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Intravenous self-administration of etonitazene alone and combined with cocaine in rhesus monkeys: comparison with heroin and antagonism by naltrexone and naloxonazine

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Abstract

Rationale—In humans, μ opioid-cocaine combinations (speedballs) have been reported to heighten pleasurable effects and result in greater abuse potential compared to either drug individually. Emerging evidence in animals suggests that the ability of μ opioids to enhance the reinforcing effects of cocaine might be independent of their μ intrinsic efficacy even though μ agonist efficacy appears to be a determinant in the reinforcing effects of μ opioids themselves.

Objectives—This study examined the relationship between agonist efficacy, self-administration and the enhancement of cocaine self-administration using the high-efficacy μ agonist etonitazene.

Methods—Rhesus monkeys self-administered cocaine, heroin, etonitazene, and opioid-cocaine combinations under a progressive-ratio schedule of IV drug injection.

Results—Unlike cocaine and heroin, etonitazene did not maintain consistent self-administration at any dose tested (0.001 – 1.0 $\mu\text{g/kg/injection}$). However, combining etonitazene (0.1 – 1.0 $\mu\text{g/kg/inj}$) with cocaine (0.01 and 0.03 mg/kg/inj) enhanced cocaine self-administration, and this enhancement was attenuated by naltrexone. These effects are similar to those obtained by combining non-reinforcing doses of heroin and cocaine. Antagonism of etonitazene-cocaine and heroin-cocaine self-administration by naloxonazine was short-lasting and was not maintained after 24hrs (when naloxonazine's purported μ_1 subtype antagonist effects are thought to predominate).

Conclusions—The results suggest that high μ agonist efficacy does not guarantee consistent drug self-administration and that the ability of μ agonists to enhance cocaine self-administration does not depend exclusively on reinforcing efficacy. Moreover, the results do not support a major role for μ_1 receptor mechanisms in either etonitazene- or heroin induced enhancement of cocaine self-administration.

Keywords

agonist efficacy; reinforcing effects; speedball; etonitazene

Introduction

An extensive body of research associates μ opioid intrinsic efficacy, determined by G-protein binding and adenylyl cyclase activity in vitro, with its effectiveness in producing analgesic and

respiratory depressant effects in vivo (Walker et al. 1993; Gerak et al. 1994). However, the degree to which agonist efficacy corresponds to the reinforcing effects of μ opioids is less understood. Emerging evidence suggests that high-efficacy μ opioids are more robustly self-administered than low-efficacy opioids. An earlier study by Mello et al. (1988) found that the high-efficacy μ opioid heroin engendered greater breakpoints under a progressive-ratio schedule of self-administration in monkeys compared to the lower-efficacy agonists buprenorphine and methadone. Winger et al. (1996) and Rowlett et al. (2002) also measured the reinforcing effects of the high-efficacy agonist alfentanil and the low-efficacy agonist nalbuphine under fixed- or progressive-ratio schedules of intravenous (IV) self-administration in monkeys. In these studies, increasing the behavioral requirement for drug delivery reduced nalbuphine self-administration, whereas alfentanil self-administration was maintained. Further, self-administration of lower-efficacy opioids appears to be more sensitive to opioid-selective antagonism compared to higher-efficacy agonists. For example, clocinnamox antagonized nalbuphine-maintained responding to a greater degree than alfentanil-maintained responding (Zernig et al. 1997). This differential sensitivity of high and low efficacy μ agonists suggests that intrinsic efficacy could be a determinant of the reinforcing effectiveness of μ opioids.

In humans, the combined use of μ opioids and cocaine (speedball) is reported to produce enhanced reinforcing effects compared to the effects of either drug alone (Walsh et al. 1996). Interestingly, both high and low efficacy μ agonists are capable of enhancing cocaine self-administration. In clinical studies of speedball self-administration, subjects reported heightened pleasurable effects when the low-efficacy agonist buprenorphine was combined with cocaine relative to the effects of cocaine alone (Foltin and Fischman 1994). Rowlett et al. (2005) also demonstrated that combining nalbuphine with cocaine enhanced drug self-administration, to about the same degree as heroin-cocaine and alfentanil-cocaine combinations. Moreover, these μ agonists enhanced cocaine self-administration at doses that did not maintain responding when self-administered alone. Collectively, these observations suggest that high agonist efficacy could be a fundamental determinant of opioid self-administration, but not of an opioid's ability to enhance cocaine self-administration.

