Clocinnamox Antagonism of the Antinociceptive Effects of Mu Opioids in Squirrel Monkeys

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ABSTRACT

The opioid agonists morphine, etorphine, buprenorphine and U50,488 were examined alone and in combination with the insurmountable opioid antagonist clocinnamox (C-CAM) in squirrel monkeys responding under a schedule of shock titration. In this procedure, shock intensity increased every 15 sec from 0.01 to 2.0 mA in 30 increments. Five lever presses during any given 15-sec shock period produced a 15-sec timeout, after which shock resumed at the next lower intensity. When given alone, each of these agonists increased the median intensity at which the monkeys maintained shock (median shock level [MSL]). At the highest dose examined alone, each agonist produced maximal increases in MSL and, except buprenorphine, decreased response rates. C-CAM dose-dependently antagonized the effects of morphine, etorphine and buprenorphine on MSL. In the presence of the higher C-CAM doses, etorphine, morphine and buprenorphine did not produce maximal effects on MSL. The effects of U50,488 were not systematically altered when tested in combination with the highest C-CAM dose. In general, C-CAM was more potent and the duration of antagonism was slightly longer against buprenorphine than against morphine and etorphine. Quantitative analysis of these data according to an extended model of Black and Leff ([1983] Proc R Soc Lond B Biol 220:141–162) yielded the following apparent affinity and efficacy estimates, respectively: etorphine (0.085 mg/kg, 117); morphine (49 mg/kg, 24) and buprenorphine (0.62 mg/kg, 7.1). Determination of the individual q values over time indicated that the receptor population recovers more quickly after C-CAM antagonism of etorphine than from C-CAM antagonism of either morphine or buprenorphine. These data suggest that C-CAM functions as a long-lasting antagonist of mu opioid agonist actions in a shock titration procedure and yields estimates of relative intrinsic efficacy with the rank order of etorphine > morphine > buprenorphine.

Receptor-selective, insurmountable antagonists are useful tools for studying pharmacological mechanisms related to the behavioral effects of opioids. For example, the mu-selective irreversible antagonist β-FNA produces a long-lasting antagonism of several behavioral effects of mu agonists but does not antagonize the effects of drugs acting at kappa opioid receptors (e.g., Dykstra et al., 1987; Zimmerman et al., 1987; Adams et al., 1990; Pitts et al., 1996). Indeed, antagonism by β-FNA generally is considered evidence that a given behavioral effect of a drug involves actions at mu opioid receptors.

Insurmountable antagonists also are used to make inferences about the relative intrinsic efficacy with which mu agonists produce their behavioral effects. In several experimental preparations, β-FNA is a more potent antagonist of the behavioral effects of mu agonists such as buprenorphine, butorphanol and nalbuphine than of mu agonists such as fentanyl, alfentanil and methadone (Zimmerman et al., 1987; Adams et al., 1990; Mjanger and Yaksh, 1991; Pitts et al., 1996). Moreover, under some conditions, opioids such as buprenorphine, butorphanol and nalbuphine do not produce a maximal behavioral effect in the presence of β-FNA (e.g., Adams et al., 1990). The greater potency of β-FNA against the effects of buprenorphine, butorphanol and nalbuphine, along with a lack of a maximal effect in the presence of β-FNA, suggests that these agonists possess relatively low intrinsic efficacy at mu receptors. That is, these opioids require occupation of a larger proportion of the mu receptor population to produce a given behavioral effect than do opioids such as fentanyl, alfentanil and methadone.

ABBREVIATIONS: β-FNA, β-funaltrexamine; C-CAM, clocinnamox; U50,488, trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide; MSL, median shock level; FR, fixed-ratio; R/sec, responses per second.
Recently, clocinnamox (C-CAM) has been characterized as an insurmountable mu receptor antagonist capable of producing a long-lasting (>24 hr) antagonism of the behavioral effects of mu, but not kappa or delta, receptor agonists (Comer et al., 1991; Burke et al., 1994; Zernig et al., 1994; Butelman et al., 1996a). Data from these studies suggest that C-CAM may be a more effective tool than b-FNA for studying mu receptor mechanisms in vivo; b-FNA produces agonist effects at kappa receptors under some conditions that could compromise interpretation of its mu antagonist effects (e.g., Ward et al., 1982; Jiang et al., 1990; Pitts et al., 1996). In contrast, C-CAM appears completely devoid of agonist activity (Comer, et al., 1991; Burke, et al., 1994; Zernig et al., 1994; Butelman et al., 1996).

