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ORIGINAL INVESTIGATION

Self-administration of cocaine–remifentanil mixtures by monkeys: an isobolographic analysis

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Abstract
Rationale Abuse of mixtures of stimulants and opioids ("speedball") is common. Although this combination has been studied in the laboratory, conclusions about the nature of the cocaine/opioid interaction have been mixed.
Objectives The objectives of the present experiment were to allow monkeys to self-administer mixtures of cocaine and the μ opioid agonist remifentanil and to quantify the interaction using the isobolographic approach. Our hypothesis was that the drugs would be super-additive in their reinforcing effects.
Materials and methods Rhesus monkeys (n = 5) prepared with i.v. catheters were allowed to self-administer cocaine or saline under a progressive-ratio schedule. When responding was stable, doses of cocaine or remifentanil were made available in test sessions. Next, mixtures of doses of the drugs were tested over a range of doses in 1:1, 1:2, and 2:1 ratios of their ED_{50}s. Results were analyzed using isobolographic techniques.
Results Both drugs alone and all drug mixtures functioned as positive reinforcers in a dose-related manner. Cocaine maintained more responding at maximum than did remifentanil, i.e., was a stronger reinforcer. The experimentally determined equi-effective dose for the 1:1 and 1:2 cocaine/remifentanil mixtures tended toward super-additivity, but the difference from additivity did not achieve statistical significance. The 2:1 mixture was super-additive. Maximum responding maintained by the mixtures was higher than that maintained by remifentanil but not different from cocaine.
Conclusions Combinations of cocaine and remifentanil can be additive or super-additive as positive reinforcers, depending on proportions of each. Interactions between stimulants and opioids may contribute to the abuse of these mixtures.

Keywords Drug abuse · Self-administration · Monkey · Cocaine · Opioid · Drug mixture · Isobologram · Additivity

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Abuse of drug mixtures is common. One highly preferred combination is the mixture of a stimulant and an opioid known as a "speedball" (Leri et al. 2003). Abuse of this mixture may be related to enhanced reinforcing effects of the combination relative to either drug alone. Although there has been some laboratory research with the stimulant–opioid mixture, conclusions about the nature of the drug interaction, if any, have been mixed. Some studies have found little evidence of an interaction between stimulants and opioids (Hemby et al. 1996; Mattox et al. 1997; Mello et al. 1995). Other studies have found an increase in potency of the combination relative to either drug alone (Duvauchelle et al. 1998; Rowlett and Woolverton 1997; Rowlett et al. 1998; Winger et al. 2006). It has also been reported that the reinforcing strength of a stimulant–opioid combination was enhanced relative to either drug alone

(Ranaldi and Munn 1998; Wang et al. 2001). Since these experiments used different behavioral conditions, it may be that the interaction between drugs as reinforcers depends upon the conditions of availability (Ward et al. 2005). Although such a conclusion would fit conceptually with our understanding of drug self-administration, firm empirical conclusions depend upon quantitation of drug interactions.

Often, interactions between drugs are quantified in reference to dose additivity using isobolographic analysis (see Loewe 1953; Tallarida 2000). Dose additivity serves as a reference point because a drug is dose-additive with itself, i.e., it is the outcome that defines no interaction. Interactions between drugs are apparent when a combination is greater than or less than dose additive, termed super-additive or sub-additive. Although this approach has been used in behavioral pharmacology (see reviews by Wessinger 1986; Woolverton 1987), it has not been widely applied to the reinforcing effects of drugs. Negus (2005) used the dose-addition model to analyze the effects of combining cocaine and heroin in rhesus monkeys allowed to choose between i.v. drug injections and food. In that study, cocaine and heroin were additive in some combinations and sub-additive in others. Super-additivity was not observed. Rowlett et al. (2007) reported additivity between cocaine and heroin in monkeys responding under a progressive-ratio schedule maintained by drug injections. Smith et al. (2006) also reported additivity between cocaine and heroin in rats.

