce a conditioned taste aversion in rats

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ABSTRACT

18-Methoxycoronaridine (18-MC), a selective antagonist of α3β4 nicotinic receptors, has been shown to reduce the self-administration of several drugs of abuse. Recently, this agent has also been shown to attenuate sucrose reward, decrease sucrose intake and prevent the development of sucrose-induced obesity in rats. The present experiments were designed to determine whether the latter effect was due to an 18-MC-induced conditioned taste aversion to sucrose. Both 18-MC (20 mg/ kg, i.p.) and control agent, lithium chloride (100 mg/kg, i.p.), reduced sucrose intake 24 h after association with sucrose; however, only lithium chloride reduced sucrose intake 72 h later. Consistent with previous data, 18-MC appears to have proactive effect for 24 h and it does not induce a conditioned taste aversion.

1. Introduction

18-MC, a synthetic congener of ibogaine, is currently being investigated as a potential therapeutic agent for drug addiction and obesity. In animal studies, 18-MC has been shown to reduce the intravenous self-administration of morphine, cocaine, methamphetamine and nicotine (Glick et al., 1996, 2000) and to alleviate several signs of opioid withdrawal in rats (Rho and Glick, 1998). In recent studies, 18-MC has also been shown to reduce sucrose intake, both in an operant model and in a free-access drinking paradigm (Taraschenko et al., 2008). Furthermore, 18-MC reduced sucrose intake, weight gain and fat deposition without altering food and water intake in rats consuming a high sucrose diet (Taraschenko et al., 2008). The mechanism for 18-MC's attenuation of sucrose drinking and its anti-obesity effect has not been elucidated; however,18-MC antagonizes α3β4 nicotinic receptors with high specificity and potency and this action could be responsible (Glick et al., 2002). For example, 18-MC could block nicotinic receptors located in the nucleus of the solitary tract, a brainstem structure responsible for recognition of basic taste qualities in rats (Dhar et al., 2000; Roussin et al., 2007). Alternatively, 18-MC could attenuate obesity by inhibiting ghrelin-induced increases in food intake (unpublished results). Ghrelin signaling has been shown to be required for the development of obesity (Zigman et al., 2005) and is known to be linked to cholinergic systems in the brain (Jerlhag et al., 2006; Jerlhag et al., 2008).

Animal studies assessing the side effect profile of 18-MC have revealed no apparent side effects. Thus, 18-MC had no effect on locomotor activity or rotorod performance in rats; neither did it affect heart rate or blood pressure (Glick et al., 1999; Maisonneuve and Glick, 2003). In contrast to its parent compound, ibogaine, 18-MC had no tremorigenic effect and no neurotoxic properties in the rat cerebellum (Glick et al., 2000). Although the effect of 18-MC on taste perception has never been assessed, such a study is necessary in light of our new discovery regarding obesity. Specifically, it is important to know whether the dose of 18-MC (i.e., 20 mg/kg) previously shown to reduce sucrose intake (Taraschenko et al., 2008) did so by eliciting an aversive response to sucrose.

Originally described by Garcia et al. (1955), the conditioned taste aversion (CTA) paradigm involves the pairing of a gustatory stimulus (conditioned stimulus) with a noxious stimulus to establish if such an association will subsequently lead to avoidance of the former (Garcia et al., 1955).Given the fact that several brain areas responsible for the processing of taste (e.g., nucleus of tractus solitarius) contain high densities of α3β4 nicotinic receptors, it is conceivable that 18-MC could bind to those receptors and act as a unconditioned stimulus for the acquisition of an aversion to a sucrose solution (Mediavilla et al., 2005; Smith and Uteshev, 2008). To test this possibility, the effect of systemic18-MC was assessed in a two-bottle paradigm (Patel and Ebenezer, 2008). In order to dissociate the previously described 18-MC-induced attenuation of sucrose intake from that produced by an aversion, additional time course experiments were carried out.