Etonitazene is a high-efficacy, potent, selective μ agonist that maintains oral self-administration in rhesus monkeys (Carroll and Meisch, 1978; Meisch, 1995) but it has not been investigated for its ability to maintain IV self-administration in monkeys. Compared to other μ agonists, etonitazene displays unusually high agonist efficacy based on its ability to stimulate [³⁵S]GTP γ S binding at cloned rat μ opioid receptors (Emmerson et al. 1996); to induce rapid endocytosis of μ receptors (Koch et al. 2005); and to increase tail withdrawal latency in monkeys even at increased stimulus intensities (Walker et al. 1993; 1995). In monkeys, etonitazene can produce greater antinociceptive and respiratory depressant effects at lower doses (up to 30 fold) compared to alfentanil (Butelman et al. 1993; Walker et al. 1993). Etonitazene acts as a selective agonist exhibiting high affinity at the μ opioid receptor ($K_i \sim 10$ pM) compared to delta or kappa opioid receptors ($\sim 10,000$ -fold) in rhesus monkey cortical membranes (Emmerson et al. 1994). Etonitazene also is reportedly selective for the putative μ_1 receptor over other μ subtypes (~ 2500 -fold), as determined by saturation binding assays using ³H-DADLE in rat brain membranes (Moolten et al. 1993). There are no reports on the involvement of μ_1 subtypes in abuse-related effects of opioids in monkeys; however there is evidence to support a role for the μ_1 subtype in rats. In particular, pretreatment with the reputed μ_1 -selective antagonist naloxonazine attenuated the discriminative stimulus effects of morphine (Suzuki et al. 1995) and morphine-induced place preference (Piepponen et al. 1997) and produced antagonist-like effects on heroin self-administration (Negus et al. 1993) at time points when naloxonazine's antagonism of μ_1 subtype effects are estimated to occur.

The present study investigated the reinforcing effects of the high-efficacy agonist etonitazene alone and in combination with cocaine. The first phase evaluated cocaine, heroin and etonitazene self-administration under a progressive-ratio schedule of IV drug injection to compare the relative reinforcing effects of individual drugs. Based on the findings that high-efficacy μ agonists generally maintain robust self-administration, etonitazene is expected to maintain consistent levels of drug self-administration. Secondly, the degree to which combining etonitazene and cocaine could enhance drug self-administration was examined. If agonist efficacy influences the ability of opioids to enhance cocaine self-administration then etonitazene, like heroin, should enhance drug self-administration when combined with cocaine. The final phase of the study investigated the ability of naloxonazine to attenuate self-administration of etonitazene-cocaine and heroin-cocaine combinations at different time points (1 and 24-hr post-treatment). Naloxonazine has been characterized as an antagonist that binds irreversibly to the μ_1 subtype and reversibly to other μ subtypes based on differential antagonism of the respiratory depressant and antinociceptive effects of opioids (Ling et al. 1985). Thus, if the enhanced reinforcing effects of opioid-cocaine combinations are mediated via μ_1 receptors, then self-administration should be attenuated by naloxonazine at both time points.

Materials and Methods

Subjects and Apparatus

Four male and 4 female adult rhesus monkeys (*Macaca mulatta*) weighing 8 to 11 kg were housed in colony rooms with a 12-h light/dark cycle (lights on at 6:30 AM) where they were fed (Teklad Monkey Diet and fresh fruit) and had unrestricted access to water. Individual stainless steel home cages also served as the testing chambers and were equipped with a panel of stimulus lights and a response lever (MED Associates, St. Albans, VT) mounted below the lights. Monkeys wore nylon mesh jackets (Lomir Biomedical Inc., Malone, NY) that were connected to 1-m stainless steel flexible tethers. Each monkey was surgically prepared with a chronic indwelling venous catheter using the general procedures described by Platt et al. (2005). The catheter was routed through the tether and connected to an injection pump located on top of the cage. Drug solutions were administered from sterile syringes that were driven by the injection pumps at an infusion rate of 0.18 ml/s. The stimulus lights, response levers, and injection pump were connected to interfaces (MED Associates) and PC-compatible computers located in an adjacent room. All procedures were conducted with the approval and under the supervision of the Harvard University Institutional Animal Care and Use Committee and conformed to recommendations described in the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996).

