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8-Carboxamidocyclazocine: A Long-Acting, Novel Benzomorphan

JEAN M. BIDLACK, DANA J. COHEN, JAY P. McLAUGHLIN, RONGLIANG LOU, YINGCHUN YE, and MARK P. WENTLAND

Department of Pharmacology and Physiology, University of Rochester, School of Medicine and Dentistry, Rochester, New York (J.M.B., D.J.C., J.P.M.); and Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York (R.L., Y.Y., M.P.W.)

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ABSTRACT

To obtain benzomorphans with a longer duration of action that may be potential therapeutics for treating cocaine abuse, 8-carboxamidocyclazocine was synthesized. The pharmacological properties of 8-carboxamidocyclazocine were compared with the parent compound cyclazocine. Changing the 8-hydroxyl group on cyclazocine to an 8-carboxamido group resulted in only a 2-fold decrease in the affinity of the compound for the κ-receptor, and no change in the affinity for the μ-opioid receptor, with both compounds having K_i values of less than 1 nM, based on radioligand binding assays. In the guanosine 5′-*O* -(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assay, the two compounds produced moderate stimulation of GTP binding to the human κ- and μ-receptors. When given by i.c.v. injection, the compounds produced less than 60% antinociception in the mouse 55°C warm-water tail-flick test. However, in the mouse writhing test, the compounds had high potency in producing antinociception. Antinociception induced by either 8-carboxamidocyclazocine or cyclazocine was mediated by both κ- and μ-opioid receptors. Cyclazocine acted as a μ-antagonist in addition to its agonist properties at the μ-receptor, as measured by the inhibition of morphine-induced antinociception. In contrast, 8-carboxamidocyclazocine did not inhibit morphine-induced antinociception, demonstrating that it was not a μ-opioid receptor antagonist in this assay. An i.p. injection of an ED_{70} dose of 8-carboxamidocyclazocine produced antinociception that lasted for 15 h in contrast to cyclazocine, which produced antinociception, lasting 2 h. 8-Carboxamidocyclazocine is a novel, long-acting benzomorphan, which possesses pharmacological properties that are distinct from the properties of cyclazocine.

A growing body of evidence suggests that κ-opioid agonists with varying activity at the μ-opioid receptor are effective in reducing cocaine self-administration in nonhuman primates (Negus et al., 1997; Mello and Negus, 1998) and rats (Glick et al., 1995). κ-Agonists and μ-antagonists inhibit dopamine release in the nucleus accumbens (Maissoneuve et al., 1994). The nucleus accumbens contains high levels of both κ-opioid receptors (Mansour et al., 1987, 1988, 1994) and dynorphin (Hokfelt et al., 1984), an endogenous opioid peptide with high affinity for κ-receptors (Chavkin et al., 1982). In contrast to cocaine, κ-agonists have been shown to decrease striatal dopamine levels in rats (DiChiara and Imperato, 1988; Spanagel et al., 1992; Devine et al., 1993). Behaviorally, the administration of κ-agonists in rodents has been reported to block or decrease cocaine-induced hyperactivity (Ukai et al., 1994; Crawford et al., 1995), sensitization to cocaine-induced hyperactivity and stereotypies (Shippenberg et al., 1996), and cocaine-induced place preferences (Suzuki et al., 1992; Crawford et al., 1995; Shippenberg et al., 1996). Further evidence for the potential of κ-agonists in treating cocaine abuse comes from studies using rhesus monkeys. Negus et al. (1997) found that in rhesus monkeys, chronic administration of ethylketocyclazocine (EKC) and U50,488 produced a dose-dependent, κ-receptor-mediated, and often sustained decrease in cocaine self-administration. U50,488 has been shown to block cocaine-stimulated activity and attenuate the development of cocaine-conditioned place preference (Crawford et al., 1995). Administration of κ-agonists has been shown to reduce the discriminative stimulus properties (Spealman and Bergman, 1992), conditioned reinforcing effects (Shippenberg et al., 1996), and self-administration of cocaine (Negus et al., 1997; Mello and Negus, 1998; Schenk et al., 1999). In an animal model of relapse, κ-agonists attenuated the reinstatement of extinguished drug-taking behavior (Schenk et al., 1999,

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ABBREVIATIONS: EKC, ethylketocyclazocine; U50,488, (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide methane-sulfonate hydrate; CHO, Chinese hamster ovary; GTPγS, guanosine-5′-*O*-(3-thio)triphosphate; 8-CAC, 8-carboxamidocyclazocine; DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; U69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl) benzeneacetamide; nor-BNI, nor-binaltorphimine; ICI 174,864, *N*,*N*-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (where Aib is α-aminoisobutyric acid); β-FNA, β-funaltrexamine; CL, confidence limit.

2000). These studies suggest that κ-agonists reduce some of the abuse-related effects of cocaine, possibly by inhibiting the release of dopamine from dopaminergic neurons.