2. Materials and methods

2.1. Animals

Naïve female Sprague–Dawley rats (230–270 g; Taconic, Germantown, NY) were housed individually and maintained on a normal 12 h

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light cycle (light on/off at 7 a.m./7 p.m.) in the colony room. For all experiments, food (normal chow) was provided ad libitum at all times except during the test sessions. The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1998). New groups of naïve animals were used for each experiment.

2.2. Drugs

18-MC (Albany Molecular Research, Albany, NY) was dissolved in 0.01 M NaH_2PO_4 (vehicle, pH = 6) and injected in a volume of 1 ml/kg. Lithium chloride (Sigma, St. Louis, MO) was dissolved in saline. All drugs were administered intraperitoneally. Sucrose (5% wt/vol, MP Biomedicals, Inc., Solon, OH) was dissolved in water.

2.3. Conditioned taste aversion paradigm

The method previously described by Patel and Ebenezer (2008) was modified as follows. Rats were water deprived for 17 h a day before each of five 30-min experimental sessions. The five sessions included three baseline sessions, one training session and one test session. Prior to each session animals were transported from the colony room to the test room, weighed and transferred to test cages where they were presented with two 100-ml graduated bottles containing 5% sucrose solution and water. The bottles were placed approximately 5 cm apart on the top of the cage and the positions of bottles with sucrose and water were alternated during the five days of study; the rats preferring water to sucrose were identified and excluded from the study. Upon completion of the session, animals were returned to their home cages and transported back to the colony room. The consumption of liquids during each session was recorded and intake during the third baseline session was used to assign animals to the three treatment groups. Different groups of naïve animals were used for each of the following three experiments; animals that did not prefer sucrose solution to water were eliminated from the analysis.

2.3.1. Treatment in the test cages and test at 24 h

This experiment was designed to test whether 18-MC, when administered acutely, produces a conditioned test aversion; the rationale is that the immediate pairing of sucrose with an aversive agent would provide an aversion to sucrose 24 h later. During the training session, rats were allowed access to sucrose and water in the test cages for 15 min, were injected with 18-MC (20 mg/kg, i.p.), lithium (100 mg/kg, i.p.), or vehicle and then allowed an additional 15 min before being transferred to their home cages. Twenty four hours later rats were returned to the test cages to assess their intake of liquids.

2.3.2. Treatment in the home cages and test at 24, 48 and 72 h

In order to distinguish a direct effect of 18-MC on sucrose intake (Taraschenko et al., 2008) from that produced by a conditioned taste aversion, the effects of a single 18-MC treatment were examined at 24 h intervals following injection. During the training session, animals were allowed access to sucrose and water in the test cages for 30 min, were returned to their home cages, transported to the colony room and injected with 18-MC (20 mg/ kg, i.p.), lithium (100 mg/kg, i.p.), or vehicle 1 h later. The animals were returned to their test cages at 24, 48 and 72 h after treatment for assessment of their sucrose and water intake.

2.3.3. Treatment in the test cages and test at 72 h

The experiment was conducted as described in Section 2.3.1 with the exception that the test session was carried out at 72 h after the administration of 18-MC, lithium or vehicle. The rationale for this study was to determine if 18-MC could produce a conditioned taste aversion when its direct pharmacological effect on sucrose intake was no longer present.

2.4. Statistical analysis

The data for sucrose intake and water intake from all experiments were analyzed by ANOVA with the treatment and sessions (baseline and test) as the two main variables. The Fisher LSD post-hoc tests were applied when appropriate.

3. Results

3.1. 18-MC and lithium reduce sucrose intake but not water intake 24 h after treatment

The baseline values of sucrose intake (ml) in the vehicle, 18-MC and lithium chloride-treated groups were as follows: 13.42 ± 0.71; 12.50 ± 0.40; 13.47 ± 1.49, while the values of water intake (ml) were 3.3 ± 0.9; 3.67 ± 0.76; 2.53 ± 0.84, respectively. The baseline intake of sucrose and water was not significantly different among the three groups.