Drug Self-Administration Procedure

Monkeys were trained to self-administer cocaine under a progressive-ratio schedule of IV drug injection similar to the schedule previously described by Rowlett et al. (2005). Experimental sessions consisted of five components, each comprising four trials, for a maximum of 20 trials per session. The initial response requirement was 100 responses and doubled across successive components to a maximum of 1600 responses. Upon completion of the response requirement, red stimulus lights were illuminated for 1-s coincident with a 1-s infusion of drug or vehicle solution. Each trial ended with either a self-administered injection or the expiration of a 30-min limited hold in the event that responding was not maintained. Under baseline conditions, cocaine (0.1–0.3 mg/kg/injection, depending on the monkey) or an equivalent volume of 0.9% saline/injection was available for self-administration on alternate days. Baseline performance was considered stable when: 1) the number of injections/session maintained by cocaine was ≥ 12 for at least three successive sessions of cocaine availability, and the number of injections/session maintained by saline was ≤ 5 for at least three successive sessions of saline availability.

and 2) no upward or downward trends in the number of injections/session were observed across either type of baseline session.

Test sessions were identical to baseline sessions except that different doses of cocaine (0.01–0.3 mg/kg/inj), heroin (0.001–0.01 mg/kg/inj) and etonitazene (0.001–1.0 µg/kg/inj) were available for self-administration. Cocaine and heroin dose ranges were selected on the basis of previous studies (Rowlett et al. 2005) to include low doses that did not maintain consistent self-administration and higher doses that maintain maximum levels of self-administration. Initially, the etonitazene doses tested ranged from 0.1–1.0 µg/kg/inj; the maximum dose was chosen because significant respiratory depression at doses > 1.0 µg/kg has been previously reported in monkeys (Butelman et al. 1993). To ensure that a broader dose range was examined, etonitazene (0.001 and 0.01 µg/kg/inj) was then tested in a subset of monkeys. Etonitazene was therefore tested across a 1000-fold dose range. In tests involving self-administration of drug combinations, selected doses of etonitazene (1.0 µg/kg/inj) or heroin (0.001 mg/kg/inj) that did not maintain significant self-administration when tested alone were combined with a range of cocaine doses (0.01–0.1 mg/kg/inj) by mixing the drugs in the same syringe. All cocaine, heroin or etonitazene doses and drug combinations were tested in irregular order, with duplicate determinations with the exception of the highest etonitazene dose (1.0 µg/kg/inj) combined with the highest cocaine dose (0.1 mg/kg/inj), which induced respiratory depression in some monkeys.

Pretreatment with Antagonists

Naltrexone (0.1 mg/kg or vehicle; i.m.) was administered 10-min before test sessions in which cocaine (0.01–0.1 mg/kg/inj) was available for self-administration alone and in combination with etonitazene (0.1 µg/kg/inj). Naloxonazine (0.3, 1.0 mg/kg or vehicle; i.m.) was administered 1-hr before test sessions in which combinations of cocaine (0.01–0.1 mg/kg/inj) + etonitazene (0.1 µg/kg/inj) or cocaine (0.01–0.03) + heroin (0.001 mg/kg/inj) were available for self-administration. An identical test session was conducted the following day to determine the effects of naloxonazine 24-hr after pretreatment (when its purported μ_1 -antagonist effects are thought to predominate). Doses of naloxonazine greater than 1.0 mg/kg were not studied to preclude potential adverse effects associated with injections of large volumes of acidic solutions (cf. Gatch et al. 1996).

Drug Preparation

Cocaine HCl, naloxonazine di-HCl and naltrexone HCl were obtained from Sigma-Aldrich, St. Louis, MO. Etonitazene HCl and heroin HCl were obtained from the National Institute on Drug Abuse (Bethesda, MD). Cocaine, etonitazene, heroin and naltrexone were dissolved in 0.9% saline solution. Naloxonazine was dissolved in a saline/0.1 N HCl (pH 5–7)/ethanol (10%) solution and filter-sterilized (0.2 µm) before administration.

