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# Glucocorticoid-reinforced responding in the rhesus monkey

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**Abstract** *Rationale:* Glucocorticoids have been reported to have rewarding effects in rats and may lead to drug-seeking behavior in humans under some circumstances. *Objectives:* The present study investigated whether glucocorticoids would be self-administered intravenously by rhesus monkeys (Macaca mulatta). *Methods:* Ten monkeys, 7 male and 3 female, were maintained on a fixed ratio 10 (30 or 100), time-out 10-s schedule for 0.1 mg/kg methohexital or saline injections. Dexamethasone (0.03–0.3 mg/kg), methylprednisolone (0.1–1.0 mg/kg) and hydrocortisone (0.3–3.0 mg/kg) were periodically substituted for methohexital or saline. *Results:* Dexamethasone (0.3 mg/kg) was self-administered by all of the male monkeys on the first, but not on subsequent occasions. It was hypothesized that suppression of hypothalamic–pituitary–adrenal (HPA) activity by these exogenous glucocorticoids following their first presentation may have interfered with their reinforcing effects on subsequent evaluation. Subsequently, plasma adrenocorticotropin and cortisol were measured in four male monkeys to ascertain that normal basal HPA activity had resumed prior to each glucocorticoid substitution. Of the ten monkeys that were tested, only one reliably self-administered dexamethasone, methylprednisolone and hydrocortisone, and he did so regardless of whether his basal HPA activity was suppressed. This monkey differed from some of the other monkeys both behaviorally and in his response to intravenous corticotropin releasing hormone. None of the three female monkeys that were tested with selected glucocorticoid doses showed any evidence of glucocorticoid reinforcement on any occasion. *Conclusions:* The results indicate that glucocorticoids were not reinforcing to the majority of monkeys in this study; nevertheless, large individual differences may exist in proclivity of monkeys to self-inject these compounds.

**Key words** Rhesus monkey · Glucocorticoid · Cortisol · ACTH · Schedule controlled responding · Self-administration · Stress · Dexamethasone · Methylprednisolone · Hydrocortisone

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## Introduction

Corticosterone, the primary glucocorticoid in the rat, has been reported to maintain self-administration behavior via both oral (Deroche et al. 1993) and intravenous routes (Piazza et al. 1993). Plasma corticosterone, measured in rats yoked to those that were self-administering corticosterone, was similar to levels of endogenously released corticosterone after exposure to a stressful stimulus (Piazza et al. 1993). Studies by Piazza and co-workers frequently assigned rats to one of two subgroups on the basis of differences in their responses to various behavioral measures. For instance, rats designated as "high responders" were those for whom lower doses of corticosterone maintained the highest rates of responding relative to the "low responders" (Piazza et al. 1993). As well as showing greater sensitivity to self-administered corticosterone, high responders also showed exaggerated behavioral responses to a novel environment as well as to drugs of abuse relative to the low responders (Piazza et al. 1991, 1993). High-responder rats also had a prolonged release of corticosterone relative to the low responders following hypothalamic–pituitary–adrenal (HPA) axis activation (Piazza et al. 1991). In addition, dopamine levels in the nucleus accumbens of high responders were found to be twice that of low responders following corticosterone administration (Piazza et al. 1996). Intraperitoneally administered dexamethasone also increased dopamine concentrations in the hypothalamus and nucleus accumbens (Rothschild et al. 1985). This is consistent with the observation that dopaminergic

mesocorticolimbic neurons contain glucocorticoid receptors (Harfstrand et al. 1986). It has been suggested that the nucleus accumbens is an important locus for the rewarding effects of glucocorticoids (Piazza et al. 1996), as well as for drugs of abuse (Wise and Bozarth 1987), and a drug's capacity to increase extracellular dopamine levels in the mesolimbic system has been proposed as a correlate of its reinforcing effect (Di Chiara and Imperato 1988).

Apart from being reinforcing in their own right, elevated levels of endogenous corticosterone that follow an acute or chronic stressor, such as exposure to novelty or physical restraint, have been shown to sensitize rats to the psychostimulant and reinforcing effects of amphetamine (Piazza et al. 1990, 1991) and cocaine (Goeders and Guerin 1996b). Indeed, the absence of corticosterone, as a result of either surgical or pharmacological adrenalectomy, can prevent acquisition of cocaine-reinforced responding as well as abolish ongoing self-administration behavior, with the latter being reinstated by restoration of corticosterone levels (Goeders and Guerin 1996a).

In the human literature, it has been reported that, in as many as 50% of cases, the use of large doses of glucocorticoids may initially produce feelings of euphoria, energy, well being, and increased alertness (Hall et al. 1979; von Zerssen 1976). Subjective reports of relief from the illness for which glucocorticoids were prescribed may precede any objective change in the patient's medical condition (Rees 1953; Kimball 1971). In some cases, patients begin to self-medicate with glucocorticoids (Goldberg and Wise 1986), escalating the dose over time to the point where the criteria for a diagnosis of physical or psychological dependence may be met (Kimball 1971; Morgan et al. 1973; Berlinger 1974). Case studies of these patients provide evidence for dependence on glucocorticoids, signs of glucocorticoid withdrawal (Dixon and Christy 1980), denial of glucocorticoid use, drug-seeking behavior and relapse after a period of abstinence (Kimball 1971; Morgan et al. 1973), all of which suggest that prolonged use of glucocorticoids at doses that are sufficient to produce subjective effects has significant abuse potential in susceptible individuals (Dixon and Christy 1980). Despite this accumulation of evidence for potential abuse associated with the use of glucocorticoids, no well-controlled studies in humans have yet been conducted.