The objective of the present experiment, then, was to study the self-administration of mixtures of a psychomotor stimulant, cocaine, and an opioid, remifentanil. Rhesus monkeys were prepared with chronic i.v. catheters and allowed to self-administer drugs under a progressive-ratio (PR) schedule of reinforcement with an inter-trial interval (ITI) between injections. A PR schedule was used because it allows measurement of both potency and strength (maximum reinforcing effect) as a reinforcer (e.g., Griffiths et al. 1975, 1978; Hoffmeister 1979; Rowlett et al. 1996). Reinforcing effects were measured for each drug alone and for combinations of fixed ratios of doses. With the "fixed-ratio" design, drug doses are combined in a particular ratio, commonly in ratios of the ED_{50}s. For a number of theoretical and statistical reasons, detailed by Tallarida (2000), the fixed-ratio approach is preferred to the fixed-dose approach of combining several doses of one drug with a fixed dose of the other. We have recently used this approach to study mixtures of drugs with comparable mechanisms of action and found these combinations to be additive (Woolverton et al. 2008). Based upon the clinical reports that the cocaine–opioid combination is a preferred mixture (e.g., Leri et al. 2003), the hypothesis of the present study was that the mixture of cocaine and the μ opioid agonist remifentanil would be super-additive in terms of reinforcing effects.

Materials and methods

All animal-use procedures were approved by the University of Mississippi Medical Center's Animal Care and Use Committee and were in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals (1996).

Animals and apparatus

The subjects were five male rhesus monkeys (*Macaca mulatta*) weighing between 9.2 and 10kg at the beginning of the study. Monkey 96R0679 had a history of self-administration of the phenyltropane RTI-31 under a fixed-ratio 1 (FR1) schedule of reinforcement (Wee et al. 2006). Monkeys RiK2, R0697, M1338, and R0463 had self-administered a variety of compounds under PR schedules similar to the one used here (Woolverton et al. 2008). All monkeys were provided with sufficient food to maintain stable body weights (140–200g/day, Teklad 25% Monkey Diet, Harlan/Teklad, Madison, WI, USA) and had unlimited access to water. Fresh fruit was provided daily, and a vitamin supplement was given three times a week. Lighting was cycled to maintain 16h of light and 8h of dark, with light on at 0600 hours.

Each monkey was fitted with a stainless-steel harness (E&H Engineering, Chicago, IL, USA) or a jacket (Lomir Biomedical, Malone, NY, USA) that was attached by a tether to the rear wall of the experimental cubicle ($1.0m^3$, Plaslabs, Lansing, MI, USA). The front door of the cubicle was made of transparent plastic, and the remaining walls were opaque. Two response levers (PRL-001, BRS/LVE, Beltsville, MD, USA) were mounted on the inside of the door. Four jeweled stimulus lights, two red and two white, were mounted above each lever. Drug injections were delivered by a peristaltic infusion pump (Cole-Parmer, Chicago, IL, USA). A Macintosh computer with custom interface and software controlled all events in an experimental session and recorded data.

Procedure

Monkeys were implanted with a silastic catheter (0.26cm o.d. × 0.076cm i.d.; Cole-Parmer) into the jugular (internal or external) or femoral vein under isoflurane anesthesia. Brachial veins were implanted with a microrenethane catheter (0.2cm o.d. × 0.1cm i.d.; Braintree Scientific, Braintree, MA, USA) heated and drawn to approximately half size at the proximal end. The proximal end of the catheter was inserted into the vein and terminated in the vena cava near the right atrium. The distal end was threaded subcutaneously to exit the back of the monkey, threaded through the tether, out the rear of the cubicle and

connected to the peristaltic pump. In the event of catheter failure, surgery was repeated using another vein, after the veterinarian confirmed the health of the monkey.

Experimental sessions began at 11:00 each day and were conducted 7days per week. Thirty minutes before each session started, catheters were filled with drugs for the sessions without infusing the drugs into monkeys. At the start of a session, the white lights were illuminated above both levers, and pressing the right lever resulted in the delivery of a drug injection for 10s. During the injection, the white lights were extinguished, and the red lights were illuminated. Responding was maintained under a progressive-ratio schedule of reinforcement comparable to that described by Wilcox et al. (2000). A session consisted of 20 trials, with one injection available per trial. The response requirement started at 50 responses per injection and doubled after every fourth trial. There was an inter-trial interval after each injection of 5min during which lights were extinguished and levers were inactive. A subject had 30min to complete a trial (limited hold 30min: LH 30′). A trial ended with a 10-s drug injection or the expiration of the LH. If the response requirement was not completed for two consecutive trials (i.e., the LH expired) or the animal self-administered all 20 injections, the session ended. After the session, catheters were filled with 0.9% saline containing heparin (40U/ml).

