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Is antagonism of α3β4 nicotinic receptors a strategy to reduce morphine dependence?

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Abstract

18-Methoxycoronaridine, a synthetic iboga alkaloid congener, has been previously shown to attenuate several signs of morphine withdrawal in rats. The recently discovered action of 18-methoxycoronaridine to block α3β4 nicotinic receptors may be responsible for this effect. To test this hypothesis the effects of non-selective α3β4 receptor antagonists, dextromethorphan, mecamylamine, bupropion, and their combinations, were assessed on of acute naltrexone-precipitated (1 mg/kg i.p.) morphine withdrawal in rats.

Dextromethorphan (5–40 mg/kg, s.c.), mecamylamine (0.25–4 mg/kg, i.p.) and bupropion (10–30 mg/kg, i.p.) alone produced variable effects on signs of withdrawal. However, two low-dose combinations, i.e., dextromethorphan (5 mg/kg, s.c.) and mecamylamine (0.25 mg/kg, i.p.), mecamylamine (0.25 mg/kg, i.p.) and bupropion (10 mg/kg, i.p.) as well as the three-drug combination significantly attenuated diarrhea and weight loss; none of the agents administered alone had these effects. The results of the present study provide evidence that α3β4 nicotinic receptors are involved in the expression of at least two signs of opioid withdrawal.

Keywords: Morphine; Naltrexone; Dextromethorphan; Mecamylamine; Bupropion; Opioid withdrawal

1. Introduction

Opioid addiction is a major public health problem in the United States. The withdrawal syndrome in opioid-dependent humans is extremely unpleasant and is manifested as tachycardia, hypertension, sweating, diarrhea, vomiting, irritability and insomnia. The symptoms arise from the loss of chronic opioid inhibition of an up-regulated cAMP pathway in a subset of neurons in the central nervous system (locus coeruleus, nucleus accumbens) and in peripheral sites (gut) (Cami and Farre, 2003; Nestler and Aghajanian, 1997; Vetulani, 2001). The abstinence syndrome frequently appears as early as 4–6 h following discontinuation of short-acting opioids (which have the highest potential for abuse) and intensifies at 24–48 h, lasting up to 14 days. The avoidance of aversive abstinence symptoms serves as a negative reinforcer that promotes drug seeking and drug taking. Despite a need for new agents designed to alleviate withdrawal symptoms, only a few treatments are currently available. The most commonly used treatment is based on cross-tolerance and involves administration of the long-acting opioid agonist methadone. This therapy is restricted to hospital use and specially licensed outpatient programs (Kosten and O'Connor, 2003; O'Brien, 2001). The second treatment option involves the use of an α2-adrenoreceptor agonist clonidine, which reduces norepinephrine release from the locus coeruleus neurons by acting on inhibitory autoreceptors (Kosten and O'Connor, 2003; O'Brien, 2001). This treatment does not reduce opioid craving and is frequently associated with hypotension. A number of other agents are being tested in preclinical studies.

18-Methoxycoronaridine, a synthetic iboga alkaloid congener, is currently being studied for the treatment of drug addiction. Reports from this laboratory have shown that 18-methoxycoronaridine reduces the self-administration of morphine (Glick et al., 1996) and attenuates several signs

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of morphine withdrawal in rats (Rho and Glick, 1998). In the nucleus accumbens, pretreatment with 18-methoxycoronaridine abolishes the sensitized increase in dopamine levels induced by chronic morphine administration (Szumlinski et al., 2000). Until recently, the exact molecular mechanism mediating 18-methoxycoronaridine's effects on dopamine release and drug self-administration remained elusive. However, recent in vitro studies have shown that 18-methoxycoronaridine is a potent antagonist of α3β4 nicotinic receptors (IC_{50}=0.75 μM) (Glick et al., 2002). In addition, a combination of a low dose (i.e., ineffective alone) of 18-methoxycoronaridine with a low dose of other agents known to block α3β4 nicotinic receptors reduced morphine self-administration (Glick et al., 2002). These results suggested that antagonism of α3β4 nicotinic receptors can modulate drug-seeking behavior.

The α3β4 nicotinic receptor antagonists referred to above included dextromethorphan (IC_{50}=8.9 μM), mecamylamine (IC_{50}=1 μM) and bupropion (IC_{50}=1.5–1.8 μM) (Hernandez et al., 2000; Fryer and Lukas, 1999; Damaj et al., 2004). All three agents are currently available on the market and have been tried, with some success, for the treatment of different aspects of psychological and physical substance dependence. Thus, dextromethorphan, also an *N*-methyl-D-aspartate (NMDA) receptor antagonist and the active ingredient of anti-cough medications, has been shown to attenuate signs of opioid withdrawal in rodents and humans (Farzin, 1999; Koyuncuoglu et al., 1990; Koyuncuoglu and Saydam, 1990). However, the phencyclidine-like behavioral activation, present at high doses (Rosen et al., 1996), may limit clinical use of dextromethorphan. Mecamylamine, an antagonist of several nicotinic receptor subtypes, is about 3-fold more potent in blocking agonist-induced responses at α3β4 neuronal nicotinic receptors than at α4β2 and α7 receptors (Chavez-Noriega et al., 1997; Hernandez et al., 2000). Previously used as an antihypertensive, mecamylamine has been shown to be effective in reducing cue-induced craving in cocaine addicts (Reid et al., 1999) and in attenuating somatic stimulant effects of alcohol (Blomqvist et al., 2002; Chi and De Wit, 2003) and tobacco (Rose et al., 1994). Bupropion, widely used as an atypical antidepressant, is a dopamine transporter blocker, and is approved by the Food and Drug Administration for non-nicotine based therapy for smoking cessation (Glover and Glover, 2001; Hurt et al., 1997). Bupropion's therapeutic value for smoking cessation has been attributed to its blockade of nicotinic receptors (Fryer and Lukas, 1999). Thus, pharmacological blockade of central α3-containing nicotinic receptors by bupropion and other drugs may play a significant role in drug dependence (Fryer and Lukas, 1999). The results indicating that α3β4 nicotinic receptors mediate 18-methoxycoronaridine's effects on drug self-administration prompted us to assess whether α3β4 nicotinic receptor antagonism might also be important for modulation of opioid withdrawal. To test this possibility we assessed the effects of dextromethorphan, mecamylamine and bupropion, as well as low-dose combinations of these agents, in a rat model of acute opioid withdrawal.