In the present study, antagonist effects of C-CAM against three mu agonists, morphine, etorphine and buprenorphine, were examined in a primate model of analgesia: a shock titration procedure with squirrel monkeys. Performance maintained under this procedure is sensitive to the antinociceptive effects of a variety of opioid analogues and provides a baseline with which to study receptor mechanisms (Yaksh and Rudy, 1977; Tang and Collins, 1985; Dykstra and Massie, 1988; Pitts and Dykstra, 1994). In the present study, dose-effect curves were obtained with morphine, etorphine and buprenorphine alone and then in combination with several doses of C-CAM. As a control, the kappa agonist U50,488 was administered alone and in the presence of the highest C-CAM dose tested.

The present experiment determined (1) whether the effects of morphine, etorphine and buprenorphine are antagonized by C-CAM, indicating mu agonist activity, and (2) the potency of C-CAM in antagonizing the effects of morphine, etorphine and buprenorphine. Potency data from C-CAM should provide information on the relative efficacy with which these opioids activate mu receptors. The time course of C-CAM antagonism also was determined because the rate of recovery of drug effects after insurmountable antagonism can be used to estimate the rate of mu opioid receptor turnover in vivo (Zernig et al., 1994; Martin et al., 1995). It has been suggested that given a constant rate of receptor turnover after receptor blockade, the relative duration of antagonism should be inversely related to the agonist's relative intrinsic efficacy (e.g., Mjanger and Yaksh, 1991; Pitts et al., 1996). Finally, apparent affinity and efficacy estimates were obtained for etorphine, morphine and buprenorphine by analyzing the data according to a model initially proposed by Black and Leff (1983) and extended by Zernig et al. (1996b) for application to behavioral data.

Methods

Animals. Five adult male squirrel monkeys (Saimiri sciureus), weighing between 0.70 and 0.95 kg, were housed individually, or in pairs, in a colony room with a 12-hr light/dark cycle. All monkeys had continuous access to water and were maintained on a high-protein monkey diet and given fresh fruit daily. Four of the monkeys had previous experience under a shock titration procedure similar to the one used in the present study, and one monkey (#290) had previous experience under a warm water tail withdrawal procedure. All monkeys previously had received various opioid compounds but had not received drugs for a minimum of 30 days before the start of the present experiment.

Apparatus. During experimental sessions, each monkey sat in a Plexiglas chair and was held in place by a waist support with its tail secured by a small stock (see Dykstra, 1985). Electric shock (110 V AC, 60 Hz) was delivered through two hinged brass plates that rested on a shaved portion of the tail. Shock intensity was adjusted through a stepping switch and a series of potentiometers.

Each chair was enclosed within a ventilated sound-attenuating chamber and was illuminated by a 10-W white houselight during experimental sessions. A lever was mounted on the right side of the front panel, 8.5 cm above the waist plate and 4.0 cm from the right side wall. During experimental sessions, pressing on the lever with a downward force of 0.15 N produced an audible click and were recorded as responses. White noise was present continuously both inside the chamber and throughout the experimental room. Experimental events were programmed and data were recorded by TRS 80 Model III microcomputers via MedState interfaces located in an adjoining room.

Behavioral Procedure. A shock titration procedure nearly identical to that described by Dykstra and Massie (1988) was used. In each session, periods during which an FR 5 schedule of shock titration was in effect alternated with periods of blackout. Each FR 5 titration period began with the illumination of the houselight and presentation of 0.01 mA shock. Shock intensity increased from 0.01 to 2.0 mA in 30 increments. Completion of the FR 5 requirement at a given shock intensity initiated a timeout during which shock was off and the houselight remained illuminated. After the 15-sec timeout, the shock resumed at the next lower intensity. If the monkey failed to complete the FR 5 during 15 sec at a given shock intensity, the intensity increased by one increment and the response requirement was reset to 5. The FR 5 titration periods usually lasted 15 min. An FR 5 period terminated automatically, however, if the shock intensity rose to the peak of 2.0 mA and the FR 5 requirement was not completed during any of five consecutive 15-sec periods. During the blackouts that separated the FR 5 titration periods, the chamber was dark, no shock was delivered and lever presses had no programmed consequences. Usually, blackouts lasted 20 min; during sessions in which U50,488 was administered, the duration of the blackouts was reduced to 10 min. Each session began with an FR 5 titration period and ended after completion of five to seven FR 5 periods.

