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ORIGINAL INVESTIGATION

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# Self-administration of methohexital, midazolam and ethanol: effects on the pituitary–adrenal axis in rhesus monkeys

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**Abstract** *Rationale:* There is disagreement in the literature with respect to how drugs of abuse affect the functioning of the hypothalamic–pituitary–adrenal (HPA) axis, and whether these changes in endocrine function may be related to the rewarding effects of these drugs. *Objectives:* To determine whether reinforcing drugs with different mechanisms of action affect HPA axis function at doses at which they serve as reinforcers. *Methods:* Seven monkeys (6 male) were randomly assigned to self-administer methohexital—a barbiturate ($n$=4), midazolam—a benzodiazepine ($n$=3), or ethanol ($n$=5). Each monkey had a surgically implanted indwelling venous catheter, and was trained to respond on a fixed ratio of 30 lever presses to receive an injection of drug or saline. Blood samples were obtained before, during, and after the self-administration sessions for the measurement of ACTH and cortisol by radioimmunoassay. *Results:* Although methohexital, midazolam, and ethanol all maintained self-administration behavior across a range of doses, they differed in their effects on ACTH and cortisol. Ethanol inhibited ACTH and cortisol secretion. Methohexital and midazolam both tended to decrease ACTH and cortisol at large doses, and increase these hormones at small doses, but the HPA effects of neither drug differed significantly from when saline was available. *Conclusions:* The neutral overall effect of methohexital and midazolam on HPA activity is consistent with other monkey and human studies, whereas the inhibitory effect of self-administered ethanol in the monkey contrasts with both the rat and human literature. The data in this study suggest that a change in HPA axis activity is not a requirement for drug-reinforced behavior in monkeys.

**Keywords** Rhesus monkey (*Macaca mulatta*) · Methohexital · Midazolam · Ethanol · Self-administration · Adrenocorticotropin · Cortisol · Reinforcement · Behavior

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## Introduction

Some drugs of abuse have a propensity for stimulating the hypothalamic–pituitary–adrenal (HPA) axis, increasing the secretion of the "stress hormones", adrenocorticotropin (ACTH) and cortisol, into the circulation. This stimulatory effect has been consistently measured following the administration of psychomotor stimulants such as cocaine in rat (Galici et al. 2000), rhesus monkey (Broadbear et al. 1999) and human studies (Mendelson et al. 2002). Although the effects of other classes of abused drugs on endocrine endpoints have also been studied, the literature contains fundamental points of disagreement. These differences may have their origin in species differences, or may have more to do with the experimental procedures that were employed. More research is needed to address these issues and enable meaningful comparisons of the HPA effects of a wider range of drugs of abuse to be made.

Barbiturates are readily self-administered by mice (Carney et al. 1991), rats (Ator and Griffiths 1987), rhesus monkeys (Winger et al. 1975; Vanover et al. 1989), baboons (Griffiths et al. 1991) and humans (Griffiths et al. 1979). A number of studies have demonstrated the reinforcing effectiveness of a wide range of barbiturates, but none have characterized the effects of self-administered barbiturates on pituitary–adrenal function. Barbiturates are thought to exert their effects by binding at a site

on the γ-amino-butyric acid ($GABA_A$) receptor complex and enhancing the inhibitory actions of GABA (Mehta and Ticku 1999). There is evidence that GABA is a potent inhibitor of CRH secretion in vivo as measured by its inhibition of ACTH release (Makara and Stark 1974). Intracerebroventricular administration of GABA to rats inhibits the release of CRH from the hypothalamus into portal blood (Plotsky et al. 1987). There is evidence that treatment with an anesthetizing dose of a barbiturate does not affect basal HPA axis activity (Buckingham 1984; D'Haese and Camu 1993) or interfere with subsequent stimulation of the HPA axis by exogenous administration of either corticotropin (ACTH; Barrett and Stockham 1965; Borner et al. 1985) or CRH (Broadbear et al. 2004a). However, other studies report that although barbiturates themselves may not affect HPA axis activity, they can attenuate the stimulatory effect of other treatments such as ether (Greer and Rockie 1968) or morphine injection (Buckingham 1984) on HPA measures.