The role of μ-antagonists in treating cocaine abuse is not clear. The μ-antagonist naloxone inhibited cocaine-induced increase in locomotor activity in one study (Houdi et al., 1989) but not in another (Schad et al., 1995). In terms of blocking the expression of cocaine conditioning, naloxone was reported to inhibit both the development and expression of cocaine-induced place preference (Houdi et al., 1989; Gerrits et al., 1995). However, the μ-selective antagonist naltrexone was ineffective in decreasing cocaine self-administration (Mello et al., 1990, 1993; Rowlett et al., 1998).

Recent studies have shown that the nonselective κ-agonist EKC, which possesses μ-receptor-mediated effects in addition to its κ-agonistic properties, decreased cocaine self-administration more effectively and with fewer undesirable side effects than highly selective κ-agonists (Negus et al., 1997). Cyclazocine, the parent compound of EKC, was partially effective as a therapeutic for heroin withdrawal (Archer et al., 1996). However, cyclazocine was ineffective in reducing cocaine self-administration in rhesus monkeys (Mello and Negus, 1998) or humans at doses up to 0.8 mg/day (Preston et al., 2001). Higher doses of cyclazocine are being tested in clinical trials for cocaine and smoking cessation (Preston et al., 2001). Like EKC, cyclazocine has μ-receptor-mediated properties in addition to its κ-agonistic activity (Bidlack and Jadrovski, 2000), although cyclazocine was not as efficacious as EKC in stimulating [^{35}S]GTPγS binding mediated by either the κ- or μ-receptors (Bidlack and Jadrovski, 2000).

Cyclazocine is *O*-glucuronidated in humans, which may account for its short duration of action (Wentland et al., 1980). In an attempt to retard this metabolic inactivation and increase its duration of action, we discovered that replacement of the 8-OH group in cyclazocine with an 8-NH_2 provided a novel compound, 8-aminocyclazocine, which had somewhat diminished antinociception potency in mice when delivered by the subcutaneous route but comparable with cyclazocine efficacy when delivered orally (Wentland et al., 1980). In addition, it was hypothesized that 8-amino derivatives of cyclazocine and EKC would have a longer duration of action than cyclazocine because the 8-amino compound would be less prone to glucuronidation (Wentland et al., 2000). Although having many pharmacological properties similar to cyclazocine and EKC, 8-amino-EKC did not produce antinociception in the mouse writhing test that lasted longer than the 90-min antinociception produced by the parent compound EKC (J. M. Bidlack, unpublished data).

In the continuing desire to produce long-acting κ-agonists with activity at the μ-opioid receptor, we synthesized 8-carboxamidocyclazocine (8-CAC) (Wentland et al., 2001b). This compound has high affinity for the κ- and μ-receptors. The present study characterizes the pharmacological properties of 8-CAC in the [^{35}S]GTPγS binding assay and in mouse antinociceptive tests. As reported in this study, 8-CAC produced antinociception that lasted for 15 h after a single systemic administration of the compound.

Materials and Methods

Synthesis of 8-CAC

8-CAC, shown in Fig. 1, was synthesized as described previously (Wentland et al., 2001b).

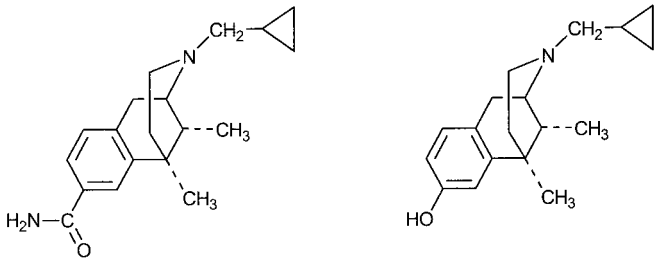

Fig. 1. Structures of 8-CAC and cyclazocine.

In Vitro Studies

Opioid Binding to Guinea Pig Brain Membranes. Guinea pig brain membranes were prepared from frozen guinea pig brains as described previously (Neumeyer et al., 2000). The affinity and selectivity of cyclazocine and 8-CAC for the multiple opioid receptors were determined by incubating the membranes with radiolabeled ligands and 12 different concentrations of the compounds at 25°C in a final volume of 1 ml of 50 mM Tris-HCl, pH 7.5. Incubation times of 60 min were used for the μ-selective peptide [^{3}H]DAMGO and the κ-selective ligand [^{3}H]U69,593. A 3-h incubation was used with the δ-selective antagonist [^{3}H]naltrindole. To determine the IC_{50} values for the inhibition of binding by the compounds, the final concentrations of [^{3}H]DAMGO, [^{3}H]naltrindole, and [^{3}H]U69,593 were 0.25, 0.2, and 1 nM, respectively. Nonspecific binding was measured by inclusion of 10 μM naloxone. Binding was terminated by filtering the samples through no. 32 glass fiber filters (Schleicher & Schuell, Keene, NH) using a 48-well cell harvester (Brandel, Gaithersburg, MD). Filters were soaked for at least 60 min in 0.25% polyethylenimine for [^{3}H]naltrindole- and [^{3}H]U69,593 binding experiments. After filtration, filters were washed three times with 3 ml of cold 50 mM Tris-HCl, pH 7.5, and were counted in 2 ml of Ecoscint A scintillation fluid. The K_i values of unlabeled compounds were calculated from the equation $K_i = IC_{50}/(1 + S)$, where S is concentration of radioligand/K_D of radioligand (Cheng and Prusoff, 1973).

