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ORIGINAL INVESTIGATION

Jillian H. Broadbear · Gail Winger · James H. Woods

Self-administration of fentanyl, cocaine and ketamine: effects on the pituitary–adrenal axis in rhesus monkeys

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Abstract *Rationale:* Drugs of abuse can affect the functioning of the hypothalamic–pituitary–adrenal (HPA) axis. Acute administration of drugs that serve as reinforcers have been observed to stimulate the rat HPA axis, leading to the suggestion that these stimulatory effects may contribute to the development of drug-maintained behaviors. *Objectives:* To determine whether reinforcing drugs that are dissimilar with respect to their mechanisms of action have similar effects on HPA axis activity at doses that are self-administered. Rhesus monkeys were randomly assigned to self-administer the μ-opioid agonist fentanyl, the psychomotor stimulant cocaine, or the NMDA antagonist ketamine. *Methods:* Each monkey was trained to press a lever in order to receive an intravenous injection of drug or saline. Blood samples were obtained before, during, and after the self-administration sessions and assayed for ACTH and cortisol by radioimmunoassay. *Results:* Fentanyl, cocaine, and ketamine were each self-administered across a range of doses. However, the three drugs differed in their effects on ACTH and cortisol. Cocaine stimulated ACTH and cortisol secretion, a finding that is consistent with previous rat and primate studies. Self-administration of both fentanyl and ketamine inhibited HPA axis activity. HPA inhibition by fentanyl is consistent with other monkey and human studies, and contrasts with the stimulatory effects of μ-opioids in rodents. The inhibitory effect of ketamine on ACTH and cortisol secretion contrasts with findings in the few primate studies that have evaluated NMDA antagonists. Neither fentanyl nor cocaine, at doses that maintained maximum rates of responding, produced significant changes in ACTH and cortisol levels. *Conclusions:* There appears to be little commonality between different classes of abused drugs and their effects on the HPA axis, which calls into question the necessity for HPA axis stimulation in the reinforcement of drug-maintained behavior.

Keywords Rhesus monkey · Cocaine · Fentanyl · Ketamine · Self-administration · Adrenocorticotropin · Cortisol · Reinforcement · Behavior

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J. H. Broadbear · G. Winger · J. H. Woods
Department of Pharmacology, University of Michigan Medical School,
1301 MSRB 3,
Ann Arbor, MI, 48109-0632, USA

Present address:
J. H. Broadbear (✉)
Department of Physiology, Monash University,
Building 13F,
Clayton, Victoria, 3800, Australia
e-mail: jillian.broadbear@med.monash.edu.au
Tel.: +61-3-99052557
Fax: +61-3-99052547

J. H. Woods
Department of Psychology, University of Michigan Medical School,
Ann Arbor, MI, USA

Introduction

Many drugs of abuse, such as psychomotor stimulants (cocaine, amphetamine, and nicotine), depressants (morphine and alcohol), and hallucinogens (LSD and *N*,*N*-dimethyltryptamine) have been shown to affect hypothalamic–pituitary–adrenal (HPA) axis activity (for review, see Sarnyai et al. 2001). Although not typically thought of as "stressors" in the aversive sense of the word, drugs of abuse nevertheless induce physiological and psychological changes that may activate homeostatic mechanisms such as the HPA axis. Over the past decade, a theoretical framework has evolved that incorporates the interaction of drug reward with activation of the HPA axis. Studies that highlight this research rely primarily on data obtained from rats. For example, rats with a heightened HPA response following exposure to novelty were reported to acquire self-administration behavior for lower doses of cocaine (Goeders and Guerin 1994), amphetamine (Piazza et al. 1991), and heroin (Shaham and Stewart 1994).

Adrenalectomized rats failed to acquire self-administration behavior, implying that glucocorticoids, the end-product of HPA axis stimulation, are necessary for the expression of self-administration behavior in the rat (Goeders and Guerin 1996). Piazza and le Moal (1996) have expanded their hypothesis, which relates the response of rats to novelty to their subsequent acquisition of drug-taking, to include humans who engage in risk-taking behaviors. This is based on the premise that risk-takers may be more likely to engage in drug experimentation, resulting in their greater vulnerability for drug abuse.

Although psychomotor stimulants such as cocaine and amphetamine generally stimulate the HPA axis regardless of the species and treatment regimen, the results of studies involving HPA effects of drugs such as opioid agonists and NMDA antagonists often conflict with one another. In the opioid literature, the fundamental nature of the disagreement appears to be species-dependent, as shown by species differences in the relationship between the endogenous opioid β-endorphin, CRH, and feeding behavior. In the rat, intracerebroventricular administration of corticotropin-releasing hormone (CRH) resulted in an increase in plasma β-endorphin levels and a decrease in food intake (Hotta et al. 1991). In primates, however, the administration of β-endorphin led to a fall in CRH secretion (Garland and Zis 1990) and an increase in food intake (Nader and Barrett 1989). Opioids decrease ACTH and cortisol secretion in humans (Gaillard et al. 1981; Delitala et al. 1983; Allolio et al. 1987), whereas acute morphine administration causes ACTH and corticosterone to rise in rat, mouse, guinea pig, and cat (see review by Pechnick 1993).