Although benzodiazepines serve as reinforcers in rats, monkeys and humans (see review by Ator and Griffiths 1987), they maintain lower rates of responding than do barbiturates (Yanagita and Takahashi 1973); short-acting benzodiazepines maintain the highest rates of responding among the benzodiazepines (Griffiths et al. 1981). Like barbiturates, benzodiazepines produce their effects at the $GABA_A$ receptor complex, although at a distinct benzodiazepine-binding site (Mehta and Ticku 1999). Although the effect of benzodiazepines on the HPA axis in rats appears to be dependent on the dose that is used (Pericic et al. 1984; Kalman et al. 1997), their effects on both monkey (Kalogeras et al. 1990) and human ACTH and cortisol secretion appear to be inhibitory (Charney et al. 1986). For example, prior treatment with temazepam significantly attenuates the stimulatory effects of exogenous CRH on ACTH and cortisol secretion in humans (Korbonits et al. 1995).

Ethanol also serves as a reinforcer in rhesus monkeys by both the intravenous (IV; Williams et al. 2004) and oral routes (Williams et al. 1998). Although ethanol's mechanism of action continues to be debated, the similarity of its effects to those of the barbiturates and benzodiazepines suggest that the $GABA_A$ receptor may also be sensitive to the actions of ethanol (Davies 2003). When ethanol is administered intraperitoneally to unanesthetized, freely moving rats, it produces an increase in plasma ACTH and corticosterone that is blocked by pretreatment with CRH-antiserum (Rivier et al. 1984). In ewes also, intravenous infusion of different doses of ethanol results in a somewhat dose-dependent stimulation of ACTH and cortisol (Cudd et al. 1996). Studies in humans variously report that ingestion of ethanol results in no effect (Stott et al. 1987; Ida et al. 1992) or in stimulation of the HPA axis (Schuckit et al. 1987; Lukas and Mendelson 1988; Schuckit et al. 1988; McCaul et al. 2001).

This study was designed to investigate whether sedative drugs, at doses that are self-administered, affect HPA axis activity in rhesus monkeys. Specifically, we have attempted to characterize the relationship between drug (methohexital, midazolam, or ethanol) intake and changes in basal ACTH and cortisol secretion. On the basis of evidence already published, it was predicted that methohexital and ethanol would fail to alter HPA axis activity, and that midazolam would dose-dependently decrease ACTH and cortisol levels.

## Materials and methods

### Subjects

Six adult male rhesus monkeys (*Macaca mulatta*), weighing between 9.8 kg 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 drug, including cocaine and methohexital. The monkeys were randomly assigned to the three drug groups; methohexital ($n$=4), midazolam ($n$=3) and ethanol ($n$=5). One monkey (3030) was tested with all three drugs, one monkey (3147) was tested with both methohexital and ethanol, one monkey (3596) was tested with both midazolam and methohexital, and one monkey (3151) was tested with both midazolam and ethanol. The remaining monkeys were tested with either ethanol (2489, 2487 female) or methohexital (monkey 2595).

### Apparatus

Each individually housed monkey had a surgically placed indwelling venous catheter in a femoral, internal, or external jugular vein. Housing and surgical details can be found in Broadbear et al. (2004b). 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 using IBM/PS2 computers located in an adjacent room. The computers were programmed using Med Associates software (Georgia, Vt., USA).

### Procedure

The procedure used in this study has been described previously (Broadbear et al. 2004b). 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), with

saline sessions randomly interspersed between drug sessions. The reinforcing effectiveness of and stress hormone response to methohexital (0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg/inj), midazolam (0.001, 0.003, 0.01, and 0.03 mg/kg/inj), and ethanol (0.003, 0.01, 0.03, and 0.1 g/kg/inj) were evaluated. Drug or saline delivery was contingent on each monkey emitting a fixed ratio of 30 lever presses (FR=30). A 1-ml aliquot of each drug concentration or saline was injected every 5 s. With the exception of ethanol, the duration of each drug injection was 5 s. Dose of ethanol (30%, w/v concentration) was regulated by varying the injection duration, which ranged from 2 s to 35 s depending on the required dose and the weight of the subject. There was a 10-s time out (TO) 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 during test sessions. A single dose of drug or saline was available each session (two sessions per day). During test sessions, blood was sampled before, during and after the session on at least two occasions for each drug dose as well as for saline. When changing to a different dose of the same drug, the new dose was made available during self-administration sessions, with blood sampling only resuming once the response stability criterion was reached (see below). The self-administration behavior of the four monkeys that were tested with more than one drug was evaluated for at least a week after switching from one drug to another, and testing did not commence until responding for the new drug had stabilized. A stable baseline of self-administration behavior was defined as consistency (<15% variability in the number of injection earned) across three sessions in responding for the drug dose of interest. The order of presentation of the drug doses was varied randomly. Blood was sampled for the measurement of ACTH and cortisol at 15 min 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. The collection, handling and storage procedures that were used have been described previously (Broadbear et al. 2004b). 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 μg/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 morning sessions 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 that the red light was illuminated. Data were averaged across subjects and plotted against each dose of each drug along with the standard error of the mean (SEM). The data were analyzed for dose dependency and differences across the sampling time.