[^{35}S]GTPγS Binding Studies to Measure Opioid Receptor Coupling to G Proteins. Membranes from Chinese hamster ovary cells stably expressing either the human κ- (L. Toll, Stanford Research Institute, Palo Alto, CA) or μ (G. Uhl, National Institute on Drug Abuse, Baltimore, MD)-opioid receptor were used in the experiments. Cells were scrapped from tissue culture plates and were centrifuged at 200*g* for 10 min at 4°C. The cells were resuspended in phosphate-buffered saline, pH 7.4, containing 0.04% EDTA. After centrifugation at 200*g* for 10 min at 4°C, the cell pellet was resuspended in membrane buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, and 1 mM EGTA, pH 7.4. The membranes were homogenized with a Dounce homogenizer, followed by centrifugation at 39,000*g* for 20 min at 4°C. The membrane pellet was resuspended in membrane buffer and the centrifugation step was repeated. The membranes were resuspended in assay buffer, which consisted of 50 mM Tris-HCl, 3 mM $MgCl_2$, 100 mM NaCl, and 0.2 mM EGTA, pH 7.4.

Either the KOR-CHO (15 μg of protein/tube) or MOR-CHO (7.5 μg of protein/tube) membranes were incubated with 12 different concentrations of the agonist in assay buffer for 60 min at 30°C in a final volume of 0.5 ml. The reaction mixture contained 3 μM GDP and 0.080 nM [^{35}S]GTPγS. Basal activity was determined in the presence of 3 μM GDP and in the absence of agonist, and nonspecific binding was determined in the presence of 10 μM unlabeled GTPγS. Then the membranes were filtered onto no. 32 glass fiber filters (Schleicher & Schuell) by vacuum filtration, followed by three washes with 3 ml of ice-cold 50 mM Tris-HCl, pH 7.5. Samples were counted in 2 ml of Ecoscint A scintillation fluid. Data are presented as the percentage of agonist-stimulation of [^{35}S]GTPγS binding over the basal

activity, defined as [(specific binding/basal binding) × 100] − 100. All experiments were repeated at least three times and were performed in triplicate.

In Vivo Studies

Animals. All antinociceptive experiments used male, ICR mice (20–24 g; Harlan, Indianapolis, IN). Mice were kept in groups of eight in a temperature-controlled room with a 12-h light/dark cycle. Food and water were available ad libitum until the time of the experiment.

Injection Techniques. Intracerebroventricular injections were made directly into the lateral ventricle. The volume of all i.c.v. injections was 5 μl, using a 10-μl Hamilton microliter syringe. The mouse was lightly anesthetized with ether, an incision was made in the scalp, and the injection was made 2 mm lateral and 2 mm caudal to bregma at a depth of 3 mm.

Tail-Flick Assay. The thermal nociceptive stimulus was 55°C water, with the latency to tail-flick or withdrawal taken as the endpoint. After determining control latencies, the mice received graded i.c.v. doses of opioid agonists or antagonists at various times. 8-CAC and cyclazocine each were given as a single i.c.v. injection at a dose of 100 nmol, with the antinociceptive effect measured 20 min after injection. In the antagonist study, various doses of the compounds were given as a single pretreatment along with 3 nmol of morphine, which produced approximately 70% antinociception when administered alone. A cutoff time of 15 s was used; if the mouse failed to display a tail-flick in that time, the tail was removed from the water and the animal assigned a maximal antinociceptive score of 100%. Mice that showed no response within 5 s in the initial control test were eliminated from the experiment. At each time point, antinociception was calculated according to the following formula: percentage of antinociception = 100 × (test latency − control latency)/(15 − control latency).

Mouse Writhing Assay. Because antinociception induced by κ-opioid agonists has been difficult to evaluate in the tail-flick test (Porreca et al., 1987), we also investigated the action of the compounds in the mouse acetic acid writhing test. After receiving graded i.c.v. doses of opioid agonists and antagonists at various times, an i.p. injection of 0.6% acetic acid (10 ml/kg) was administered to each mouse. Five minutes after administration, the number of writhing signs displayed by each mouse was counted for an additional 5 min. Antinociception for each tested mouse was calculated by comparing the test group to a control group in which mice were treated with i.c.v. vehicle solution. In the receptor selectivity studies, the κ-selective antagonist nor-BNI or the δ-selective antagonist ICI 174,864 was given with the agonist in the same injection. β-FNA, the μ-selective antagonist, was injected 24 h before agonist injection.

