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The effect of the irreversible μ-opioid receptor antagonist clocinnamox on morphine potency, receptor binding and receptor mRNA

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Abstract

In these experiments, the effect of the irreversible μ-opioid receptor antagonist clocinnamox on the potency of morphine, opioid receptor binding and μ-opioid receptor mRNA was examined. Mice were injected with clocinnamox (0.32–12.8 mg/kg) and the analgesic potency of morphine was examined 24 h later. Clocinnamox produced a dose-dependent decrease in the potency of morphine; and at the higher dose of clocinnamox the maximal analgesic effect was not observed following doses of morphine in excess of 500 mg/kg s.c. In saturation binding studies in brain, clocinnamox (0.32–25.6 mg/kg) dose-dependently decreased μ-opioid ([^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin; DAMGO) receptor B_{max} with relatively minimal effects on K_d. Binding to δ-opioid receptor ([^{3}H][D-Pen^2,D-Pen^5]enkephalin; DPDPE) and κ-opioid receptor ([^{3}H](5,7,8)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl) benzeneacetamide; U69,593) was not affected by clocinnamox. The effect of clocinnamox was time-dependent in that the greatest changes in morphine potency and μ-opioid receptor density were observed within 24 h of administration and decreased with time (336 h). Although μ-opioid receptor density was decreased to less than 30% of control 24 h following clocinnamox (12.8 mg/kg) and had increased to 80% by 5 days, a solution hybridization assay for μ-opioid receptor mRNA transcript revealed no changes in the steady-state levels of this mRNA. These studies indicate that clocinnamox is an irreversible antagonist at the μ-opioid receptor since it appears to selectively affect receptor density with minimal effects on affinity. Furthermore, clocinnamox produces time- and dose-dependent changes in B_{max} and these changes appear to be unrelated to changes in μ-opioid receptor mRNA. It is possible that the repopulation of brain by μ-opioid receptors following clocinnamox is mediated by an existing pool of receptors that are activated following treatment.

Keywords: Clocinnamox; Morphine; Opioid receptor antagonist; DAMGO ([D-Ala^2,MePhe^4,Gly-ol^5]enkephalin); DPDPE ([D-Pen^2,D-Pen^5]enkephalin); U69,593; Opioid receptor alkylation; (Irreversible antagonist); Opioid receptor binding; mRNA; Solution hybridization; Analgesia

1. Introduction

Clocinnamox has been shown to be an irreversible, competitive antagonist at the μ-opioid receptor. Early studies indicated that clocinnamox displaced [^{3}H]-etorphine binding and interacted with μ-, δ- and κ-opioid receptors as determined in the mouse vas deferens assay (Aceto et al., 1989; Lewis et al., 1989). Initial in vivo studies suggested that clocinnamox had a long duration of antagonist action in the tail flick assay (Aceto et al., 1989). Recent in vivo studies have shown that clocinnamox will block the analgesic effects of morphine, alfentanil, fentanyl and etonitazene (Comer et al., 1992; Zernig et al., 1994; Burke et al., 1994). Clocinnamox does not appear to alter κ (U-50,488H) agonist potency (Zernig et al., 1994). The duration of antagonist action is at least 7–8 days following a dose of clocinnamox (Comer et al., 1992; Zernig et al., 1994). In binding studies following in vivo administration, clocinnamox selectively decreased the density (B_{max}) of [^{3}H][D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (DAMGO) and [^{3}H]naltrexone binding sites without

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altering the affinity (K_d) of either ligand (Burke et al., 1994). Both the decreases in opioid agonist potency and opioid receptor density are dependent on the dose of clocinnamox and the time following administration (Comer et al., 1992; Burke et al., 1994; Zernig et al., 1994).

In the present study we have examined the time- and dose-dependent effect of clocinnamox on the in vivo potency of morphine in the mouse. In parallel experiments, the effects of clocinnamox on the binding of μ-, δ- and κ-opioid receptor ligands have been determined in mouse brain. To evaluate if changes in μ-opioid receptor density are associated with changes in μ-opioid receptor gene expression, the abundance of μ-opioid receptor mRNA in mouse brain has been evaluated following clocinnamox.

2. Materials and methods

2.1. Subjects

Male, Swiss-Webster mice (22–24 g) obtained from Taconic Farms (Germantown, NY, USA) were used throughout. The animals were maintained 5–10 per cage with free access to food and water and housed for at least 24 h prior to experimentation. Mice were used only once.

