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Effects of kappa opioid agonists alone and in combination with cocaine on heart rate and blood pressure in conscious squirrel monkeys

Charles W. Schindler, Zofi Graczyk, Joanne P. Gilman, S. Stevens Negus, Jack Bergman, Nancy K. Mello, and Steven R. Goldberg

Preclinical Pharmacology Section, Behavioral Neuroscience Branch, DHHS/NIH/NIDA Intramural Research Program, Baltimore MD (CWS, ZG, JPG, SRG) McLean Hospital, Harvard Medical School, Belmont MA (SSN, JB, NKM)

Abstract

As kappa agonists have been proposed as treatments for cocaine abuse, the cardiovascular effects of the kappa opioid receptor agonists ethylketocyclazocine (EKC) and enadoline were investigated in conscious squirrel monkeys. Both EKC and enadoline increased heart rate with little effect on blood pressure. This effect appeared to be specific for kappa receptors as the mu opioid agonist morphine did not mimic the effects of the kappa agonists. The opioid antagonist naltrexone, at a dose of 1.0 mg/kg, blocked the effect of EKC. An action at both central and peripheral receptors may be responsible for the heart rate increase following kappa agonist treatment. The ganglionic blocker chlorisondamine partially antagonized the effect of EKC on heart rate, suggesting central involvement, while the peripherally-acting agonist ICI 204,448 ((±)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride) also increased heart rate, supporting a peripheral site of action. When given in combination with cocaine, EKC produced effects that were sub-additive, suggesting that the kappa agonists may be used safely as cocaine abuse treatments.

Keywords

Kappa Opioid; Blood Pressure; Heart Rate; Cocaine; Squirrel Monkey

1. Introduction

Kappa receptors are one of the original three classes of opioid receptors described by Martin and his colleagues (e.g., Martin et al., 1984). Kappa receptors were named for the first proposed agonist-ketocyclazocine. Dynorphin, an endogenous ligand for kappa receptors, has been isolated and two subtypes of kappa receptors have been described (Wollerman et al., 1993). The subtypes are best distinguished by the chemical classes of the drugs that bind to them. Kappa-1 receptor subtypes bind primarily arylacetamides, while kappa-2 subtypes bind the benzomorphan kappa agonists. It has also been proposed that these subtypes do not represent two distinct receptors, but simply the same receptor in different affinity states (Rusovici et al., 2004).

Corresponding Author: Charles Schindler Preclinical Pharmacology Section NIH/NIDA Intramural Research Program 5500 Nathan Shock Dr. Baltimore, MD 21224 (410) 550-1454 (410) 550-1648 (fax) cschindl@helix.nih.gov.

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It was originally hoped that kappa receptor agonists would function as analgesics without the abuse potential observed with mu opioid agonists such as morphine. However, it soon became clear that the “side effects” of these drugs would limit their use (Husbands, 2004). These side effects included diuresis and sedation, but the most troubling side effect was the incidence of reported psychomimetic effects. To avoid the psychomimetic effects, recent research on the use of these drugs for analgesia has focused on peripherally acting compounds (Riviere, 2004).

The use of kappa agonists for the treatment of drug abuse, and in particular cocaine abuse, has been promoted by a number of authors (e.g., Archer et al., 1996; Mello and Negus, 2000). The rationale for this treatment comes from studies showing that kappa agonists act presynaptically on dopamine terminals in the nucleus accumbens to inhibit dopamine release. Treatment with kappa antagonists can increase dopamine levels in the nucleus accumbens, while treatment with kappa agonists decrease these levels and block increases in dopamine following the administration of cocaine (see Herz, 1998, for a review). Since these neurons are important for cocaine self-administration in animals, kappa agonists may be useful in inhibiting the actions of cocaine that lead to its abuse. A variety of animal studies have demonstrated that kappa agonists can suppress or alter behavior related to cocaine abuse (Bowen et al., 2003). For example, Mello and Negus (2000) reported that a variety of arylacetamide and benzomorphan kappa agonists could suppress cocaine self-administration in rhesus monkeys. Some of these drugs were also behaviorally specific, in that they could suppress cocaine self-administration at doses that did not suppress responding for a food reinforcer.