Statistics. IC_{50} values were calculated by least-squares fit to a logarithm-probit analysis. Saturation binding data were analyzed by nonlinear regression analysis using the LIGAND program (Munson and Rodbard, 1980). All dose-response lines were analyzed, using the regression methods described by Tallarida and Murray (1986). Regression lines, ED_{50} (dose producing 50% antinociception) values, and 95% confidence limits were determined with each individual data point (Tallarida and Murray, 1986). All data points shown are the mean of 7 to 10 mice, with standard error of the mean represented by error bars. Statistical analysis of the [^{35}S]GTPγS binding data and the antinociceptive data used the Student's *t* test.

Chemicals. 8-CAC was synthesized as described previously (Wentland et al., 2001a) and converted to a hydrochloride salt. Cyclazocine was obtained as a methane sulfonate salt from Sanofi-Snythelabo (Paris, France). Both compounds were dissolved in distilled, deionized water, which served as the vehicle control in the antinociception experiments. [^{3}H]DAMGO (60 Ci/mmol) and [^{3}H]U69,593 (64 Ci/mmol) were purchased from Amersham Biosciences (Piscataway, NJ). [^{3}H]Naltrindole (48.6 Ci/mmol) was obtained from PerkinElmer Life Sciences (Boston, MA). Morphine sulfate was purchased from Mallinckrodt (St. Louis, MO). U50,488, nor-BNI, ICI 174,864, and β-FNA were purchased from Sigma/RBI (Natick, MA).

Results

In Vitro Studies

Affinity, Selectivity, and Efficacy of 8-CAC, Cyclazocine, and U50,488. The binding of the novel benzomorphan 8-CAC (Fig. 1) to the multiple opioid receptors was measured and compared with the parent compound cyclazocine and the κ-selective compound U50,488. As shown in Table 1, 8-CAC, cyclazocine, and the κ-selective agonist U50,488 had K_i values of less than 0.5 nM for inhibiting the binding of [^{3}H]U69,593 to the κ-receptor in guinea pig brain membranes. The replacement of the hydroxyl group on the C8-position with a carboxamido group decreased the affinity of the compound for the κ-receptor by only 2-fold. The two compounds had the same affinity for the μ-opioid receptor, having K_i values of 0.3 nM. Both compounds were relatively nonselective, particularly between the κ- and μ-receptors. Cyclazocine and 8-CAC had a 6- and 12-fold, respectively, lower affinity for the δ-receptor than the κ-receptor. U50,488 had similar affinity as cyclazocine and 8-CAC for the κ-receptor.

To characterize the relative efficacy of 8-CAC, cyclazocine, and U50,488, the [^{35}S]GTPγS assay was used with CHO membranes that had been stably transfected with either the κ- or μ-opioid receptors. Table 2 shows that 8-CAC and cyclazocine produced the same maximal stimulation of [^{35}S]GTPγS binding. The κ-selective agonist U50,488 produced slightly greater stimulation of [^{35}S]GTPγS binding than 8-CAC or cyclazocine ($P < 0.05$). In this assay, cyclazocine had the lowest EC_{50} value, whereas 8-CAC and U50,488 had EC_{50} values that were not statistically different from each other. The rank order of the EC_{50} values correlates with the K_i values obtained for the compounds in the binding assays with [^{3}H]U69,593.

8-CAC and cyclazocine produced similar stimulation of [^{35}S]GTPγS binding induced by the μ-opioid receptor, indicating that both compounds were agonists at the μ-receptor in addition to being agonists at the κ-receptor. However, the E_{max} values for 8-CAC and cyclazocine were considerably lower than the E_{max} value for the full μ-agonist DAMGO ($P < 0.01$). Like the K_i values, the EC_{50} values of the two compounds for stimulating [^{35}S]GTPγS binding were similar. As expected, the κ-selective compound did not stimulate [^{35}S]GTPγS binding induced by the μ-opioid receptor.

TABLE 1

K_i values for the inhibition of μ-, δ-, and κ-opioid binding to guinea pig brain membranes by 8-CAC, cyclazocine, and U50,488

Membranes were incubated with varying concentrations of the compounds in the presence of either 0.25 nM [^{3}H]DAMGO, 0.2 nM [^{3}H]naltrindole, or 1 nM [^{3}H]U69,593 to measure binding to μ-, δ-, or κ-sites, respectively. After equilibrium binding was reached, membranes were filtered onto glass fiber filters, as described under *Materials and Methods*. Data are expressed as the mean K_i value ± S.E. for three determinations performed in triplicate.

Compounds	K_i		
	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)
	nM ± S.E.		
8-CAC	0.34 ± 0.01	4.9 ± 0.80	0.42 ± 0.02
Cyclazocine	0.32 ± 0.02	1.1 ± 0.04	0.18 ± 0.020
U50,488	220 ± 5.6	2500 ± 170	0.36 ± 0.056

TABLE 2

E_{max} and EC_{50} values for stimulation of [^{35}S]GTPγS binding to human κ- and μ-opioid receptors

In a final volume of 0.5 ml, 12 different concentrations of each compound were incubated with 7.5 μg of CHO cell membranes that stably expressed the human μ-opioid receptor. The assay buffer consisted of 50 mM Tris-HCl, pH 7.4, 3 mM $MgCl_2$, 0.2 mM EGTA, 3 μM GDP, and 100 mM NaCl. The final concentration of [^{35}S]GTPγS was 0.080 nM. Nonspecific binding was measured by inclusion of 10 μM GTPγS. Binding was initiated by the addition of the membranes. After an incubation of 60 min at 30°C, the samples were filtered through no. 32 glass fiber filters (Schleicher & Schuell).