The present study was designed to investigate whether glucocorticoids would maintain self-administration behavior in rhesus monkeys. The glucocorticoids that were tested were hydrocortisone (the endogenous glucocorticoid in monkeys and humans) and the synthetic glucocorticoids, methylprednisolone and dexamethasone. In humans, dexamethasone is potent, long-acting and selective for glucocorticoid over mineralocorticoid receptors. Methylprednisolone is somewhat less potent and selective than dexamethasone, and has a shorter half-life. Hydrocortisone is short-acting and non-selective (Berlinger 1974). The effects of these glucocorticoids on mood, adrenocorticotropin (ACTH) suppression and sodium retention (an indication of their mineralocorticoid activity) are summarized in a review by von Zerssen (1976), who described dexamethasone as having a "very marked" effect on mood and ACTH suppression relative to the "marked" or "slight" effects of methylprednisolone and cortisol (hydrocortisone). Psychotropic effects of glucocorticoids are more easily evoked following intramuscular or intravenous injection than after oral administration, indicating that it is probably the rapidity of the change in hormone level rather than the dose per se that leads to CNS effects (Lidz et al. 1952).

Intravenous dexamethasone appeared to be an effective reinforcer in some monkeys, but only on the first exposure (data reported in this paper). Given intramuscularly, dexamethasone (0.5 mg/kg) suppressed basal ACTH and cortisol release in male rhesus monkeys for 3–7 days (Broadbear et al. 1999c). This suggested the possibility that dexamethasone's prolonged suppression of HPA axis activity may have interfered with its reinforcing effectiveness on subsequent exposures. One of the aims of the present study was to explore this hypothesis. Following sessions in which dexamethasone, methylprednisolone and hydrocortisone were available for self-administration, plasma cortisol and ACTH were measured in several monkeys to ascertain whether normal basal HPA activity had resumed prior to the next glucocorticoid substitution. Of the ten monkeys tested, one monkey reliably self-administered all three glucocorticoids regardless of whether his basal HPA activity was suppressed due to earlier glucocorticoid self-administration. The sensitivity of the monkeys' HPA axis to an infusion of corticotropin releasing factor (CRF) was explored in order to determine whether there were differences between the monkey that reported glucocorticoids to be reinforcing and other monkeys that did not, that might place him in the "high-responder" category described above.

## Materials and methods

### Subjects

Seven adult male rhesus monkeys (Macaca mulatta), six intact and one (Monkey 1583) orchiectomized, weighing between 9.0 kg and 14 kg, and three intact female monkeys, weighing between 6.0 kg and 7.5 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. Each monkey was individually housed in a stainless-steel cage measuring 83.3×76.2×91.4-cm (Bryan Research Equipment Corporation, Bryan, Tex.) located in a laboratory that contained a total of 24 similarly housed monkeys. The temperature in the room was maintained at 72°F, and lights were illuminated from 0630 hours until 1930 hours daily. The monkeys were fed 8–12 Purina Monkey Chow biscuits twice daily to maintain normal adult weight, and 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 exited the monkey. The external portion

of the catheter was protected inside the cage by a flexible stainless steel tether, with one end attached to the double layer polyester jacket (Lomir, New York, N.Y.) worn by the monkey and the other end bolted to the rear of the cage.

*HPA response following CRF infusion*

Of the ten monkeys tested in the glucocorticoid self-administration study, three male (RN23, 1583 and 2900) and two female (2487 and 2083) monkeys were tested in the CRF study. At the time this study was conducted, all subjects had at least several months' experience with drug self-administration.

## Apparatus

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 infusion, 1 ml over a period of 5 s. During each 10-s 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.).

## Procedure

*Glucocorticoid self-administration*

Drug self-administration sessions were scheduled twice daily for 130 min, starting at approximately 1000 hours and 1600 hours. Drug delivery was contingent on the monkey emitting the required number of lever presses [fixed ratio (FR)10 ($n$=8), FR30 (monkey 1583) or FR100 (monkey RN23)], and there was a 10-min time out between response opportunities. Differences in the FR values among subjects were based entirely on the response requirement needed to obtain a reliable difference between the number of methohexital and saline injections administered by each monkey. Saline, 0.1-mg/kg methohexital and glucocorticoid drugs were made available for self-administration during different sessions. Saline was substituted on a frequent basis (25–50% of sessions). The subjects in this study had months (monkeys RN23 and 1583) to years (monkeys 2900 and 3577) of experience with methohexital on this schedule. A stable baseline of self-administration behavior in this study was defined as consistency in both response rates and total number of methohexital injections across sessions (variability between sessions ≤10%).

Monkeys were also required to show a reliable decrease in both response rate and injection number during the first session that saline was substituted for methohexital (total saline injections numbering less than 30% of methohexital injections). Three synthetic glucocorticoids were made available for self-administration during the course of this study: dexamethasone (0.03–0.3 mg/kg; Gensia Laboratories Ltd., Irvine, Calif.); methylprednisolone (0.1–1.0 mg/kg; Solu-Medrol, Pharmacia and Upjohn Company, Kalamazoo, Mich.); and hydrocortisone (0.3–3.0 mg/kg; Solu-Cortef, The Upjohn Company, Kalamazoo, Mich.). For the male monkeys, testing began with either dexamethasone (0.3 mg/kg, $n$=3) or hydrocortisone (0.03 mg/kg, $n$=4). Thereafter, the order of dose and glucocorticoid varied randomly. Glucocorticoids were substituted no more than once every 3 days (six sessions). In the four male monkeys for which basal cortisol and ACTH were able to be measured, glucocorticoids were substituted when basal HPA activity had recovered to pre-study levels (usually 3–10 days after a glucocorticoid substitution, depending on the dose and glucocorticoid involved), except when the effects of HPA suppression by prior dexamethasone administration were being evaluated in monkey RN23. All glucocorticoid substitutions were made during the morning session, following one or two saline sessions the previous day. Each glucocorticoid dose was substituted on two or more occasions for each subject until consistency between tests was obtained. Four monkeys (RN23, 2900, 1583 and 0351) had single blood samples taken via their intravenous catheters between 0900 hours and 1000 hours Monday to Friday for the measurement of basal ACTH and cortisol levels.