2.2. Procedure

Clocinnamox dose-response

Mice were injected i.p. with saline or clocinnamox (0.32–12.8 mg/kg). Twenty-four hours post-injection, mice were weighed, a baseline tailflick latency determined and then tested in morphine dose-response studies (see below). Other mice were treated with clocinnamox (0.32–25.6 mg/kg) or saline but 24 h later mice were killed, whole brain removed and binding studies conducted (see below).

Clocinnamox time action profile

Mice were injected i.p. with saline or clocinnamox (12.8 mg/kg) and 1, 2, 4, 24, 72, 96, 120, 168, or 336 h post-injection, binding or analgesia studies were conducted. Beginning 24 h following clocinnamox, mice were weighed and a baseline tailflick latency determined. Mice were then tested for analgesia following s.c. morphine using a cumulative dose-response protocol (see below). Other mice were treated as above with clocinnamox or saline but at each time point beginning 1 h following clocinnamox mice were killed, whole brain removed and binding studies conducted (see below). At 24 and 120 h following clocinnamox treatment, mice were killed for determination of μ-opioid receptor mRNA by solution hybridization (see below).

2.3. Analgesia assay

Analgesia (antinociception) was determined using the tailflick assay in which a beam of light was focused on the dorsal tail surface approximately 2 cm from the tip of the tail. The intensity of the light was adjusted so that baseline flick latencies determined prior to morphine administration were 2–4 s. If a mouse failed to flick by 10 s following morphine administration, the test was terminated and a latency of 10 s was recorded. Mice that had a latency of 10 s were defined as analgesic. Mice were tested for analgesia 30 min following morphine. All testing was conducted in a blind manner.

2.4. Cumulative dose-response protocol

A cumulative dose-response protocol was used for all studies. All mice in a treatment group (typically 7–10 per group) were injected s.c. with a starting dose of morphine and tested for analgesia 30 min later. All mice that were not analgesic were given a second dose of morphine within 5 min of testing and then tested for analgesia again 30 min later. This cumulative dose-response protocol was continued until at least 75% of the mice were analgesic. The actual morphine doses used in the cumulative dose-response protocol were determined in a previous study (Duttaroy and Yoburn, 1995). Data from cumulative dosing are presented such that the percent of mice that are analgesic are plotted against the total (cumulative) dose administered.

2.5. Brain opioid receptor binding

Mice were killed and whole brain rapidly removed, weighed and then homogenized in 80 volumes of ice-cold 50 mM Tris buffer (pH 7.4). Homogenates were then centrifuged at 15000 rpm for 15 min, the supernatant discarded and the pellet resuspended in buffer and centrifuged again. The pellet was resuspended and incubated (30 min at 25°C), centrifuged a third time and finally resuspended in 20–80 volumes of 50 mM phosphate buffer (pH 7.2). An aliquot (200 μl) of the homogenate was then assayed in triplicate in tubes containing 0.04–5.0 nM [^{3}H]DAMGO, 0.06–9.0 nM [^{3}H][D-Pen^2,D-Pen^5]enkephalin (DPDPE) or 0.05–7.0 nM [^{3}H](5α,7α,8β)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl) benzeneacetamide (U69, 593). Nonspecific binding was determined in the presence of 1000 nM levorphanol for [^{3}H]DAMGO and [^{3}H]DPDPE, and 10 μM naloxone for [^{3}H]U69,593. Homogenates were incubated for 90 min at 25°C. Incubation was terminated by the addition of ice-cold phosphate buffer and the samples were filtered over GF/B glass fiber filters. Filters were washed 3 times with cold buffer, transferred to vials, scintillation cocktail added

Table 1
The effect of clocinnamox dose on morphine analgesia

Treatment	Morphine	
	ED_{50} (mg/kg)	ED_{50} shift
Saline	2.82 (± 0.78)	
0.32 mg/kg clocinnamox	6.83 (± 1.46)	2.53 [a] (±0.18)
Saline	2.72 (± 0.71)	
3.2 mg/kg clocinnamox	17.95 (± 2.95)	7.00 [a] (±0.75)
Saline	3.29 (± 0.43)	
6.4 mg/kg clocinnamox	21.54 (± 3.79)	6.60 [a] (±0.84)
Saline	2.91 (± 0.40)	
12.8 mg/kg clocinnamox	135.65 (±41.7)	44.38 [a] (±8.22)