ACTH and cortisol levels that were measured in plasma during saline, methohexital, midazolam, and ethanol self-administration are presented as mean±SEM. The effects of saline self-administration on ACTH and cortisol secretion among the drug-taking groups were compared, prior to comparisons of the HPA effects of saline and each drug dose within each of the drug-taking groups. 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 samples 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 prior to and subsequent to transformation to area under curve (AUC) calculation. AUC values are an estimate of the total ACTH (pg min/ml) and cortisol (μg min/dl) released during the session relative to pre-session levels. AUC values were calculated according to the trapezoidal rule (e.g. Tallarida and Murray 1987). Plasma ACTH and cortisol AUCs were analyzed for dose-related drug effects on HPA axis hormones.

Analysis of variance (ANOVA) 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

Methohexital was purchased from Ace Surgical Supplies (Brockton, Mass., USA) and diluted with sterile water. Midazolam (50 mg/10 ml) was purchased from Abbott Laboratories (N. Chicago, Ill., USA). Ethanol (95%, 190% proof) was purchased from Pharmco (Brookfield, Conn., USA). Methohexital and midazolam dilutions were made

using sterile saline. Ethanol (95%) was diluted in saline to make a 30% (w/v) solution.

## Results

*Saline* ACTH and cortisol secretion was compared during the sessions in which saline was available for self-administration by three groups of monkeys that alternatively had access to one of methohexital, midazolam or ethanol, with "group" as the between-subjects factor. The secretory patterns of ACTH and cortisol when saline was available did not differ among the three groups, although there was an effect of sampling time [ACTH: $F(7,63)=6.72$, $P<0.05$; cortisol: $F(7,63)=5.83$, $P<0.05$] which indicated that ACTH and cortisol levels for all three groups were elevated at the 130, 165, 200 and 260 min time points relative to the 25, 60, and 320 min sampling times. In addition, the secretory pattern of cortisol for the three drug groups differed as indicated by a group–sampling time interaction [$F(14,63)=2.13$, $P<0.05$]. During saline availability, the rise in cortisol levels in monkeys in the midazolam group was smaller than those measured in the methohexital and ethanol groups during saline availability.

*Methohexital* Responding for methohexital generated a bell-shaped function, with a significant effect of dose [$F(5,10)=58.02$, $P<0.05$]. A maximum response rate of 0.46 ±0.03 responses/s was maintained by injections of 0.1 mg/kg/inj methohexital. Total intake of methohexital increased as a function of the available dose, with peak intake ranging from 17.0 mg/kg to 25.3 mg/kg when the largest dose (1.0 mg/kg/inj) of methohexital was available (Fig. 1A).

Baseline ACTH and cortisol levels prior to sessions during which methohexital was tested were 6.6±0.60 pg/ml and 13.7±0.74 μg/dl, respectively. Self-administration of methohexital did not significantly affect the secretion of ACTH or cortisol relative to saline during the session. However, there was a tendency for the smaller methohexital doses to increase and for the larger doses to suppress ACTH secretion [$F(35,105)=2.24$, $P<0.05$; Fig. 1B,C]. There was some evidence for a rebound effect, as there was a trend towards an increase in ACTH and cortisol levels, relative to saline and the smaller doses, following sessions in which 0.3 mg/kg/inj and 1.0 mg/kg/inj methohexital were available (Fig. 1B,C). When the cumulative release of both cortisol and ACTH during the self-administration session was examined, the larger doses appeared to suppress hormone secretion relative to the smaller doses [ACTH: $F(5,15)=9.40$, $P<0.05$; cortisol: $F(5,15)=4.15$, $P<0.05$; Fig. 1D]. In the case of ACTH, 1.0 mg/kg/inj methohexital resulted in reduced ACTH levels relative to when 0.01 mg/kg/inj methohexital was available ($P<0.05$). Similarly for cortisol, self-administra-