Non-competitive NMDA antagonists such as ketamine and phencyclidine (PCP) also inhibit dopamine uptake, although the majority of their behavioral effects appear to be related to their activity at NMDA receptors (e.g. Koek et al. 1989). With respect to the HPA axis effects of NMDA antagonists, the findings are not always in agreement even within species. NMDA itself has been shown to stimulate ACTH release in rats (Iyengar et al. 1990; Farah et al. 1991; Jezova et al. 1991) and primates (Gay and Plant 1987; Reyes et al. 1990). NMDA antagonists such as AP5 and MK-801 block the stimulatory effect of NMDA on ACTH in rats (Farah et al. 1991; Jezova et al. 1991). However, anesthetizing doses of the NMDA antagonists PCP or ketamine produced increases in ACTH and cortisol when administered to rhesus monkeys (Setchell et al. 1975; Elvidge et al. 1976) or humans (Adams et al. 1992). It is possible that the paradoxical effect of NMDA antagonists on HPA axis function in primates may be based on the magnitude of the dose and the duration of its administration.

Although there is some understanding of the ways in which acute administration of drugs of abuse may alter HPA axis function, there is an almost complete absence of information as to how these drugs, at doses that are self-administered, interact with stress hormone secretion. If there is a role for the HPA axis in drug reward, it is perhaps most meaningful to study it under conditions in which the dose and delivery are guided by the subject's perception of the rewarding properties of the drug under study. In the present paper, we have examined the behavioral and HPA effects of fentanyl, a μ-selective opioid agonist, cocaine, a psychomotor stimulant, and ketamine, a non-competitive NMDA antagonist. Our general hypothesis is that each of these drugs will produce self-administration behavior over a range of doses, and that despite their differing mechanisms of action, they will produce similar effects on ACTH and cortisol secretion.

Materials and methods

Subjects

Eight adult male rhesus monkeys (*Macaca mulatta*), weighing between 9.9 and 15.4 kg, and one female monkey, weighing 8.2 kg, were the subjects for this study. All subjects had an extensive self-administration history with two or more classes of drugs, including cocaine and methohexital. The monkeys were randomly assigned to the three drug groups; cocaine (n=4), fentanyl (n=4), and ketamine (n=4). One monkey (2489) was tested with all three drugs, one monkey (female: 2487) was tested with both fentanyl and ketamine, and one monkey (3151) was tested with both cocaine and fentanyl. The remaining monkeys were tested with only a single drug; cocaine (monkeys 3603 and 3596), fentanyl (monkey 4395), and ketamine (monkeys 3147 and 3579).

Apparatus

Each monkey was individually housed in a stainless steel cage measuring 83.3×76.2×91.4 cm deep (Bryan Research Equipment Corporation, Bryan, Tex., USA) located in a laboratory that contained a total of 24 similarly housed monkeys. The temperature in the room was maintained at 21°C, and lights were illuminated from 0630 hours until 1930 hours daily. The monkeys were fed 8–12 Purina Monkey Chow biscuits twice daily and fruit once daily to maintain normal adult weight; water was freely available. Each monkey had an indwelling venous catheter in a femoral, internal, or external jugular vein. Catheters were inserted during aseptic surgery under ketamine (10 mg/kg) and xylazine (2 mg/kg) anesthesia. Following placement in the vein, the catheter was guided subcutaneously to the midscapular region where it was externalized. The outer portion of the catheter was protected inside the cage by a flexible stainless steel tether, with one end attached to a double layer polyester jacket (Lomir, New York, N.Y., USA) worn by the monkey and the other bolted to the rear of the cage. Each cage had a 15×20 cm panel fixed to its right wall. Each panel had three stimulus lights, two red and one central green light, placed above two response levers. The red stimulus light over the right lever signaled drug availability. The green center light was illuminated for the duration of the drug or saline injection, 1 ml per

5 s. During a time out, all stimulus lights were extinguished and responding had no programmed consequences. The experiment was controlled by IBM/PS2 computers located in an adjacent room. The computers were programmed using Med Associates software (Georgia, Vt., USA).

Procedure

Drug self-administration sessions were scheduled twice daily for 130 min starting at approximately 10 a.m. and 4 p.m. Saline was substituted on a frequent basis (25–50% of sessions). The reinforcing effectiveness of and stress hormone response to cocaine (0.001, 0.003, 0.01, 0.03, and 0.1 mg/kg per injection), fentanyl (0.03, 0.1, 0.3, 1.0, and 3.0 μg/kg per injection), and ketamine (0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg per injection) were evaluated. Drug or saline delivery was contingent on each monkey emitting a fixed ratio of 30 lever presses (FR30) while the red stimulus light was illuminated. Following completion of the ratio, the red light was extinguished and the green light was illuminated for the duration of the intravenous injection of either drug or saline. There was a 10-s time out between the end of an injection and the next response opportunity. The 2 h 10 min session was divided into four 25-min components, each separated by a 10-min time out during which venous blood was drawn. A single dose of drug or saline was made available each session (two sessions per day; 10 a.m. and 4 p.m.), and blood was sampled during self-administration of each drug dose during at least two morning self-administration sessions. Blood was drawn after the lever press responses for each drug dose had stabilized, usually after 4–7 days of drug dose availability. The self-administration behavior of the three monkeys that were tested with more than one drug was allowed to stabilize for at least a week after switching from one drug to another, and testing did not commence until monkeys were responding reliably for the new drug. A stable baseline of self-administration behavior was defined as consistency (<15% variability in the number of injection earned) across sessions. The order of presentation of the drug doses was varied randomly. Blood was sampled for the measurement of ACTH and cortisol at 15 and 5 min prior to the self-administration session, as well as during (25, 60, 95, and 130 min) and after the session (2 h 45 min, 3 h 20 min, 4 h 20 min, and 5 h 20 min). All experiments reported in this study were conducted during the morning self-administration session.