Mice were injected with saline or clocinnamox (0.32–12.8 mg/kg i.p.) and 24 h later cumulative dose-response studies were conducted using morphine. The ED_{50} shift is the ED_{50} for the clocinnamox group divided by the ED_{50} for the saline control. Data are means (±S.E.M.) of 2–3 independent studies for each dose. [a] Significant ($P < 0.05$) change in ED_{50}.

and then counted. For assays of [^{3}H]U69,593 binding, filters had been presoaked in polyethylenamine (0.1%) for 2 h. Counts per minute (cpm) were converted to disintegrations per minute (dpm) using the external standard method. Protein was determined using a microassay technique based on the method of Bradford (1976) using reagent purchased from Bio-Rad (Richmond, CA, USA). Each binding experiment was conducted at least twice.

2.6. Solution hybridization assay for μ-opioid receptor mRNA

Mice were killed 1 or 5 days following clocinnamox (12.8 mg/kg) or saline treatment. Brains were rapidly removed and total cellular RNA was extracted with phenol and precipitated with ethanol according to a procedure which routinely yields RNA recoveries of 77.0 ± 7.2% (S.D.) (Zhu et al., 1992). The solution hybridization assay was conducted as previously described by Brodsky et al. (1994). A ^{32}P-labeled riboprobe complementary to the μ-opioid receptor (MOR-1) mRNA (specific activity 4.6×10^8 dpm/mg) was transcribed from a pSP73 plasmid containing the HindIII fragment of the RAT-MOR-1 cDNA, a gift from Dr. L. Yu, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA (see Chen et al., 1993). This fragment includes all of the open reading frame (1194 bp) with small 5′ and 3′ untranslated sequences, of 213 and 38 bp respectively. The transcript was purified by use of CF11 columns as described by Zhu et al. (1992).

The details of the solution hybridization assay have been described elsewhere (Zhu et al., 1992). Briefly,

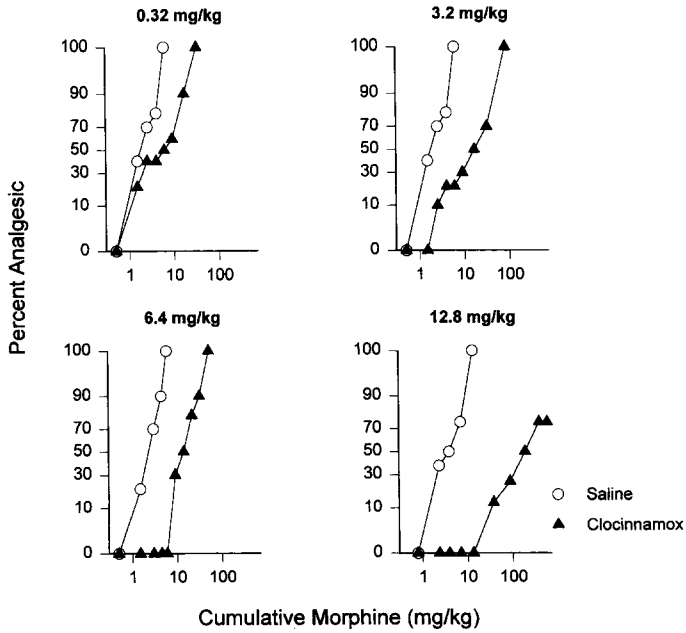

Fig. 1. The effect of clocinnamox on morphine analgesia. Mice were injected with saline or clocinnamox (0.32–12.8 mg/kg) and 24 h later cumulative dose-response studies conducted. Each panel is a representative independent experiment. See Table 1 for summary.

Table 2
The time-action profile of clocinnamox on morphine analgesia

Hours following treatment	Morphine ED_{50} (mg/kg)		ED_{50} shift
	Saline	Clocinnamox	
24	2.91 (±0.40)	135.65 (±41.7)	44.43 [a] (±8.25)
72	2.65 (±0.11)	31.78 (±3.80)	12.17 [a] (±1.94)
96	3.50 (±0.66)	9.67 (±0.45)	3.02 [a] (±0.70)
120	3.56 (±0.30)	8.43 (±0.49)	2.43 [a] (±0.34)
168	3.95 (±0.45)	7.30 (±0.06)	1.89 [a] (±0.20)
336	3.18 (±0.05)	4.49 (±0.13)	1.42 (±0.07)