In baseline sessions, injections of cocaine or saline were available. The baseline dose of cocaine was the lowest dose that maintained the maximum injections in individual monkeys, between 0.1 and 0.4mg/kg per injection. For initial training, the baseline dose of cocaine or saline was available under a double-alternation schedule, i.e., two consecutive daily cocaine sessions were followed by two consecutive daily saline sessions. This sequence was sometimes modified to allow extra sessions for responding to be maintained by cocaine or to extinguish with saline injection, according to the behavior of the individual monkey. Responding was considered stable in baseline sessions when injections per session varied by no more than two for both cocaine and saline for at least two consecutive double-alternation sequences. At this point, test sessions were inserted to the daily sequence between two saline or two cocaine sessions. To prevent monkeys from learning this session sequence, a randomly determined saline or cocaine baseline session was inserted after every other test session. Thus, the final daily sequence of sessions was C, S, T, S, C, T, R, C, S, T, S, C, T, R, where “C”, “S”, “R” and “T”, respectively, represent a cocaine baseline, a saline, a randomly determined cocaine/saline, and a test session.

Seven doses of cocaine (6–400μg/kg per injection) and five doses of remifentanil (0.025–0.8μg/kg per injection), in 1/3 or 1/6 log unit intervals, were made available to each monkey in test sessions that were otherwise identical to baseline sessions. Remifentanil was tested first in all of the monkeys, and doses of both drugs were tested in an irregular order. After a test session, a monkey was returned to baseline conditions until responding again met stability criteria or a new stable baseline was established. All doses were tested at least twice in each monkey, once with a saline session the day before and once with a cocaine session the day before. When the two test sessions of a dose showed high variability (each of the two determinations ≥mean ± three injections), the dose was re-tested twice, once after a saline and once after a cocaine baseline session.

After testing individual drugs, ED_{50}s were calculated for individual monkeys using non-linear regression (GraphPad Prism 4.0). Monkeys were then tested with mixtures of cocaine and remifentanil with doses combined in fixed ratios of their individual ED_{50}s. In theory, any fixed ratio combination of constituents can be used. Since the nature of the interaction (super- or sub-additivity) between two drugs can change with the dose ratio, three ratios were tested. Because it is desirable for statistical purposes (variance is smaller) to test proportions that yield points in the central region of the isobologram (see Fig. 3), combinations were tested in 1:1, 2:1, and 1:2 ratios of their ED_{50}s. The dose of a mixture is the sum of the doses of the two component drugs in the mixture. For example, if the ED_{50} of cocaine were 100μg/kg per injection and the ED_{50} of remifentanil were 10μg/kg per injection, one possible dose in a 1:1 ratio would be the mixture of the ED_{50}s for a total dose of 110μg/kg per injection. Other doses in the 1:1 dose–response function were selected using the same log interval as doses for the individual drugs, e.g., 55μg/kg per injection, 27.5μg/kg per injection, and so on. This process was repeated for the 1:2 and 2:1 ratios. Order of testing of the ratios was counterbalanced across monkeys.

Data analysis

The mean number of injections per session was calculated individually from the two test sessions at a dose, and mean values were calculated for the group. The mean dose–effect data for each drug alone and for mixtures were fitted by nonlinear regression as described by Tallarida (2000). The effects of drug mixtures were compared to the prediction of additivity using isobolographic analysis. The isobole of additivity is the curve of dose pairs predicted to give a constant effect (injections per session), in this case 50% of the maximum for cocaine. It should be noted that any effect level in the range of effects of cocaine could have been used. We chose the half maximal effect of the most effective drug, a typical choice. Predicted additive combinations are derived using a computational procedure based on the concept of dose equivalence (see Fig. 1; Grabovsky and Tallarida 2004; Tallarida 2000).