Compounds	E_{max}	EC_{50}
	% maximal stimulation	*nM ± S.E.*
KOR		
8-CAC	190 ± 8.1	8.8 ± 1.7
Cyclazocine	190 ± 7.5	1.6 ± 0.60
U50,488	240 ± 12	7.0 ± 4.3
MOR		
8-CAC	140 ± 3.5	4.9 ± 2.3
Cyclazocine	150 ± 6.0	4.5 ± 0.50
DAMGO	220 ± 12	110 ± 12

In Vivo Studies

Antinociceptive Effects of 8-CAC and Cyclazocine in Mouse Warm-Water Tail-Flick and Writhing Tests. Because both 8-CAC and cyclazocine had high affinity for the μ-opioid receptor, as measured in the receptor binding assays, the antinociceptive properties of the compounds were characterized in the 55°C warm-water tail-flick test. 8-CAC and cyclazocine produced less than 60% antinociception after an i.c.v. dose of 100 nmol at 20 min after injection. Because κ-agonists do not usually produce full dose-response curves in the warm-water tail-flick test (Porreca et al., 1987), the effect of 8-CAC and cyclazocine were characterized in the writhing test. As shown in Fig. 2, 8-CAC had an ED_{50} value and 95% CL of 0.21 (0.09–0.5) nmol in this test. Cyclazocine had an ED_{50} value and 95% CL of 2.9 (1.4–6.1) nmol. In the writhing test, 8-CAC was 10-fold more potent in producing antinociception than cyclazocine. The selectivity of the agonist effect produced by 8-CAC and cyclazocine in the writhing test was determined by the use of selective antagonists. The μ-selective antagonist β-FNA and the κ-selective antagonist nor-BNI both reduced the antinociception induced by 8-CAC (Fig. 3A), suggesting that 8-CAC was an agonist at both the κ- and μ-receptors. Likewise, the antinociception induced by cyclazocine was inhibited by the μ-and κ-antagonists, but not the δ-selective antagonist (Fig. 3B).

Antagonist Properties of 8-CAC and Cyclazocine. Many benzomorphans act as partial agonists at the μ-opioid receptor, in addition to being κ-agonists. Experiments were performed to determine whether 8-CAC and cyclazocine could inhibit antinociception induced by morphine and measured in the 55°C warm-water tail-flick test. Increasing doses of either 8-CAC (Fig. 4A) or cyclazocine (Fig. 4B) were coinjected with 3 nmol of morphine, which produced approximately 70% antinociception. Figure 4A shows that 8-CAC did not significantly antagonize morphine-induced antinociception. In contrast, cyclazocine at a dose of 1 nmol potently inhibited morphine-

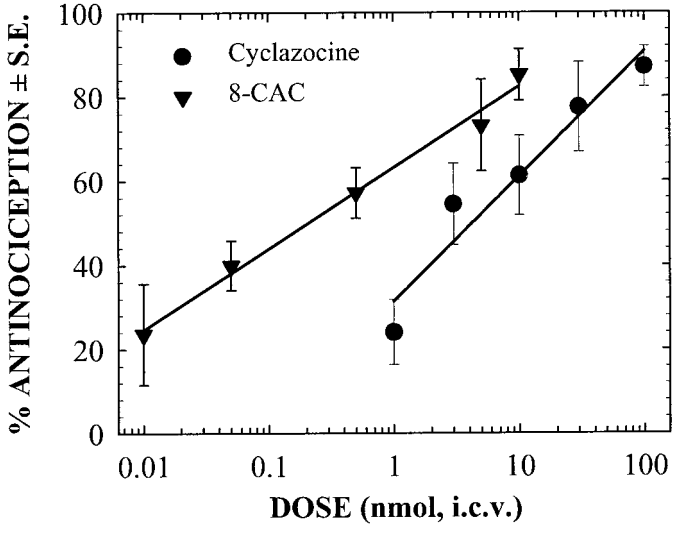

Fig. 2. Dose-response lines for i.c.v. 8-CAC and cyclazocine in the mouse writhing test. Testing occurred 20 min after the injection of the compounds.