Pharmacological Procedure. Dose-effect curves for morphine, etorphine, buprenorphine and U50,488 were obtained by a cumulative-dosing procedure. Under this procedure, vehicle (saline) was injected 20 min before the first FR 5 period (10 min during sessions in which U50,488 was tested). On completion of the first FR 5 period (at the onset of the first blackout), the lowest agonist dose was injected and its effects were assessed in the following FR 5 period. At the onset of each subsequent blackout, an amount of drug that increased the cumulative dose by 0.25 to 0.5 log unit was injected. Injections continued according to this procedure until a cumulative dose was reached that suppressed lever pressing such that the FR 5 period terminated automatically (i.e., the monkey allowed the shock intensity to rise to 2.0 mA and did not complete an FR 5 during five consecutive 15-sec periods). Under some conditions, agonist doses did not decrease response rates substantially (e.g., when buprenorphine was given alone and when the mu agonists were given in combination with the higher doses of C-CAM). In these cases, cumulative administration continued until consecutive doses failed to produce appreciable increases in MSL or until it was feared that the safety of the monkey might be compromised. Under conditions in which an agonist dose-effect curve was flattened substantially in the presence of C-CAM, cumulative dosing was always continued until a dose was reached that was at least 1 log unit higher than the highest dose tested alone (in most cases, this dose was more than 1 log unit higher). Drug administrations usually occurred on Tuesdays and Fridays, and saline (nondrug control) injections were given before some or all of the FR 5 periods on Thursdays. Due to a long duration of action, consecutive determinations of the buprenorphine dose-
effect curve were spaced by a minimum of 1 week. Dose-effect curves for each agonist alone were determined at least three times before initiation of testing with C-CAM.

Dose-effect curves for morphine, etorphine and buprenorphine also were obtained in the presence of two or three doses of C-CAM (0.01–0.1 mg/kg). Note that buprenorphine was not be studied in the presence of 0.1 mg/kg C-CAM because of its solubility limits at higher doses. Dose-effect curves for U50,488 were obtained in the presence of the largest C-CAM dose (0.1 mg/kg). For each agonist, C-CAM was injected 4 hr before determination of the dose-effect curve. Subsequently, the agonist dose-effect curve was redetermined 3, 10, 17, 24 and 31 days after C-CAM administration. Usually, C-CAM doses were administered in combination with each agonist once. However, to determine the effects of buprenorphine at each of the designated time points after C-CAM administration, each C-CAM dose had to be administered twice. After the first administration, the buprenorphine dose-effect curve was determined 4 hr, 10 days and 24 days later; after the second administration, the buprenorphine curve was determined 3, 17 and 31 days later. All drugs were injected intramuscularly except C-CAM, which was injected subcutaneously. Injection volumes were 0.5 ml/kg.

Morphine sulfate, buprenorphine hydrochloride, etorphine hydrochloride and U50,488 (trans-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)benzene acetamide) (all provided by the National Institute on Drug Abuse) were dissolved in sterile saline. Clocinnamox mesylate (generously donated by J. H. Woods, University of Michigan) was dissolved in distilled water. Doses of all compounds are expressed in terms of the forms listed above.

Data analysis. MSLs and response rates were determined under all conditions for individual monkeys. MSL was expressed as the shock intensity below which the monkey kept the shock 50% of the time. Response rates (R/sec) during shock and during the timeouts were calculated separately for each monkey by dividing the total number of responses during shock (or timeout) by the total time spent in shock (or timeout). Because of possible warm-up effects (see Hoffman, 1966), data from the first 5 min of each FR 5 titration period were eliminated from the analysis.

For each agonist, an ED50,control value was determined for MSL. This was done by averaging two or three control dose-response curves for each monkey and fitting logistic dose-response curves to the data points using InPlot (GraphPAD, San Diego, CA). Individual ED50,control values were averaged for each agonist to obtain a group ED50,control (referred to as the observed ED50,control). For the quantitative comparisons, control dose-response curves for individual monkeys receiving a given agonist, as well as the dose-response curves obtained 4 hr, 3 days and 10 days after C-CAM administration, were analyzed simultaneously using the model proposed by Black and Leff (1985) and applied to behavioral data according to methods described extensively by Zernig et al. (1996b). These analyses yielded in vivo estimates of agonist affinity (Ka), agonist efficacy (tau, an operational definition of efficacy) and the fraction of receptors available for interaction with an agonist after treatment with C-CAM (q). These values were determined using the model by Black and Leff (1983) extended by q as defined by Furchgott (1966) according to the following equation:

\[ E = E_{ml} / \left[ \left( 10^{(\log(K_A) - \log(A))} + 1 \right)/(q\tau_{u,\text{control}}) \right] + 1 + c \]

where E is the effect (MSL), Em is the maximum attainable response, A is the agonist concentration, n is the slope factor of the transducer function and c is the control MSL; \( \tau_u \) is represented as (q\tau_{u,\text{control}}).