Blood collection and handling

Blood samples were collected from the monkeys in the self-administration study via their indwelling venous catheters. Prior to drawing each blood sample, a 3 ml syringe was used to empty the contents of the catheter and this fluid was discarded. Then each blood sample (1.1–1.4 ml) was placed in a 2-ml Vacutainer (Becton Dickinson and Company, Franklin Lakes, N.J., USA) containing 0.04 ml of 7.5% EDTA and immediately placed on ice. After a blood sample was drawn, 1.5–3 ml of 30 IU/ml heparin saline solution was injected into the catheter. For blood samples drawn during the self-administration session, drug solution sufficient to fill the catheter lumen was also injected. Blood samples were centrifuged at 4000 rpm for 5 min at 4°C and then the plasma (0.7 ml) was pipetted into 2-ml Cryovials (Corning Incorporated, Corning, N.Y., USA) and stored at −80°C until assay. ACTH and cortisol levels were determined using commercially available radioimmunoassay kits (ACTH: Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA; cortisol: Diagnostic Products Corporation, Los Angeles, Calif., USA). The limit of detection of the cortisol assay was 0.2 ug/dl, while the intra-assay and inter-assay coefficients of variation were 5 and 6.5%, respectively. The limit of detection for the ACTH assay was 0.5 pg/ml, with intra-assay and inter-assay coefficients of variation of 3 and 7%, respectively.

Data analysis

Self-administration data (responses per second, mean number of injections and drug intake) were obtained from mornings during which blood samples were collected. Response rate was calculated by dividing the total number of lever presses executed during the session by the cumulative time during which the red light was illuminated. Data were averaged across subjects and plotted against each dose of each drug. The data were analyzed for dose dependency and for differences across the sampling time.

ACTH and cortisol levels that were measured in plasma during saline, fentanyl, cocaine, and ketamine self-administration are presented as mean±standard error of the mean (SEM). Initially the effects of saline self-administration on ACTH and cortisol secretion among the drug-taking groups were compared to ascertain that there were no underlying differences. Then comparisons of the HPA effects of saline and each drug dose within each of the drug-taking groups were made. There was some individual variation in the pre-session ACTH and cortisol measurements. Therefore, the raw ACTH and cortisol data for each drug dose were standardized by subtraction of the averaged pre-session ACTH or cortisol value from the subsequent data points prior to graphing and statistical analysis. Except in the case of the saline comparison, within-drug (but not between-drug) data analysis was carried out.

Summary data are shown subsequent to calculation of area under curve (AUC) values. AUC values are an estimate of the total ACTH (pg.min/ml) and cortisol (ug.min/dl) release during the self-administration session relative to pre-session levels. AUC values were calculated according to the trapezoidal rule (e.g. Tallarida and Murray 1987). The dose-related effects of cocaine,

fentanyl, and ketamine on ACTH and cortisol secretion were examined separately for each drug.

Analysis of variance was conducted for all of the comparisons described above, using one or two within-subject variables (dose and sampling time) and one between-subject variable (in the case of the saline comparison across the different drugs). Where appropriate, post hoc pairwise comparisons using the Tukey Honest Significant Difference test of significance ($P<0.05$) were carried out (Statistica v.5.0, Statsoft, Tulsa, Okla., USA).

Drugs

Cocaine and fentanyl were obtained from the National Institute of Drug Abuse (Bethesda, Md., USA). Ketamine was purchased from Vetpo (Holland, Mich., USA). Each drug dilution was made using sterile saline.

Results

Fentanyl Responding for fentanyl generated a bell-shaped function, with a significant effect of dose [$F(5,15)=6.78$, $P<0.05$]. The maximum response rate was 1.04 ±0.12 responses/s for injections of 0.1 μg/kg per injection fentanyl. Total intake of fentanyl increased as a function of the available dose, with peak intake ranging from 38 to 79 μg/kg when the highest dose (3.0 μg/kg per injection) of fentanyl was available (Fig. 1a). ACTH and cortisol secretion decreased during self-administration of 0.3, 1.0, and 3.0 μg/kg per injection fentanyl [ACTH: $F(5,15)=5.84$, $P<0.05$; Fig. 1b] and 1.0 and 3.0 μg/kg per injection fentanyl [cortisol: $F(5,15)=4.95$, $P<0.05$; Fig. 1c] relative to when only saline was available. When the cumulative release of ACTH and cortisol was examined, the AUC for both hormones diminished as the dose of fentanyl increased [ACTH: $F(5,10)=5.28$, $P<0.05$; cortisol: $F(5,10)=4.86$, $P<0.05$; Fig. 1d]. Overall ACTH and cortisol secretion were significantly attenuated when total fentanyl intake equaled or exceeded 18.9±3.3 μg/kg. Self-