Mice were injected with saline or clocinnamox (12.8 mg/kg i.p.) and 24–336 h later cumulative dose-response studies were conducted using morphine. The ED_{50} shift is the ED_{50} for the clocinnamox group divided by the ED_{50} for the saline control. Data are means (±S.E.M.) of two independent studies for each dose. [a] Significant ($P < 0.05$) change in ED_{50}.

total RNA extracts were incubated with 150000 dpm of the MOR-1 riboprobe for 4 h at 75°C, and then subjected to RNase digestion for 1 h at 30°C. The RNase resistant duplexes which formed between the ^{32}P-labeled riboprobe and the complementary RNA were precipitated with trichloroacetic acid, collected on glass microfiber filter paper and counted by liquid scintillation. A standard calibration curve was included in each assay to permit quantitation. The 1.4 kb unlabelled sense transcript of the MOR-1-pSP73 construct was obtained with the SP6 transcription system and served as the standard for MOR-1 mRNA quantitation. Total cellular RNA in each sample was quantified by UV absorbance at 260 nm. Since the solution hybridization assay utilized a rat-derived ribroprobe, the relative estimates of μ-opioid receptor mRNA transcript levels in the treated and control mice are expressed in 'pg equivalents' of sense transcript, normalized by the amount of total cellular RNA in each sample. Successful use of such heterologous RNA assays has been reported (Franklin et al., 1993; Brodsky et al., 1994).

2.7. Drugs and drug administration

Morphine sulfate was obtained from Penick Laboratories (Newark, NJ, USA). Clocinnamox was generously supplied by Dr. James Woods, Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA and Dr. James Lewis, School of Chemistry, University of Bristol, Bristol, UK. Morphine was dissolved in 0.9% saline and doses are expressed as free base. Clocinnamox was dissolved in dH_2O and doses expressed as the salt. All saline, morphine and most

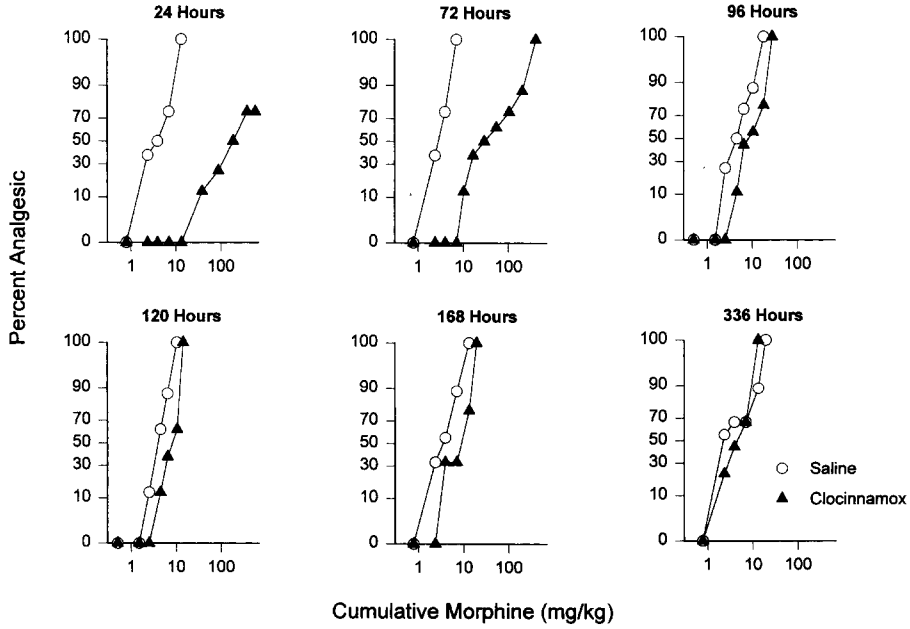

Fig. 2. The effect of clocinnamox on morphine analgesia at various times following administration. Mice were injected with saline or clocinnamox (12.8 mg/kg) and tested for analgesia 24–336 h later. Each panel is a representative independent experiment. See Table 2 for summary.