All dose-response curves obtained with a given agonist were fitted to the above equation using a nonlinear fitting program (Zernig and Issacvitch, 1995) and the general mathematical software package Mathematica (Wolfram Research, Champaign, IL; Wolfram, 1991).

The derived values determined by the mathematical analysis of Black and Leff (1983) can be used in the equation reported by Black et al. (1985):

\[ ED_{50,\text{control}} = K_A / \left( \left( 2 + \tau_u \right)^{1/n} - 1 \right) \]

to determine the control ED50 of an agonist. This “back-calculated” ED50 (referred to as the calculated ED50,control) was then compared with the observed ED50,control as a measure of internal consistency.

Variance estimates for a given variable are represented as S.E.M. Variance estimates for the q values for 0.01 mg/kg C-CAM in combination with buprenorphine are represented as a range because only two monkeys participated in this portion of the experiment. Statistical calculations were done on an IBM PC with the Prism and InStat computer package (GraphPAD). Statistical differences among q values for each agonist in combination with 0.03 mg/kg C-CAM were determined separately at the 4-hr, 3-day and 10-day time points by a one-factor ANOVA followed, when appropriate, by Student-Newman-Keuls Multiple Comparisons Test. Statistical differences between individual q values for 0.01 or 0.1 mg/kg C-CAM were determined by Student’s t test. A one-way ANOVA also was performed on the n values of each agonist (slope of the transducer function). Significance was set at P < .05.

Results

Control performance. Table 1 shows the MSL range and average rate of responding obtained for each monkey after saline injections. MSL for the five monkeys ranged between 0.02 and 0.45. For all but one monkey (711), response rates in the presence of shock exceeded response rates during the postshock timeouts.

Effects of etorphine, morphine, buprenorphine and U50,488 administered alone. Figure 1 shows average dose-effect curves for morphine, etorphine, buprenorphine and U50,488 for MSL and rate of responding. Each agonist produced dose-dependent increases in MSL with maximal effects occurring at 5.6 mg/kg morphine, 0.0017 mg/kg etorphine, 0.56 mg/kg buprenorphine and 5.6 mg/kg U50,488. Morphine, etorphine and U50,488 dose-dependently decreased response rates, whereas buprenorphine generally did not decrease response rates in the dose range studied.

Effects of morphine in combination with C-CAM. Figure 2 shows the effects of morphine on MSL in individual monkeys. Morphine’s effects are shown before C-CAM administration and 4 hr, 3 days, 10 days and 17 days afterward. In general, at 4 hr after administration, C-CAM (0.01–0.03 mg/kg) dose-dependently antagonized the effects of morphine.

TABLE 1

<table>
<thead>
<tr>
<th>Monkey</th>
<th>N</th>
<th>MSL range</th>
<th>R/sec during shock (S.E.)</th>
<th>R/sec during timeout (S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1909</td>
<td>34</td>
<td>0.04–0.45</td>
<td>0.23 (0.0030)</td>
<td>0.063 (0.010)</td>
</tr>
<tr>
<td>282</td>
<td>34</td>
<td>0.02–0.25</td>
<td>0.25 (0.0050)</td>
<td>0.021 (0.019)</td>
</tr>
<tr>
<td>290</td>
<td>15</td>
<td>0.02–0.06</td>
<td>0.33 (0.011)</td>
<td>0.23 (0.027)</td>
</tr>
<tr>
<td>711</td>
<td>10</td>
<td>0.03–0.15</td>
<td>0.30 (0.016)</td>
<td>0.42 (0.10)</td>
</tr>
<tr>
<td>713</td>
<td>10</td>
<td>0.08–0.15</td>
<td>0.27 (0.011)</td>
<td>0.083 (0.0080)</td>
</tr>
</tbody>
</table>