*HPA response following CRF infusion*

Five monkeys received intravenous CRF (1 µg/kg and 10 µg/kg; human/rat CRF, Calbiochem, La Jolla, Calif.). Testing commenced between 0900 hours and 1000 hours, and blood was sampled at –20 min, –10 min and immediately prior to CRF infusion. Samples continued to be drawn at 10-min intervals until 90 min post-infusion, and then at 2, 2.5, 3 and 4 h.

*Blood collection and handling*

Prior to drawing each blood sample, a 3-cc 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.) containing 0.04 ml of 7.5% ethylene diamine tetraacetic acid (EDTA) and immediately placed on ice. After a blood sample was drawn, 1.5–3 ml of 30-U/ml heparin saline solution was infused into the catheter.

Blood samples were centrifuged at 2860 *g* (Beckman-Coulson JA-21 rotor, 5000 rpm) for 5 min at 4°C and the plasma (0.7 ml) was pipetted into 2-ml Cryovials (Corning) and stored at –80°C until assay. Samples were sent on dry ice to Washington University, Mo., where ACTH and cortisol levels were determined using radioimmunoassay kits (cortisol: Diagnostic Products Corporation, Los Angeles, Calif.; ACTH: Nichols Institute Diagnostics, San Juan Capistrano, Calif.).

## Data analysis

*Glucocorticoid self-administration*

Glucocorticoid self-administration data for the seven male monkeys are presented as raw data or as percentage of methohexital injections, where the average number of methohexital injections taken by each monkey during a session is designated as 100%. This was necessary as the average number of methohexital injections taken by individual monkeys ranged from 63±3 to 113±4. Only the results of the saline and 0.1-mg/kg methohexital tests that occurred immediately prior to each glucocorticoid substitution were used in the data analysis. Mean and individual data are presented for each glucocorticoid dose tested. The data were analyzed for individual differences and dose dependency and, in the case of dexamethasone, for differences in the number of injections taken during the first versus subsequent presentations. The results for the three female monkeys, tested with a more limited range of glucocorticoid doses, are reported in tabular form.

Mean cortisol and ACTH data for four monkeys, representing the time course of changes in basal levels subsequent to dexamethasone substitutions, are also presented. Measurements of basal HPA activity that were obtained on days following infusions of 0.03, 0.1 and 0.3 mg/kg dexamethasone were compared with cortisol and ACTH levels from samples taken on days prior to each test (0 mg/kg dexamethasone). Data for the four monkeys were pooled for each substitution of the different doses of dexamethasone.

*HPA response following CRF infusion*

The raw time course data of the HPA response to CRF infusion were plotted for presentation. However, data were transformed for each subject prior to statistical analysis due to variability in indi-

vidual basal release of ACTH and cortisol, both between and within subjects. This was achieved by averaging the cortisol and ACTH values obtained from samples taken prior to CRF administration and then subtracting these mean values from post-CRF infusion levels. Summary data are shown subsequent to transformation to area under curve (AUC) calculation. AUC values are an estimate of the total cortisol (µg×min/dl) or ACTH (pg×min/ml) release relative to basal levels during the 4-h sampling time following the CRF infusion. AUC values were calculated according to the trapezoidal rule (Tallarida and Murray 1987). The plasma cortisol and ACTH AUCs were analyzed for individual differences.

### Statistics

All data are presented as either individual data or as mean±SEM. One- or two-way repeated analyses of variance (ANOVA; self-administration data) or MANOVA (time course data) were conducted on raw data except where stated otherwise, and, where appropriate, post-hoc pair-wise comparisons using the Tukey Honest significant-difference test of significance ($P$<0.05) were carried out (Statistica v.5.0, Statsoft, Tulsa, Okla.).

## Results

### Glucocorticoid self-administration

Male monkeys were tested on more occasions and with a wider variety of glucocorticoid doses. The data from male and female monkeys was therefore treated separately.

#### *Male monkeys (n=7)*

On average, male monkeys self-administered more injections of dexamethasone than of either methylprednisolone or hydrocortisone ($F_{2,32}$=14.38, $P$<0.001, Fig. 1). There was a significant dose effect in the number of injections that were taken of dexamethasone and methylprednisolone. Both 0.1 mg/kg and 0.3 mg/kg dexamethasone were injected with a higher frequency than 0.03 mg/kg dexamethasone or saline ($P$<0.001), and 0.3 mg/kg methylprednisolone was injected a greater number of times more than the 0.1-mg/kg dose ($P$<0.005) or saline ($P$<0.05). There was no dose dependence in the number of injections of hydrocortisone that were taken. However, most of these effects were due to one monkey, RN23, which self-administered a greater number of glucocorticoid injections, expressed as a percentage of his methohexital baseline, relative to the other six male monkeys ($P$<0.001, Fig. 2). RN23 took significantly more injections of the "middle" and "high" doses of each glucocorticoid than the other monkeys did ($P$<0.05). Dexamethasone, at the dose that maintained the highest number of infusions in RN23 (0.3 mg/kg), maintained less self-administration behavior than the baseline drug, 0.1 mg/kg methohexital (Table 1).