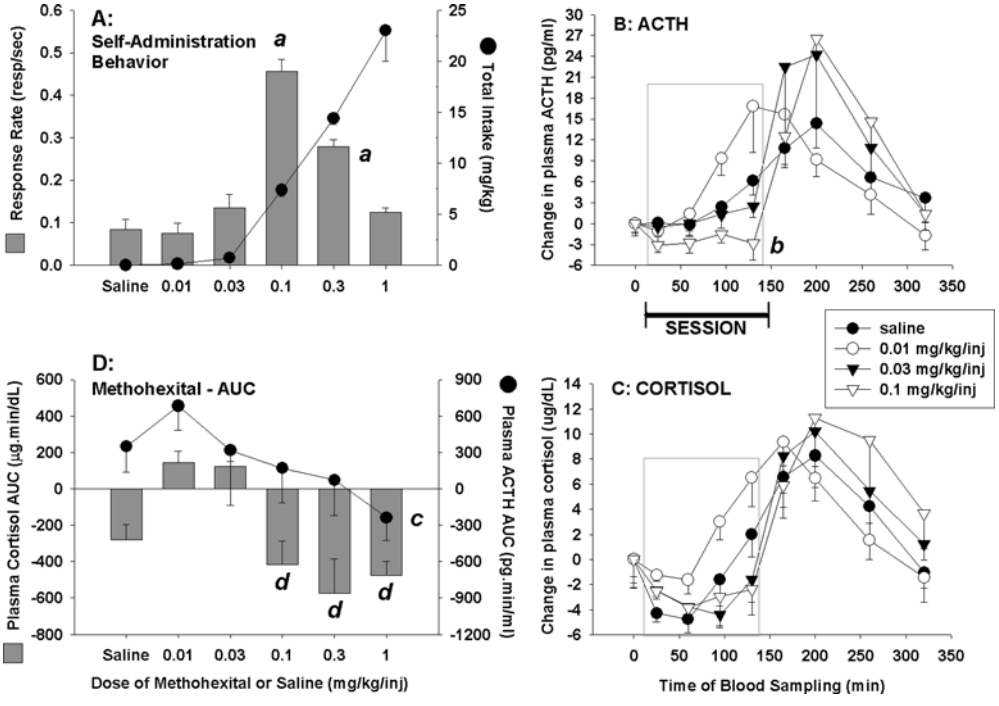


**Fig. 1** **A** Response rate (responses/s) and drug intake (mg/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of the barbiturate, methohexital (mg/kg/inj) or saline ($n$=4). **B**, **C** Change in plasma concentrations of ACTH (**B**; baseline = 6.6±0.60 pg/ml) and cortisol (**D**; baseline = 13.7±0.74 μg/dl) measured in venous blood samples obtained before, during (*in box*) and after sessions during which saline or methohexital was available for self-administration. **D** Cumulative release of ACTH (pg min/ml) and cortisol (μg min/dl) during self-administration of methohexital 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 (saline), 0.01, 0.03 and 1.0 mg/kg/inj methohexital, *b* ACTH levels significantly different from 0.01 mg/kg/inj and 0.03 mg/kg/inj methohexital (not shown), *c* ACTH levels significantly different from 0.01 mg/kg/inj methohexital, *d* cortisol levels significantly different from 0.01 mg/kg/inj methohexital

tion of 0.01 mg/kg/inj and 0.03 mg/kg/inj methohexital resulted in an increase in cortisol secretion relative to when 0.1, 0.3 and 1.0 mg/kg/inj methohexital were available ($P<0.05$).

*Midazolam* Responding for midazolam also generated a bell-shaped function, with a significant effect of dose [$F(4,8)=13.05$, $P<0.05$]. A maximum response rate of 0.15 ±0.02 responses/s was maintained by injections of 0.01 mg/kg/inj midazolam. Total intake of midazolam increased as a function of the available dose, with peak intake ranging from 0.62 mg/kg to 0.87 mg/kg when the largest dose (0.03 mg/kg/inj) of midazolam was available (Fig. 2A).

Baseline ACTH and cortisol levels prior to sessions during which midazolam was tested were 5.9±0.87 pg/ml and 11.0±0.57 μg/dl, respectively. There was a significant effect of midazolam dose across sampling times; smaller doses increased, and larger doses decreased ACTH concentrations during the session [$F(4,8)=5.16$, $P<0.05$]. The smallest dose of midazolam (0.001 mg/kg/inj) increased ACTH secretion relative to when the largest dose (0.03 mg/kg/inj) of midazolam was available ($P<0.05$; Fig. 2B). There was a similar trend for the effect of midazolam on cortisol, with the smallest dose producing a higher concentration of cortisol than did the largest dose ($P<0.05$), but neither dose differed significantly from saline in its effect on cortisol (Fig. 2C). When the cumulative release of both ACTH and cortisol during the self-administration session were examined, the AUC for both hormones decreased as the dose of midazolam increased [ACTH: $F(4,8)=10.97$, $P<0.05$; cortisol: $F(4,8)=3.53$, $P<0.05$; Fig. 2D]. This decrease in cumulative cortisol and ACTH secretion was significant relative to the stimulatory effect of the smaller doses of midazolam but not to the ACTH and cortisol responses when saline was available.