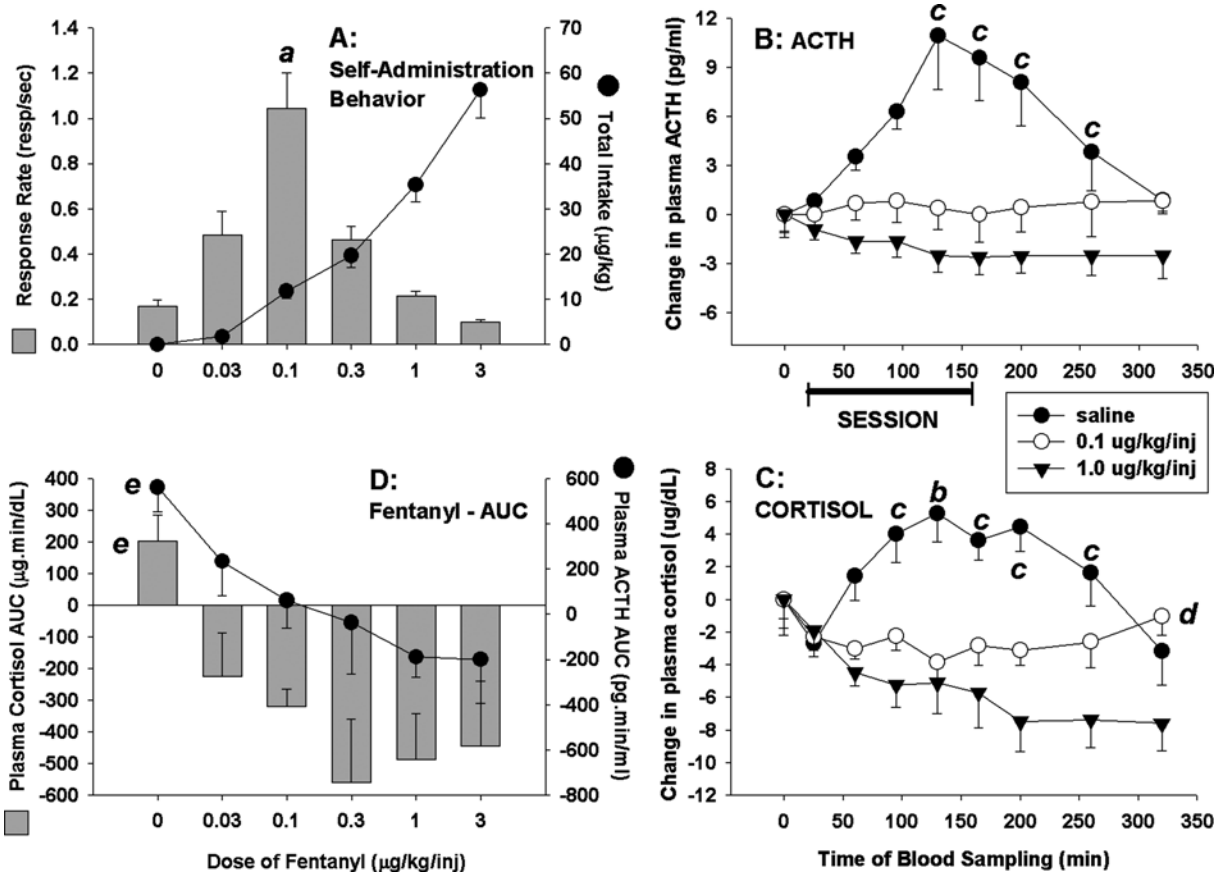

Fig. 1 **a** Response rate (responses/s) and drug intake (μg/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of the μ-opioid agonist, fentanyl (μg/kg per injection) or saline ($n=4$). **b**,**c** Plasma concentration of ACTH (**b**; baseline = 6.6±0.43 pg/ml) and cortisol (**c**; baseline = 12.3±0.88 μg/dl) measured in venous blood samples obtained before, during, and after sessions during which saline or fentanyl was available for self-administration. **d** Cumulative release of cortisol (μg.min/dl) and ACTH (pg.min/ml) during self-administration of fentanyl or saline. The zero line on the *Y*-axis indicates the average pre-session hormone levels. Key to symbols: (*a*) self-administration rates significantly different from 0.03, 1.0, and 3.0 μg/kg per injection fentanyl; (*b*) ACTH and cortisol levels significantly different from 0.1, 0.3, and 1.0 and (*c*) 3.0 μg/kg per injection fentanyl; (*d*) ACTH and cortisol levels significantly different from 0.03 μg/kg per injection fentanyl; (*e*) ACTH and cortisol levels significantly different from 0.3, 1.0, and 3.0 μg/kg per injection fentanyl, $P<0.05$

administration of the three largest doses of fentanyl (0.3, 1.0, and 3.0 μg/kg per injection) generated average intakes equal to or greater than 18.9 μg/kg, and resulted in an overall decrease in cortisol and ACTH secretion relative to when saline was available. The inhibitory effects of fentanyl intake on the HPA axis persisted for up to 2 h following the cessation of the session.