2. Materials and methods

2.1. Drugs

Morphine sulfate and naltrexone hydrochloride (1 mg/kg) (Research Biochemicals Inc., Natick, MA) were dissolved in saline and injected subcutaneously and intraperitonealy, respectively. Dextromethorphan hydrobromide (5, 10, 20, 30 and 40 mg/kg, Sigma, St. Louis, MO) was dissolved in saline and injected subcutaneously. Mecamylamine hydrochloride (0.25, 0.5, 1, 2 and 4 mg/kg, Sigma) and bupropion hydrochloride (10, 20 and 30 mg/kg, Sigma) were dissolved in saline and injected intraperitonealy. For two-drug combinations the injection of dextromethorphan (0.5 mg/kg and 10 mg/kg) was immediately followed by an injection of mecamylamine (0.25 mg/kg and 0.5 mg/kg) or bupropion (10 mg/kg and 20 mg/kg); administration of mecamylamine (0.25 mg/kg and 0.5 mg/kg) was promptly followed by an injection of bupropion (10 mg/kg and 20 mg/kg). For the three-drug combination the injection of dextromethorphan (0.5 mg/kg, s.c.) was immediately followed by an injection of mecamylamine (0.25 mg/kg, i.p.) and bupropion (10 mg/kg, i.p.).

2.2. Animals

Naïve female Spraque–Dawley rats (Taconic, Germantown, NY), weighting 250–300 g, were housed individually and maintained on a normal 12:12–h light/dark cycle (light on at 7 a.m., light off at 7 p.m.). Food and water were provided ad libitum. All animal experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Academy of Sciences, 1996).

2.3. Morphine treatment

Morphine was administered subcutaneously twice a day (9.30 a.m. and 3.30 p.m.) for 7 days according to the following schedule: 10 and 20 mg/kg on the first day, 40 and 60 mg/kg on the second day, and 60 and 80 mg/kg on the third and subsequent days. This schedule was previously used by Rho and Glick (1998) to induce morphine dependence in rats.

2.4. Naltrexone-precipitated withdrawal

On the day of testing, pretreatment with either drug(s) or saline was followed 30 min later by injection of naltrexone. Signs of the precipitated withdrawal syndrome were observed and counted continuously for each 15-min interval for 2 h; the observations were made blindly. The following signs were monitored: "wet dog" shakes, rearing, grooming,

teeth chattering, burying, diarrhea. Rats were weighed twice: immediately prior to injection of naltrexone and 2 h later.

2.5. Data analysis

Withdrawal scores were expressed as percents of the appropriate control means and were analyzed with two-way analysis of variance (ANOVA) with dose and sign as the two factors. This analysis was followed by post hoc comparison tests (Fisher LSD). The analysis of time-course effects of the drugs was performed using repeated-measures ANOVA followed by post hoc comparison tests when appropriate.

3. Results

Naltrexone immediately precipitated a withdrawal syndrome in morphine-dependent rats which was characterized by "wet-dog" shaking, rearing, grooming, teeth chattering, burying, diarrhea and weight loss. The average withdrawal scores in all control saline-pretreated animals ($n=31$) were as follows (mean incidents per 2 h ± S.E.M., except for the weight loss in g): "wet-dog" shakes, 54.4 ± 5.3; rearing, 38.0 ± 4.9; grooming, 14.0 ± 1,4; teeth chattering, 21.2 ± 2.1; burying, 12.8 ± 1.6; diarrhea, 4.4 ± 0.3; and weight loss, 11.2 ± 0.5. The mean scores for four out of six withdrawal signs (i.e., "wet-dog" shakes, rearing, burying and diarrhea) decreased while teeth chattering increased during the first hour. The mean score for grooming was unchanged during the 2-h period of observation.

3.1. Single-drug treatments

Mean withdrawal scores in control rats ($n=11$) for Figs. 1–3 were as follows (mean incidents per 2 h ± S.E.M., except for weight loss in g): "wet-dog" shakes, 60.1 ± 8.4; rearing, 50.6 ± 10.3; grooming, 13.8 ± 2.1; teeth chattering, 26.1 ± 4.5; burying, 13.5 ± 2.6; diarrhea, 3.5 ± 0.3; and weight loss, 10.4 ± 0.4.

3.1.1. Dextromethorphan alone (Fig. 1)

Two-way ANOVA with dose of dextromethorphan and sign as the two factors revealed a significant main effect of treatment and a significant treatment × sign interaction [$F(5,33)=6.35$, $P<0.00031$ and $F(30,198)=2.27$, $P<0.0005$, respectively]. Further post hoc tests showed that three dosages of dextromethorphan (i.e., 5, 10 and 30 mg/kg) reduced the incidence of rearing, while two dosages (i.e., 30 and 40 mg/kg) reduced grooming. At 5 and 20 mg/kg dextromethorphan significantly attenuated and increased teeth chattering, respectively. The highest tested dose of dextromethorphan (i.e., 40 mg/kg) significantly attenuated burying. Dextromethorphan had no effect on "wet dog" shakes, diarrhea and weight loss.

3.1.2. Mecamylamine alone (Fig. 2)

Two-way ANOVA revealed a significant main effect of treatment and a significant treatment × sign interaction [$F(5,35)=7.56$, $P<0.00007$ and $F(30,210)=2.66$, $P<0.00003$, respectively]. Further post hoc tests showed that at 0.5 mg/kg and 1 mg/kg mecamylamine significantly increased "wet-dog" shakes. Three dosages (0.25, 0.5 and 2 mg/kg) reduced rearing, while two dosages (i.e., 0.5 and 2 mg/kg) attenuated teeth chattering. At 1 and 0.5 mg/kg mecamylamine significantly increased grooming and attenuated burying, respectively. All dosages except 0.25 mg/kg attenuated diarrhea, while three dosages (i.e., 0.5, 2 and 4 mg/kg) reduced weight loss.