If kappa receptors are to be used as treatments, it is important to understand the cardiovascular effects of these drugs. In particular, if kappa agonists are to be used in the treatment of cocaine abuse, it is important to study potential interactions between cocaine and the kappa agonists. With any pharmacological treatment for drug abuse, it is inevitable that the treatment drug and the abused drug will be used in combination in some individuals that relapse to drug use. While a number of studies have looked at the effects of kappa agonists on cardiovascular function, most of these studies have been performed in isolated organ preparations (e.g., Kannan and Seip, 1986; Maeda et al., 1987; Wong et al., 1990) or anesthetized animals (Laurent and Schmitt, 1983; Punnen and Sapru, 1986; Rochford et al., 1991). The purpose of this study was to determine the cardiovascular effects of two representative kappa opioid agonists in a conscious, non-human primate model of cardiovascular function. The two drugs chosen for study represent the two chemical classes of kappa opioid agonists. Enadoline is an arylacetamide and ethylketocyclazocine (EKC) is a benzomorphan kappa opioid agonist. Squirrel monkeys were studied as they have been used extensively to study both the behavioral and cardiovascular effects of abused drugs (Schindler, 1996).

2. Methods

2.1. Animals

Subjects were 16 adult male squirrel monkeys (*Saimiri sciureus*) housed in individual cages in rooms in which light, temperature and humidity were controlled. Fresh water was continuously available. The monkeys' daily food intake was restricted to maintain a constant body weight throughout the course of the experiment (800–1000 gms). Monkeys were implanted with both a venous catheter (internal iliac vein) for the delivery of drug and an arterial catheter (internal iliac artery) for the measurement of blood pressure. Catheters were implanted during a single sterile surgery. The general surgical procedure has been described in detail elsewhere (Herd et al., 1969). In brief, polyvinyl chloride catheters (inside diameter, 0.38 mm; outside diameter, 0.76 mm) were implanted during anesthesia with flurothane (2–2.5%)-oxygen mixtures. The distal ends of the catheters were passed subcutaneously (s.c.) out through the skin in the middle of the back. Monkeys wore nylon jackets at all times to protect the

catheters. Following a 2-week recovery period, experiments were begun. Catheters were flushed with heparinized saline (5 units/ml) at least twice weekly and sealed with stainless steel obturators when not in use. If an arterial catheter failed, it was removed and replaced with a second arterial catheter on the opposite side. If a venous catheter failed, it was removed and replaced with a jugular vein catheter.

All animals used in this study were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care and experiments were conducted in accordance with guidelines of the Institutional Animal Care and Use Committee of the Intramural Research Program, NIDA, NIH, and Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003).

2.2. Apparatus

During experimental sessions, the monkeys sat in Plexiglas chairs (Hake and Azrin, 1953) and were loosely restrained in the seated position by a waist lock. The chairs were enclosed in ventilated, sound-attenuating chambers (model AC-3; Industrial Acoustics Co., Bronx NY) that were provided with continuous white noise to mask extraneous sounds. The monkeys were fully adapted to the chair and acoustic chamber prior to surgery. The distal end of the arterial catheter was connected via polyethylene tubing to a blood pressure transducer (no. T42-20, Coulbourn Instruments, Lehigh Valley, PA). The transducers were calibrated regularly between experimental sessions with a hand-held manometer. During the sessions, the arterial catheter was continuously flushed with saline at a rate of 0.03 ml/min. The transducer was connected to an associated amplifier (no. S72-25, Coulbourn Instruments) and blood-pressure processor (no. S77-34, Coulbourn Instruments) outside the experimental chamber.

The blood-pressure processor analyzed the raw transducer signal, giving analog outputs of systolic, diastolic and mean pressure at the end of each cardiac cycle. The signal for the end of the cardiac cycle was fed into an Apple Macintosh computer. For each cycle, the computer measured the time between cycles with a resolution of 1 msec and read the analog signals for pressure from the blood pressure processor with a resolution of 1 mm of Hg. These values were summed and averaged over periods of 30 sec for subsequent analysis. Only mean arterial pressure (diastolic + [(systolic – diastolic)/3]) and heart rate were used for statistical analysis. The distal end of the venous catheter was passed outside the experimental chamber via polyethylene tubing and connected to a syringe for the injection of drugs.