Cocaine Responding for cocaine generated a bell-shaped function, with a significant effect of dose [$F(5,15)=8.62$, $P<0.05$]. The maximum response rate was 0.81 ±0.06 response/s for injections of 0.01 mg/kg per injection cocaine. Total intake of cocaine increased as a function of the available dose, with peak intake ranging from 2.55 to 3.75 mg/kg when the highest dose (0.1 mg/kg per injection) of cocaine was available (Fig. 2a). Cocaine (0.03 and 0.1 mg/kg per injection) significantly increased the secretion of ACTH relative to when saline, 0.001, 0.003, and 0.01 mg/kg per injection cocaine were available ($P<0.05$; Fig. 2b). Cocaine, at the same doses (0.03 and 0.1 mg/kg per injection), increased cortisol secretion relative to when saline was available for self-administration [$F(5,15)=4.09$, $P<0.05$; Fig. 2c]. When the cumulative release of ACTH and cortisol was examined, the AUC for both hormones increased as the dose of cocaine increased [ACTH: $F(5,15)=3.14$, $P<0.05$; cortisol: $F(5,15)=7.53$, $P<0.05$; Fig. 2d]. Overall, ACTH and cortisol secretion was significantly increased when total cocaine intake equaled or exceeded 1.84±0.28 mg/kg. Self-administration of the two largest doses of cocaine (0.03 and 0.1 mg/kg per injection) generated average intakes equal to or greater than 1.84 mg/kg, and resulted in an overall increase in cortisol and ACTH secretion relative to when saline (ACTH and cortisol) or 0.003 mg/kg per injection cocaine were available (cortisol only). The stimulatory effects of cocaine intake on cortisol at the 0.03 and 0.1 mg/kg per injection doses persisted following the cessation of the session, with post-session AUC significantly elevated for these doses relative to when saline, 0.001, 0.003, and 0.01 mg/kg per injection cocaine had been self-administered [$F(5,15)=5.20$, $P<0.05$; data not shown].

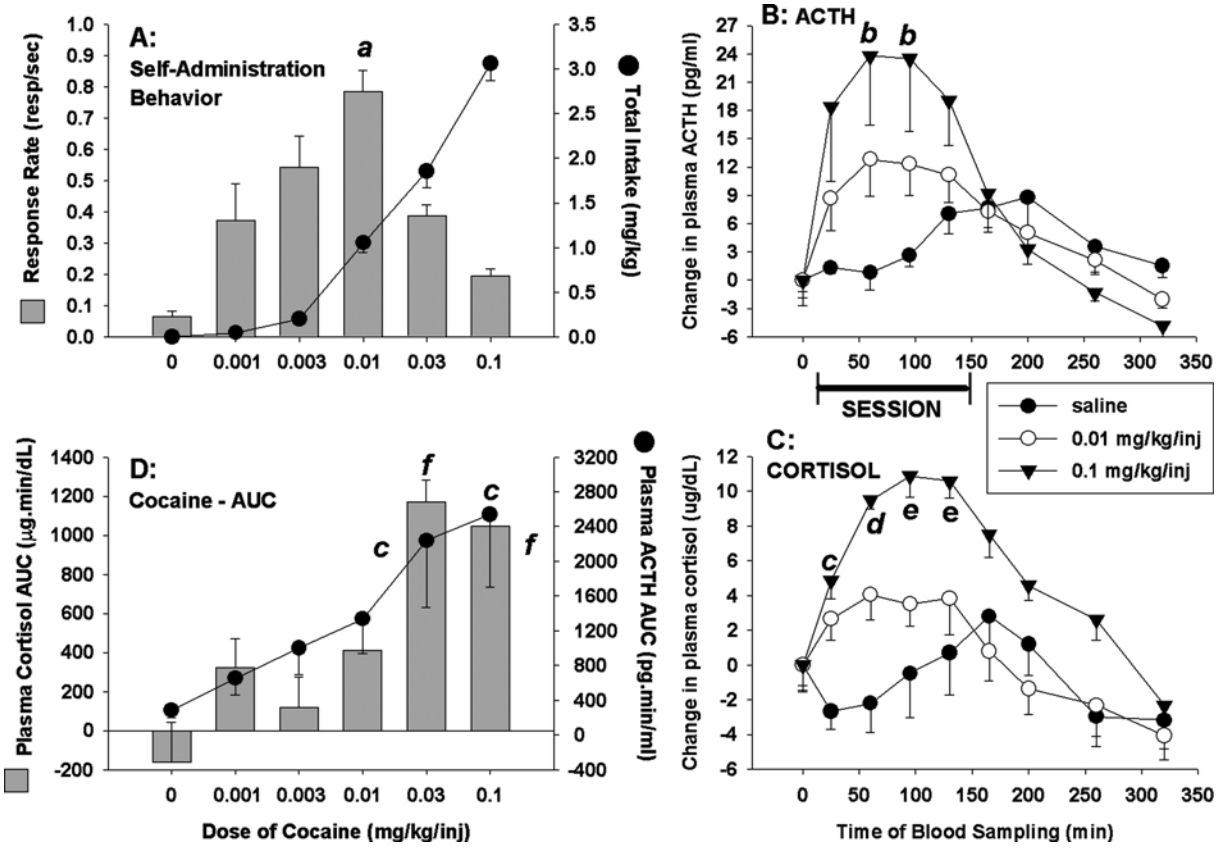

Fig. 2 **a** Response rate (responses/s) and drug intake (mg/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of the psychomotor stimulant, cocaine (mg/kg per injection) or saline ($n=4$). **b**,**c** Plasma concentration of ACTH (**b**; baseline = 6.7±0.74 pg/ml) and cortisol (**c**; baseline = 11.3±0.56 μg/dl) measured in venous blood samples obtained before, during, and after sessions during which saline or selected doses of cocaine were available for self-administration. **d** Cumulative release of cortisol (μg.min/dl) and ACTH (pg.min/ml) during self-administration of cocaine or saline. Key to symbols: (*a*) self-administration rates significantly different from saline, 0.001, 0.03, and 0.1 mg/kg per injection cocaine; (*b*) ACTH and cortisol levels significantly different from saline, 0.001 or 0.003 mg/kg per injection cocaine; ACTH and cortisol levels significantly different from saline (*c*) 0.001, 0.003 (*d*), and (*e*) 0.01 mg/kg per injection cocaine; (*f*) Cortisol levels significantly different from saline and 0.003 mg/kg per injection cocaine, $P<0.05$