As shown in Fig. 1 all rats preferred sucrose solution to water. Consistent with previous reports (Patel and Ebenezer, 2008), when compared to baseline, the consumption of sucrose was significantly reduced during the test session conducted 24 h after association of sucrose solution with injection of an aversive agent, lithium chloride (100 mg/kg, i.p.) (Treatment × Session interaction: $F_{2,10} = 8.56$, $P < 0.007$; post-hoc tests). Assessed in the same experiment, the consumption of sucrose was also reduced 24 h after association with an injection of 18-MC (20 mg/ kg, i.p.). This effect could be due to either the direct pharmacological action of 18-MC on sucrose intake (Taraschenko et al., 2008) or to an aversion to sucrose. Sucrose intake was not altered in vehicle-injected control rats. Consumption of water was not altered by association with either 18-MC, lithium chloride or vehicle in the same animals (Treatment × Session interaction: $F_{2,10} = 3.02$, $P > 0.1$).

3.2. 18-MC but not lithium chloride has a protracted effect on sucrose intake

The baseline values of sucrose intake (ml) in the vehicle, 18-MC and lithium chloride-treated groups were as follows: 8.75 ± 0.21;

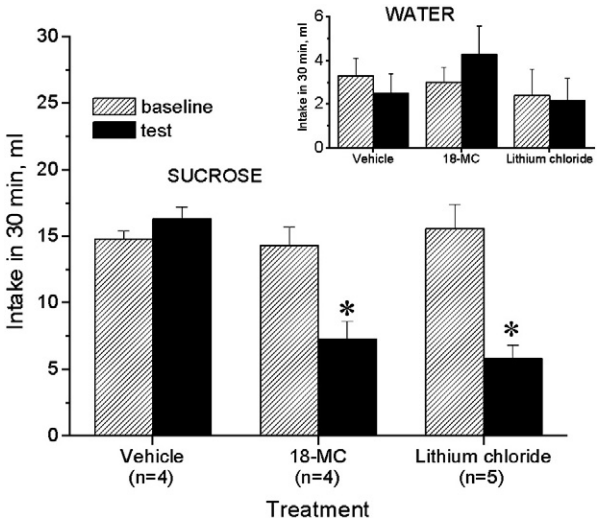

Fig. 1. Effects of 18-MC (20 mg/kg, i.p.), lithium chloride (100 mg/kg , i.p.) and vehicle on intake (mean ml ± SEM) of sucrose solution (5%) and water (insert) at 24 h after immediate (in test cages) association with sucrose.

9.58 ± 0.34; 8.83 ± 1.62, while the values of water intake (ml) were 2.83 ± 1.15; 1.42 ± 0.55; 2.92 ± 1.10, respectively. The baseline intake of sucrose and water was not significantly different among the three groups.

In order to discern a protracted effect of 18-MC on sucrose intake 24 h later from that produced by a conditioned taste aversion, the pairing of sucrose solution with injection of either 18-MC, lithium chloride or vehicle was delayed. Thus, a single injection of either treatment took place an hour after sucrose intake session in a different environment (i.e., home cages), and all groups of animals were tested at 24, 48 and 72 h after injection. The time course of effects is shown in Fig. 2. Lithium chloride (100 mg/ kg, i.p.) reduced intake of sucrose at 24 h but not at 48 and 72 h after injection (Treatment × Session interaction: $F_{2,27} = 4.93$, $P < 0.002$; post-hoc tests). In contrast, 18-MC reduced intake of sucrose at both 24 and 48 h indicating that its effects were protracted; animals consumed 54% and 28% less sucrose at 24 and 48 h, respectively, compared to baseline. Interestingly, animals injected with vehicle had a significantly increased intake of sucrose at 72 h after injection. Water consumption measured in the same experiment was not altered in any treatment group (Treatment × Session interaction: $F_{2,27} = 0.44$, $P > 0.85$).