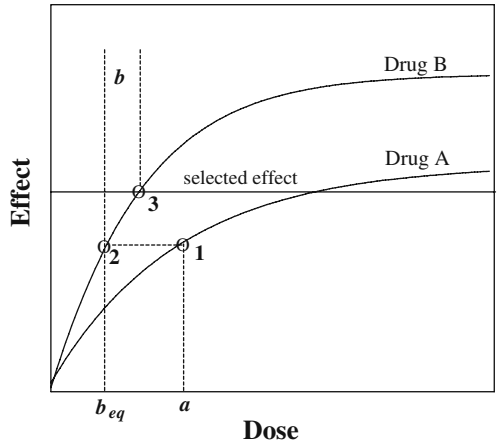

Fig. 1 Constructing the isobole for drug A and drug B. The graph illustrates how dose pairs (a, b) define the additive isobole for the selected effect. To achieve the selected effect level (e.g., ten injections per session), consider an arbitrary dose a of drug A (*point 1*). This dose has a drug B-equivalent, b_{eq} (*point 2*). Either dose alone would produce an effect that is less than the selected effect. To achieve the selected effect, one needs to add to dose a of drug A a dose b of drug B indicated by the dose interval labeled b, the difference between the doses at *points 3* and *2*. The set of dose pairs (a, b) defined in this way are the doses that would be predicted to have the selected effect if the drugs were dose-additive. The population of these pairs defines the isobole. If the potency ratio is constant, the isobole is a straight line. If not, the isobole is curved. In either case, it is a curve (or line) of dose pairs predicted to have the selected effect if the drugs were additive. Its equation is given by Grabovsky and Tallarida (2004)

Specifically, the dose–effect data for each agent were fit to equations of the form given below:

Drug A (remifentanil) with dose a and effect E:

$$E = \frac{E_A a^q}{a^q + C_A^q},$$

where the parameters, E_A, C_A, and q (Hill coefficient) are determined by standard nonlinear regression.

Drug B (cocaine) with dose b and effect E: $E = \frac{E_B b^p}{b^p + C_B^p}$

with parameters E_B, C_B and p, also determined by nonlinear regression. Using these equations, the equi-effective doses were determined by equating their right-hand sides, yielding b in terms of a and, thus, the b-equivalent of dose a:

$$b_{eq} = \frac{C_B}{\left[\frac{E_B}{E_A}\left(1 + \frac{C_A^q}{a^q}\right) - 1\right]^{1/p}}$$

This equivalency allowed a determination of the dose pairs (a, b) that would be expected to give the same effect when both drugs are given if the drugs were additive. This follows from the selection of the effect level of interest, E_i, and the dose of drug B alone, denoted B_i, that gives this effect (often the half maximum effect so that B_i = its ED_{50}). Thus, the dose combination (a, b) was converted into a quantity of drug B, i.e., $a + b = b_{eq} + b$, using the above, and this sum was equated to B_i. Using the equation $\frac{C_B}{\left[\frac{E_B}{E_A}\left(1+\frac{C_A^q}{a^q}\right)-1\right]^{1/p}} + b = B_i$ the (a, b) pairs define the isobole of additivity (Grabovsky and Tallarida 2004; Tallarida 2006, 2007). It is called additive because of the addition of b and b_{eq} (dose addition), and it provides the basis used by Loewe (1953) for distinguishing super- or sub-additivity from additivity. It is important to note that an isobole equation is generally non-linear. It is linear only when there is a constant potency ratio between the two drugs (i.e., in the special case in which $E_B = E_A$ and $p = q$, which yields the constant potency ratio = C_A/C_B. In the present experiment, the parameters of the curve fit revealed a curvilinear isobole given by the above equation largely due to the different maximum effects of the drugs.

Briefly restated, this procedure uses the ED_{50} of cocaine to establish a selected effect and determines the dose combinations of drug A remifentanil and drug B cocaine that would be predicted to have the same effect if the drugs were additive. Dose of remifentanil (a) was converted into its b-equivalent (b_{eq}) and then added to the dose b of cocaine such that $b_{eq} + b = ED_{50}$ (Tallarida 2006, 2007). The additive isobole for the selected effect provides a graphical view to aid in selection of proportions of constituent drugs to be tested and for assessing departures from additivity. Actual experimental combinations (a, b) that had the selected effect were determined from each animal's dose–effect data by linear regression (GraphPad Prism 4.0). Those combinations whose coordinates plot below the isobole are super-additive, whereas points above the isobole are sub-additive. To distinguish non-additive and additive interactions, the mean total experimental dose ($a + b$) was statistically compared to the mean total additive dose using the t test for unequal variances (Tallarida 2000) and using the criterion $p < 0.05$.