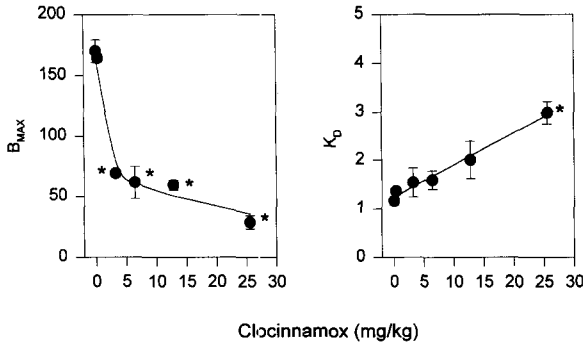

Fig. 3. The effect of clocinnamox on μ-opioid receptor binding in mouse brain. Mice were injected with saline or clocinnamox (0.32–25.6 mg/kg) and 24 h later killed and saturation binding studies conducted using [^{3}H]DAMGO. B_{max} is in fmol/mg protein. K_d is expressed as nanomolar. Data presented are means ± S.E.M. of 3–5 independent studies for each dose. * Significantly different from control ($P < 0.05$).

clocinnamox injection volumes were 10 ml/kg. However, due to solubility limits of clocinnamox, mice were injected with 20 ml/kg of a 0.64 mg/ml solution for the 12.8 mg/kg dose and 40 ml/kg for the 25.8 mg/kg dose.

2.8. Data analysis.

Quantal dose-response data were analyzed by probit analysis (Finney, 1973) using a computerized program (BLISS 21, Department of Statistics, University of Edinburgh) that estimates ED_{50} values, 95% confidence limits and relative potency.

B_{max} and K_d values were determined from saturation studies using nonlinear regression (Inplot ver. 4.0, Graphpad Software, San Diego, CA, USA). In all cases binding data were fit best by a one-site model. Significant differences for data from binding and mRNA studies were analyzed using analysis of variance with appropriate post-hoc comparisons.

3. Results

3.1. Pharmacodynamics

Clocinnamox treatment did not significantly alter baseline tailflicks (data not shown). Clocinnamox produced a dose-dependent shift in the ED_{50} for morphine 24 h following treatment ($F = 14.2$, $P < 0.01$) (Table 1). Dose-response data from representative experiments are presented in Fig. 1, where it can be seen that morphine did not produce a maximal analgesic effect at 590 mg/kg cumulative morphine following the 12.8 mg/kg dose of clocinnamox. Since this dose of clocinnamox (12.8 mg/kg) necessitated that morphine doses near the limits of solubility for morphine be used, higher doses of clocinnamox were not studied.

The shift in the ED_{50} for morphine as a function of time following clocinnamox (12.8 mg/kg) is shown in

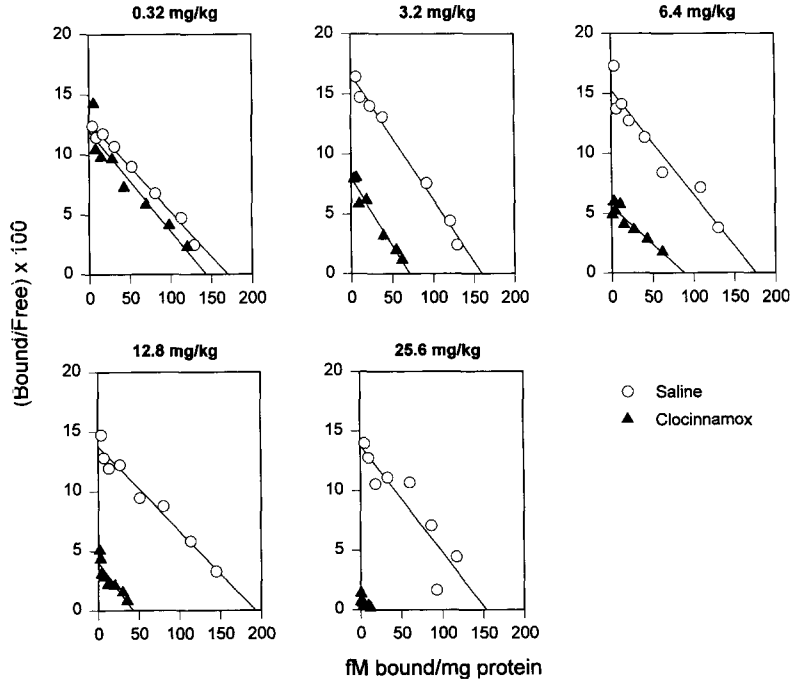

Fig. 4. Representative Scatchard plots of the effect of clocinnamox on μ-opioid receptor binding in mouse brain. Mice were injected with saline or clocinnamox (0.32–25.6 mg/kg) and 24 h later killed and saturation binding studies conducted using [^{3}H]DAMGO. Each panel presents a representative independent experiment. See Fig. 3 for summary.