Ketamine Responding for ketamine generated a bell-shaped function, with a significant effect of dose [$F(5,15)=3.39$, $P<0.05$]. The maximum response rate was 1.77 ±0.16 responses/s for injections of 0.03 mg/kg per injection ketamine. Total intake of ketamine increased as a function of the available dose, with peak intake ranging from 10.1 to 18.9 mg/kg when the highest dose (0.3 mg/kg per injection) of ketamine was available (Fig. 3a). ACTH secretion decreased during self-administration of 0.03, 0.1, and 0.3 mg/kg per injection ketamine [$F(5,15)=3.61$, $P<0.05$; Fig. 3b] relative to when saline (0.1 mg/kg per injection dose only) or 0.003 mg/kg per injection ketamine was available. Cortisol secretion decreased during self-administration of 0.1 and 0.3 mg/kg per injection ketamine ($P<0.05$; Fig. 3c) relative to when 0.003 mg/kg per injection ketamine was available. When the cumulative release of ACTH and cortisol was examined, the AUC for both hormones diminished as the dose of ketamine increased [ACTH: $F(5,15)=6.97$, $P<0.05$; cortisol: $F(5,15)=5.41$, $P<0.05$; Fig. 3d]. Self-administration of 0.1 mg/kg per injection ketamine (total intake = 10.2 ±2.3 mg/kg) resulted in a decrease in cortisol and ACTH release relative to when saline, 0.003 and 0.01 mg/kg per injection ketamine were available. The inhibition of cortisol and ACTH measured while ketamine was being actively self-administered did not persist once the session had ended.

Saline ACTH and cortisol secretion were compared during the sessions in which saline was self-administered by monkeys from the fentanyl, cocaine, and ketamine groups, with drug group as the between-subjects factor. The secretory patterns of ACTH and cortisol during saline self-administration did not differ among the drug groups, although there was an effect of sampling time [ACTH: $F(7,56)=6.48$, $P<0.05$; cortisol: $F(7,56)=7.14$, $P<0.05$] which indicated that both ACTH and cortisol levels were elevated relative to the pre-session levels from 95 to 200 min (i.e. from half way through the session until 70 min after the session). This effect of saline was unrelated to the drug being tested in these monkeys.