When the data analysis was repeated without monkey RN23, there was a difference in the number of injections taken for the different glucocorticoids ($F_{2,32}$=3.47, $P$<0.05), with more injections of the high dose of dexa-

**Table 1** Dexamethasone self-administration: individual data for male monkeys ($n$=7)

| Monkey | Methohexital infusions (mean±SEM) | Response rate (responses/s) | Saline infusions (mean±SEM) | Response rate (responses/s) | Dexamethasone self-administration (mg/kg) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.03: Infusions | | Rate (responses/s) | | 0.1: Infusions | | Rate (responses/s) | | 0.3: Infusions | | Rate (responses/s) | |
| | | | | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| [a]RN23 | 59.40±2.22 | 0.86±0.01 | 13.2±1.59 | 0.17±0.02 | 16 | 15 | 0.21 | 0.20 | 58 | 36 | 0.84 | 0.50 | 52 | 57 | 0.74 | 0.84 |
| 2900 | 69.7±6.05 | 0.10±0.01 | 20.0±1.98 | 0.03±0.00 | 25 | 11 | 0.03 | 0.01 | 12 | 12 | 0.02 | 0.02 | 26 | 15 | 0.04 | 0.02 |
| [a]1583 | 66.4±3.19 | 0.30±0.02 | 15.2±3.40 | 0.06±0.01 | 12 | 11 | 0.05 | 0.04 | 21 | 8 | 0.08 | 0.03 | 27 | 17 | 0.11 | 0.07 |
| O351 | 89.4±2.01 | 0.14±0.00 | 8.2±1.20 | 0.01±0.00 | 8 | 7 | 0.01 | 0.01 | 25 | 10 | 0.03 | 0.01 | 26 | 3 | 0.04 | 0.00 |
| 3577 | 113.2±4.43 | 0.19±0.01 | 12.9±1.50 | 0.02±0.00 | 23 | 10 | 0.03 | 0.01 | 13 | 25 | 0.02 | 0.03 | **40** | 24 | 0.23 | 0.03 |
| 3589 | 91.0±6.70 | 0.14±0.01 | 17.9±1.62 | 0.03±0.00 | 5 | 11 | 0.01 | 0.02 | 17 | 18 | 0.02 | 0.03 | 58 | 14 | 0.08 | 0.02 |
| 2642 | 102.3±4.11 | 0.50±0.02 | 11.0±1.98 | 0.04±0.01 | 9 | 15 | 0.04 | 0.06 | 29 | 6 | 0.12 | 0.03 | 46 | 7 | 0.19 | 0.03 |
| | | | | Monkey RN23 excluded | 13.7 | 10.8 | 0.028 | 0.025 | 19.5 | 13.2 | 0.048 | 0.025 | 37.2 | 13.3 | 0.115 | 0.028 |
| | | | | Mean ($n$=6) SEM ($n$=6) | 3.4 | 1.0 | 0.007 | 0.008 | 2.8 | 2.9 | 0.017 | 0.003 | 5.4 | 3.0 | 0.032 | 0.009 |

[a] Fixed ratio was 10 lever presses except for monkeys 1583 (FR 30) and RN23 (FR 100). *Bold value* indicates case in which the dexamethasone solution ran out prior to the session end

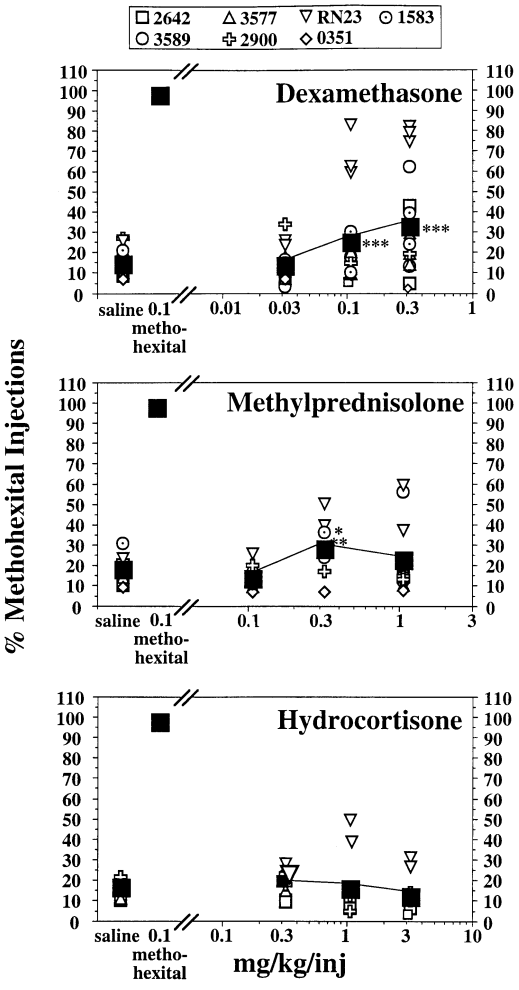


**Fig. 1** Glucocorticoid self-administration showing mean data for male monkeys (*filled squares*, *n*=7) and individual data (*open symbols*) for dexamethasone (*upper panel*), methylprednisolone (*center panel*) and hydrocortisone (*lower panel*). The extent to which saline and the different glucocorticoids were self-administered during the 130-min session is expressed as a percentage of methohexital injections, where methohexital was the baseline drug. Monkeys worked on a fixed ratio (FR)10 (FR30 or FR100, *n*=1 for each), TO 10-s schedule twice a day, which commenced at approximately 1000 hours and 1600 hours daily. ***$P$<0.001, 0.3 mg/kg and 0.1 mg/kg vs 0.03 mg/kg dexamethasone and saline. **$P$<0.005, 0.3 mg/kg vs 0.1 mg/kg methylprednisolone. *$P$<0.05, 0.3 mg/kg vs saline