Data Analysis

The number of injections/session and break point (BP; the highest response requirement reached during a session) were determined each session for individual monkeys. Grouped data for injections/session were analyzed using one- or two-way repeated measures analysis of variance (RM ANOVA). For analysis of individual subject data, the number of injections/session at each dose of cocaine, heroin and etonitazene was compared to the number of saline injections/session averaged over the two saline baseline sessions that preceded and the two saline baseline sessions that followed the test. A dose of a drug was considered to maintain a significant level of self-administration if the mean number of injections/session at that dose exceeded the mean number of saline injections/session by > 2 standard deviations. Because BP data for individual monkeys had non-normal distributions that violated the assumption of homogeneity of variance, the data were subjected to an inverse sine transformation (cf. Rowlett

et al. 1996) and then analyzed using RM ANOVA. Planned comparisons were made using Bonferroni t-tests; the significance level was set at $p < 0.05$.

Results

Cocaine, heroin and etonitazene self-administration

Under baseline conditions in which cocaine and saline were available for IV self-administration on alternate days, cocaine maintained stable levels of self-administration, with number of injections/session ranging from 12 to 20 for individual monkeys and BP values ranging from 800 to 1600 responses/injection. Saline, on the other hand, maintained only low levels of self-administration, with number of injections/session ranging from 0 to 5 across individual monkeys and BP values ranging from 100 to 200 responses/injection. Under test conditions in which different doses of cocaine were substituted for the baseline dose, the mean number of injections/session increased as a function of cocaine dose (fig. 1a, top), as did the mean BP (fig. 1a, bottom). ANOVAs revealed a significant effect of cocaine dose on both the number of injections/session ($F_{4, 28} = 49$; $p < 0.001$) and BP ($F_{4, 28} = 6.2$; $p < 0.001$). Planned comparisons showed a significantly greater number of injections/session and BP ($p < 0.05$; Bonferroni t-tests) maintained by self-administration of cocaine (0.1 and 0.3 mg/kg/inj) compared to saline.

When heroin was made available for self-administration during test sessions, the lowest dose of heroin (0.001 mg/kg/inj) maintained an average of 6 injections/session, whereas the higher doses (0.003 and 0.01 mg/kg/inj) maintained averages of 10 or 11 injections/session (figure 1b). ANOVAs revealed a significant effect of heroin dose on both the number of injections/session ($F_{3, 18} = 20$; $p < 0.001$) and BP ($F_{3, 18} = 6.7$; $p = 0.003$). Planned comparisons showed that 0.003 and 0.01 mg/kg/inj of heroin maintained significantly greater injections/session and BP than did saline ($p < 0.05$).

In contrast to cocaine and heroin, etonitazene (0.1 – 1.0 $\mu\text{g/kg/inj}$) did not maintain levels of self-administration that differed significantly from the level maintained by saline (fig. 1c). ANOVAs revealed no significant effect of etonitazene dose on either the number of injections/session ($F_{3, 21} = 0.6$; $p > 0.05$) or BP ($F_{4, 21} = 0.18$; $p > 0.05$). Further analysis of data for individual subjects showed that compared to saline no dose of etonitazene maintained significant levels of self-administration in five of the eight monkeys studied (Table 1). In the remaining three, etonitazene was effective at only a single dose (0.1 $\mu\text{g/kg/inj}$ in Monkey 427, 0.3 $\mu\text{g/kg/inj}$ in Monkey 162 and 1.0 $\mu\text{g/kg/inj}$ in Monkey 296). By comparison, one or more doses of both cocaine and heroin maintained a significantly greater number of injections/session compared to saline in all subjects. As shown in fig. 1d, low doses of etonitazene (0.001 and 0.01 $\mu\text{g/kg/inj}$) also had no significant effect on either the number of injections/session ($F_{2, 11} = 2.6$; $p > 0.05$) or BP ($F_{2, 11} = 0.9$; $p > 0.05$) in the subset of four monkeys tested at these doses. Analysis of data for individual subjects showed that compared to saline, neither low dose of etonitazene maintained significant self-administration in any of the four monkeys (not shown).

Self-administration of etonitazene + cocaine

To investigate the degree to which drug self-administration might be enhanced as a result of combining etonitazene with cocaine, we redetermined the effects of etonitazene (0.1 and 1.0 $\mu\text{g/kg/inj}$) and cocaine (0.01–0.1 mg/kg/inj) alone (fig. 2, unfilled symbols) and then compared these effects with those of the etonitazene + cocaine dose combination (fig. 2, filled symbols). Combining etonitazene with cocaine resulted in an overall upward shift of the cocaine dose-response function for both injections/session (fig. 2, top) and BP (fig. 2, bottom). Two-way RM ANOVA revealed a significant effect of cocaine ($F_{3, 42} = 37$; 11 ; $p < 0.001$), etonitazene ($F_{3, 42} = 23$; 7.1 ; $p < 0.05$) and cocaine $\times$ etonitazene interaction ($F_{6, 42} = 5.4$; 2.0 ; $p < 0.05$) on the number of injections/session and BP respectively (note the F values for injections/session and