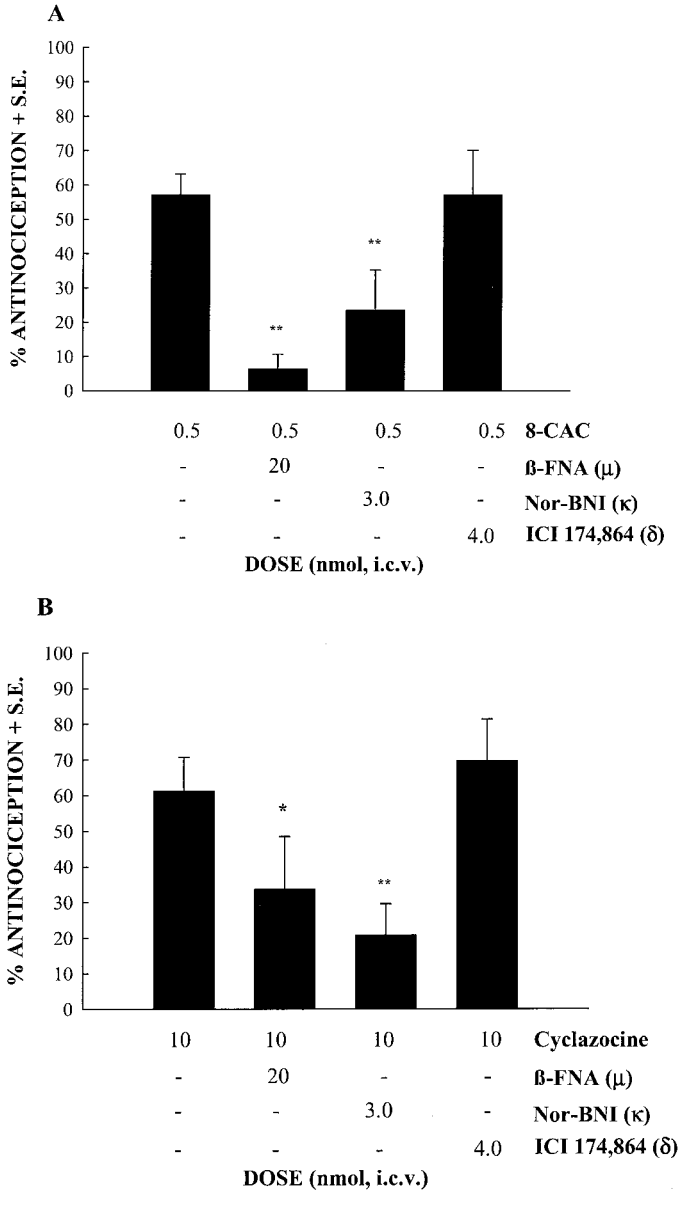

Fig. 3. Dose-response lines for i.c.v. 8-CAC (A) and cyclazocine (B) in the presence or absence of either i.c.v. ICI 174,864 (4 nmol), nor-BNI (1 nmol), or β-FNA (20 nmol, −24 h) in the mouse writhing test. Testing occurred 20 min after the injection of the agonists. ⋆, significant from the agonist alone, $P < 0.05$; ⋆⋆, $P < 0.01$.

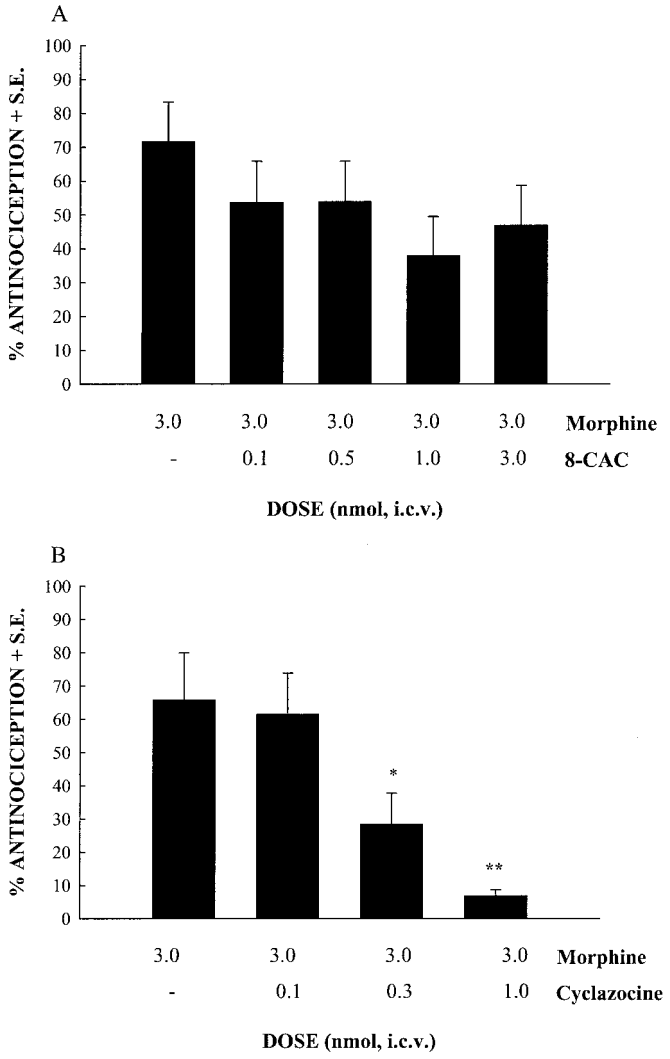

Fig. 4. Antinociceptive effects of i.c.v. morphine (3 nmol) in mice treated with i.c.v. doses of 8-CAC (A) and cyclazocine (B). Morphine was coinjected with either 8-CAC or cyclazocine. Antinociception was measured in the mouse 55°C warm-water tail-flick test at 20 min after the injection of the compounds. Ten mice were used for each data point. ⋆⋆, significant from morphine alone, $P < 0.01$; ⋆, $P < 0.05$.

induced antinociception. These findings demonstrate that 8-CAC is an agonist at both the κ- and μ-opioid receptors, and is devoid of μ-antagonist properties in the antinociception assays. However, cyclazocine is an agonist at the κ-receptor, and a partial agonist at the μ-opioid receptor.