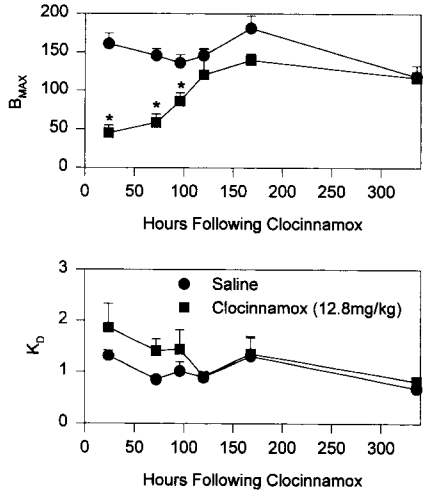

Fig. 5. The time-action profile of clocinnamox on μ-opioid receptor binding in mouse brain. Mice were injected with saline or clocinnamox (12.8 mg/kg) and killed at various times (24–336 h) following dosing. Saturation binding studies were conducted in whole brain using [^{3}H]DAMGO. B_{max} is in fmol/mg protein. K_d is expressed as nanomolar. Data presented are means ± S.E.M. of 2–4 independent studies for each time point. *Significantly different from control ($P < 0.05$).

Table 2. Clocinnamox (12.8 mg/kg) produced a significant time-dependent effect on morphine potency ($F = 11.5$, $P < 0.05$). Representative data for the time action profile of clocinnamox on morphine analgesia are shown in Fig. 2.

3.2. Binding

The effect of clocinnamox dose on binding 24 h following administration is presented in Fig. 3. Clocinnamox produced a dose-dependent decrease in B_{max} for [^{3}H]DAMGO 24 h following treatment ($F = 26.7$, $P < 0.01$). There was a significant decrease in B_{max} for all doses compared to saline controls except the lowest clocinnamox dose (0.32 mg/kg). Clocinnamox also produced a decrease in affinity ($F = 11.4$, $P < 0.01$); although only the 25.6 mg/kg dose was significantly different from saline controls. Representative Scatchard plots for the effect of clocinnamox on binding are shown in Fig. 4. In a related binding study, mice were injected with 12.8 mg/kg clocinnamox and the binding of δ-opioid receptor ([^{3}H]DPDPE) and κ-opioid receptor ([^{3}H]U69,593) ligands was determined 24 h later in saturation studies. There was no significant effect of clocinnamox on B_{max} (fmol/mg protein) or K_d (nM) for [^{3}H]U69,593 (B_{max}: 69.0 ± 2.6, 65.5 ± 3.6; K_d: 1.4 ± 0.1, 1.3 ± 0.2; mean ± S.E.M.; saline and clocinnamox respectively) or [^{3}H]DPDPE (B_{max}: 85.1 ± 3.8, 90.4 ± 2.7; K_d: 1.3 ± 0.2, 1.1 ± 0.1; saline and clocinnamox respectively).

The time course of clocinnamox (12.8 mg/kg) effects on [^{3}H]DAMGO B_{max} and K_d are shown in Fig. 5. B_{max} and K_d estimates could not be reliably determined 1–4 h post 12.8 mg/kg clocinnamox, since binding was reduced by 91–95% compared to control.