BP within brackets). Planned comparisons showed that combinations of cocaine (0.01 and 0.03 mg/kg/inj) and etonitazene (0.1 and 1.0 µg/kg/inj) maintained a significantly greater number of injections/session than did the corresponding doses of cocaine alone ($p < 0.05$; asterisks, fig. 2, top). Similarly, most combinations of cocaine + etonitazene maintained a significantly greater number of injections/session than did etonitazene alone ($p < 0.05$; crosses). BP values engendered by some combinations of cocaine + etonitazene were also significantly higher than BP values maintained by the respective doses of cocaine and etonitazene alone ($p < 0.05$; asterisks and crosses, fig.2, bottom).

Effects of naltrexone on etonitazene + cocaine self-administration

The effects of pretreatment with naltrexone on etonitazene + cocaine self-administration were evaluated by combining a range of cocaine doses (0.01–0.10 mg/kg/inj) with a low dose of etonitazene (0.1 µg/kg/inj) that significantly enhanced self-administration compared to cocaine and/or etonitazene alone (cf. fig.2). As shown in figure 3, pretreatment with naltrexone (0.1 mg/kg) produced an overall reduction in both the number of injections/session and BP maintained by etonitazene + cocaine self-administration. With the dose of etonitazene held constant at 0.1 µg/kg/inj, RM ANOVA revealed a significant effect of naltrexone ($F_{1, 12} = 23$; 6.9; $p < 0.05$) and cocaine ($F_{1, 12} = 21$; 7.2; $p < 0.05$) and a non-significant naltrexone $\times$ cocaine interaction ($F_{2, 12} = 3.4$; 2.1; $p > 0.05$) on both the number of injections/session and BP. Planned comparisons showed that, compared to vehicle, naltrexone pretreatment resulted in a significantly reduced number of injections/session maintained by etonitazene + cocaine (0.03 or 0.1 mg/kg/inj) as well as significantly reduced BP values maintained by etonitazene + cocaine (0.1 mg/kg/inj) ($p < 0.05$). Pretreatment with naltrexone did not, however, alter the dose-response curve for either injections/session or BP maintained by cocaine self-administration in the absence of etonitazene (fig.3, insets).

Effects of naloxonazine on etonitazene + cocaine self-administration

Compared to vehicle, pretreatment with naloxonazine (0.3 or 1.0 mg/kg) 1-hr prior to testing resulted in an overall attenuation of self-administration of etonitazene + cocaine combinations (fig. 4a). RM ANOVA revealed a significant effect of naloxonazine ($F_{2, 28} = 12$; 17; $p < 0.05$) and cocaine ($F_{2, 28} = 15$; 13; $p < 0.05$) but not of their interaction ($F_{4, 28} = 1.0$; 1.0; $p > 0.05$) on the number of injections/session and BP respectively. Planned comparisons showed that the number of injections/session maintained by most cocaine + etonitazene combinations were significantly reduced after 1-hr administration of 1.0 mg/kg naloxonazine ($p < 0.05$). Twenty-four hours after naloxonazine treatment (fig.4b), RM ANOVA revealed a significant effect of cocaine ($F_{2, 28} = 35$; 11; $p > 0.05$), but not of naloxonazine ($F_{2, 28} = 1.3$; 0.5; $p > 0.05$) nor a naloxonazine $\times$ cocaine interaction ($F_{4, 28} = 1.0$; 0.8; $p > 0.05$) on either the number of injections/session or BP.

Self-administration of heroin + cocaine

To compare the enhanced self-administration of etonitazene combined with cocaine to that of heroin combined with cocaine, low, ineffective doses of cocaine (0.01 and 0.03 mg/kg/inj; cf. fig.1) were made available for self-administration alone and in combination with an ineffective dose of heroin (0.001 mg/kg/inj). Both heroin + cocaine combinations maintained a significantly greater number of injections/session than did the corresponding doses of either cocaine or heroin alone ($p < 0.05$; Bonferroni-corrected t-tests; asterisks and crosses, respectively) and a similar trend was observed with BP values ($p < 0.05$; Student t-tests) (fig. 5a).

