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ORIGINAL ARTICLE

Dehydroepiandrosterone Attenuates Cocaine-Seeking Behaviour Independently of Corticosterone Fluctuations

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The neurosteroid dehydroepiandrosterone (DHEA) is involved in the pathophysiology of several psychiatric disorders, including cocaine addiction. We have previously shown that DHEA attenuates cocaine-seeking behaviour, and also that DHEA decreases corticosterone (CORT) levels in plasma and the prefrontal cortex. Previous studies have found that rats demonstrate cocaine-seeking behaviour only when the level of CORT reaches a minimum threshold. In the present study, we investigated whether the attenuating effect of DHEA on cocaine seeking is a result of it reducing CORT levels rather than a result of any unique neurosteroid properties. Rats received either daily DHEA injections (2 mg/kg, i.p.) alone, daily DHEA (2 mg/kg, i.p.) with CORT infusion (to maintain stable basal levels of CORT; 15 mg/kg, s.c.) or vehicle (i.p.) as control, throughout self-administration training and extinction sessions. We found that both DHEA-treated and DHEA + CORT-treated groups showed a significantly lower number of active lever presses compared to controls throughout training and extinction sessions, as well as at cocaine-primed reinstatement. DHEA-treated rats showed lower CORT levels throughout the experimental phases compared to DHEA + CORT-treated and control rats. Additionally, we show that DHEA administered to cocaine-trained rats throughout extinction sessions, or immediately before reinstatement, attenuated cocaine seeking. These findings indicate that DHEA attenuates cocaine-seeking behaviour independently of fluctuations in CORT levels.

Key words: dehydroepiandrosterone, corticosterone, cocaine self-administration

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Cocaine stimulates the hypothalamic-pituitary-adrenal (HPA) axis, increasing the secretion of hormones including corticosterone (CORT), in man, nonhuman primates and rodents (1–5). CORT was shown to be requisite for cocaine self-administration. Acquisition, although not maintenance, was totally blocked by bilateral adrenalectomy, and was dose-dependently decreased by metyrapone, which inhibits CORT synthesis, and was also partly reversed by adding CORT to drinking water in rats (6,7). Goeders and Guerin (8) also found that self-administration does not occur unless plasma CORT is increased above a critical threshold needed for reward. Moreover, increasing CORT levels augments sensitivity to low cocaine doses in this model (9,10). Glucocorticoids facilitate the reinforcing effect of cocaine (11) and pretreatment with CORT facilitates the acquisition of cocaine self-administration (12). Several studies have also shown that stress and elevated glucocorticoid levels increased the acquisition and maintenance of cocaine self-administration (3,13). Circulating CORT secretion is increased during cocaine withdrawal (14–17). For reinstatement, it was found that CORT plays a role in stress-induced, cue-induced, environmental-cue-induced and food deprivation-induced reinstatement, whereas it only has a partial role in cocaine-induced reinstatement (14–17).

Corticosterone also plays a role in cocaine-induced neuroplasticity that contributes to addiction (17). CORT itself is self-administered by rats (18), although it does not produce conditioned place preference in rats (19). In addition to CORT, it has also been reported that corticotrophin-releasing hormone, both hypothalamic (stress-induced) and extra-hypothalamic, may be involved in the behavioural and physiological aspects of drug-seeking motivation (20), and is critical for cue-induced reinstatement after extinction (9).

The neurosteroid dehydroepiandrosterone (DHEA) is a natural steroid produced from cholesterol by the adrenal glands. DHEA is also produced in the gonads, adipose tissue and the brain. It is

structurally similar to (and is a precursor of) androstenedione, testosterone and oestrogens (21). DHEA is involved in the pathophysiology of several psychiatric disorders, including cocaine addiction (21–26). We previously found that chronic treatment with DHEA attenuated cocaine acquisition during self-administration training, reduced cocaine seeking during extinction and blocked cocaine-primed reinstatement (27–29). In addition, we found that daily administration of DHEA decreased both plasma and prefrontal cortex CORT levels (30). Thus, DHEA may be involved in drug-seeking behaviour by affecting various neural mechanisms, including the HPA axis.