N, Number of saline injections; MSL, median shock level; R/sec, responses per second.
on MSL. The lowest C-CAM dose (0.01 mg/kg) produced a small shift in the dose-effect curve for #282 and flattened the curve for #290 such that 30 mg/kg morphine produced only a slight increase in MSL; this C-CAM dose did not antagonize the effects of morphine on MSL in #1909. At the 4-hr time point, 0.03 mg/kg C-CAM shifted the morphine dose-effect curve rightward in #1909 and 282 and flattened the dose-effect curve in #290. In the presence of 0.03 mg/kg C-CAM, maximal increases in MSL were produced by 56 mg/kg morphine in #1909 and 282, whereas doses of morphine up to 100 mg/kg did not increase MSL in #290. At 4 hr after administration of 0.1 mg/kg C-CAM, the dose-effect curve for etorphine was flattened in all monkeys; doses of etorphine that produced maximal increases in MSL no longer increased MSL at all. Although 0.1 mg/kg C-CAM produced a greater antagonism of the etorphine dose-effect curves than did 0.03 mg/kg C-CAM, the time required for recovery of the etorphine curve was similar at both of these C-CAM doses. In all but one case (#1909 at 0.1 mg/kg C-CAM), the etorphine dose-effect curve returned to within 0.25 log unit of control values by 3 days after C-CAM administration.

Clocinnamox also produced a dose-dependent antagonism of the response rate-decreasing effects of etorphine; at the highest dose of C-CAM, etorphine did not decrease response rates at any of the doses tested (data not shown).

**Effects of buprenorphine in combination with C-CAM.** Figure 4 shows effects of buprenorphine on MSL in individual monkeys at 4 hr, 3 days, 10 days, 17 days, and 24 days after administration of C-CAM (0.01–0.03 mg/kg). At 4 hr after administration, C-CAM produced a dose-dependent antagonism of the effects of buprenorphine on MSL. The 0.01 mg/kg C-CAM dose shifted the buprenorphine dose-effect curve to the right by ~1.5 log units in all monkeys and the dose-effect curve returned to within 0.25 log unit of control values by day 10 after administration of 0.01 mg/kg C-CAM for both #1909 and #282. After administration of 0.03 mg/kg C-CAM, the buprenorphine dose-effect curve returned to within 0.25 log unit of control values by 24 days (#713).

Note that buprenorphine typically did not decrease response rates appreciably when given alone and that it did not decrease response rates when given in combination with C-CAM.
Effects of U50,488 in combination with C-CAM. In general, 0.1 mg/kg C-CAM did not appreciably alter the average effects of U50,488. For the three monkeys tested, the average ED$_{50}$ (95% CL) for U50,488 on MSL alone was 1.9 (1.6–2.2) mg/kg and 4 hr after C-CAM administration was 2.7 (1.4–5.2) mg/kg. At 3, 10 and 17, days after C-CAM administration, these values were 2.4 (1.5–3.9), 2.7 (0.75–9.5) and 2.6 (0.80–8.3) mg/kg, respectively. The 0.1 mg/kg dose of C-CAM did not attenuate the maximal effect of U50,488 on MSL in any of the monkeys at any time point. This C-CAM dose also did not appreciably alter the dose-effect curve or the maximal effect of U50,488 on response rate (data not shown).

Determination of affinity and efficacy estimates. The data obtained for morphine, etorphine and buprenorphine (see figs. 2–4) were analyzed to obtain estimates for the apparent in vivo dissociation constant, $K_A$ (which is inversely proportional to the agonist affinity), efficacy ($\tau$) (Black and Leff, 1983) and the fraction of receptors remaining after C-CAM treatment ($q$) (Furchgott, 1966). The data for etorphine yielded the highest affinity estimate ($K_A = 0.085$ mg/kg) and the highest efficacy estimate ($\tau = 117$). Morphine showed the lowest affinity estimate ($K_A = 49$ mg/kg) and an intermediate efficacy estimate ($\tau = 24$). Buprenorphine showed an intermediate affinity estimate ($K_A = 0.62$ mg/kg) and the lowest efficacy estimates ($\tau = 7.1$). The derived values determined by mathematical analyses were used to calculate control ED$_{50}$ values for agonists in the absence of C-CAM to assess the internal consistency of the model. These back-calculated ED$_{50}$ values for control dose-response curves were similar to observed ED$_{50}$ values (table 2).

Values for $q$, an estimate of the fraction of receptors available for agonist interaction after insurmountable antagonism, are presented in table 3. This analysis indicates that 4 hr after administration, 0.01, 0.03 and 0.1 mg/kg C-CAM reduced the receptor population by an average of 33%, 96% and 99%, respectively (see average $q$ values presented in table 3). Determination of the $q$ values over time for morphine and buprenorphine indicated that on average, the receptor population returns to normal by 10 days after treatment with 0.01 mg/kg C-CAM. There was a significant difference across agonists in the rate of receptor recovery after treatment with 0.03 mg/kg C-CAM. That is, the ANOVA revealed a significant difference in $q$ values across agonists at 3 days after treatment with 0.03 mg/kg C-CAM days ($F(2,6) = 5.7; P < 0.041$). The source of this variation was that the $q$ value for etorphine was significantly different from those for morphine and buprenorphine; the $q$ value for etorphine had returned to near base line by 3 days after treatment with 0.03 mg/kg C-CAM. This statistical difference
disappeared by day 10, despite the fact that \( q \) values for morphine and buprenorphine were still \( \leq 50\% \) or below. The \( q \) value for buprenorphine after 0.03 mg/kg C-CAM at 4 hr was smaller than those for etorphine and morphine, although this finding was not statistically significant due to the amount of variability at this time point. Finally, there was no difference in the rates of recovery of the receptor population as indicated by the individual \( q \) values for etorphine and morphine after 0.1 mg/kg C-CAM.