To compare the effects of naloxonazine on self-administration of etonitazene + cocaine combinations with its effects on self-administration of heroin + cocaine combinations, naloxonazine (1.0 mg/kg or vehicle) was administered as a pretreatment before test sessions

in which combinations of heroin (0.001 mg/kg/inj) and cocaine (0.01 or 0.03 mg/kg/inj) were available for self-administration (fig. 5b and c). Planned comparisons showed that compared to vehicle, pretreatment with naloxonazine (1.0 mg/kg) 1 hr prior to self-administration of both heroin + cocaine combinations produced significant decreases in the number of injections/session and BP ($p < 0.05$; asterisks in both panels of fig. 5b). In contrast, 24 hrs after pretreatment, naloxonazine had no significant effect on either the number of injections/session or BP maintained by the heroin + cocaine combinations (fig. 5c).

Discussion

The present study examined the relationship between agonist efficacy, self-administration and the enhancement of cocaine self-administration using the high-efficacy μ agonist etonitazene. Reference experiments were conducted with heroin to afford direct comparison with etonitazene in the same group of monkeys. The first major finding is that the high-efficacy μ agonist etonitazene failed to maintain consistent self-administration under the conditions in this study. This outcome demonstrates that high μ agonist efficacy does not necessarily result in significant IV drug self-administration under the PR schedule. Despite this lack of self-administration, combining etonitazene with cocaine enhanced self-administration of the drug combination, compared to that of etonitazene or cocaine alone. This finding implies that the ability of μ agonists to enhance cocaine self-administration does not depend on its ability to maintain self-administration on its own. Thirdly, pretreatment with naltrexone (also see Rowlett et al. 1998) and naloxonazine resulted in similar antagonism profiles for etonitazene-cocaine and heroin-cocaine self-administration, indicating that the enhanced self-administration of the two opioid-cocaine combinations were mediated via similar μ receptor mechanisms. Finally, to the extent that etonitazene and naloxonazine are viable tools for examining μ_1 receptor mechanisms, the finding that antagonism of self-administration of μ opioid-cocaine combinations by naloxonazine was short-lasting does not support a role for μ_1 receptor mechanisms in opioid enhancement of cocaine self-administration.

Self-administration of etonitazene

In contrast to the consistent reinforcing effects of cocaine and heroin in our study, etonitazene did not maintain significant levels of self-administration over a 10-fold (8 monkeys) or 1000-fold (4 monkeys) range of doses. Moreover, analysis of data from individual subjects showed that only two of seven monkeys self-administered significant levels of etonitazene and only at a single dose each. Our finding that etonitazene had weak and inconsistent reinforcing effects by the IV route in rhesus monkeys is seemingly at odds with previous reports that etonitazene can function as a reinforcer by the oral, IV and other routes of administration in rodents (Carroll et al., 1979; Ahlgren-Beckendorf et al. 1998; Braida et al. 1998). It is noteworthy, however, that the ability of etonitazene to maintain self-administration in rats is strain-dependent (Carroll et al. 1986; Suzuki et al. 1992), typically requires food deprivation (Carroll et al. 1979; Meisch and Kliner 1979), and often relies on special training procedures involving schedule-induction of drinking (McMillan and Leander 1976), limiting alternative sources of fluid (Carroll and Meisch 1978; Carroll et al. 1979, 1986) or pairing of etonitazene with other reinforcers (Ahlgren-Beckendorf et al. 1998). In the only previous studies conducted in rhesus monkeys, oral self-administration of etonitazene was induced by a combination of food deprivation, manipulation of food available to induce drinking, and gradual substitution of etonitazene for either water (Carroll and Meisch 1978) or an ethanol solution (Meisch 1995). Although self-administration of etonitazene eventually was established in the majority of subjects in these experiments, the levels of responding maintained by etonitazene were considerably less than the level maintained by a reference ethanol solution in the same subjects (Meisch 1995), suggesting that etonitazene has comparatively weak reinforcing effects in the oral self-administration model as well. Compared to heroin, etonitazene Examination of the time course

of etonitazene's μ agonist effects in ongoing drug discrimination experiments revealed that the discriminative stimulus effects of etonitazene are actually half as short-acting as heroin, lasting up to approximately 80-minutes and exhibiting no delayed onset of action (DM Platt, unpublished findings).