2.3. Procedure

The subjects were placed in the chamber once a day every weekday. Typically, injections of drugs were given on Tuesdays and Fridays and saline vehicle was given on all other training days. The order of doses was non-systematic. For all sessions, the subjects were placed in the chamber for a period of 90 min, with the intravenous (i.v.) injection of a kappa agonist (EKC, enadoline or ICI 204,448 {(±)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol hydrochloride}) or saline (1.0 ml/kg) given, if scheduled, 30 min after the start of the session. For studies investigating the pretreatments and drug combinations, EKC was chosen for study as it had the lowest incidence of side-effects. Naltrexone was given i.m. just prior to the experimental session and chlorosondamine was given i.v. 20 min prior to EKC. For kappa-agonist/cocaine drug combination studies, the EKC was given i.v. 5 min prior to i.v. cocaine. There were also control sessions where a pretreatment drug vehicle was given prior to cocaine and sessions where a pretreatment drug was followed later by saline. These control sessions were no different from baseline drug or saline sessions and therefore their results were combined for analysis.

2.4. Drugs

Ethylketocyclazocine, enadoline (N. Mello, Harvard Medical School, Boston, MA) and chlorisondamine diiodide (Tocris, Ellisville, MO) were dissolved in sterile water. Cocaine hydrochloride (NIDA, Baltimore, MD), ICI 204,448 (Sigma Chemical, St. Louis, MO) and morphine sulfate (NIDA, Baltimore, MD) were dissolved in sterile, physiological saline. The i.v. injections were given over a period not exceeding 15 sec and in a volume of 1.0 ml/kg. Naltrexone hydrochloride (Sigma Chemical, St. Louis, MO) was dissolved in saline and injected i.m. in a volume of 0.3 ml/kg.

2.5. Data Analysis

Heart rate and blood pressure data were averaged over 1-min periods from the 30-sec periods averaged by the computer. The use of 30-sec means leads to a greater amount of variability in blood pressure and heart rate, even following saline administration. This variability most likely reflects movement by the animal. Therefore, the use of the 1-min mean gives a more accurate measure of any drug-induced change in both the blood pressure and heart rate measures.

Average change was calculated using the 1-min mean change scores, where the 5-min period prior to drug was used as the baseline. As peak effects on blood pressure typically occurred within 5 min of drug administration and peak effects on heart rate typically occurred within 30 min, mean measures are reported for the 30-min period following the last injection of the session. While some drugs had more persistent effects, the 30-min measure insured that peak effects of both the pretreatment and test drug were captured for the drug combination studies. Error bars are the standard error of the mean. All data were subjected to a least squares analysis-of-variance with Tukey follow-up tests to determine individual effects (JMP, SAS Software or PRISM, Graphpad Software).

3. Results

Prior to injection of saline, the mean blood pressure was 103.7 ± 8.9 mm Hg and heart rate was 253.3 ± 29.9 beats/min. These values were not different from those seen prior to drug treatments. For 30 min following treatment with saline, the average blood pressure and heart rate were virtually unchanged (Fig. 1). Treatment with EKC or enadoline did not significantly alter blood pressure, although at the highest dose of enadoline tested, there was a slight, but not significant, increase in blood pressure. A higher dose of enadoline was not tested because of severe side effects observed at this dose. These side effects included sedation, salivation and vomiting. In contrast to blood pressure, heart rate was significantly increased by both EKC ($F_{5,18} = 3.64$, $P < 0.05$) and enadoline ($F_{5,33} = 4.89$, $P < 0.01$) at the 0.01 mg/kg dose. Enadoline appeared to be more efficacious than EKC at increasing heart rate. In comparison, the mu agonist morphine did not significantly alter either blood pressure or heart rate.

Fig. 2 shows the time course for the effects of both EKC and enadoline at the 0.01 mg/kg dose in animals that had received both drugs at this dose. For comparison, the effects of saline are also presented. Both EKC and enadoline produced small increases in blood pressure immediately after administration, with the effect of enadoline being slightly larger. This led to a significant interaction between drug condition and time ($F_{23,132} = 2.52$, $P < 0.01$), although none of the individual effects reached significance. Both EKC and enadoline increased heart rate throughout the testing period, leading to a significant drug effect ($F_{2,132} = 7.51$, $P < 0.01$).