**Discussion**

In the present study, the \( \mu \) opioids morphine, etorphine and buprenorphine and the \( \kappa \) opioid U50,488 all dose-dependently increased MSL in squirrel monkeys responding under a shock titration procedure. Similar results have been reported previously for morphine, buprenorphine and U50,488 (Craft and Dykstra, 1990; Dykstra, 1985; Negus and Dykstra, 1988; Pitts and Dykstra, 1994); however, to our knowledge this is the first report of etorphine’s effects under a shock titration procedure. In general, increases in MSL after morphine, etorphine and U50,488 were accompanied by decreases in rates of responding during shock (see Dykstra and McMillan, 1977; Smith and McKealney, 1977). In contrast, buprenorphine increased MSL without decreasing rates of responding. These data with buprenorphine are particularly noteworthy because they illustrate that effects on MSL in this preparation are not necessarily accompanied by changes in rate of responding (see Dykstra, 1979).

The insurmountable \( \mu \) receptor antagonist C-CAM did not affect MSL when administered alone, but dose-dependently antagonized the effects of morphine, etorphine and buprenorphine on MSL. These findings are consistent with previous reports that C-CAM antagonizes other effects of \( \mu \) opioids, including their antinociceptive, reinforcing and response rate-decreasing effects (Butelman *et al*., 1996; Burke *et al*., 1994; Zernig *et al*., 1995a, 1995b, 1997). In contrast, the largest C-CAM dose did not systematically alter the effects of U50,488 on MSL; C-CAM did not alter the ability of U50,488 to produce a maximal effect on MSL.

Although C-CAM produced a dose-dependent antagonism of the effects of morphine, etorphine and buprenorphine on MSL, the pattern of C-CAM antagonism differed across ago-
Of the agonists tested, buprenorphine clearly was the most sensitive to antagonism by C-CAM. For example, 4 hr after treatment with 0.03 mg/kg C-CAM, buprenorphine doses of up to 1.5 log units higher than those examined alone did not produce a maximal effect in any of the monkeys tested. In contrast, 4 hr after treatment with 0.03 mg/kg C-CAM, both morphine and etorphine produced a maximal, or near-maximal effect in at least two of three monkeys tested. These data suggest that buprenorphine increases MSL with a lower efficacy (i.e., by requiring the activation of more receptors) than does morphine and etorphine. This conclusion is supported by the results of the quantitative analysis. The efficacy estimate for buprenorphine was substantially lower \( \tau = 7.1 \) than for morphine and etorphine \( \tau = 24 \) and 117, respectively.

The present results with buprenorphine relative to morphine and etorphine are in keeping with those from other studies. For example, \( \beta \)-FNA produced larger shifts in the dose-effect curve for buprenorphine than for morphine or etorphine in a rat analgesia procedure (Zimmerman, et al., 1998).
1987). Furthermore, morphine-tolerant rats revealed a larger degree of cross tolerance to buprenorphine than to etorphine or fentanyl in a tail flick analgesia assay (Paronis and Holtzman, 1992). It has been proposed that the degree of cross tolerance among mu opioids and the magnitude of shift produced by irreversible antagonists such as β-FNA are inversely related to intrinsic activity. Thus, the data from the present study, along with those previous studies, provide compelling evidence that buprenorphine acts with lower intrinsic activity than morphine and etorphine in a variety of behavioral preparations and across different species.