3.1.3. Bupropion alone (Fig. 3)

The overall two-way ANOVA with dose of bupropion and sign as the two factors showed a significant main effect

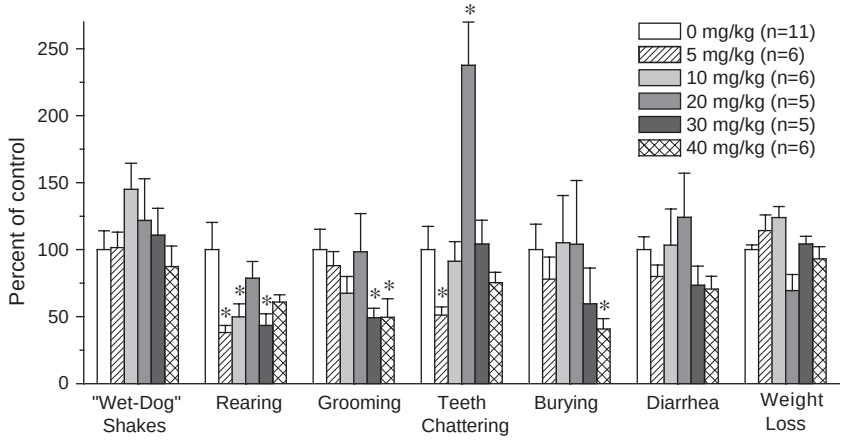

Fig. 1. Effects of dextromethorphan on morphine withdrawal signs. Dextromethorphan or saline were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, dextromethorphan versus saline.

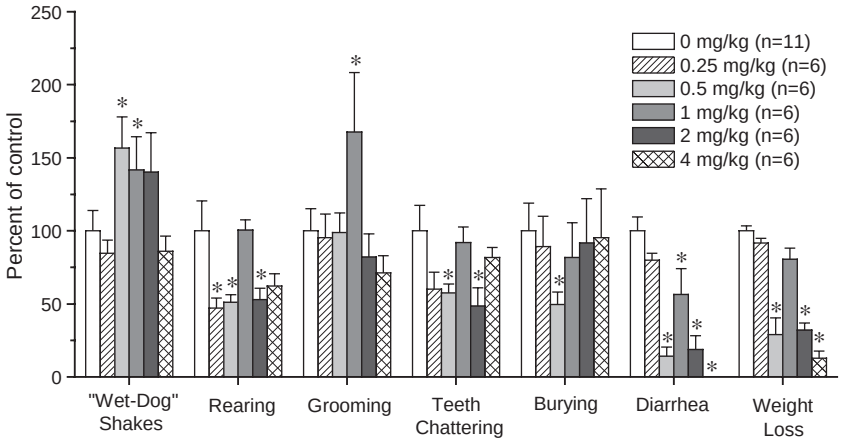

Fig. 2. Effects of mecamylamine on morphine withdrawal signs. Mecamylamine or saline were administered 30 min before naltrexone (1 mg/kg, i.p.) and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, mecamylamine versus saline.

of treatment and a significant treatment × sign interaction [$F(3,23)=9.84$, $P<0.00023$ and $F(18,138)=2.53$, $P<0.001$, respectively]. Bupropion (10 mg/kg) significantly attenuated rearing. At higher doses (i.e., 20 and 30 mg/kg) bupropion decreased grooming and burying while all dosages decreased teeth chattering. Bupropion had no effects on "wet-dog" shakes, diarrhea and weight loss.

3.2. Combination treatments

3.2.1. Dextromethorphan–mecamylamine combinations (Figs. 4–6)

Mean withdrawal scores in control rats ($n=18$) were as follows (mean incidents per 2 h ± S.E.M., except for weight loss in g): "wet-dog" shakes, 66.9 ± 6.6; rearing, 42.9 ± 7.0; grooming, 13.6 ± 1.6; teeth chattering, 23.3 ± 3.07; burying, 11.0 ± 1.9; diarrhea, 3.6 ± 0.3; and weight loss, 11.0 ± 0.6.

The effects of pretreatment with dextromethorphan (5 mg/kg, s.c.), mecamylamine (0.25 mg/kg, i.p.) and their combination are shown in Fig. 4. An overall two-way ANOVA with treatment and sign as the two factors revealed a significant main effect of treatment ($F(3,34)=9.72$, $P<0.00009$). Post hoc tests showed that rearing and teeth chattering were significantly attenuated by each drug alone as well as their combination, while diarrhea and weight loss were attenuated by combination treatment only. The effects of combination were different from the effects of dextromethorphan alone for weight loss.

The effects of dextromethorphan (10 mg/kg), mecamylamine (0.5 mg/kg) and their combination are shown in Fig. 5. The overall two-way ANOVA showed a significant main effect of treatment and a significant treatment × sign interaction [$F(3,32)=15.01$, $P<0.00001$ and $F(18,192)=3.34$, $P<0.00002$, respectively].