In the present study, we examined whether the DHEA-induced decrease in CORT levels is involved in the attenuating effect of DHEA on cocaine-seeking behaviour. We examined the effect of DHEA treatment, with or without maintaining steady CORT levels, on the various stages of cocaine self-administration: acquisition, maintenance, extinction and reinstatement. In addition, we examined CORT levels throughout these phases.

Materials and methods

Animals

Male Sprague–Dawley rats, weighing 250–350 g (Bar-Ilan University, Ramat Gan, Israel), were housed three or four per cage, under a 12 : 12 h reversed light/dark cycle (lights on 20.00 h). Food and water were available *ad lib.* The study was approved by the Animal Care and Use Committees of the Felsenstein Medical Center, Faculty of Medicine, Tel-Aviv University and of Bar Ilan University, Ramat Gan, Israel.

Study design

Figure 1 depicts the combined scheme of three separate experiments. In the first experiment, rats received either daily DHEA injections (2 mg/kg, i.p., n = 6), DHEA injections (i.p.) concomitantly with CORT minipump infusion (s.c.) (n = 7) or vehicle (i.p.) (n = 7). Treatments began 3 days before, and continued throughout cocaine self-administration training (days 1–8), extinction sessions (days 9–17) and reinstatement (day 18). Injections were given 90 min before placement of rats in the self-administration chambers, at all stages (and given at the same time of day on the 3 days prior to commencement of training). The pump stopped releasing CORT on day 14. For measurement of CORT levels, blood was drawn (50 μl, through the jugular vein catheter) on days 8 (maintenance), 9 (extinction) and 18 (reinstatement), immediately after conclusion of the sessions.

In the second experiment, rats were trained for cocaine self-administration (days 1–8) and received vehicle injections throughout training (90 min before placement in self-administration chambers). After conclusion of training, rats were divided into two subgroups that received either daily DHEA injections (2 mg/kg, i.p, n = 6) or vehicle (n = 7), 90 min before the daily extinction sessions (days 9–17) and 90 min before reinstatement (day 18). Blood was drawn (50 μl, through the jugular vein catheter) on day 9 (extinction), immediately after conclusion of the sessions.

In the third experiment, rats underwent cocaine self-administration training (days 1–8) and extinction sessions (days 9–17) and received daily vehicle injections throughout (90 min before placement in self-administration chambers). Rats were then divided into two subgroups that received either a single DHEA injection (n = 8) or vehicle (n = 6), 90 min before entering the self-administration chamber for cocaine-primed reinstatement (day 18).