Time Course for Antinociception Produced by 8-CAC and Cyclazocine in Writhing Assay When Compounds Were Given by Systemic Administration. When 8-CAC was administered by an i.p. injection, with testing taking place 30 min after administration, 8-CAC was a potent agonist in the writhing test. 8-CAC had an ED_{50} value and 95% CL of 0.19 (0.03–1.2) mg/kg. Cyclazocine had an ED_{50} value of 0.36 (0.10–2.1) mg/kg. Figure 5 shows the time course of antinociception produced by 8-CAC and cyclazocine after an i.p. injection of 1 mg/kg. Mice were tested for antinociception in the writhing test at varying times after the administration of 8-CAC and cyclazocine. Cyclazocine produced antinociception for 2 h, whereas 8-CAC produced antinociception for up to 15 h after a single injection of 8-CAC, demonstrating that

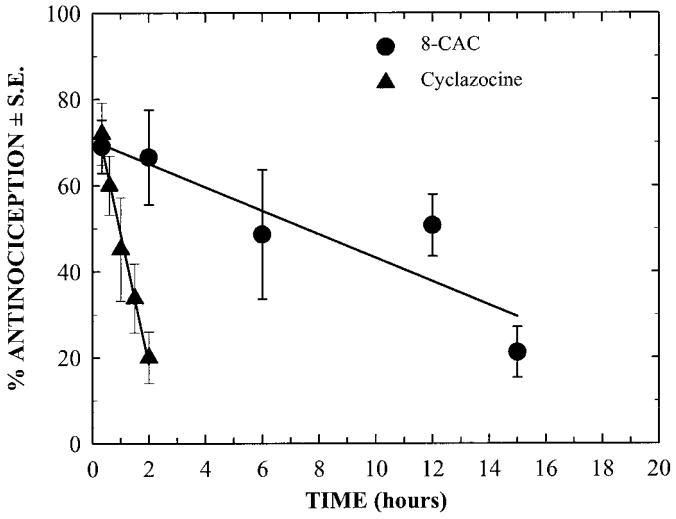

Fig. 5. Time course for the effect of 8-CAC and cyclazocine in producing antinociception in the mouse writhing test. Mice were injected with either 8-CAC or cyclazocine (1.0 mg/kg), given by i.p. administration. At varying times, antinociception was measured using the acetic acid writhing test. Data are the mean ± S.E. from 10 mice/data point.

8-CAC had a much longer duration of action than cyclazocine.

Discussion

The purpose of synthesizing 8-CAC was to determine whether it was possible to substitute a carboxamido group for the prototypic 8-OH group on cyclazocine and still retain high-affinity binding to the opioid receptors. We hypothesized that the carboxamido group would not be metabolized as rapidly as the phenolic group and therefore, it might be possible to obtain a benzomorphan with a longer duration of action. There is considerable interest in the benzomorphans as potential therapeutics for treating cocaine abuse. Cyclazocine is in clinical trials for cocaine (Preston et al., 2001) and smoking (Pickworth et al., 2001). EKC is the most potent compound to date to block cocaine self-administration in rhesus monkeys with minimal effect on food intake (Negus et al., 1997; Mello and Negus, 1998). Therefore, if an analog of cyclazocine could be synthesized that was more resistant to metabolism than cyclazocine, but still possessed many of the same pharmacological properties of the parent compound, this compound would open new avenues of research and would be a potential pharmacotherapeutic for treating cocaine abuse and other drugs of abuse.

This current study demonstrated high affinity for the multiple opioid receptors was observed with a novel derivative of cyclazocine, 8-CAC, which had the prototypic 8-OH replaced by a carboxamido group. 8-CAC had K_i values similar to cyclazocine in receptor binding assays. In the [^{35}S]GTPγS binding assay, both 8-CAC and cyclazocine stimulated [^{35}S]GTPγS binding mediated by the κ- and μ-opioid receptors, and the two compounds had identical E_{max} values. The κ-selective agonist U50,488 had a greater E_{max} value than 8-CAC and cyclazocine, suggesting that U50,488 is more efficacious at the κ-receptor than the 8-CAC and cyclazocine. This finding correlates with previous studies, which have shown that the arylacetamides were more potent in stimulating [^{35}S]GTPγS binding than most benzomorphans, except EKC (Bidlack and Jadrovski, 2000).