Following determination of the dose-effect function for EKC and enadoline, a subset of monkeys was treated with 0.01 mg/kg EKC alone and following various pretreatments. Fig. 3 shows that 0.01 mg/kg EKC alone produced a significant ($F_{5,18} = 10.0$, $P < 0.01$) increase in heart rate above saline values. While blood pressure was slightly elevated, this effect was not significant. The opioid antagonist naltrexone by itself did not affect heart rate or blood pressure,

but it did reverse the effects of EKC. At the time point at which EKC was injected, the ganglionic blocker chlorisondamine was producing no further changes in blood pressure or heart rate in any of the monkeys tested. Chlorisondamine did, however, partially reverse the effects of EKC. The chlorisondamine/EKC group was not significantly different from either the saline or the EKC alone group. To further investigate the role of peripheral kappa receptors in the cardiovascular effects of kappa agonists, the peripherally active kappa receptor agonist ICI 204,448 was also administered to conscious squirrel monkeys. Fig. 4 shows that ICI 204,448 had little effect on blood pressure, but like EKC and enadoline did significantly ($F_{3,12} = 3.5$, $P < 0.05$) increase heart rate.

Since kappa agonists have been proposed as treatments for cocaine abuse, 0.003 mg/kg EKC was given as a pretreatment before cocaine (0.03 to 1.0 mg/kg) to a subgroup of the monkeys. A dose below the peak of the dose-effect function for EKC was chosen to allow for the observation of any potentiating effects. Fig. 5 shows the results of this experiment. EKC produced small increases in blood pressure and larger increases in heart rate when given alone. When combined with cocaine, the blood pressure effects of EKC in combination with cocaine were actually lower than cocaine alone. These small differences failed to reach significance. For heart rate, the effects of EKC in combination with cocaine were more similar to the effects of EKC alone. As a result, the effects of the drug combination on heart rate were significantly different from cocaine alone ($F_{1,65} = 17.5$, $P < 0.01$).

4. Discussion

The most prominent effect of the kappa agonists was an increase in heart rate. Neither EKC nor enadoline significantly affected blood pressure in conscious squirrel monkeys in the dose range tested. The results of previous studies on the effects of kappa agonists on heart rate are mixed. Some studies of kappa agonists and dynorphin have reported decreases in heart rate (e.g., Gautret and Schmitt, 1985; Laurent and Schmitt, 1983; Wu and Martin, 1989). However, all of these studies involved anesthetized animals or injections into limited regions of the CNS. Other studies have reported an increase in heart rate (e.g., Hassen et al., 1983; Punnen and Sapru, 1986; Rochford et al., 1991), but again these studies also involved anesthesia and central injections. In conscious dogs, Wu and Martin (1989) reported that EKC had no effect on heart rate at doses that exceed those tested here. As the dose-effect function may be turning down at higher doses of EKC, this may not be surprising. Human studies have investigated the mixed μ /kappa agonist nalbuphine (Mello et al., 2005) and the kappa agonist niravoline (Bellissant et al., 1996). No changes in heart rate were noted in either of these studies. While further studies are needed to confirm these results, they do suggest that kappa agonist treatment in conscious primates will increase heart rate significantly above baseline values.

The heart rate increasing effects of EKC and enadoline occurred at doses that are well above the behaviorally active doses. At the 0.01 mg/kg dose where the peak effects on heart rate were observed, both EKC and enadoline would completely suppress schedule-controlled behavior (Mello and Negus, 2000), with enadoline being the more potent of the two drugs. Therefore, if these drugs were to be used clinically, they would most likely be used at doses below those that increased heart rate in this study, although the high end of the behaviorally effective dose range typically extends to the doses used here.

The cardiovascular effects of EKC were most likely mediated by kappa opioid receptors. The μ agonist morphine did not mimic the effects of the kappa agonists, having no significant effect on either blood pressure or heart rate. Further, the opioid antagonist naltrexone, at a dose of 1.0 mg/kg that has been shown to block other effects of kappa receptor agonists (Leander, 1982), completely antagonized the effects of EKC.

The location of the receptors mediating the heart rate increases produced by EKC and enadoline are not clear. The ganglionic blocker chlorisondamine partially antagonized the effects of EKC, a result that suggests central autonomic control. The dose of chlorisondamine used should have been sufficient to block any central cardiovascular response. In comparison to the saline baseline, chlorisondamine decreased baseline blood pressure by nearly 20 mm Hg (23.0 ± 7.4 mm Hg). This dose (1.0 mg/kg) will also completely block the heart rate decrease observed following an injection of norepinephrine (Tella et al., 1993), indicating complete blockade of the baroreceptor reflex response. Various locations in the central nervous system have been associated with a heart rate increase following kappa receptor treatment. In urethane-anesthetized rats, Rochford et al. (1991) reported that intrathecal administration of dynorphin increased heart rate. However, this effect was not blocked by an opioid antagonist and was not seen following administration of the specific kappa receptor agonist U50,488. Since the heart rate increase observed in conscious squirrel monkeys after EKC administration was blocked by naloxone, it is unlikely that this effect was mediated by spinal kappa receptors. Punnen and Sapru (1986) report that microinjections of dynorphin into areas of the ventrolateral medulla can increase heart rate and blood pressure in urethane-anesthetized rats. In artificially-ventilated, pentobarbital-anesthetized rats, Hassen et al. (1983) reported that microinjections of the kappa agonist MRZ 2549 into the nucleus tractus solitarius increased heart rate without altering blood pressure. Thus, it seems likely that kappa agonists acting in the hindbrain cardiovascular control regions could contribute to the increased heart rate observed in conscious squirrel monkeys.