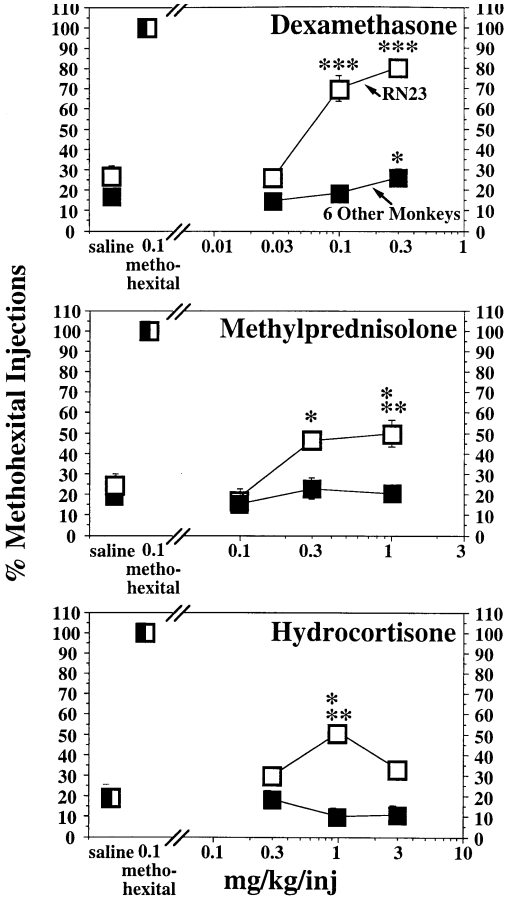


**Fig. 2** Self-administration of dexamethasone (*upper panel*), methylprednisolone (*center panel*) and hydrocortisone (*lower panel*) by monkey RN23 (*open squares*) compared with the remaining male monkeys in this study (*n*=6). *Upper panel*: *$P$<0.01, 0.3 mg/kg vs 0.03 mg/kg dexamethasone. ***$P$<0.001, 0.3 mg/kg and 0.1 mg/kg dexamethasone vs 0.03 mg/kg (RN23) and all doses of dexamethasone (*n*=6). *Center panel*: *$P$<0.05, RN23 vs mean (*n*=6). **$P$=0.05, 1.0 mg/kg vs 0.1 mg/kg methylprednisolone (RN23). *Lower panel*: *$P$<0.05, 1.0 mg/kg vs 0.3 mg/kg and 3 mg/kg hydrocortisone (RN23). **$P$<0.01, RN23 vs mean (*n*=6). Details as for Fig. 1

methasone (0.3 mg/kg) being taken than the doses of 0.03 mg/kg dexamethasone, saline, 0.1 mg/kg methylprednisolone or 1.0 mg/kg and 0.3 mg/kg hydrocortisone ($P$<0.05 for all comparisons; Fig. 2).

There was a significant difference in the extent to which 0.3 mg/kg dexamethasone was self-administered on the first and second occasion that it was offered to monkeys other than RN23 (*n*=6), with a greater number of injections being taken on the first occasion that dexamethasone was made available ($F_{1,10}$=18.3, $P$<0.005; Table 1). This phenomenon was not evident for other doses of dexamethasone, nor for methylprednisolone or hydrocortisone. Dexamethasone was made available on more than two occasions to several monkeys at some doses when there was a discrepancy in the number of injections taken during different tests. However, any difference in the numbers of injections always appeared between the first and second exposures. Subsequent exposures merely replicated the saline-like effect observed after the second occasion in monkeys other than RN23.

Figure 3 shows the basal levels of cortisol and ACTH measured between 0900 hours and 1000 hours on days prior to and following dexamethasone self-administration. Except on day 0, samples were drawn prior to saline or 0.1 mg/kg methohexital self-administration sessions, neither of which had any carry-over effects on basal HPA activity. Dexamethasone suppressed cortisol and ACTH basal activity for up to 3 days relative to basal levels prior to dexamethasone self-administration ($P$<0.05). In the case of cortisol, 0.1 mg/kg dexamethasone (intake: 2.16±0.32 mg/kg) reduced cortisol on day

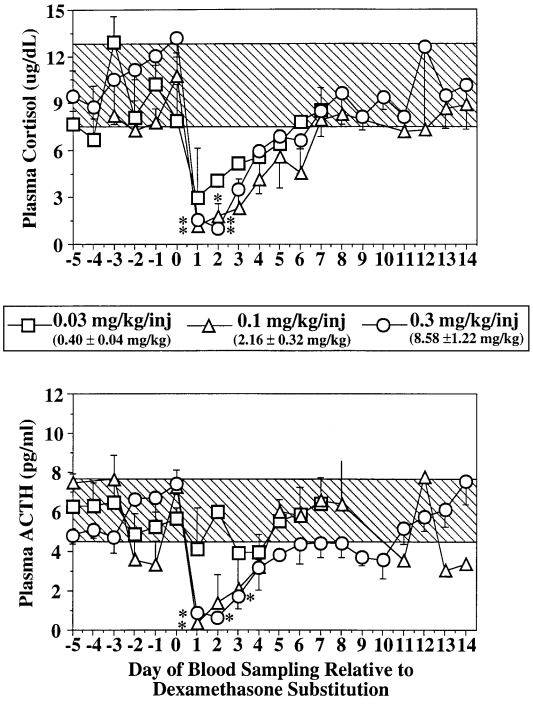


**Fig. 3** Comparison of basal cortisol (*upper panel*) and adrenocorticotropin (ACTH) (*lower panel*) levels on mornings before and after self-administration of 0.03, 0.1 and 0.3 mg/kg dexamethasone (mean intake±SEM; monkeys RN23, 1583, 2900 and 0351). Blood was sampled via an indwelling intravenous catheter from minimally disturbed monkeys between 0900 hours and 1000 hours. "*Day 0*" marks the day on which dexamethasone was available for self-administration. On all other days, either 0.1 mg/kg methohexital or saline was subsequently available for self-administration. **P*<0.05. See text for results of statistical comparisons

1 and day 2 relative to levels measured prior to dexamethasone ($P<0.05$), whereas 0.3 mg/kg dexamethasone (intake: 8.58±1.22 mg/kg) suppressed cortisol for 3 days ($P<0.05$). In the case of ACTH, both 0.1 mg/kg and 0.3 mg/kg dexamethasone self-administration suppressed basal ACTH activity on day 1 relative to levels measured prior to dexamethasone ($P<0.05$), and 0.3 mg/kg dexamethasone continued to suppress ACTH activity on day 2 and day 3 ($P<0.05$).