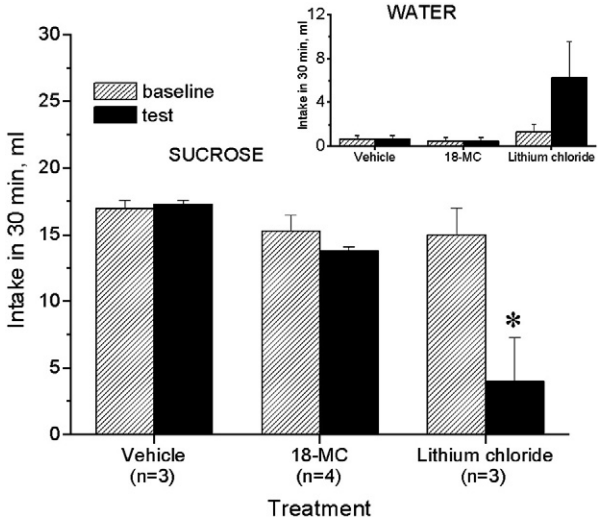

Fig. 3. Effects of 18-MC (20 mg/kg, i.p.), lithium chloride (100 mg/kg , i.p.) and vehicle on intake (mean ml ± SEM) of sucrose solution (5%) and water (insert) at 72 h after immediate (in test cages) association with sucrose.

3.3. 18-MC does not produce a conditioned taste aversion

The baseline values of sucrose intake (ml) in the vehicle, 18-MC and lithium chloride-treated groups were as follows: 12.56 ± 1.25; 12.42 ± 1.54; 12.00 ± 1.84, while the values of water intake (ml) were 1.22 ± 0.48; 1.45 ± 0.98; 2.78 ± 1.54, respectively. The baseline intake of sucrose and water was not significantly different among the three groups.

As indicated above, 18-MC has no proactive effect on intake of sucrose at 72 h after injection. Therefore, in the present experiment, animals were tested at 72 h after the immediate association of 18-MC and lithium chloride with sucrose solution (Fig. 3). Lithium chloride clearly reduced intake of sucrose while 18-MC had no effect (Treatment × Session interaction: $F_{2,7} = 13.83$, $P < 0.004$; post-hoc tests). Water consumption measured in the same session was not significantly altered in any treatment group (Treatment × Session interaction: $F_{2,7} = 4.38$, $P > 0.06$) .

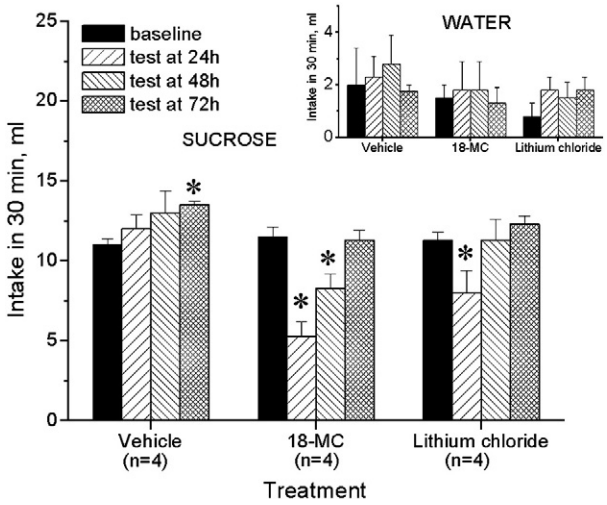

Fig. 2. Time course of effects of a single injection of 18-MC (20 mg/kg, i.p.), lithium chloride (100 mg/kg , i.p.) and vehicle on intake (mean ml ± SEM) of sucrose solution (5%) and water (insert) at 24, 48 and 72 h after treatment; treatments were administered in home cages 1 h after sucrose session.