The data for morphine and etorphine are particularly interesting. The quantitative analysis indicates that etorphine increased MSL with higher efficacy than did morphine (τ was 117 for etorphine and 24 for morphine), although such a difference is not easily discerned by comparing figures 2 and 3. This difference in τ likely was due, at least in part, to the data obtained with #290. The effects of morphine on MSL for this monkey were particularly sensitive to antagonism by C-CAM. The potency of C-CAM against morphine and etorphine appeared similar in the two monkeys that received both agonists (#1909 and #282). Note, however, that in the presence of 0.1 mg/kg C-CAM at 4 hr, at least one dose of etorphine increased MSL above the control range with both 1909 and 282. In contrast, none of the doses of morphine tested increased MSL above control values in the presence of 0.1 mg/kg C-CAM. Thus, these results prompt a tentative conclusion that etorphine increased MSL in the present study with greater efficacy than morphine. The fact that the average recovery time for the etorphine dose-effect curve after C-CAM administration was shorter than for morphine is consistent with this notion.

Interestingly, some previous studies have provided data showing greater intrinsic activity for etorphine than morphine in vivo. For example, compared with morphine, some investigators report a smaller degree, or a lack of tolerance to etorphine’s antinociceptive effects (Paronis and Holtzman, 1992) and discriminative stimulus (Paronis and Holtzman, 1994) effects in morphine-tolerant rats, and to its response rate-decreasing effects in morphine-tolerant squirrel monkeys (Hughes et al., 1995). Also, using a tail-withdrawal procedure with rats, Walker et al. (1998) reported that 10 mg/kg C-CAM produced a greater attenuation of the antinociceptive effects of morphine than etorphine. On the other hand, Zimmerman et al. (1987) reported that β-FNA antagonized the antinociceptive effects of morphine and etorphine to a similar degree in rats, and Young et al. (1991) reported a similar degree of tolerance to the discriminative stimulus effects of morphine and etorphine in morphine-tolerant pigeons. Thus, the exact conditions under which etorphine shows greater intrinsic activity than morphine in vivo remain to be identified.

Clocinnamox antagonism data yielded the following apparent affinity estimates (Kᵢ): etorphine (0.085 mg/kg); morphine (49 mg/kg) and buprenorphine (0.62 mg/kg). These data indicate that etorphine and buprenorphine, high and low efficacy agonists, respectively, have high affinity for their receptors. Similarly, in radioligand binding studies, buprenorphine and etorphine show high affinity for mu opioid receptor sites (Richards and Sadee, 1985; Sadée et al., 1982). Morphine had the lowest affinity estimate. The values calculated for morphine in the present study were similar to in vivo Kᵢ estimates for morphine in antinociceptive assays in rhesus monkeys (13–60 mg/kg; Zernig et al., 1994; Walker et al., 1995), mice (29 mg/kg, Zernig et al., 1995b) and rats (22–50 mg/kg, Blasig et al., 1979; Porreca et al., 1982; Tallarida and Cowan, 1982; Walker et al., 1998). Previous affinity estimates for buprenorphine were 0.042 mg/kg in rats (Walker et al., 1998) and ranged from 0.12 to 0.19 mg/kg in rhesus monkeys (Walker et al., 1995); these values indicate greater affinity than the 0.62 mg/kg value obtained in the present study for squirrel monkeys. Interestingly, buprenorphine and other oripavines typically produce agonist effects in squirrel monkeys at doses that are much lower than those required to produce similar effects in rhesus monkeys and rats (Dykstra, 1983; DeRossett and Holtzman, 1986; Dykstra and Negus, 1995). Furthermore, characterization of buprenorphine is complicated by demonstrations that it can function as an antagonist in squirrel monkeys (Negus and Dykstra, 1988) and rhesus monkeys (Walker et al., 1995). Given the difficulty in making species comparisons based on different studies that each use different apparatus, stimuli, responses and behavioral procedures, more direct comparisons are required to determine the nature of the differences in sensitivity to buprenorphine’s behavioral effects across species in vivo.

In addition to affinity and efficacy estimates, C-CAM antagonism experiments allow estimation of the fraction of receptors available for agonist interaction after each dose of C-CAM (q). The effect of C-CAM on q was dose related; q values at 4 hr after C-CAM administration indicate that 0.01,
0.03 and 0.1 mg/kg reduced the \( \mu \) receptor population by an average of 33\%, 96\% and 99\%, respectively. On occasion, however, the variance obtained for \( q \) in the present study was considerable, implying that variables other than the fraction of receptors available can affect \( q \) values. This effect occurred mainly at the higher C-CAM doses (0.03 and 0.1 mg/kg), indicating that it may be difficult to quantify \( q \) precisely when the receptor population has been dramatically reduced. Other investigators also have reported variation in \( q \) values that may relate to factors other than the fraction of receptors available (Zernig et al., 1994). Note that \( q \) estimates in the present study indicate that receptor reappearance after 0.03 mg/kg C-CAM occurs more quickly for etorphine than for morphine and buprenorphine; a significant difference in \( q \) values occurred among agonists on day 3. Differences in \( q \) were not obtained after the higher dose of C-CAM or between morphine and buprenorphine at any time point. Ex vivo binding studies in mice indicate that the recovery of \( \mu \) receptors after C-CAM treatment appeared to be due to de novo synthesis of \( \mu \) receptors (Zernig et al., 1996a) and that this recovery of receptors should be independent of the agonist used to measure it. It is not clear why a difference in \( q \) across agonists occurred on day 3 after 0.03 mg/kg C-CAM. Further studies with other agonists and C-CAM in squirrel monkeys are needed to determine the sources of control over \( q \) values obtained from behavioral data and whether such values are useful estimates of the fraction of receptors available after C-CAM administration.