Monkey RN23 was offered 0.1 mg/kg dexamethasone on two consecutive days, when it was known from previous experience that his basal ACTH and cortisol levels would be suppressed on the second day from his dexamethasone intake the previous day. RN23 took a similar number of infusions of 0.1 mg/kg dexamethasone on both occasions (36 on the first day, 38 on the second day, relative to 19 saline infusions injected during the intervening evening session). The duration of the suppression of HPA activity for RN23 following glucocorticoid self-administration was similar to the other three monkeys from which blood samples were obtained. There was no evidence that disruption of basal HPA activity following glucocorticoid injections had any effect on methohexital or saline responding in any of the subjects.

**Table 2** Glucocorticoid self-administration: individual data for female monkeys ($n$=3)

| Monkey | Methohexital infusions (mean±SEM) | Response rate (responses/s) | Saline infusions (mean±SEM) | Response rate (responses/s) | Dexamethasone | | | | Methylprednisolone | | | | Hydrocortisone | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0.3: Infusions | | Rate(responses/s) | | 1.0: Infusions | | Rate(responses/s) | | 3.0: Infusions | | Rate(responses/s) | |
| | | | | | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd | 1st | 2nd |
| [a]2487 | 156±11.3 | 0.77±0.03 | 17±3.9 | 0.07±0.03 | 19 | 9 | 0.08 | 0.04 | 13 | 8 | 0.05 | 0.03 | 32 | 19 | 0.16 | 0.08 |
| O58F | 113±12.7 | 0.19±0.03 | 7±1.5 | 0.01±0.00 | 2 | 8 | 0 | 0.01 | 6 | 7 | 0.01 | 0.01 | 3 | – | 0 | – |
| 2083 | 91±10.2 | 0.14±0.02 | 14.5±2.5 | 0.02±0.00 | 18 | 11 | 0.02 | 0.01 | 12 | 11 | 0.02 | 0.01 | 14 | 11 | 0.02 | 0.01 |
| | | | | Mean ($n$=3) | 13 | 9.333 | 0.033 | 0.02 | 10.33 | 8.667 | 0.027 | 0.017 | 16.33 | 15 | 0.06 | 0.045 |
| | | | | SEM ($n$=3) | 5.508 | 0.882 | 0.024 | 0.01 | 2.186 | 1.202 | 0.012 | 0.007 | 8.452 | 4 | 0.05 | 0.035 |

[a]Fixed ratio was 10 lever presses except for monkey 2487 (FR 30)

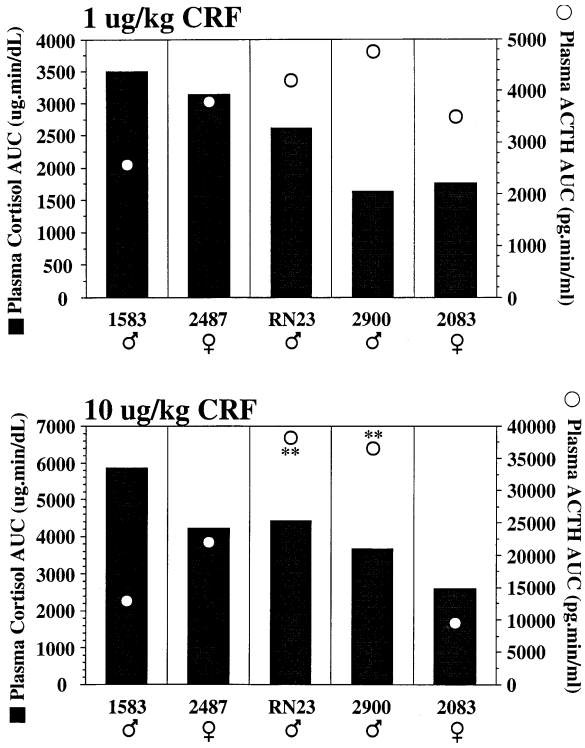


**Fig. 4** Cumulative release of plasma cortisol and adrenocorticotropin (ACTH) over a 4-h period following intravenous infusion of 1 µg/kg (*upper panel*) and 10 µg/kg (*lower panel*) corticotropin releasing factor (CRF) to male ($n$=3) and female ($n$=2) rhesus monkeys. All of these monkeys took part in the glucocorticoid self-administration study (1583, RN23, 2900, 2487 and 2083). Beginning between 0900 hours and 1000 hours, blood was sampled via an indwelling intravenous catheter from minimally disturbed monkeys, with sampling every 10 min commencing 20 min prior to CRF infusion until 90 min post-infusion, then at 120, 150, 180 and 240 min post-infusion. 10 µg/kg CRF produced significantly larger cortisol and ACTH areas under curves (AUCs) than 1 µg/kg CRF. **$P$<0.05, ACTH AUC for monkeys 2900 and RN23 following 10 µg/kg CRF vs the other monkeys

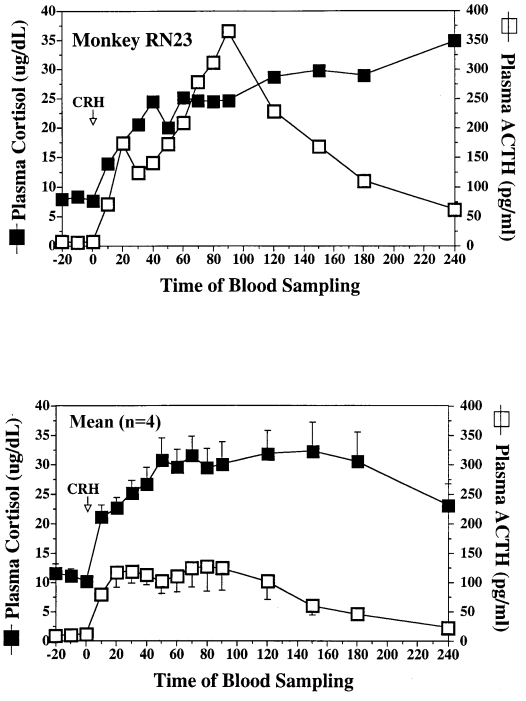


**Fig. 5** Time course of effects of an intravenous infusion of 10 µg/kg corticotropin releasing factor (CRF) on cortisol and adrenocorticotropin (ACTH) levels in monkey RN23 (*upper panel*) compared with other monkeys in this study ($n$=4; 1583, 2900, 2487 and 2083). Other details as for Fig. 4