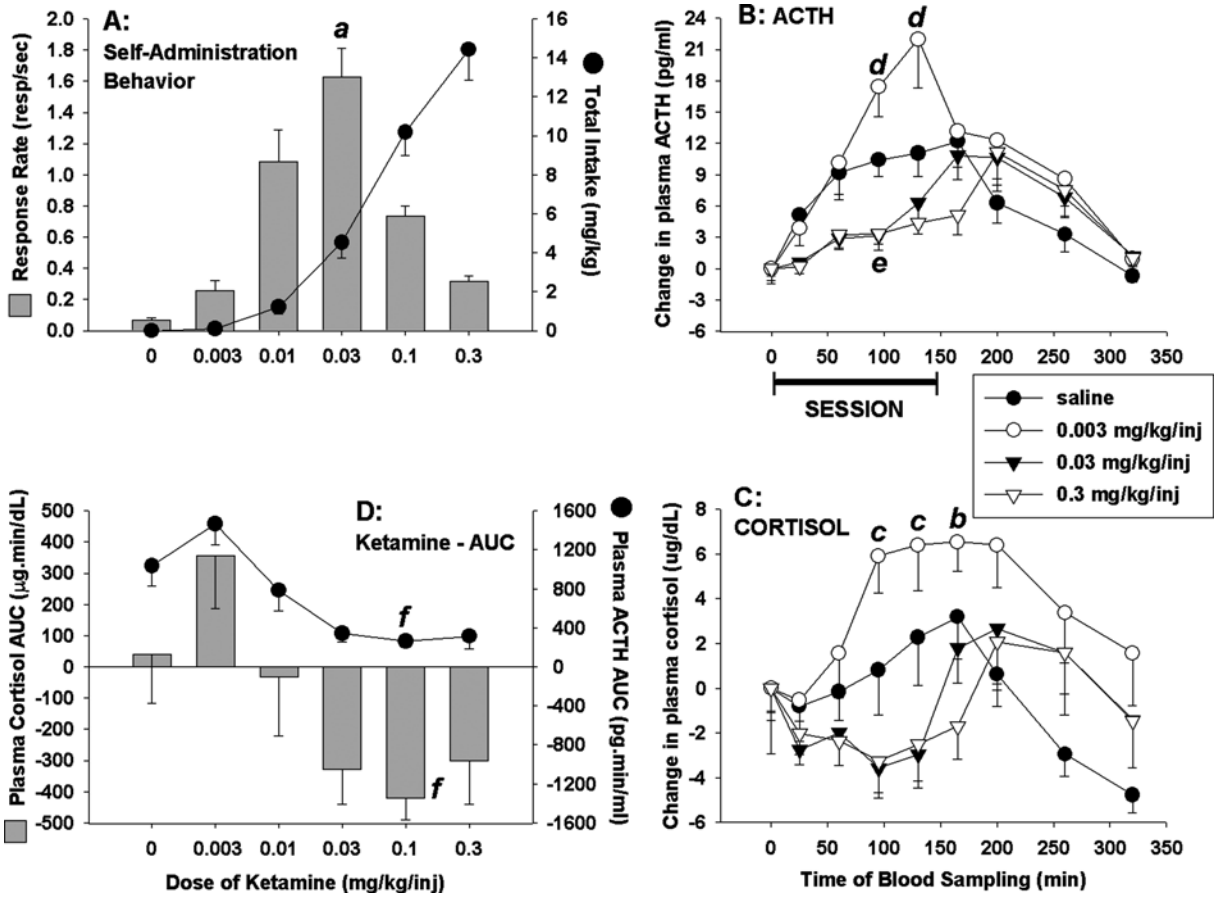

Fig. 3 **a** Response rate (responses/s) and drug intake (mg/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of the NMDA antagonist, ketamine (mg/kg per injection) or saline ($n=4$). **b**,**c** Plasma concentration of ACTH (**b**; baseline = 6.2±0.42 pg/ml) and cortisol (**c**; baseline = 15.1±0.57 µg/dl) measured in venous blood samples obtained before, during, and after sessions during which saline or selected doses of ketamine were available for self-administration. **d** Cumulative release of cortisol (µg.min/dl) and ACTH (pg.min/ml) during self-administration of ketamine or saline. Key to symbols: (*a*) self-administration rates significantly different from saline, 0.003 and 0.3 mg/kg per injection ketamine; ACTH and cortisol levels significantly different from (*b*) 0.3, (*c*) 0.1, and (*d*) 0.03 mg/kg per injection ketamine; (*e*) ACTH levels significantly different from saline; (*f*) ACTH and cortisol levels significantly different from saline, 0.003 and 0.01 mg/kg per injection ketamine, $P<0.05$

Discussion

This study was designed to measure the effects of self-administered drugs of abuse on the HPA axis hormones, ACTH and cortisol, in rhesus monkeys. The monkeys tested in this study reliably self-administered fentanyl, cocaine, and ketamine, generating a bell-shaped function of response rates across a range of doses for each drug. In addition, each self-administered drug changed the plasma concentrations of the HPA axis hormones, ACTH and cortisol. Intake of the psychomotor stimulant, cocaine, resulted in stimulation of ACTH and cortisol secretion, whereas the μ-opioid agonist, fentanyl, and the NMDA antagonist, ketamine, both attenuated HPA axis activity. Despite the differences in their modulation of HPA axis activity, neither fentanyl nor cocaine, at doses that maintained maximum rates of responding, produced significant changes in ACTH and cortisol levels. These findings are at odds with our hypothesis that drugs of abuse, at doses that are self-administered, would stimulate HPA axis activity regardless of their mechanism of action.

Saline self-administration appeared to stimulate the secretion of both ACTH and cortisol in monkeys also self-administering fentanyl, a stimulatory effect that is probably due to the increase in behavioral activity measured at the start of all sessions. However, a comparison of ACTH and cortisol secretion during saline availability in monkeys trained to self-administer cocaine, ketamine, or fentanyl, showed that there were no differences in their hormone responses to saline. Hence for the monkeys in each drug group, the HPA response to saline was used as the basis for comparison for the effects of drug on ACTH and cortisol secretion. Each of the monkeys tested in the present study had a similar drug history, having had the opportunity to self-administer both cocaine and methohexital on previous occasions. The four subjects in each group were consistent with respect to their drug and dose-appropriate behavioral and endocrinological responses to each of the test drugs. It is unlikely that differences in drug history affected the results of the present study since subjects were randomly assigned for testing with each of the drugs.

Self-administration of fentanyl reduced both ACTH and cortisol secretion, and this effect persisted for several hours after the session had ended. There appeared to be a floor to this effect, however, as intakes exceeding 19 μg/kg produced similar reductions in the release of both hormones. Tolerance and dependence to fentanyl were unlikely to have developed during this study. The doses that were available were small, and the drug sessions were alternated with saline on a regular basis (saline was available during approximately 50% of the sessions). It has been demonstrated in our laboratory that a dose of 0.04 mg/kg fentanyl given every 6 h produces dependence after a month (J. Woods, personal communication). In our study, monkeys earned between 0.04 and 0.08 mg/kg fentanyl only when the largest dose (3.0 ug/kg per injection) was available, but this dose was self-administered only once every 24 h during the weeks in which it was being tested, with saline available during the remaining session each day. While no comparable study has been carried out in non-human primates, our finding that a μ-opioid agonist such as fentanyl inhibits the release of ACTH and cortisol is consistent with work published in humans (Gaillard et al. 1981; Rittmaster et al. 1985; Allolio et al. 1986; Garland and Zis 1989). For example, intravenous morphine blunted the effect of intravenous oCRH in humans (Rittmaster et al. 1985), and an intramuscular injection of the enkephalin analog, FK 33-824, attenuated the stimulatory effect of h/rCRH on ACTH, β-endorphin, and cortisol secretion in humans (Allolio et al. 1986). In work done using rodents, there is general agreement that acute administration of opioid agonists in vivo increases rather than decreases ACTH and corticosterone (e.g. Hayes and Stewart 1985). To date, there have been no studies in rats that describe the HPA effects of doses of opioids that reinforce behavior. On the basis of our findings in the present study, we would predict that small opioid doses that serve as reinforcers in the rat would fail to stimulate plasma concentrations of ACTH and corticosterone.