The finding that chlorisondamine partially blocked the effects of EKC does not necessarily mean that the effect was centrally mediated. Proper functioning of postganglionic nerves is necessary for that effect. In addition, the fact that the effect of EKC was not completely blocked by chlorisondamine suggests the involvement of peripheral kappa receptors as well. Kappa receptors (Krumins et al., 1985) and dynorphin (Spampinato and Goldstein, 1983) have been observed in the rat heart, leaving open the possibility of peripheral control for the cardiovascular effects of kappa agonists. Kappa receptors are found throughout the heart, but are most prominent in the right atrium and are functionally altered in response to hemorrhagic shock in the right atrium and ventricle (Krumins et al., 1985). Activation of kappa receptors in ventricular myocytes leads to a release of intracellular calcium (Wong et al., 1995).

To investigate the possibility of peripheral action further, the peripherally acting kappa agonist ICI 204,448 was administered. ICI 204,448 is approximately equipotent to EKC in peripheral tissue (Barber et al., 1994; Hunter et al., 1990), but is over 150 times less potent than EKC in behavioral measures that should involve central control (Carey and Bergman, 2001). ICI 204,448 significantly increased heart rate in squirrel monkeys and was only slightly less potent than EKC. This result indicates that actions at peripheral kappa receptors may also contribute to the cardiovascular effects of the kappa agonists.

The site of action for the kappa agonists in the periphery is even less clear than for the central nervous system. No studies have reported an increase in heart rate following kappa agonist treatment due to peripheral mechanisms. The data that is available points to inhibitory actions of peripheral kappa receptors on the cardiovascular system. Ensinger et al. (1984) reported that EKC treatment decreased the release of norepinephrine in the periphery. Gautret and Schmitt (1985) showed that dynorphin produced bradycardia in pentobarbital-anesthetized rats, even following bilateral vagotomy, suggesting a direct action on the heart. Further support for a direct inhibitory action of kappa agonists on the heart comes from a study by Maeda et al. (1987), who showed a decrease in beat frequency in the isolated rat atrium following treatment with EKC and dynorphin. In this study, the kappa agonists did produce a positive inotropic effect, indicating that kappa agonists can have some excitatory effects in the periphery. Kappa agonists can also be arrhythmogenic in the isolated rat heart (Wong et al., 1990). Nevertheless,

none of these studies points to a peripheral mechanism that can explain the heart rate increases observed in conscious squirrel monkeys following kappa agonist treatment.

Some of these inconsistencies may be related to differences in kappa receptor structure and function between rats and primates. For example, kappa agonist treatment differentially regulated rat and human kappa receptors in vitro, and this difference was related to species differences in a single amino acid of the kappa receptor (Li et al., 2002). Species differences in the distribution and density of kappa receptors have also been observed, with humans and non-human primates possessing a far greater proportion of kappa receptors than rats (Mansour et al., 1988). These differences in opioid receptor anatomy may contribute to functional differences in opioid receptor modulation of neural circuits. In humans, release of norepinephrine from cortical slices was inhibited by kappa and delta agonists, but not by a mu agonist (Berger et al., 2006). Exactly the opposite was true in cortical slices from rat brain, with norepinephrine release inhibited by a mu agonist, but not by kappa or delta agonists (Berger et al., 2006). Differences in behavioral effects have also been noted for kappa opioid agonists. For example, EKC is self-administered by rats, but not by monkeys (Woods, 1983; Young and Khazan, 1983).