*Female monkeys (n=3)*

Various glucocorticoid doses were made available to three female monkeys also on a FR10, TO 10-s schedule of reinforcement for 0.1 mg/kg methohexital or saline. The doses were selected on the basis of those that had maintained responding in monkey RN23. Dexamethasone (0.3 mg/kg), methylprednisolone (1.0 mg/kg) and hydrocortisone (3.0 mg/kg) were tested on one or two occasions, and overall the substituted glucocorticoids did not maintain responding above saline rates (Table 2). However, vomiting was observed during sessions where 1.0 mg/kg methylprednisolone (monkey 2083) or 3.0 mg/kg hydrocortisone (monkey 2487) was available. Despite the fact that the intake (mg/kg) of the glucocorticoids in these female monkeys was similar to the intake of the male monkeys, glucocorticoid-related vomiting was never observed in the male monkeys.

HPA effects of CRF infusion

Intravenous infusion of 10 µg/kg CRF resulted in a larger release of ACTH than a dose of 1 µg/kg ($F_{1,12}$=55.37, $P$<0.001). Monkeys RN23 and 2900 had a larger ACTH response to CRF than did the other monkeys for the 10-µg/kg dose ($P$<0.05; Fig. 4).

Intravenous infusion of 10 µg/kg CRF also resulted in a larger release of cortisol than a dose of 1 µg/kg ($F_{1,12}$=6.77, $P$<0.05), although there were no significant individual differences in the overall release of cortisol during the 4-h sampling period following the CRF infusion (Fig. 4). However, when examining the duration of the cortisol response, estimated from the time at which cortisol levels peaked following the 10-µg/kg CRF infusion, monkey RN23 may have had a later peak time (at or after 240 min, the last sampling time) than the other monkeys (mean=120±20 min; Fig. 5). This contrasts with the cortisol responses of the other monkeys, which had declined to 56±8% (23 µg/dl) of the peak cortisol level (33 µg/dl) relative to the increase over the mean basal level (10 µg/dl), by the 240-min (final) sampling time, the time at which the peak cortisol level for RN23 was measured.

## Discussion

Three glucocorticoids, dexamethasone, methylprednisolone and hydrocortisone, were made available to ten rhe-

sus monkeys for self-administration under a FR 10 (30 or 100), TO 10-s schedule of reinforcement. These glucocorticoids served reliably as reinforcers in only one of these ten monkeys. Monkey RN23 self-administered dexamethasone, methylprednisolone and hydrocortisone. In addition, suppression of RN23's basal ACTH and cortisol activity from earlier dexamethasone self-administration did not affect subsequent dexamethasone-reinforced responding. Also, there was an indication that monkey RN23 showed an enhanced cortisol response to intravenous administration of 10 µg/kg CRF relative to the other monkeys. For six of the other nine monkeys, dexamethasone (0.3 mg/kg), the most glucocorticoid receptor-selective, potent and long-acting of the three glucocorticoids tested, was self-administered more frequently when it was made available for the first time than on subsequent occasions. The initial test with 0.3 mg/kg dexamethasone was separated from the subsequent test with this dose by 13–56 days. It should be noted that lower doses of dexamethasone, as well as other glucocorticoids, were tested during this intervening time. For the three remaining monkeys, all females, responses for the substituted glucocorticoids were similar to saline responding. Infusions of 1.0 mg/kg methylprednisolone or 3.0 mg/kg hydrocortisone were associated with vomiting in two of the female subjects, an effect that was not observed in the male subjects with any of the glucocorticoids tested. The choice of methohexital rather than, for example, cocaine, as a baseline for the evaluation of the reinforcing effects of glucocorticoids was based more on the observation that methohexital is a less efficacious reinforcer than cocaine (Winger 1993) and thus a methohexital baseline may provide a more sensitive context for evaluating drugs that may have limited reinforcing efficacy.

The results from the present study contrast with the findings of similar studies done in rats. Two studies have demonstrated that corticosterone is self-administered more frequently than saline via both oral (Deroche et al. 1993) and intravenous routes (Piazza et al. 1993) over a range of doses, and this difference was maintained over several days. There is also a previous report that two analogs of ACTH, one of which was a peptidic precursor to corticosterone synthesis, were self-administered by about 60% of rats that were tested (Jouhaneau-Bowers and Magnen 1979). Their observation that $ACTH_{4\text{-}10}$, which does not stimulate adrenocortical steroid release, also maintained self-administration behavior in ten of eighteen rats, suggested that the ACTH may have direct central effects independent of glucocorticoid release. Involvement of the opioid system was implicated in the reinforcing effects of ACTH, as naloxone administration was found to attenuate ACTH self-administration.

In contrast to the reinforcing effects of ACTH and corticosterone in rats, intracerebroventricular (i.c.v.) and sub-cutaneous (s.c.) administration of CRF has been reported to induce place aversion in rats (Cador et al. 1992). This apparently aversive effect of i.c.v. CRF was ameliorated by the co-administration of the CRF antagonist, a-helical CRF, but appears to be unrelated to the stimulation of ACTH and corticosterone in plasma, as doses of s.c. CRF that were necessary to produce place aversion resulted in greater stimulation of ACTH and corticosterone than did i.c.v. CRF. Populations of CRF-immunoreactive neurons have been identified in neural structures unrelated to the HPA system, such as in the amygdala, stria terminalis, prefrontal cortex and brainstem (Olschowka et al. 1982; Cummings et al. 1983; Swanson et al. 1983). Central administration of CRF reduces appetitive behaviors, such as feeding, drinking and sexual activity, and facilitates processes that energize the organism, such as activation of sympathetic NS. Thus, CRF appears to inhibit the expression of behaviors that are extraneous to the organism's successful response to a stressor. The slower, CRF-induced release of glucocorticoids, however, is thought to produce effects that compensate for the physiological changes that occur in the initial response to stress, thus preventing these adaptive mechanisms "overshooting" and causing harmful effects such as hypoglycemia and autoimmune disease (Munck et al. 1984). Therefore, the observation that glucocorticoids may maintain responding in a self-administration paradigm does not necessarily imply that "stress" per se is a consequence associated with the reinforcement process, as increasing glucocorticoid levels in the absence of a rise in CRF may circumvent the aversive effects associated with activation of the HPA axis and produce effects that are quite dissimilar to the stress state. For instance, systemically administered glucocorticoids have been reported to increase extracellular dopamine levels in the nucleus accumbens (Piazza et al. 1996), an effect that is often associated with reinforcing stimuli (Wise and Bozarth 1987).