Several important issues must be considered carefully when interpreting the results of quantitative pharmacological analyses of behavioral data. First, such analyses usually entail some critical assumptions (see discussions by Dykstra et al., 1987; Zernig et al., 1996b). For example, the present analysis assumes a linear relation between the administered dose of the agonist and its concentration in equilibrium at the receptor sites. In addition, the model proposed by Black and Leff (1983) assumes that the concentration of receptors is greater than the concentration of signal transduction molecules. Although the degree to which the first of these assumptions was met cannot be determined in vivo, the pharmacological and behavioral parameters chosen to approximate these conditions were based on those used in other studies employing similar methods (e.g., Dykstra and Massie, 1988; Pitts and Dykstra, 1994; Zernig et al., 1994). Second, because behavioral effects of drugs are affected by a variety of non-pharmacological variables (see Barrett 1987; Branch, 1991), it is likely that estimates of \( K_\alpha \), \( \tau \) and \( q \) obtained from behavioral data are influenced by such variables. Thus, demands are placed on the researcher to achieve a high degree of experimental control across pharmacological conditions. Caution is therefore warranted when applying quantitative pharmacological analyses to behavioral data. Despite these important considerations, the similarity of the results from present analyses to those from other studies cannot be dismissed. The affinity and efficacy estimates obtained in the present study resemble those from other in vivo studies and the rank order of agonist efficacy obtained here corresponds to that predicted from previous studies (e.g., Walker et al., 1995, 1998; Zernig et al., 1994, 1995b).

Finally, because of its long duration of action, investigations of buprenorphine in combination with C-CAM followed a different time course than investigations of morphine and etorphine. Although the dose-effect curves for morphine and etorphine were determined at 4 hr, 3 days and then at 7-day intervals (i.e., 10, 17, 24 and 31 days after C-CAM administration), the buprenorphine dose-effect curve was determined no more often than once every 10 days. Thus, the C-CAM/buprenorphine interactions were first examined at 4 hr after C-CAM administration and then 10 and 24 days later. Subsequently, the same dose of C-CAM was administered a second time and the buprenorphine dose-effect curve was determined at 3, 17 and 31 days after C-CAM administration. Zernig et al. (1994) suggested that each determination of an agonist dose-effect curve might extend the time needed for recovery of receptor populations. Because buprenorphine was tested less frequently after each C-CAM administration, it is possible that the recovery time of the buprenorphine curve was underestimated relative to morphine and etorphine. Thus, had it been possible to test buprenorphine at the same intervals used to test morphine and etorphine, recovery time for the buprenorphine dose-effect curve may have been even longer. Nevertheless, it is important to note that despite the longer intervals between successive determinations of the buprenorphine dose-effect curve, the buprenorphine curve still showed the slowest recovery time of the three agonists. For example, after administration of 0.03 mg/kg C-CAM, the etorphine dose-effect curve required ~3 days to recover, the morphine curve required ~10 days, and buprenorphine curve required as many as 24 days.

In summary, the \( \mu \)-selective antagonist clocinnamox produced a dose-dependent, long-lasting and insurmountable antagonism of the effects of the \( \mu \) agonists morphine, etorphine and buprenorphine on MSL under a shock titration procedure with squirrel monkeys. The effects of buprenorphine on MSL were the most sensitive to C-CAM antagonism, suggesting that buprenorphine displays the lowest intrinsic activity of these agonists under these conditions. On average, the effects of morphine were slightly more sensitive to C-CAM antagonism than those of etorphine, suggesting that etorphine may possess greater intrinsic activity than morphine under the present conditions. The results of the present study suggest that under appropriate experimental conditions, in vivo affinity and efficacy estimates can be used for drug classification as well as for making inferences about the relation of efficacy to the behavioral actions of opioid agonists.

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References