Post hoc analysis showed that "wet-dog" shakes were significantly increased by dextromethorphan and mecamylamine alone but not by their combination; these effects of the individual drugs were also significantly different from the effect of their combination. The time-course of these effects is shown in Fig. 6. The overall repeated-measures ANOVA with treatment and time as the two factors revealed a significant main effect of the treatment and a significant treatment × time

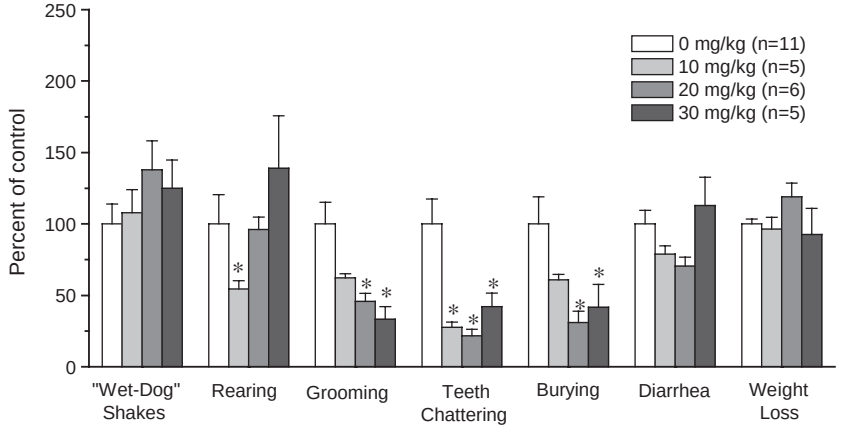

Fig. 3. Effects of bupropion on morphine withdrawal signs. Bupropion or saline were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, bupropion versus saline.

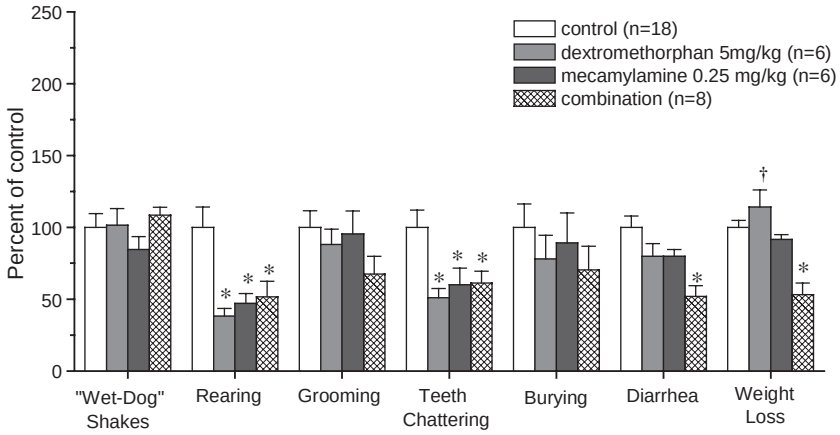

Fig. 4. Effects of the combination of dextromethorphan (5 mg/kg, s.c.) and mecamylamine (0.25 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

interaction [$F(3,32)=5.71$, $P<0.003$ and $F(21,224)=2.81$, $P<0.00008$, respectively]. Further post hoc tests showed that dextromethorphan and mecamylamine alone significantly increased "wet-dog" shaking at all time points except 120 min after naltrexone. However, the combination of these agents significantly attenuated "wet-dog" shakes at 15 and 60–105 min after naltrexone. The effect of the combination was significantly different from the effect of each drug alone at all time points except 120 min. In contrast to the results for the combined 2-h means (Fig. 5), this analysis demonstrated that the combination treatment significantly attenuated wet-dog shaking at five out of eight time points after naltrexone.

Post hoc tests following the initial overall ANOVA showed that neither dextromethorphan or mecamylamine affected grooming, but their combination significantly attenuated it; the effect of the combination was also significantly different from that of mecamylamine alone (Fig. 5). Teeth chattering was significantly attenuated by mecamylamine alone and by combination. For this sign the effects of combination were different from those of dextromethorphan alone (Fig. 5). Rearing was attenuated by either drug alone, but not by their combination while burying was reduced by mecamylamine only. Diarrhea and weight losses were significantly attenuated by mecamylamine alone and by combination. For those two signs the effects of combination were different from those of dextromethorphan alone.

3.2.2. Mecamylamine–bupropion combinations (Figs. 7 and 8)

Mean withdrawal scores in control rats ($n=18$) were as follows (mean incidents per 2 h ± S.E.M., except for weight loss in g): "wet-dog" shakes, 52.3 ± 6.0; rearing, 42.9 ± 7.2; grooming, 12.8 ± 1.5; teeth chattering, 22.9 ± 3.0; burying, 14.9 ± 2.1; diarrhea, 3.8 ± 0.4; and weight loss, 10.6 ± 0.5.

The effects of mecamylamine (0.25 mg/kg, i.p.), bupropion (10 mg/kg, i.p.) and their combination are shown in Fig.

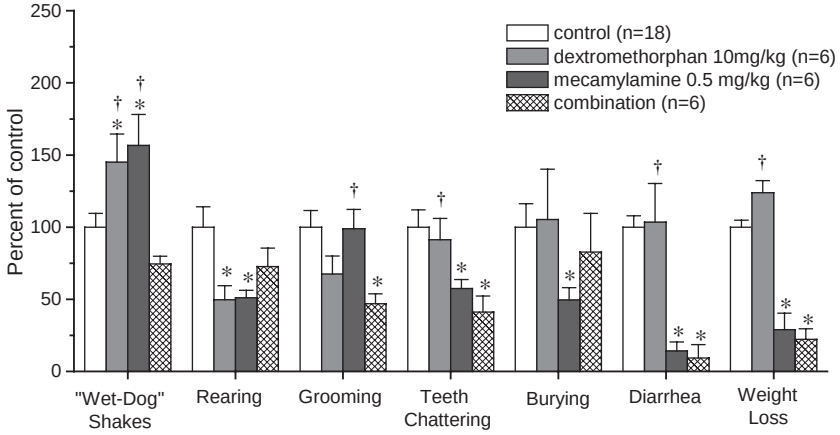

Fig. 5. Effects of the combination of dextromethorphan (10 mg/kg, s.c.) and mecamylamine (0.5 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