Studies have shown that μ agonists with high intrinsic efficacy exhibit more robust analgesic and respiratory depressant effects compared to μ agonists with low intrinsic efficacy (Walker et al. 1993; Gerak et al. 1994). However etonitazene, which possesses very high intrinsic activity at the μ receptor (Emmerson et al. 1996) does not produce robust self-administration. The contrasting effects of etonitazene compared to heroin in the present study and to alfentanil in a previous study using similar procedures (Rowlett et al. 2002) suggest that etonitazene may be exceptional among high-efficacy μ agonists in terms of its low reinforcing effectiveness; it appears that high intrinsic efficacy at the μ opioid receptor does not insure robust drug self-administration, at least under the PR schedule we used. Based on displacement binding studies using ^3H -DAMGO in monkey cortical membranes, Emmerson et al. (1994) showed that etonitazene exhibits ~10,000-fold higher affinity at the μ opioid receptor while high-efficacy agonists that are self-administered e.g. morphine and fentanyl display μ receptor selectivity in the 300-fold range. It is possible that high μ receptor specificity of etonitazene might influence its reinforcing properties.

Etonitazene can also be distinguished from other commonly abused μ agonists based on its interaction with the Ca^{2+} channel blocker diltiazem. Whereas diltiazem significantly enhanced the potency of heroin and morphine in the rhesus monkey warm water (50°C) tail-withdrawal assay, diltiazem did not affect the analgesic potency of etonitazene (Kishioka et al. 2000). Etonitazene also produces less tolerance and behavioral sensitization compared to other high-efficacy μ agonists in rats. Chronic treatment with morphine resulted in tolerance to its antinociceptive effects and sensitization to its locomotor stimulating effects whereas neither tolerance nor locomotor sensitization was observed after chronic etonitazene administration (Grecksh et al. 2006). Failure of etonitazene to induce behavioral sensitization, a process associated with drug-seeking behavior (Vanderschuren and Kalivas 2000), conceivably could have contributed to its weak reinforcing effects in the present study.

Self-administration of opioid-cocaine combinations

Despite the absence of clear-cut reinforcing effects, combining etonitazene with low doses of cocaine resulted in significant enhancement of self-administration compared to cocaine alone. These results closely resemble those obtained in comparable studies in which either heroin (present study; Rowlett et al. 2005) or alfentanil (Rowlett et al. 2005) was combined with cocaine. In each case, enhanced self-administration of the opioid-cocaine combinations was observed at doses that did not themselves maintain significant self-administration. Like etonitazene, the μ partial agonist nalbuphine exhibited little or no reinforcing effects when tested alone (Rowlett et al. 2002), and under the same conditions enhanced drug self-administration when combined with cocaine (Rowlett et al. 2005). Given that non-reinforcing doses of μ agonists can enhance cocaine self-administration, it appears that self-administration of opioid-cocaine combinations may not depend on the reinforcing effects of μ agonists, at least under PR schedules. Furthermore, combining cocaine with both low-efficacy (nalbuphine) and high-efficacy (heroin, alfentanil and etonitazene) agonists enhanced cocaine self-administration to similar degrees, suggesting that μ agonist efficacy might not be the primary factor determining an opioid's ability to enhance cocaine self-administration.

Antagonism of opioid-cocaine combinations

The finding that naltrexone blocked self-administration of etonitazene-cocaine combinations while having no effect on self-administration of cocaine alone is consistent with the selective

antagonism of heroin-cocaine, but not of cocaine self-administration shown in a previous study (Rowlett et al. 1998). This outcome clearly supports a role for μ opioid receptors in the enhanced reinforcing effects of opioid-cocaine combinations. Antagonism of etonitazene-cocaine and heroin-cocaine self-administration by naloxonazine strengthens the hypothesis that μ receptor mechanisms are important in mediating the “speedball” effect. Additionally, our similar findings with etonitazene and heroin suggest that the two drugs enhance self-administration of cocaine via similar mechanisms. The purported high agonist selectivity of etonitazene at the μ_1 receptor subtype could imply that the enhanced self-administration, evident when etonitazene and cocaine were combined, involves μ_1 receptor subtype mechanisms. However, the finding that naloxonazine failed to attenuate self-administration of either the etonitazene-cocaine or heroin-cocaine combinations at the 24-hr time point does not support such a simple conclusion. In rats, naloxonazine irreversibly (i.e. after 24 hrs) blocks the antinociceptive but not the respiratory depressant effects of morphine implicating the involvement of different μ receptors, namely μ_1 subtype mechanisms in the antinociceptive but not respiratory depressant effects of morphine (Ling et al. 1985). Thus, our finding that antagonism of self-administration of μ opioid-cocaine combinations by naloxonazine was short-lasting does not support a role for μ_1 receptor mechanisms in opioid enhancement of cocaine self-administration.