*Ethanol* Responding for ethanol generated a bell-shaped function, with a significant effect of dose [$F(4,12)=5.72$, $P<0.05$]. A maximum response rate of 0.45 ±0.08 responses/s was maintained by injections of 0.03 g/kg/inj ethanol. Total intake of ethanol increased as a function of the available dose, with peak intake ranging from 1.2 g/kg to 3.0 g/kg when the largest dose (0.1 g/kg/inj) of ethanol was available (Fig. 3A).

Baseline ACTH and cortisol levels prior to sessions during which ethanol was tested were 6.0±0.41 pg/ml and 12.1±0.58 μg/dl, respectively. ACTH and cortisol secretion decreased during self-administration of 0.01 (cortisol only), 0.03 g/kg/inj and 0.1 g/kg/inj ethanol [ACTH: $F(4,12)=4.16$, $P<0.05$; Fig. 3B, and cortisol: $F(4,12)=13.91$, $P<0.05$; Fig. 3C] relative to when saline, or 0.003 g/kg/inj ethanol (in the case of cortisol) were available for

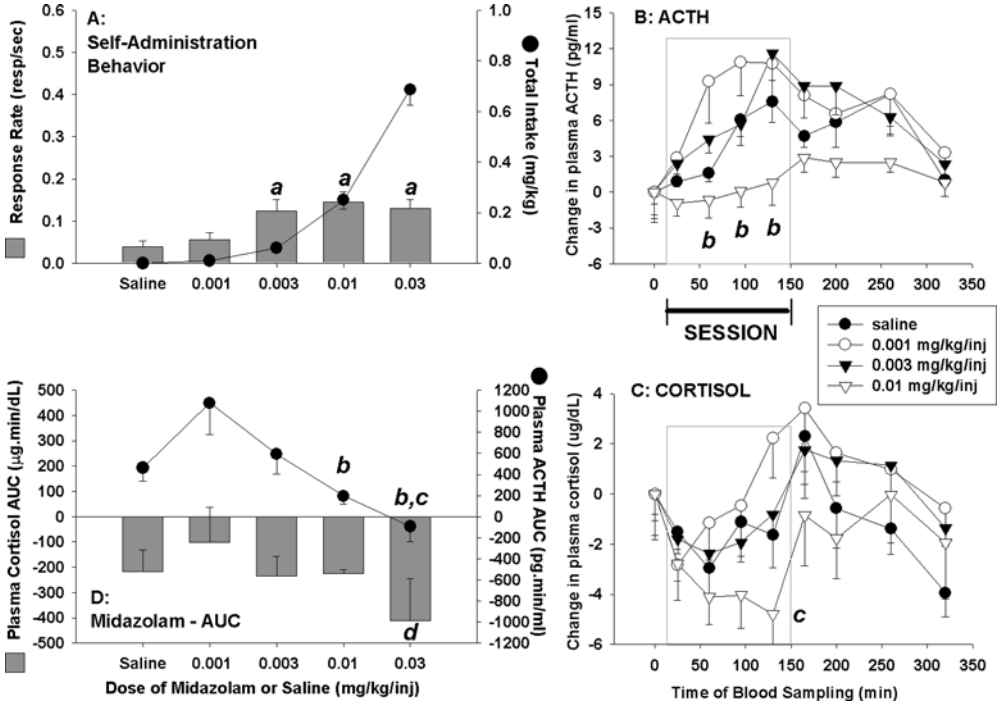


**Fig. 2** **A** Response rate (responses/s) and drug intake (mg/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of the benzodiazepine, midazolam (mg/kg/inj) or saline ($n=3$). **B**, **C** Change in plasma concentrations of ACTH (**B**; baseline = 5.9±0.87 pg/ml) and cortisol (**C**; baseline=11.0±0.57 μg/dl) measured in venous blood samples obtained before, during (*in box*) and after sessions during which saline or midazolam was available for self-administration. **D** Cumulative release of ACTH (pg min/ml) and cortisol (μg min/dl) during self-administration of midazolam or saline. Key to symbols: *a* Rates of responding significantly different from 0 (saline) and 0.001 mg/kg/inj midazolam, *b* ACTH response significantly different from 0.001 mg/kg/inj midazolam, *c* ACTH and cortisol levels significantly different from 0.003 mg/kg/inj midazolam, *d* Cortisol response significantly different from 0.001 mg/kg/inj midazolam