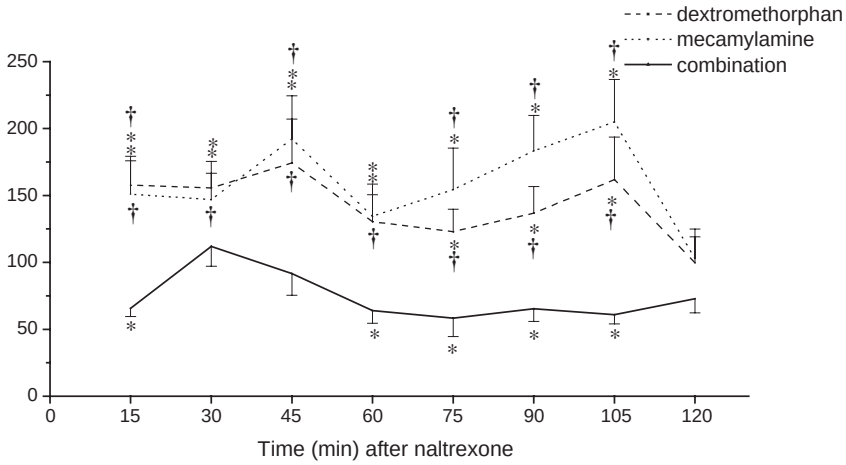

Fig. 6. Time-course of the effects of dextromethorphan (10 mg/kg, s.c., $n=6$), mecamylamine (0.5 mg/kg, i.p., $n=6$) and their combination ($n=6$) on "wet-dog" shakes. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.), and signs of withdrawal (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

7. An overall two-way ANOVA with treatment and sign as the two factors revealed a significant main effect of treatment and a significant treatment × sign interaction [$F(3,32)=5.37$, $P<0.004$ and $F(18,192)=2.86$, $P<0.0002$, respectively]. Post hoc tests revealed that rearing was significantly attenuated by either mecamylamine or bupropion, while grooming was increased by combination treatment. For these signs the effects of either drug alone were significantly different from the effects of combination. Teeth chattering was significantly attenuated by either drug alone and by their combination, while diarrhea and weight loss were reduced by combination treatment only. The effects of the combination treatment for diarrhea were also significantly different from those of the individual treatments. Neither treatment affected "wet-dog" shaking and burying.

The effects of mecamylamine (0.5 mg/kg, i.p.), bupropion (20 mg/kg, i.p.) and their combination are shown in Fig. 8. The overall two-way ANOVA demonstrated a significant main effect of treatment and a significant treatment × sign interaction [$F(3,33)=11.12$, $P<0.00003$ and $F(18,198)=5.48$, $P<0.00001$, respectively]. Post hoc tests showed that "wet-dog" shaking was increased by mecamylamine and bupropion alone but not by their combination. Mecamylamine alone significantly attenuated rearing, while combination treatment increased this sign; the effect of mecamylamine for rearing was significantly different from that of the combination. Grooming was attenuated by bupropion only, while teeth chattering was reduced by both mecamylamine and bupropion; the combination treatment was not effective for those signs. The effect of bupropion on teeth chattering was also significantly different from that of combination. All three treatments (i.e., mecamylamine, bupropion and their combination) attenuated burying, while two treatments (i.e., mecamylamine and combination) attenuated diarrhea and weight loss. The effects of dextromethorphan on diarrhea and weight loss were also significantly different from those of their combination.

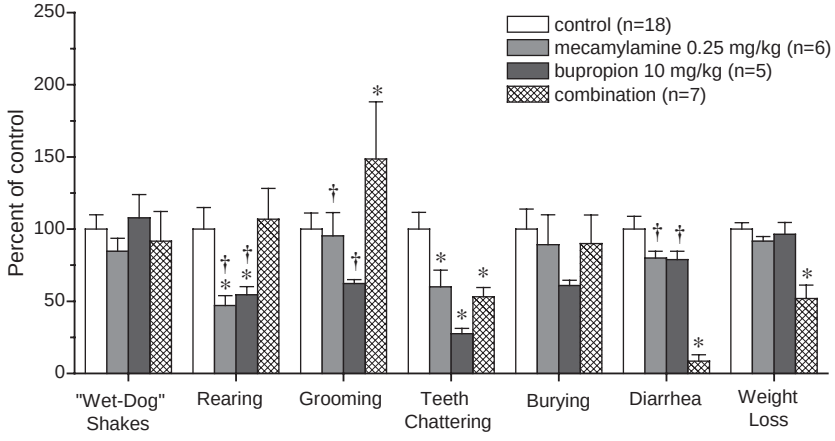

Fig. 7. Effect of the combination of mecamylamine (0.25 mg/kg, i.p.) and bupropion (10 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.) and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

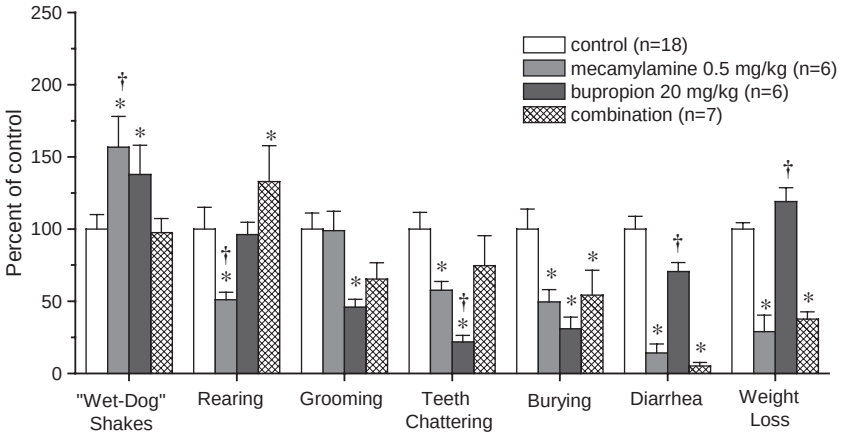

Fig. 8. Effects of the combination of mecamylamine (0.5 mg/kg, i.p.) and bupropion (20 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.) and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

3.2.3. Dextromethorphan–bupropion combinations (Figs. 9 and 10)

Mean withdrawal scores in control rats ($n=18$) were as follows (mean incidents per 2 h ± S.E.M., except for weight loss in g): "wet-dog" shakes, 52.3 ± 6.0; rearing, 42.9 ± 7.2; grooming, 12.8 ± 1.5; teeth chattering, 22.9 ± 3.0; burying, 14.9 ± 2.1; diarrhea, 3.8 ± 0.4; and weight loss, 10.6 ± 0.5.