Additionally, the maximum number of injections, regardless of dose, was used as a measure of reinforcing strength in an individual subject, and mean group maximums were calculated for each drug and mixture. Statistical significance of differences was analyzed using paired t test for drugs alone or one-way analysis of variance for repeated measures for the five subjects tested in all conditions for each drug pairing.

Drugs

Cocaine hydrochloride was provided by the National Institute on Drug Abuse (Rockville, MD, USA), and remifentanil hydrochloride was purchased commercially. Final solutions were prepared using 0.9% saline. Doses were expressed as the salt forms of the drugs.

Results

Cocaine and remifentanil maintained responding in all monkeys (Fig. 2), with asymptotic dose–response functions that reached different maxima. Parameters for best fit curve for cocaine were $E_B = 19.3$, $C_B = 39.3$, and $p = 1.00$ and for remifentanil were $E_A = 13.2$, $C_A = 0.16$, and $q = 2.14$. The doses producing an effect equal to ½ the maximum of cocaine (9.67 injections per session) were 39.3 ± 6.1μg/kg per injection for cocaine and 0.26 ± 0.07μg/kg per injection for remifentanil.

The equation for the isobole was $b = 39.3 - 39.3a^{2.14}/(0.46a^{2.14} + 0.029)$. All ($a$, b) values along this isobole would be predicted to have the same effect, i.e., 9.67 injections per session, if the drugs were additive. The curvature of the additive isobole (Fig. 3) is due to the varying potency ratio. The combinations that were tested experimentally were based on the relative potencies and contained mean cocaine/remifentanil proportions, i.e., the ratio of totalμg/kg in an injection, of 0.997:0.003 for 2:1 (I, Fig. 3); 0.994:0.006 for 1:1 (II, Fig. 3); and 0.989:0.011 for 1:2 (III, Fig. 3). Mixtures also maintained a dose-related increase in responding in all monkeys. All experimental equi-effective doses fell below the curve of additivity, indicating super-additivity. However, only group I containing the highest proportion of cocaine was found to be significantly different from additivity ($p < 0.05$; Table 1). While experimental groups II and III had mean values numerically less than the additive values, the variances were sufficiently large to preclude statistical significance.

The mean maximum responding maintained by cocaine was 17.5 (±0.5, SEM) injections per session and occurred at doses of 200 or 400μg/kg per injection in different monkeys. The mean maximum maintained by remifentanil was 12.7 (±0.85, SEM) injections per session at doses of 0.4 or 0.8μg/kg per injection. Note that these values are

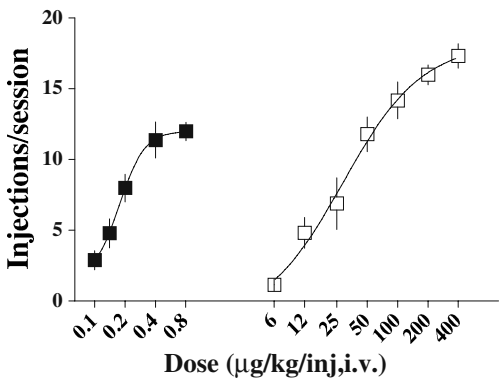

Fig. 2 Self-administration of cocaine and remifentanil by monkeys responding under a PR schedule of reinforcement. Each data point represents the mean injections per session of each dose for two to five monkeys. *Vertical lines* represent SEM