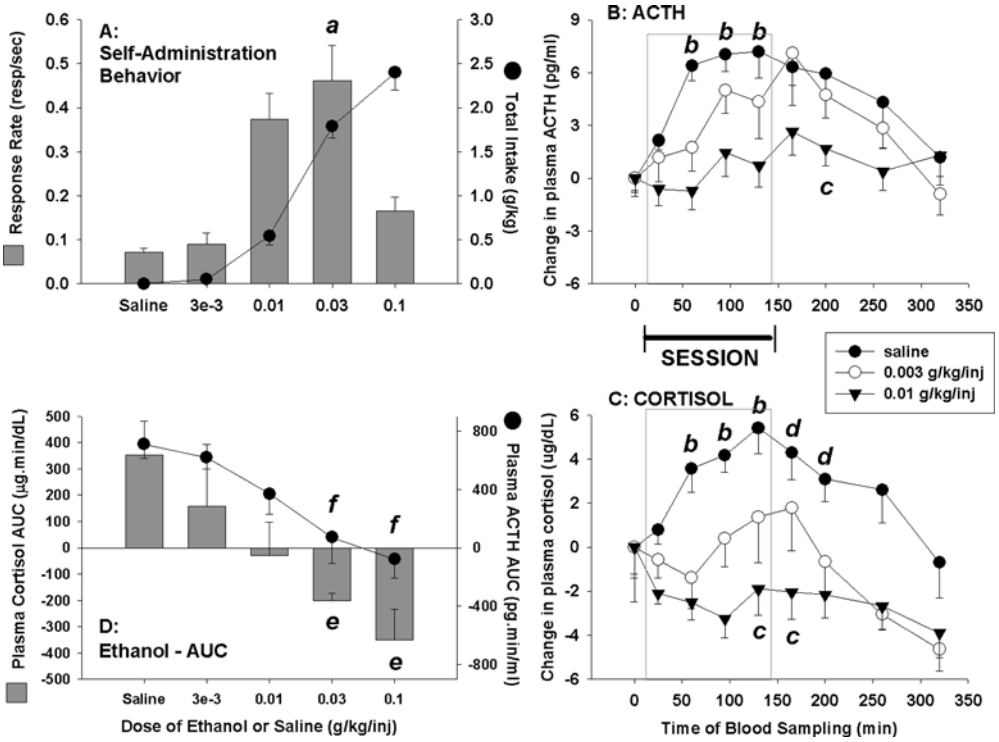


**Fig. 3** **A** Response rate (responses/s) and drug intake (g/kg) for monkeys trained to lever press on a fixed ratio of 30 responses for each injection of ethanol (g/kg/inj) or saline ($n$=5). **B**, **C** Change in plasma concentration of ACTH (**B**; baseline=6.0±0.41 pg/ml) and cortisol (**C**; baseline=12.1±0.58 µg/dl) measured in venous blood samples obtained before, during (*in box*) and after sessions during which saline or ethanol was available for self-administration. **D** Cumulative release of ACTH (pg min/ml) and cortisol (µg min/dl) during self-administration of ethanol or saline. Key to symbols: *a* Response rate significantly different from saline, 0.003 g/kg/inj and 0.1 g/kg/inj ethanol, *b* ACTH and cortisol levels significantly different from 0.03 g/kg/inj (not shown) and 0.1 g/kg/inj ethanol, *c* ACTH and cortisol levels for 0.003 g/kg/inj ethanol (not shown) significantly different from 0.1 g/kg/inj ethanol, *d* Cortisol levels for saline significantly different from 0.1 g/kg/inj ethanol, *e* Cortisol levels significantly different from 0 (saline), (f) ACTH levels significantly different from 0 (saline) and 0.003 g/kg/inj ethanol

injection. This ethanol-related inhibition of ACTH and cortisol secretion continued for several hours following the end of the session ($P$<0.05). When the cumulative release of both cortisol and ACTH during the self-administration session was examined, the AUC for both hormones diminished during self-administration of larger doses of ethanol [ACTH: $F(4,12)=20.33$, $P$<0.05; cortisol: $F(4,12)=5.15$, $P$<0.05; Fig. 3D]. Both 0.03 g/kg/inj and 0.1 g/kg/inj ethanol attenuated ACTH release relative to when saline, 0.003 g/kg/inj or 0.01 g/kg/inj ethanol were available ($P$<0.05). Self-administration of 0.03 g/kg/inj and 0.1 g/kg/inj ethanol resulted in decreases in cortisol release relative to when saline was available ($P$<0.05).