The effects of dextromethorphan (5 mg/kg, s.c.), bupropion (10 mg/kg, i.p.) and their combination are shown in Fig. 9. The overall two-way ANOVA with treatment and sign as the two factors revealed a significant main effect of treatment and a significant treatment × sign interaction [$F(3,32)=4.77$, $P<0.007$ and $F(18,192)=1.73$, $P<0.04$, respectively]. Post hoc tests showed that dextromethorphan alone attenuated rearing although the combination treatment had no effect; the effects of either drug alone were also significantly different from that of the combination. Teeth chattering was reduced by both dextromethorphan and bupropion but not by their combination; the effect of bupropion was also significantly different from that of combination. Burying was significantly reduced by bupropion alone and by the combination. Neither treatment had an effect on "wet-dog" shaking, diarrhea and weight loss.

The effects of dextromethorphan (10 mg/kg, s.c.), bupropion (20 mg/kg, i.p.) and their combination are shown in Fig. 10. The overall two-way ANOVA demonstrated a significant treatment × sign interaction ($F(18,198)=2.53$, $P<0.001$). Post hoc analysis revealed that combination treatment significantly increased rearing, while only dextromethorphan alone was effective in reducing this sign. The effects of either drug alone were also significantly different from that of the combination. Grooming, teeth chattering and burying were significantly attenuated by bupropion only but not by the combination treatment. The effect of bupropion on teeth chattering was also significantly different from that of the combination. Neither treatment had an effect on "wet dog " shaking, grooming, diarrhea and weight loss.

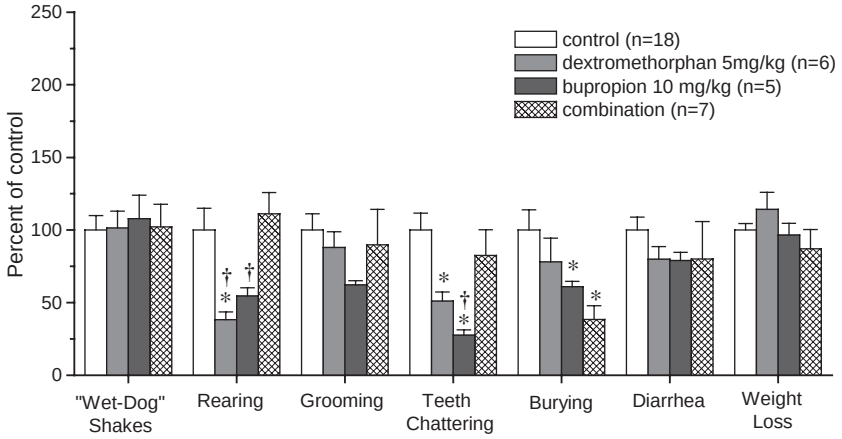

Fig. 9. Effects of the combination of dextromethorphan (5 mg/kg, s.c.) and bupropion (10 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; †$P<0.05$, LSD test, individual drug versus drug combination.

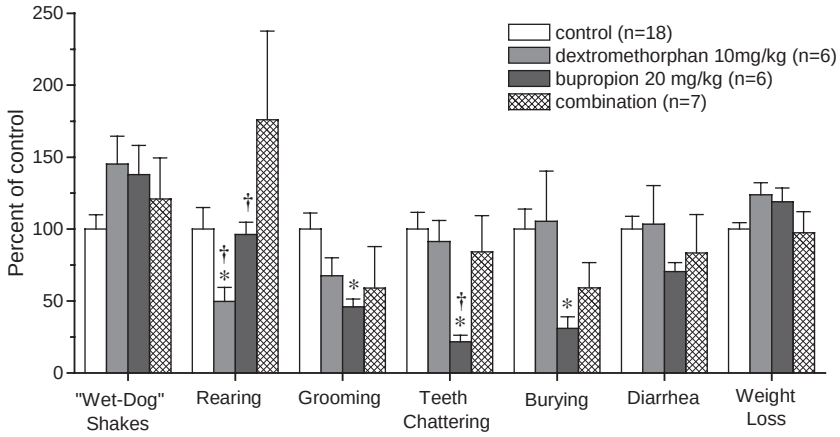

Fig. 10. Effects of the combination of dextromethorphan (10 mg/kg, s.c.) and bupropion (20 mg/kg, i.p.) on morphine withdrawal signs. Drug treatments or vehicle (saline) were administered 30 min before naltrexone (1 mg/kg, i.p.), and withdrawal signs (percent of control ± S.E.M.) were assessed for 2 h. *$P<0.05$, LSD test, drug versus control; $^{\dagger}P<0.05$, LSD test, individual drug versus drug combination.

3.2.4. Dextromethorphan–mecamylamine–bupropion combination (Fig. 11)

The effects of triple-agent combination treatment on diarrhea and weight loss are shown on Fig. 11. The mean withdrawal scores in control rats ($n=17$) were as follows (mean incidents per 2 h ± S.E.M., except for weight loss in g): "wet-dog" shakes, 50.6 ± 7.4; rearing, 45.9 ± 7.8; grooming, 20.1 ± 4.6; teeth chattering, 23.4 ± 3.4; burying, 15.3 ± 2.5; diarrhea, 6.9 ± 1.7; and weight loss, 9.2 ± 0.5.

The overall two-way ANOVA with treatment and sign as the two factors revealed a significant main effect of treatment and a significant treatment × sign interaction [$F(4,35)=3.37$, $P<0.02$) and $F(24,210)=1.90$, $P<0.001$, respectively]. Post hoc tests revealed that rearing was attenuated by either drug alone but not by their combination; the effects of individual drugs were also significantly different from those of the combination. Three drugs (i.e., dextromethorphan, mecamylamine and bupropion) significantly reduced teeth chattering. The combination of three agents significantly attenuated grooming, diarrhea and weight loss, although none of the individual drugs had an effect. The effect of mecamylamine alone on all three signs was also significantly different from that of combination. Likewise, the effects of dextromethorphan and bupropion on weight loss were significantly different from those of combination.