Currently, it is not clear whether greater efficacy at the κ-and/or μ-receptors renders a compound a better candidate for blocking cocaine self-administration in nonhuman primates, and ultimately, would reduce cocaine abuse in humans. Because μ-antagonists decrease dopamine release in the nucleus accumbens (Maissoneuve et al., 1994), it has been speculated that μ-antagonistic properties are important in the development of medications to treat cocaine abuse (Archer et al., 1996). As shown in the mouse tail-flick test, cyclazocine antagonized morphine-induced antinociception, whereas 8-CAC was not an antagonist in this assay. It is possible that a metabolite of cyclazocine, and not cyclazocine itself, is responsible for the antagonism of morphine-induced antinociception. Although having different properties in the mouse antinociception assays, the two compounds were μ-agonists in the [^{35}S]GTPγS assay, with considerably lower efficacy than the full μ-agonist DAMGO. There is a desire to correlate pharmacological properties of compounds with the behavioral effects of the compounds to predict which compounds will be effective pharmacotherapeutics for treating drug abuse.

Both 8-CAC and cyclazocine produced full dose-response curves in the writhing but not the warm-water tail-flick test. The antinociception produced by these two compounds in the writhing test was blocked by both κ- and μ-antagonists, demonstrating that both compounds acted as agonists at these two receptors. These results correlate with results observed with other benzomorphans. For example, pentazocine, 8-amino-cyclazocine, and 8-phenylamino-cyclazocine produced antinociception that was mediated by both the κ- and μ-receptors (Bidlack et al., 2000). In addition to cyclazocine, pentazocine, 8-amino-cyclazocine, and 8-phenylamino-cyclazocine antagonized morphine-induced antinociception (Bidlack et al., 2000). Surprisingly, 8-CAC did not significantly antagonize morphine-induced antinociception (Fig. 4A). This finding indicates that the addition of a carboxamido group in place of the 8-OH group resulted in a compound that was an agonist at the μ-receptor, instead of a partial agonist like many of the other benzomorphans. These findings may explain why 8-CAC had a 10-fold lower ED_{50} value than cyclazocine in the writhing assay. Alternatively, 8-CAC may have a slower rate of clearance from the brain than cyclazocine. However, in the [^{35}S]GTPγS binding assay, 8-CAC and cyclazocine were both agonists, producing significantly less stimulation of [^{35}S]GTPγS binding than the full μ-agonist DAMGO.

An earlier study showed that 8-amino-cyclazocine had an oral-to-parental ratio that was considerably better than the ratio observed with cyclazocine, suggesting that replacement of the 8-OH with a group that might not be metabolized as rapidly may increase the bioavailability of these compounds (Wentland et al., 1980). Future studies will address the bioavailability of 8-CAC.

We had hypothesized that replacing the 8-OH group on cyclazocine with 8-carboxamido group would result in a compound with long duration of activity. The data presented in Fig. 5 confirm this hypothesis. 8-CAC produced antinociception in the mouse writhing assay for up to 15 h after a single i.p. injection of 1 mg/kg. In contrast, under the same conditions, cyclazocine produced antinociception for only 2 h. These findings confirm that the replacement of the 8-OH group on cyclazocine with a carboxamido group resulted in an agonist with a duration of activity that was considerably longer than cyclazocine. The longer duration of action is probably due to the slower metabolism and/or clearance of 8-CAC in comparison with cyclazocine. The addition of an 8-carboxamido group to benzomorphans results in a new series of opioid ligands, which will probably have longer durations of action than the parent compounds.

Recently, we have reported the synthesis of 3-carboxamido derivatives of morphine and naltrexone (Wentland et al., 2001a). Replacing the 3-OH group on morphine with a carboxamido group resulted in a 39-fold decrease in the affinity of the compound for the μ-receptor in comparison with morphine, as measured in a radioligand binding assay. Nevertheless, 3-carboxamido-naltrexone had a K_i value of 1.9 nM for the inhibition of the μ-selective ligand [^{3}H]DAMGO, whereas naltrexone had an 11-fold greater affinity with a K_i value of 0.17 nM. However, a K_i value of 1.9 nM still means that the 3-carboxamido derivative of naltrexone had high affinity for the μ-receptor (Wentland et al., 2001a), and suggests that it may be possible to synthesize some morphinans that have the 3-OH group replaced with a carboxamido group. Like 8-CAC, these compounds may also be longer acting than the parent compounds.

In summary, 8-CAC is a high-affinity benzomorphan that acts as an agonist at the κ- and μ-receptors. In contrast to the parent compound cyclazocine, 8-CAC did not antagonize morphine-induced antinociception. Although cyclazocine produced antinociception that lasted for only 2 h, 8-CAC produced antinociception that lasted for 15 h after a single i.p. injection of 1 mg/kg into mice. This increased duration of action is probably due to a decrease in the rate of metabolism of 8-CAC in comparison with cyclazocine. The addition of a carboxamido group to benzomorphans and morphinans will produce a new series of compounds that may have longer durations of action than the parent compounds.

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Address correspondence to: Dr. Jean M. Bidlack, Department of Pharmacology and Physiology, Box 711, University of Rochester, School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642-8711. E-mail: jean_bidlack@urmc.rochester.edu