## Discussion

In this study, we measured the effects of self-administered methohexital, midazolam or ethanol on the secretion of ACTH and cortisol in rhesus monkeys. The pre-session (basal) ACTH and cortisol levels were low as previously reported (Broadbear et al. 1999), indicating that the monkeys were habituated to their surroundings and to the experimental procedure. Each of these drugs maintained behavior across a range of doses, as evident from the bell-shaped curves generated in response to drug availability. The predictions regarding the effects of drug intake on HPA axis activity were not supported by the results of this study. Originally it was postulated that midazolam would attenuate HPA axis activity, and that neither methohexital nor ethanol would change ACTH and cortisol secretion relative to when saline was available. Instead, for both methohexital and midazolam, self-administration of small doses stimulated ACTH and cortisol relative to the apparent attenuation of secretion that was observed with self-administration of the larger doses. Neither the small nor the large doses differed significantly from saline in their effects on ACTH and cortisol. Conversely, at the doses self-administered in this study, ethanol dose-dependently inhibited the secretion of ACTH and cortisol. The biphasic changes in hormone secretion measured during saline self-administration are likely to be a consequence of diurnal variation as they also occur in the absence of the self-administration session (Broadbear et al. 1999).

Our finding that self-administration of methohexital had no effect on HPA axis activity relative to when saline was available is consistent with other reports of a neutral effect of larger, anesthetizing doses of barbiturates on stress axis hormones (Buckingham 1984; D'Haese and Camu 1993). During the course of other studies in this laboratory, we examined the effect of brief anesthetization with metho-

hexital on the subsequent HPA stimulation produced by intravenous administration of CRH in rhesus monkeys. Prior treatment with methohexital had no effect on the ACTH or cortisol response following the CRH challenge (Broadbear et al. 2004a). However, the differences in HPA response when small and large doses of methohexital were available for self-administration were unexpected. Rates of responding maintained by the small doses did not differ from responding maintained by saline; these doses increased stress hormone levels but not enough for statistical significance. On the other hand, larger doses that did maintain self-administration behavior produced an attenuation of ACTH and cortisol secretion. Intake of methohexital equal to or greater than 7.5 mg/kg corresponded to a reduction in pituitary–adrenal activity relative to when smaller doses were available. Unlike earlier studies, in which the HPA effects of single bolus doses of barbiturates have been examined (Buckingham 1984; D'Haese and Camu 1993), this study examined a range of doses, none of which was sufficient to produce complete anesthetization. Thus, the contrast between the effects of small and large doses on ACTH and cortisol secretion has not been previously reported.

Although no dose of midazolam, a short-acting benzodiazepine, differed from saline in its effects on the HPA axis in the present study, the large and small doses of midazolam also varied from one another in their effects on ACTH and cortisol secretion. As with methohexital, the smallest dose of midazolam appeared to stimulate ACTH and cortisol secretion relative to when the largest dose was self-administered. Dose-related differences in the effect of benzodiazepines in rats have been reported, although they are opposite from what we report here. In rats, small doses of diazepam decreased HPA activity (Pericic et al. 1984), whereas larger doses stimulated HPA hormone secretion (Kalman et al. 1997). In vitro studies using rat hypothalami showed that alprazolam inhibited CRH release via a serotonergic mechanism (Kalogeras et al. 1990), a result that is consistent with the in vivo findings using small doses of benzodiazepines in rats. Alprazolam in monkeys (Kalogeras et al. 1990), and temazepam (Beary et al. 1983) and alprazolam (Charney et al. 1986) in humans, tended to decrease basal levels of cortisol and ACTH as was found in the present study. The experimental procedure and findings of both the present study and that of Kalogeras et al. (1990) are similar. In both studies, monkeys with indwelling catheters were tested in their home cages. Kalogeras et al. (1990) measured a clearly dose-dependent reduction in ACTH and cortisol following IV alprazolam administration, although alprazolam was administered as a single bolus dose, as opposed to the pattern of self-injected doses taken by the monkeys in the present study.