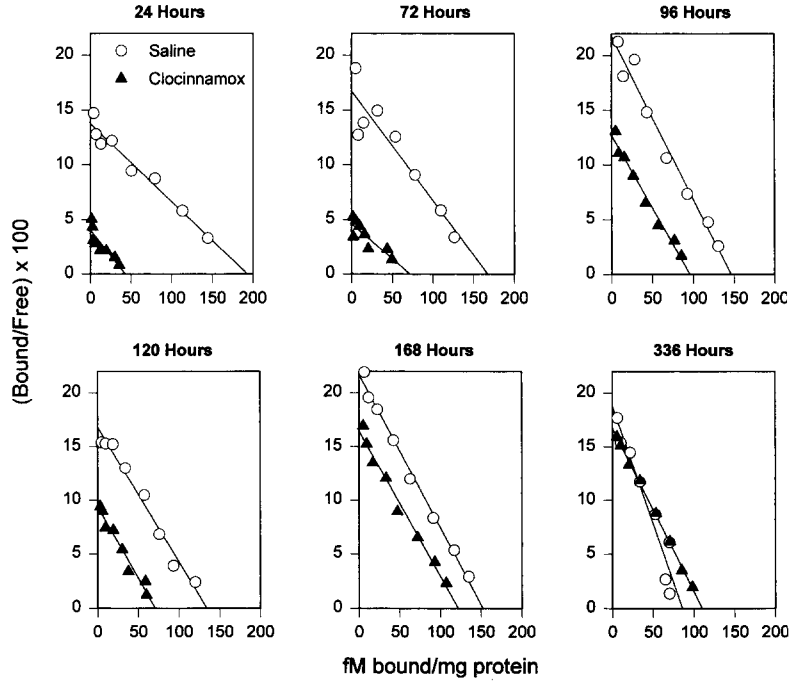

Fig. 6. Representative Scatchard plots of the time-action profile of clocinnamox on μ-opioid receptor binding in mouse brain. Mice were injected with saline or clocinnamox (12.8 mg/kg) and killed at various times (24–336 h) following dosing. Saturation binding studies were conducted using [^{3}H]DAMGO. Each panel presents a representative independent experiment. See Fig. 5 for summary.

Table 3
The effect of clocinnamox on μ-opioid receptor mRNA

Treatment	mRNA transcripts (pg equivalents/μg RNA)
24 h	
Saline	0.29 ± 0.04
Clocinnamox	0.28 ± 0.03
120 h	
Saline	0.26 ± 0.08
Clocinnamox	0.22 ± 0.03

Mice were treated with saline or clocinnamox (12.8 mg/kg) and 24 or 120 h later killed and RNA extracted and μ-opioid receptor mRNA transcript levels determined by solution hybridization (n = 6/group/time point). Data are means ± SD.

μ-Opioid receptor density was decreased by more than 70% 24 h following clocinnamox and subsequently increased as function of time. There was a significant effect of clocinnamox treatment on B_{max} ($F = 8.09$, $P < 0.0001$). Post-hoc tests indicated that the clocinnamox-treated group's B_{max} was significantly different from control 24–96 h following clocinnamox. No significant effect of clocinnamox was found for affinity (F = 1.86, $P > 0.10$). Representative binding data for the time action profile of clocinnamox are shown in Fig. 6.

3.3. mRNA

Assay of mRNA for the μ-opioid receptor by solution hybridization at 24 and 120 h following clocinnamox (12.8 mg/kg) indicated no significant changes (Table 3). These data contrast with the changes in binding and function observed at these time points.

4. Discussion

The present results confirm and extend the findings of others (Aceto et al., 1989; Lewis et al., 1989; Comer et al., 1992; Zernig et al., 1994; Burke et al., 1994) that clocinnamox is an irreversible, systemically active, μ-opioid receptor antagonist. In the present binding studies, clocinnamox produced a profound decrease in μ-opioid receptor density within 1 h of administration and there was a time-dependent return of receptors. Clocinnamox dose-dependently reduced the B_{max} for the μ-opioid receptor ligand [^{3}H]DAMGO. At all but the highest dose of clocinnamox (25.6 mg/kg), there was no significant effect of clocinnamox on [^{3}H]-DAMGO affinity. The effects of clocinnamox were selective for μ-opioid receptors since a dose of clocinnamox (12.8 mg/kg) which significantly decreased [^{3}H]DAMGO B_{max}, did not affect the affinity and density of κ-opioid ([^{3}H]U69,593) and δ-opioid ([^{3}H]DPDPE) binding sites. Concurrent with μ-opioid receptor binding changes, clocinnamox dose-dependently increased the ED_{50} for morphine and, following the 12.8 mg/kg dose, the maximal analgesic effect of morphine could not be produced even using doses in excess of 500 mg/kg s.c. In addition, clocinnamox produced a time-dependent change in morphine's analgesic ED_{50} that reflected the changes in μ-opioid receptor density. Taken together with the results from previous studies, these functional and binding data strongly suggest that clocinnamox is a specific, irreversible μ-opioid receptor antagonist. However, it should be noted that, given the small effect on affinity at the highest dose of clocinnamox, the binding data in the present study do not allow us to exclude the possibility that the slow, time-dependent reappearance of μ-opioid receptors may reflect dissociation of clocinnamox.