There were clear individual differences among the monkeys in this study with respect to the extent to which they self-administered glucocorticoids. The observation that only one in ten monkeys responded reliably for a number of different glucocorticoids is consistent with reports of individual differences in other species with regard to the reinforcing effects of glucocorticoids. While it is possible that drug history may have influenced the results of this study, it should be noted that the drug histories of monkeys RN23 and 1583 were identical despite the lack of agreement in their glucocorticoid-directed behavior. Overall, each of the monkeys in this study had similar self-administration histories; the main difference between them being duration of exposure to each drug (months versus years of exposure). Rats have been designated "high" or "low" responders on the basis of differences in both their behavioral reactivity to novelty and sensitivity to drugs of abuse (Piazza et al. 1991, 1993). Hallmarks of high responders include a prolonged HPA (corticosterone) response to stress and enhanced sensitivity to the reinforcing effects of corticosterone (Piazza et al. 1993). There is some evidence that monkey RN23 in this study may fit some of the criteria that would classify him as a high responder. RN23 appeared more sensitive to the effects of intravenous CRF when

compared with the other monkeys, as he had a more sustained cortisol response to 10 µg/kg CRF. RN23 was also more susceptible to the reinforcing effects of glucocorticoids than any of the other monkeys. RN23 required a substantially larger FR than most of the other monkeys in order to obtain a reliable difference between his saline and methohexital responding [FR100 vs FR10 ($n$=7) and FR30 ($n$=2)]. In a previous study, RN23 was one of three monkeys whose HPA response when saline was available for self-administration was similar to his HPA response to different doses of cocaine. Both saline and cocaine self-administration resulted in cortisol and ACTH levels that were in excess of basal release during the same times of day (Broadbear et al. 1999a, 1999b). This may imply that it was the self-administration behavior itself and not just cocaine intake that was generating cortisol and ACTH release, and the present study provides evidence that glucocorticoids are reinforcing in this monkey.

There is also the question of why some of the monkeys in this study initially self-administered 0.3 mg/kg dexamethasone, but responded at or below saline levels for each subsequent test with this dose. It is possible that going to still higher doses would have led to an increase in responding; however, the fact that a single exposure to high-dose dexamethasone "desensitized" the majority of subjects to dexamethasone's reinforcing effect, when it was subsequently tested days or weeks later, may suggest that simply increasing the dose would not have reinstated dexamethasone self-administration. A possible explanation for the lack of replicability is that any positive effects associated with the initial intake of large doses of glucocorticoids in humans are usually short lived, only lasting a few days or weeks (Brody 1952), and reports of persistent positive effects, which may apply in the case of monkey RN23, are rare (Hall et al. 1979). After experience with a variety of glucocorticoids at different doses over a number of months, any reinforcing effects that may have been present early in the study may no longer be present for the majority of subjects. Thus, the factors controlling continued administration of glucocorticoids over time may be unrelated to the positive subjective effects that may have been present initially.

Glucocorticoids may be used as therapeutic agents in a variety of clinical conditions (Ling et al. 1981). Patients and normal volunteers alike may experience positive subjective effects in association with glucocorticoid administration. It has been speculated that this may lead to self-medication, with an escalation of the dose being taken over the course of several years (Morgan et al. 1973). Overdosage with glucocorticoids may lead to mental stimulation, marked feelings of well-being, hyperactivity, increased appetite, reduced sleep and, sometimes, tension and irritability. These effects are more likely when high doses of the more potent glucocorticoids (e.g. dexamethasone rather than methylprednisolone) are given (von Zerssen 1976). This is consistent with the finding that for the majority of monkeys in the present study, dexamethasone was the only glucocorticoid for which the number of infusions taken on any occasion reliably exceeded the infusions earned when saline was available. The reasons for a person's continued use of glucocorticoids beyond what is clinically indicated may include alleviation of symptoms of the underlying disease for which glucocorticoids were originally prescribed, adrenal insufficiency, as well as for amelioration of steroid withdrawal symptoms such as weakness, arthralgias and mood swings (Von Zerssen 1976; Dixon and Christy 1980). Although there is one report that glucocorticoid abuse is more prevalent in women than in men (Ling et al. 1981), none of the three female monkeys that were tested in this study showed any propensity to self-administer dexamethasone, even when it was made available for the first time; neither did they self-administer either methylprednisolone or hydrocortisone at the doses tested.

In summary, dexamethasone, methylprednisolone and hydrocortisone were made available for self-administration to rhesus monkeys in order to determine whether glucocorticoids would serve as reinforcers. Under the conditions used in this study, only one of the ten monkeys reliably self-administered dexamethasone, methylprednisolone and hydrocortisone in a dose-dependent manner. Suppression of basal HPA axis activity from previous dexamethasone intake did not alter subsequent responding for dexamethasone in this monkey. This same monkey also showed an enhanced cortisol response to intravenous CRF, indicating that his HPA axis may have been relatively hyper-responsive to stimulation in comparison with other monkeys. Therefore, it would appear that, in the majority of monkeys, intravenous glucocorticoids do not maintain self-administration behavior.

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