In addition to reducing basal HPA activity, treatment with benzodiazepines has been shown to attenuate the HPA response to subsequent challenges such as insulin-induced hypoglycemia in monkeys (VanVugt et al. 1997), and exogenous CRH (Korbonits et al. 1995) and a "mental stress task" in humans (Rohrer et al. 1994). In the latter study, Rohrer and colleagues found that although treatment with alprazolam blunted the HPA response to a stressful task, it did not attenuate the HPA response to exogenous CRH. They concluded that alprazolam modulated CRH secretion rather than pituitary responsiveness to CRH. In contrast to the inhibitory modulation of the HPA axis by benzodiazepine agonists, the opposite effect could be measured following administration of inverse agonists at the benzodiazepine receptor. Drugs such as β-carboline-3-carboxylic acid ethyl ester (β-CCE) have been shown to stimulate the HPA axis in rhesus monkeys (Insel et al. 1984; Takada et al. 1986; unpublished work from our laboratory), further evidence that modulation of the HPA axis by benzodiazepines is a receptor-based phenomenon.

Ethanol had a clearly inhibitory effect on ACTH and cortisol secretion in the present study, as larger doses decreased both hormones relative to smaller doses as well as to saline. Although AUC cortisol levels appeared higher when saline was available to monkeys in the ethanol self-administration group, statistical analysis of the data at each sampling time found no significant difference in the ACTH or cortisol responses to saline in the monkeys in any of the three drug groups. Despite this, it is possible that when the drug being tested (ethanol in this case) has a tendency to inhibit basal stress hormone release, a conditioned, compensatory rise in hormone secretion may be revealed when saline is substituted (i.e. when drug is absent) during testing. A similar effect was also observed during the self-administration of fentanyl, another drug that inhibited stress hormone secretion, when fentanyl was tested under the same experimental conditions (Broadbear et al. 2004b).

We recently published a study evaluating the behavioral and hormonal effects of intravenously self-administered ethanol (0.03 g/kg/inj) following pretreatment with the opioid antagonist, naltrexone, in rhesus monkeys (Williams et al. 2004). Although the ethanol intake was similar to what is reported for this dose in the current study, ACTH secretion did not differ significantly from when saline was available. However, even though pretreatment with naltrexone reduced ethanol intake from 1.9 g/kg to 0.3 g/kg, this intake of ethanol was sufficient to attenuate the stimulatory effect of the antagonist on ACTH levels, suggesting that ethanol had an inhibitory effect on the naltrexone-activated HPA axis (Williams et al. 2004; see also Cami et al. 1988), which is consistent with the inhibitory effect of ethanol on basal hormone levels reported in the present study.

The human literature generally describes ethanol as having a stimulatory or neutral effect on HPA axis activity. The maximum intake of intravenous ethanol in the present study (2.4±0.2 g/kg when 0.1 g/kg/inj was available for self-administration), was approximately double the amount of ethanol ingested orally in the human studies. The range of doses tested in humans was 0.7 g/kg (Lukas and Mendelson 1988) to 1.0 g/kg or slightly higher (Stott et al. 1987; Ida et al. 1992; McCaul et al. 2001). In the majority of human studies, ethanol was consumed orally within a designated time (between 10 min and 60 min). In

the present study, 63% of the total intake of ethanol (0.1 g/kg/inj) was self-administered during the first 25 min component of the session (1.51±0.18 g/kg), and 85% of the total intake was consumed by the end of the second component (60 min after the start of the session), so the pattern of intake of ethanol was similar to the human subject studies. However, there remains the question of how route of administration (oral versus intravenous) may influence the effect of ethanol on the HPA axis. Davis and Jeffcoate (1983) compared the effects of oral (1.0 g/kg) and intravenous ethanol (0.81 g/kg) on cortisol in normal males. However, these comparatively small doses of ethanol had no effect on plasma cortisol in men following administration by either the oral or intravenous route.

Historically, comparisons of the HPA effects of drugs of abuse have been complicated by the myriad of procedural differences between studies. The advantage of the current study is that a variety of drugs were tested under the same experimental conditions. The addition of data for methohexital, midazolam and ethanol to our earlier study in which cocaine, fentanyl and ketamine were examined (Broadbear et al. 2004b), has revealed clear differences in the ways in which different drugs of abuse, at doses that are self-administered, affect basal HPA function in rhesus monkeys. Despite the fact that all six drugs tested in these two studies served as reinforcers, cocaine was the only drug to reliably stimulate ACTH and cortisol release. Fentanyl, ketamine and ethanol, despite their different mechanisms of action, all decreased HPA axis activity at the higher doses. Neither methohexital nor midazolam had a systematic effect on basal HPA axis function.

Therefore it appears that stimulation of the HPA axis may be a unique property of psychomotor stimulants such as cocaine. Other classes of abused drugs do not appear to share a common HPA effect when tested under the same conditions, suggesting that there is a separation between the stress hormone consequences of drug taking and the capacity of a drug to maintain self-administration behavior in rhesus monkeys.

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