In order to assess if the reappearance of μ-opioid receptors following clocinnamox was associated with a change in gene expression, the abundance of mRNA in whole mouse brain was determined at two time points using a sensitive solution hybridization assay. The density of μ-opioid receptors was less that 30% of control 24 h following clocinnamox and had increased to 80% of control by 120 h following treatment. Although clear increases in μ-opioid receptor density were observed between 24 h and 120 h following clocinnamox, the relative abundance of μ-opioid receptor mRNA was not different between the clocinnamox- and saline-treated controls. These data indicate that μ-opioid receptor mRNA is not a rate-limiting step in the reappearance of receptors following clocinnamox and that turnover of receptors is independent of mRNA. These results raise the possibility that increases in μ-opioid receptor density as measured in binding studies do not depend upon de novo synthesis of receptors and may reflect changes in the rate of post-translational processing such as activation of an existing pool of 'cryptic' receptors. Support for this suggestion comes from studies which show that upregulation of opioid receptors by chronic antagonist treatment in vitro is not affected by a protein synthesis inhibitor (Tempel et al., 1986). Further support comes from in vivo studies which indicate that the lack of adrenal or sex steroids which may be important modulators of gene expression does not alter antagonist-induced receptor upregulation (Candido et al., 1992). Furthermore, recent studies indicate that chronic in vivo opioid antagonist treatment, which increases μ-, δ- and κ-opioid receptor density, does not alter, or may actually decrease, μ-opioid receptor mRNA (Brodsky et al., 1995; Unterwald et al., 1994). In contrast, in vitro studies have shown that δ-opioid receptor mRNA is increased in NG108 cells following treatment with ethanol or naloxone, both of which induce increases in δ-opioid receptor density (Charness et al., 1993; Jenab and Inturrisi,

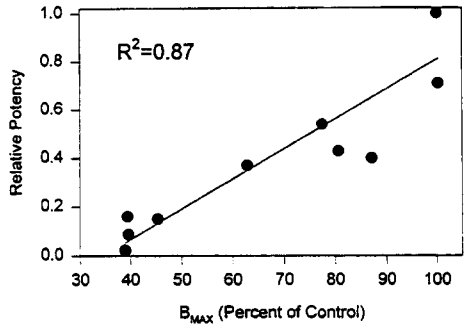

Fig. 7. Correlation between B_{max} (as percent of control) and relative potency ($1/(ED_{50}$ shift)) of morphine. The figure combines all data from both the dose-dependency (Table 1, Fig. 3) and time-dependency (Table 2, Fig. 5) studies for clocinnamox. The line was fit by linear regression.

1994). At present, it is not clear why alterations in in vitro receptor density are mirrored by changes in message, while receptor changes in vivo are not related to message. However, a relationship between in vivo sensitivity to morphine and μ-opioid receptor mRNA has been demonstrated. Using the same solution hybridization assay employed in the present experiments, Brodsky et al. (1994) found lower steady-state levels of μ-opioid receptor mRNA transcript in CNS areas associated with analgesia in the μ-opioid receptor deficient and morphine insensitive CXBK mouse strain compared to a morphine sensitive (CD-1) strain.

The relationship between binding (B_{max}) and functional effects (relative potency of morphine) of clocinnamox is suggested by the close association between the two indices. Fig. 7 presents the correlation between μ-opioid receptor B_{max} (as percent of control) and the relative potency ($1/(ED_{50}$ shift)) of morphine. The figure combines all data from both the dose-dependency (Table 1, Fig. 3) and time-course (Table 2, Fig. 5) studies. There was a significant correlation between the two measures ($r^2 = 0.87$). These data strongly suggest that the change in morphine's potency is associated with the decrease in μ-opioid receptor density and consequently that the change in receptors is the substrate for the changes in the functional effects of morphine.

In summary, we have examined the dose- and time-dependent effects of clocinnamox in the mouse. The results indicate that unlike other putative irreversible μ-opioid receptor antagonists (e.g. β-funaltrexamine, see Martin et al., 1993; Liu-Chen et al., 1991; Adams et al., 1987), clocinnamox produces parallel changes in receptor density and agonist potency with relatively minimal effects on receptor affinity. Furthermore, clocinnamox, while producing dramatic decreases in the density of μ-opioid receptors, does not appear to alter mRNA levels in mouse brain. These findings raise interesting questions concerning the mechanism of the rebound in μ-opioid receptors.

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