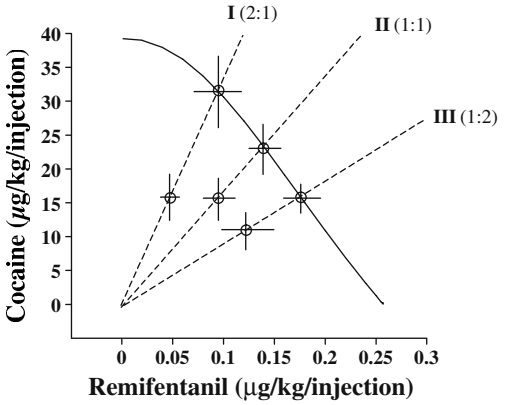

Fig. 3 Isobologram representing the self-administration of mixtures of remifentanil (*abscissa*) and cocaine (*ordinate*). The *solid line* connects the ED_{50} of cocaine alone to the remifentanil dose calculated to have the same effect as the ED_{50} of cocaine. This *curve* represents combinations of doses that would be predicted to have this same effect if the drugs were additive, i.e., the additive isobole. *Dashed radial lines* (*I*, *II*, *III*) represent the three different cocaine/remifentanil dose ratios that were tested. *Symbols on the radial lines* represent the additive (on the additive line) and the experimentally determined equi-effective doses of those mixtures. The *horizontal and vertical lines* through these points represent the SEM8

different from maxima calculated for the mean dose–response functions (Fig. 2) because they are means of individual subject maxima, regardless of dose, rather than a calculated mean for each dose. Mean maximum responding for all of the mixtures was 16.6 ± 0.94, 17.4 ± 0.56, and 17 ± 1.04 for, respectively, the 1:1, 1:2, and 2:1 cocaine/remifentanil mixtures. The values were higher than the maximum for remifentanil ($p < 0.05$ in all cases) but not different from the maximum maintained by cocaine.

Discussion

Both cocaine and remifentanil functioned as positive reinforcers under the present PR schedule in monkeys. This result is consistent with a previous report that both of these drugs served as positive reinforcers under these or similar conditions (Ko et al. 2002; Woolverton et al. 2008). Absolute and relative potencies of these compounds were comparable to what was reported in those studies. Under the present PR schedule, cocaine was clearly a stronger reinforcer than remifentanil, maintaining higher maximum responding under a PR schedule. Previous studies comparing heroin and cocaine under a PR schedule have generally found cocaine to be the stronger reinforcer in both monkeys (Rowlett and Woolverton 1997; Rowlett et al. 1998) and in rats (Duvauchelle et al. 1998). On the other hand, Rowlett et al. (2005) studied monkeys under conditions similar to those used in the present experiment and did not find substantial differences in maximum responding maintained by cocaine and either heroin or the mu agonist alfentanil.

Table 1 Comparison of predicted additive and experimental total dose combinations yielding the selected effect

Group proportions cocaine/remifentanil	Additive total dose	Experimental total dose	Statistical analysis
(I, 2:1) 0.997:0.003	31.7±5.08	15.8±2.37	$p<0.05$
(II, 1:1) 0.994:0.006	23.2±2.94	15.8±4.61	n.s.
(III, 1:2) 0.989:0.011	16.0±2.24	11.1±2.53	n.s.

The additive total dose is the total dose of the two drugs in the indicated proportions predicted by additivity to yield the selected effect, i.e., $^1/_2\ E_B$, the ED_{50} of cocaine. The experimental total dose is the experimentally determined total dose of the two drugs in the indicated proportions that actually yielded the selected effect. Values are mean total μg/kg per injection ±SEM. The right column indicates the statistical comparison between the two.

Winger et al. (2006) recently used a behavioral economic approach to compare demand curves of cocaine and remifentanil. In that study, demand curves for cocaine were less elastic than those for remifentanil in two of three monkeys, indicating cocaine was the stronger reinforcer in those animals. However, in group comparisons, there was no difference in demand curves for cocaine and remifentanil. For heroin, it has been proposed that pharmacokinetic factors may have contributed to lower responding relative to cocaine. Heroin has a longer half-life than cocaine, may accumulate over the course of a session, and suppress responding. Remifentanil, however, is a shorter-acting compound than heroin, making pharmacokinetic differences unlikely to account for the present results.