4. Discussion

This study demonstrated for the first time that 18-MC, a selective antagonist of α3β4 nicotinic receptors and a potential anti-obesity agent does not induce a conditioned taste aversion in rats. The same dose of 18-MC used here previously attenuated sucrose reward, decreased consumption of palatable fluids, and when given repeatedly, reduced sucrose-induced weight gain and fat deposition in rats (Taraschenko et al., 2008).Since 18-MC has no effect on motor activity in rats (Maisonneuve and Glick, 2003) or on intake of water, the present findings suggest that the inhibition of sucrose drinking by 18-MC was behaviorally-specific.

In the present experiments, lithium chloride consistently reduced sucrose intake in control rats in all three experimental paradigms, i.e., at 24 h and 72 h after the immediate association with sucrose as well as at 24 h after the delayed association with sucrose. These findings are in agreement with other reports (Rozin, 1969; Domjan, 1977). A lithium-induced aversion developing after immediate association with a conditioned stimulus can occur as quickly as 5–10 min after injection and can be maintained for prolonged periods of time (Domjan, 1977). On the other hand, a CTA can also be successfully established with a delay of several hours between the presentation of sucrose and the lithium injection (Rozin, 1969). Only one pairing of conditioned and unconditioned stimuli is sufficient for CTA learning to occur, and it is more robust when the conditioned stimulus is a novel taste [for review, see (Yamamoto et al., 1994)].

Nicotinic receptors in the brain have been shown to be important for the processing of taste and for CTA acquisition (Stolerman, 1988; Shoaib et al., 2002). For example, administration of a cholinergic agonist, carbachol, into the insular cortex facilitates acquisition of a CTA to saccharin in rats (Clark and Bernstein, 2009). Likewise, injection of nicotine into the nucleus accumbens or interpeduncular nucleus produces a CTA in rats; the former effect could be blocked by systemic pretreatment with the nicotinic antagonist mecamylamine (Shoaib and Stolerman, 1995; Clark and Bernstein, 2009).Taken collectively, these findings suggest that the novelty of a taste and the formation of a CTA can be manipulated through the nicotinic cholinergic receptors (Clark and Bernstein, 2009). The lack of a CTA response to 18-MC in the present experiment suggests that α3β4 nicotinic receptors are not likely to be involved in aversion learning in sucrose drinking rats.

In the present study, a single intraperitoneal injection of 18-MC reduced consumption of sucrose but not water for as long as 48 h after treatment. Previously, 18-MC was shown to produce protracted effects on variety of behavioral and neurochemical responses in rodents [for review, see (Maisonneuve and Glick, 2003)]. For example, the effect of 18-MC on sensitized dopamine release in the nucleus accumbens of morphine-experienced rats lasts up to 19 h, while its effects on morphine and cocaine self-administration last up to 48 h. Furthermore, the effects of repeated injections of 18-MC on fat deposition in rats consuming a high sucrose diet were significant for 7 days after the last treatment (Taraschenko et al., 2008). These effects, lasting well beyond the presence of the drug in plasma (Glick et al., 1999) were thought to be due to the drug's deposition in fat and subsequent mobilization (Maisonneuve and Glick, 2003). Reduced sucrose drinking after18-MC in the present studies (Fig. 2) is consistent with such an interpretation. Interestingly, in the same experiment, vehicle-injected rats increased their sucrose intake at 72 h compared to baseline. The previously described phenomenon of "attenuation of neophobia" could be responsible for this observation (Domjan, 1976). Thus, if ingestion of a novel taste is not repulsive, subsequent presentation of the same taste will lead to increased consumption.

In conclusion, data obtained in this study indicate that 18-MC does not induce a conditioned taste aversion in rats and that $\alpha 3\beta 4$ nicotinic receptors are not likely to be involved in the establishment of a CTA. Thus, the previously demonstrated reduction of sucrose intake and sucrose-induced obesity in rats treated with 18-MC was not due to an aversion to sucrose.

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