4. Discussion

Nicotinic receptors may be important targets for novel medications for opioid dependence. 18-Methoxycoronaridine and dextromethorphan, both antagonists of α3β4 nicotinic receptors, have previously been shown to reduce the expression of opioid withdrawal in animals and humans (Farzin, 1999; Koyuncuoglu et al., 1990; Koyuncuoglu and

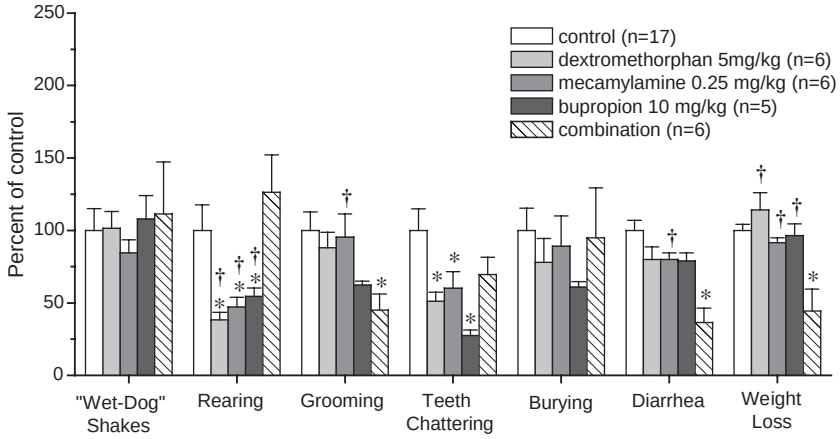

Fig. 11. Effects of dextromethorphan (5 mg/kg, s.c.), mecamylamine (0.25 mg/kg, i.p.), bupropion (10 mg/kg, i.p.) and their combination on morphine withdrawal signs. Drug treatments or vehicle (saline, $n=17$) were administered 30 min before naltrexone (1 mg/kg, i.p.). *$P<0.05$, LSD test, drug versus control; $^{\dagger}P<0.05$, LSD test, individual drug versus drug combination.

Saydam, 1990; Manning et al., 1996; Rho and Glick, 1998). In the present study, the effects of the non-selective α3β4 nicotinic receptor antagonists dextromethorphan, mecamylamine, bupropion and their combinations were assessed on signs of acute naltrexone-precipitated opioid withdrawal in rats.

As expected, chronic morphine administration resulted in the development of opioid dependence such that injection of naltrexone precipitated acute withdrawal, characterized by the presence of the following signs: "wet-dog" shakes, rearing, grooming, teeth chattering, burying, diarrhea and weight loss. Consistent with previous work (Rasmussen and Vandergriff, 2003), the majority of withdrawal signs (i.e., "wet-dog" shakes, rearing, burying and diarrhea) were expressed maximally during the first 15 min after naltrexone, decreasing thereafter (data not shown). However, teeth chattering tended to increase over the first hour while grooming occurred at a constant rate during the first hour.

The opioid withdrawal syndrome has been linked to multiple brain sites and neurotransmitter systems. Rearing and teeth chattering have been associated with the overactivity of noradrenergic neurons in the nucleus locus coeruleus and periaqueductal gray (Maldonado et al., 1992). "Wet-dog" shakes have been attributed to the activation of the anterior hypothalamus and nucleus raphe magnus (Maldonado et al., 1992). The aversive motivational aspects of opiate withdrawal appear to involve the nucleus accumbens (Koob et al., 1992). The expression of somatic signs of opioid withdrawal in morphine-dependent rats does not correlate with the magnitude of withdrawal-induced aversion as measured with conditioned place preference (Hand et al., 1988; Mucha, 1987). The possible effects of α3β4 antagonists and their combinations on motivational aspects of morphine withdrawal remain to be investigated.

Dextromethorphan, mecamylamine and bupropion each have unique actions (dextromethorphan blocks NMDA receptors, (Trujillo, 2000); mecamylamine blocks other subtypes of nicotinic receptors, (Papke et al., 2001); bupropion is an inhibitor of catecholamine uptake (Learned-Coughlin et al., 2003). However, the only action they have in common is to block nicotinic α3β4 receptors (Fryer and Lukas, 1999; Hernandez et al., 2000; Papke et al., 2001). It was therefore reasoned that combinations of low doses of these agents would produce an additive effects at the common site (i.e., α3β4 receptors) without producing effects mediated by their other actions. The results for only two out of seven signs of withdrawal, i.e., diarrhea and weight loss, were consistent with this premise. Thus, diarrhea and weight loss were attenuated by combinations of dextromethorphan and mecamylamine (5 mg/kg, s.c. and 0.25 mg/kg, i.p., respectively, Fig. 4), mecamylamine and bupropion (0.25 mg/kg, i.p. and 10 mg/kg, i.p., respectively, Fig. 7) as well as by the three-drug combination (Fig. 11). None of the agents administered alone had an effect on these signs. Attenuation of diarrhea by the combined treatments in this study is likely mediated by actions of the drugs on both centrally and peripherally located α3β4 nicotinic receptors. In the brain, α3β4 receptors are present in the cerebellum, dorsal tegmentum, subiculum, anteroventral thalamic nucleus and the locus coeruleus (Perry et al., 2002; Whiteaker et al., 2002), although the highest densities of these receptors are in the nuclei of the habenulo–interpeduncular pathway (Mulle et al., 1991; Quick et al., 1999; Sheffield et al., 2000). The locus coeruleus is known to mediate the expression of diarrhea associated with morphine withdrawal (Taylor et al., 1988; Kimes et al., 1990) and may mediate attenuation of diarrhea produced by the combination treatments in this study. Furthermore, antagonists of α3β4 nicotinic receptors could act in the dorsal motor nucleus of the vagus. This brain site expresses modest α3β4-like binding (Perry et al., 2002) and controls parasympathetic activity in the gastrointestinal tract. In the peripheral nervous system, α3β4 nicotinic receptors are densely expressed in the myenteric neurons of the gut (Zhou et al., 2002). Nicotinic receptors containing α3 and β4 subunits may mediate excitatory cholinergic neurotransmission in the gut and regulate intestinal peristalsis. Previous reports have shown that peristaltic contractions of murine ileal segments were attenuated by mecamylamine, a potent antagonist of myenteric α3β4 nicotinic receptors (IC_{50}=0.1 μM) (Ren et al., 2003; Zhou et al., 2002). Thus, the attenuation of diarrhea and associated weight loss in the present study could be attributed to the action of the drugs at those peripheral receptor sites.