When cocaine was combined with remifentanil, the mixture containing the highest proportion of cocaine relative to remifentanil was super-additive, while the two mixtures with lower proportions of cocaine did not differ significantly from additivity. Taken at face value, this result suggests that the interaction between cocaine and remifentanil in self-administration can be super-additive and depends upon the proportions of the drugs in the mixture. It is well known that the nature of a drug interaction (super-additive, sub-additive) is not simply an effect of combining drugs but also depends on the ratio of constituents (see Tallarida 2000; Wessinger 1986; Woolverton 1987), and the present results would extend that conclusion to reinforcing effects. Although not statistically significant, there was a trend toward super-additivity for both of the other mixtures, suggesting that super-additivity, though less pronounced, may exist for these combinations as well. Three other studies have used isobolographic analysis to study the interaction between self-administered cocaine and heroin. Negus (2005) studied monkeys given a choice between a drug injection and food and used a fixed-ratio drug combination approach comparable to the one used here to study three mixtures. Consistent with the present results, the nature of the cocaine–heroin interaction also varied with the drug proportions, and the combination was most effective when the proportion of cocaine was the highest. In contrast to the present results, however, the combination was additive at that mixture and sub-additive for the other mixtures. Rowlett et al. (2007), using a PR schedule comparable to the one used here and a fixed-dose approach, also reported that cocaine and heroin were additive. Smith et al. (2006) studied a single cocaine/heroin ratio in rats and found the mixture to be additive. Thus, the present study has been the only one to this point to report super-additive cocaine–opioid combinations in self-administration. In a recently published study using an approach identical to the one used in the present study, we found mixtures of drugs with comparable mechanism of action to be additive (Woolverton et al. 2008). Considered in the context of that study, the super-additivity found in the present experiment is even more distinctive. We have also reported that that the mixture of cocaine with the antihistamine diphenhydramine can be super-additive in combinations of 1:1, 1:2, and 2:1 ED_{50} mixture ratios (Wang and Woolverton 2007). On balance, it seems reasonable to suggest based on currently available data that the interaction between cocaine and opioids can vary with the dose proportions but that other conditions that may influence the nature of the interaction are, as yet, unclear.

Another important consideration is the maximum responding that was maintained by the various drugs and mixtures. Under a PR schedule, maximum responding is generally considered a measure of relative strength, or efficacy, of a reinforcer. In the present study, cocaine alone and all mixtures maintained more responding than remifentanil alone, consistent with the conclusion that cocaine and cocaine–opioid mixtures are stronger reinforcers than opioids alone. On the other hand, maximum responding maintained by cocaine alone was not different from that maintained by the mixtures, suggesting equivalent reinforcing strength. This conclusion is consistent with the findings of Winger et al. (2006) using a behavioral economic approach. In studies with the combination of heroin and cocaine, increases in maximum responding under a PR schedule relative to cocaine and heroin alone have been reported in some cases (Duvauchelle et al. 1998; Ranaldi and Munn 1998) but not in others (Rowlett et al. 1998; Rowlett and Woolverton 1997; Ward et al. 2005). The mechanism(s) that determine these differences across studies are not clear. Species differences may play a role as increases

in maximum responding have been reported in rats but not in monkeys. The present study and those of Rowlett and Woolverton (1997) and Rowlett et al. (1998) used a relatively long ITI between injections. The ITI allows time for the non-specific rate-altering effects of the drugs to dissipate, so that responding is more clearly determined by reinforcing effects (e.g., Griffiths et al. 1978; Winger 1993; Rowlett et al. 1996). It may be that limiting the influence of non-specific rate-altering effects affected maximum responding. It is also important to note that in the present study cocaine and the mixtures maintained responding at or near the procedural maximum of 20 injections. That is, any differences that may exist in maximum responding may be obscured by a ceiling effect (see also Rowlett and Woolverton 1997; Rowlett et al. 1998). It will be of interest to empirically test that hypothesis.

Taken together, the studies that have been conducted to date would support the conclusion that cocaine and opioids can be super-additive as reinforcers under certain conditions, at least in terms of the potency changes measured using the isobolographic technique. The mixture appears to have greater relative reinforcing strength that opioids alone, though the comparison to cocaine is less clear in this regard. Experimental conditions that may influence the nature of the interaction are not completely clear. The relative proportions of the drugs that are combined may be important: quantitatively more substantial interactions have been reported with combinations with a relatively higher proportion of cocaine. Species and conditions of drug availability may also be important factors. Nevertheless, super-additive interactions in terms of reinforcing effects predict that combining the drugs would be more reinforcing than simply increasing the dose of one or the other constituent, an effect that would be expected to contribute to the abuse of cocaine–opioid combinations.

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