It is important to note that any conclusions implicating μ_1 mechanisms are based on limited evidence for naloxonazine and etonitazene as pharmacological agents with μ_1 subtype selectivity. Regarding etonitazene, there is only in vitro evidence for its μ_1 selectivity and this has not been defined in monkeys. Moreover, etonitazene produces robust respiratory depressant effects in monkeys (present study; Butelman et al. 1993) despite its reputed 1000-fold selectivity for μ_1 receptors. Failure of naloxonazine to antagonize the reinforcing effects of opioid-cocaine combinations after 24-hrs is reminiscent of the study by Gatch et al. (1996) in which initial attenuation of levorphanol-induced antinociceptive and respiratory depressant effects in monkeys by naloxonazine was not maintained 24 hours later. Since naloxonazine's μ_1 selectivity in monkeys is not well-defined, it is possible that naloxonazine was not studied here under conditions in which it might function as a μ_1 -selective antagonist in monkeys. Nevertheless, the only two reports using naloxonazine to examine μ_1 subtype mechanisms in monkeys (Gatch et al. 1996; present study) both found that naloxonazine functions as a short-acting antagonist with no apparent μ_1 selectivity in monkeys.

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Figure 1.

Cocaine, heroin and etonitazene self-administration in rhesus monkeys under a PR schedule of IV drug injections. Data are means $\pm$ SEM for number of injections/session (top) and break points (bottom). Panel a: cocaine (n=8); Panel b: heroin (n=7); Panel c: etonitazene (n=8); Panel d: etonitazene low doses (n=4). *p<0.05 comparing mean number of injections/session or mean BP (Bonferroni t-test) following individual drug doses versus vehicle.

Figure 2.

Self-administration of cocaine, etonitazene and cocaine + etonitazene combinations under a PR schedule of IV drug injections (n=8). Points above “Etz” show the effects of etonitazene alone. *p<0.05 comparing effects of cocaine + etonitazene versus cocaine alone. +p<0.05 comparing effects of cocaine + etonitazene versus etonitazene alone.

Figure 3.

Effects of pretreatment with naltrexone (0.1 mg/kg) on i.v. self-administration of cocaine combined with etonitazene or cocaine alone (inset). * $p < 0.05$ comparing effects of naltrexone versus vehicle pretreatment.

Figure 4.

Effects of pretreatment with naloxonazine (0.3 and 1.0 mg/kg) on self-administration of cocaine + etonitazene. Panel a: after 1 hr; Panel b: after 24 hrs. * $p < 0.05$ comparing effects of naloxonazine versus vehicle pretreatment.

Figure 5.

Panel a: Self-administration of cocaine, heroin and cocaine + heroin combinations. Bars above “Veh” and “Her” show effects of saline and heroin alone, respectively. * $p < 0.05$ comparing mean number of injections/session and mean BP following combinations of cocaine + heroin versus cocaine alone. + $p < 0.05$ comparing mean number of injections/session and mean BP following combinations of cocaine + heroin versus heroin alone. Panels b and c: Effects of pretreatment with naloxonazine on self-administration of cocaine + heroin after 1 hr (Panel b) or 24 hrs (Panel c). * $p < 0.05$ comparing effects of naloxonazine versus vehicle pretreatment.

Table 1

Doses of cocaine, heroin and etonitazene that maintained significant self-administration in individual subjects (see *Methods*). Doses expressed as mg/kg/injection.

Subject #	Cocaine	Heroin	Etonitazene
162	0.1, 0.3	0.003, 0.01	0.0003
216	0.1, 0.3	0.003, 0.01	-
296	0.1, 0.3	0.01	0.001
301	0.1, 0.3	0.003, 0.01	-
302	0.1, 0.3	0.003, 0.01	-
303	0.1, 0.3	0.01	-
307	0.1, 0.3	n.t.	-
427	0.1, 0.3	0.003, 0.01	0.0001

n.t., not tested