Antagonist-precipitated morphine withdrawal in rodents produces a variety of metabolic and endocrine effects that may induce weight loss. For example, opioid withdrawal in rats activates the hypothalamus–pituitary–adrenal axis via enhanced noradrenegic input, and causes an overproduction of corticotropin-releasing factor as well as an elevation of plasma corticosteroid levels (Gonzalvez et al., 1994; Ignar and Kuhn, 1990; Milanes et al., 1998). Major noradrenergic afferents in the hypothalamus arrive from the hippocampus and brain stem nuclei that are enriched in α3β4 nicotinic receptors. Therefore, by acting on those afferents, α3β4 nicotinic antagonists could prevent activation of the hypothalamus during withdrawal and thus attenuate weight loss.

Consistent with an α3β4 mechanism, grooming was attenuated by the higher-dose combination of dextromethorphan and mecamylamine (10 mg/kg, s.c. and 0.5 mg/kg, i.p., respectively, Fig. 5) as well as by the triple-drug combination (Fig. 11); the same doses of the individual drugs had no effect on grooming. The expression of grooming during withdrawal in morphine-dependent rats has been linked to the substantia nigra (Baumeister et al., 1992). Since this brain area does not express α3β4 nicotinic receptors or expresses very low densities (Perry et al., 2002; Whiteaker et al., 2002), it

may have been affected indirectly. At the same time, although "wet-dog" shakes were significantly increased by either dextromethorphan (10 mg/kg, s.c.) or mecamylamine (0.5 mg/kg, i.p.) alone (Fig. 5), it was significantly attenuated by combination of those agents at 15 and 60–105 min after naltrexone (Fig. 6). The expression of "wet-dog" shakes during opioid withdrawal has been linked to the nucleus raphe magnus (Yap and Taylor, 1983), where there are moderate levels of α3β4 nicotinic receptors (Perry et al., 2002).

The other effects produced by the individual drugs alone may have been due to their other actions. Previous studies have demonstrated that central glutamatergic mechanisms play an important role in the physiology of opioid withdrawal (for review, (Nestler and Aghajanian, 1997). The up-regulation of glutamate receptors is one of the neuroadaptative processes associated with repeated opioid treatment. A variety of glutamatergic antagonists have been shown to attenuate opioid withdrawal (Farzin, 1999; Gonzalez et al., 1997; Herman et al., 1995; Manning et al., 1996; Popik and Skolnick, 1996). In previous studies, injection of dextromethorphan prior to naloxone treatment attenuated six signs of withdrawal in morphine-dependent mice (Farzin, 1999). Similarly, dextromethorphan reduced two out of eight signs of withdrawal in morphine-dependent rats (Koyuncuoglu et al., 1990). Such effects of dextromethorphan were attributed to the blockade of the effects of glutamate in brain sites responsible for the expression of withdrawal. The attenuation of rearing, grooming and burying by dextromethorphan alone in the present study were consistent with this mechanism (Fig. 1). Interestingly, 5 and 20 mg/kg of dextromethorphan significantly decreased and increased teeth chattering, respectively, whereas 30 and 40 mg/kg were ineffective. A biphasic dose-dependent effect of dextromethorphran on teeth chattering during opioid withdrawal was previously reported by Manning et al. (1996). The biphasic effect of dextromethorphan on rearing was also demonstrated in this study (Fig. 1). The mechanism of this effect remains unclear at this time.

Although mecamylamine has repeatedly been tested in nicotine and alcohol dependence paradigms (Blomqvist et al., 2002; Chi and De Wit, 2003; O'Dell et al., 2004; Rose et al., 1994), it has not previously been evaluated for opioid withdrawal. This drug may have promise for clinical use. While mecamylamine has affinity for the most prevalent nicotinic receptors in the brain, i.e., α4β2 and α7 (IC_{50}=2.5 μM and 6.9 μM, respectively), it is most potent at the α3β4 site (IC_{50}=0.64 μM) (Papke et al., 2001). The differential dose-dependent effects of mecamylamine alone in this study may be due to its actions on different nicotinic receptor subtypes.

Bupropion's effects were most probably mediated by its central actions (Suckow et al., 1986). In the brain bupropion is nearly equally potent at blocking α3β4 nicotinic receptors and blocking dopamine and norepinephrine uptake (Damaj et al., 2004). In the present study, when administered alone, bupropion significantly reduced several signs of withdrawal (i.e., rearing, grooming, teeth chattering and burying, Fig. 3). At the same time, the action of bupropion on dopamine uptake in certain brain areas may have dominated over its action on nicotinic receptors; thus, the increase in extracellular dopamine may have prevented the attenuation of some signs of withdrawal. For example, increased extracellular dopamine in the nucleus accumbens or medial prefrontal cortex has been associated with excessive rearing (Narita et al., 2003) and "wet-dog" shaking (Bassareo et al., 1995), respectively.

As discussed earlier, different signs of opioid withdrawal appear to be mediated by different neural systems. The results of the present study provide evidence that α3β4 nicotinic receptors are involved in the expression of at least two signs of opioid withdrawal.

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