Kappa agonists have been proposed as treatments for cocaine abuse. As such, it is important to understand the interaction of the kappa agonists with cocaine. Cocaine is known to increase both blood pressure and heart rate in squirrel monkeys (Schindler, 1996; Tella et al, 1993). As the kappa agonists also increase heart rate, the potential for additive or synergistic effects when the drugs are given in combination must be a concern. The blood pressure effects of the drug combinations were similar to those observed for cocaine alone or were actually reduced. For heart rate, the effects of the drug combinations were intermediate between cocaine alone and the kappa agonist alone, but tended to be more similar to EKC alone. These results suggest that any cocaine use that does occur during treatment with kappa agonists should not lead to any further increases in heart rate beyond those observed for either drug alone (sub-additive). While we are aware of no other studies in primates investigating the interaction of kappa opioid drugs and cocaine on cardiovascular function, Saland et al. (2002) investigated the interaction of morphine and cocaine on coronary artery constriction in humans. They found that morphine actually reversed cocaine-induced coronary artery constriction. While morphine most likely works through mu opioid receptors, these results provide further evidence that combination of opioids and cocaine can be used in combination without exacerbating either drug's effects on cardiovascular function. Since, peripherally acting kappa agonists are also being investigated as analgesics, the results of this study clearly indicate that the potential cardiovascular effects of these drugs should be investigated further.

In conclusion, kappa receptor agonists produced clear increases in heart rate when administered i.v. to conscious squirrel monkeys. There were no changes observed in blood pressure. The increase in heart rate was antagonized by an opioid antagonist, suggesting the involvement of opioid receptors. The site of action for the kappa agonists may be in either the central or peripheral nervous system. While the increase in heart rate produced by the kappa agonists EKC and enadoline were moderate in size, when EKC was given in combination with cocaine no further exacerbation of the cardiovascular effects of either drug were observed, indicating that kappa agonists may be used safely in the treatment of cocaine abuse.

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Fig. 1.

Dose-effect functions for the effects of the kappa agonists EKC ($n = 2-5/\text{point}$) and enadoline ($n = 4-8/\text{point}$), and the mu agonist morphine ($n = 4-8/\text{point}$) given i.v. in conscious squirrel monkeys. Each point represents the mean change in blood pressure (top panel) or heart rate (bottom panel) over the first 30 min following drug administration. Points above "saline" are from multiple tests following saline administration during the determination of each dose-effect function. Doses are mg/kg. Only one dose was administered per day and at least 2 days without drug separated each dose test. Error bars are $\pm$ S.E.M.. * $P < 0.05$ from respective saline.

Fig. 2.

Time course for the effects of 0.01 mg/kg EKC and enadoline given i.v. in conscious squirrel monkeys. For comparison, the time course for saline is shown. All monkeys ($n = 5$) included in this figure were tested with both EKC and enadoline. Error bars are $\pm$ S.E.M..

Fig. 3.

Effects of EKC (0.01 mg/kg, i.v.) alone and following various pretreatments in conscious squirrel monkeys ($n = 4$). Each bar represents the mean change in blood pressure (top panel) or heart rate (bottom panel) over the first 30 min following the second drug administration using the 5-min period prior to the second drug administration as the control. Naltrexone (Nal), 1 mg/kg, was administered i.m. just before the session (30 min before EKC). Chlorisondamine (Chlor), 1 mg/kg, was administered i.v. 20 min prior to EKC (10 min into the session). Doses are mg/kg. Only one test was administered per day and at least 2 days without drug separated each dose test. Error bars are $\pm$ S.E.M.. * $P < 0.05$ from all other treatments except Chlor. + EKC. Chlor. + EKC was not significantly different from any other treatment.

Fig. 4.

Dose-effect function for the peripherally active kappa agonist ICI 204,448 in conscious squirrel monkeys ($n = 4$). Each bar represents the mean change in blood pressure (top panel) or heart rate (bottom panel) over the first 30 min following i.v. drug administration. Only one dose was administered per day and at least 2 days without drug separated each dose test. Error bars are $\pm$ S.E.M.. * $P < 0.05$ from saline.

Fig. 5. Effects of EKC (0.003 mg/kg, i.v.) alone and in combination with various i.v. doses of cocaine in conscious squirrel monkey ($n = 4-8$). Each point represents the mean change in blood pressure (top panel) or heart rate (bottom panel) over the first 30 min following i.v. drug administration. Points (open symbols) above "saline" are from multiple tests following saline administration during the determination of each dose-effect function and from EKC given alone. There were no significant differences between the drug combinations and cocaine alone for blood pressure. For heart rate, the effects of the drug combinations were significantly different from the effects of cocaine alone. Doses are mg/kg. Only one test was administered per day and at least 2 days without drug separated each dose test.