Cocaine stimulated ACTH and cortisol release at the two highest doses, both of which were contained in the descending limb of the dose–response function. This stimulation occurred following total drug intakes in excess of 1.8 mg/kg. This finding is similar to what we have published previously using a different schedule of reinforcement (Broadbear et al. 1999a). Another interesting similarity between our present and previous studies is that both studies feature doses of cocaine that maintained self-administration behavior yet did not result in significant activation of the HPA axis. In the present study, 0.01 mg/kg per injection cocaine maintained the highest rates of responding but did not differ from saline in its effects on ACTH and cortisol. Total cocaine intake at this dose was 1.1±0.18 mg/kg. Activation of the HPA axis by cocaine has also been reported in rats (Moldow and Fischman 1987; Rivier and Vale 1987; Galici et al. 2000) and in human subjects (Mendelson et al. 1989; Vescovi et al. 1992; Heesch et al. 1995). Cocaine has been shown to cause the release of CRH in hypothalamic cells in vitro (Calogero et al. 1989). While there is evidence supporting the potential importance of HPA hormone feedback during cocaine self-administration in rats (Shaham et al. 1998; Goeders and Guerin 2000), few studies have attempted to address the role of HPA hormones in rhesus monkeys and humans. In the monkey, it has been shown that acute pharmacological antagonism of cortisol synthesis as well as antagonism of CRH at the level of the pituitary did nothing to disrupt ongoing self-administration of cocaine (Broadbear et al. 1999b). Similarly, pretreatment with ketoconazole, a cortisol synthesis inhibitor, prior to administration of cocaine did not diminish the subjective reinforcing effects of cocaine in experienced users (Ward et al. 1998). Studies designed to address the role of HPA axis hormones in the acquisition or reinstatement of cocaine-maintained behavior in humans or non-human primates have yet to be conducted.

Ketamine, at doses that maintained responding, inhibited ACTH and cortisol secretion. This inhibition appeared maximal at intakes exceeding 4.5 mg/kg. As with fentanyl, the low basal levels of ACTH and cortisol prior to the session may have precluded the measurement of further decreases as intake increased. There are some data in rhesus monkeys (Setchell et al. 1975; Elvidge et al. 1976) and humans (Adams et al. 1992) with which to compare our findings. In these studies, the HPA axis effects of large doses of ketamine or phencyclidine, sufficient to produce anesthetization, were reported. Anesthetic doses and self-administered intake of ketamine are remarkably similar. The average intake of ketamine at the largest dose (0.3 mg/kg per injection) was in the vicinity of 14 mg/kg in the present study. A single injection of 10 mg/kg ketamine is sufficient to produce anesthesia in rhesus monkeys (e.g. present study). In the study by Setchell et al. (1975), a fall in cortisol was measured in all subjects during the first 60 min after phencyclidine injection, which is consistent with the findings of the present study. It is only after a more prolonged period of anesthetization (and additional dosing with phencyclidine) that the cortisol levels began to increase. It is therefore possible that drug dose and duration of action are important determinants in the effects that NMDA antagonists have on ACTH and cortisol secretion.

Although data from this study indicate that different drugs of abuse may have opposing effects on HPA axis activity, they do not exclude the possibility that interactions with the HPA axis still do underlie some aspects of the rewarding properties of these drugs (for instance, to promote normalization of the HPA axis during or following the application of a stressor). There is also the possibility that drug-associated reinforcement may correlate with the activation of ascending CRH pathways to brain reward areas such as the nucleus accumbens (Lu et al. 2003), and this activation may not be reflected by a corresponding rise in pituitary–adrenal hormones. Although answering this question is beyond the scope of the present study, the lack of evidence for HPA activation in the peripheral circulation does not discount a possible role for CRH in the central nervous system. Our findings in rhesus monkeys do, however, undermine the premise that a stimulation of the HPA axis is necessary for mediating drug reward (Sarnyai et al. 2001). Clearly, two of the three drugs tested in this study attenuated rather than stimulated basal ACTH and cortisol secretion over a range of self-administered doses. In the primate, as well as the rat, drug-taking history may be critical with respect to the role of the HPA axis in the reinforcement of drug-taking behavior. The case for critical involvement of the HPA axis is far stronger with respect to acquisition and reinstatement of self-administration behavior in rats (e.g. Piazza and le Moal 1996; Shaham et al. 1998). Since no one has addressed these aspects of drug-maintained behavior in primates, our conclusion that there is no apparent role for the HPA axis in the self-administration of drugs of abuse may only apply to the case in which drug-maintained behavior is already established and remains stable over time.

The present study takes our understanding of reinforcement and HPA axis activation to the next level with the addition of fentanyl and ketamine data to our current and earlier work with cocaine. In contrast to cocaine, both fentanyl and ketamine inhibited the HPA axis, which makes a simple relationship between the stress axis and substance abuse unlikely. As with cocaine, the intake of fentanyl at doses that maintained peak levels of responding did not produce a significant change in either ACTH or cortisol. It was only the higher doses on the descending limb of the dose–response function that produced significant change. This suggests a connection between the rate-limiting properties of each drug and a change in ACTH and cortisol release, but in terms of reinforcement, there is no change in HPA axis activity that appears to be a requirement for the self-administration of these mechanistically different drugs of abuse.

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