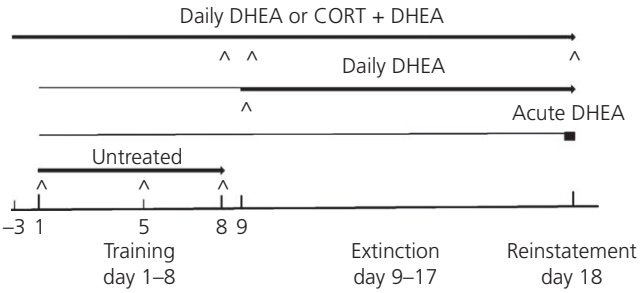

Fig. 1. Experimental design. In the first experiment (top line in figure), rats received either daily dehydroepiandrosterone (DHEA) injections (2 mg/kg, i.p., n = 6), DHEA injections (i.p.) concomitantly with corticosterone (CORT) minipump infusion (s.c.) (n = 7) or vehicle (i.p.) (n = 7). Treatments began 3 days before, and continued throughout cocaine self-administration training (days 1–8), extinction sessions (days 9–17) and reinstatement (day 18). Injections were given 90 min before placement of rats in the self-administration chambers. The pump stopped releasing CORT on day 14. Blood was drawn on days 8 (maintenance), 9 (extinction) and 18 (reinstatement), immediately after conclusion of the sessions. In the second experiment (middle line), rats were trained for cocaine self-administration (days 1–8), and received vehicle injections throughout (90 min before placement in self-administration chambers). After conclusion of training, rats were divided into two subgroups that received either daily DHEA injections (2 mg/kg, i.p, n = 6) or vehicle (n = 7), 90 min prior to extinction daily sessions (days 9–17) and before reinstatement (day 18). Blood was drawn on day 9 (extinction), after conclusion of the session. In the third experiment (bottom line), rats underwent cocaine self-administration training (days 1–8) and extinction sessions (days 9–17), and received daily vehicle injections throughout (90 min before placement in self-administration chambers). Rats were then divided into two subgroups that received either a single DHEA injection (n = 8) or vehicle (n = 6), 90 min before entering the self-administration chamber for cocaine-primed reinstatement (day 18). Another group of untreated rats was trained for cocaine self-administration, and blood was drawn at acquisition (days 1 and 5) and maintenance (day 8), immediately after conclusion of the sessions. Arrows denote days of blood draws.

Another group of untreated rats was trained for cocaine self-administration, and blood was obtained immediately after conclusion of the acquisition (days 1 and 5) and maintenance (day 8) sessions (50 μl plasma, through the jugular vein catheter).

All self-administration experiments and all blood draws were conducted during the dark cycle (00.00–02.00 h). For the diurnal experiment, blood was drawn during the light cycle (00.00 h) and during the dark cycle (12.00 h).

DHEA treatment

In all experiments, DHEA was dissolved in dimethyl sulphoxide (DMSO) and diluted with saline to a final concentration of 1.0%, and was injected i.p. at a dose of 2 mg/kg. For controls, the same vehicle was used.

Preliminary experiments: the effect of DHEA treatment on CORT levels

First, we examined the effect of daily DHEA treatment (2 mg/kg) on plasma CORT levels. Rats received daily injections of DHEA (2 mg/kg) or vehicle as control (n = 10 for each), for three consecutive days. Blood was drawn from the edge of the tails (after local anaesthesia with ethyl chloride) 2.5 h after the last injection and the level of CORT in plasma was determined [to

determine the CORT concentration required to reverse CORT levels to normal range (to be administered by minipump, in subsequent experiments)].

One week later, the two groups were combined and then randomly divided into three subgroups. Each subgroup was treated for 3 days, with either vehicle (controls; n = 6), DHEA (2 mg/kg, i.p; n = 7) or with DHEA (2 mg/kg, i.p.) concomitantly with CORT via a minipump (n = 7). The dose of CORT for the minipump (CORT concentration needed to reverse CORT level to the basal level) was calculated taking into account the difference between CORT levels at baseline and after DHEA treatment, volume of blood, treatment mode (s.c.) and pump rate. After the 3 days of treatment, rats were decapitated (2.5 h after the last injection), plasma was separated and CORT levels were assessed to determine whether the minipump implantation can normalise CORT levels that were decreased by DHEA injections. It was found that 15 mg/kg of CORT administered by the minipump is sufficient to counteract the effect of DHEA on plasma CORT level, namely to restore it to the normal range.

Cocaine self-administration

Surgery

An 11-cm long silastic catheter (inner diameter 0.02″, outer diameter 0.037″; Spectrum Chromatography, Houston, TX, USA) was implanted into the right jugular vein of rats anaesthetised i.p. with xylazine and ketamine (10 mg/kg and 100 mg/kg, respectively (Merck, Darmstadt, Germany) as described previously (31). The catheter was secured to the vein with silk sutures and was passed s.c. to the top of the skull where it exited into a connector (a modified 22-gauge cannula; Plastics One, Roanoke, VA, USA) mounted to the skull with MX-80 screws (Small Parts, Inc., Miami Lakes, FL, USA) and dental cement (Yates & Birds, Chicago, IL, USA). Carprofen (2 mg/kg, i.p.) was injected post-surgery. Rats were allowed to recover from surgery for 5 days prior to cocaine exposure.

Self-administration training

Rats were trained for cocaine self-administration by an FR1 schedule for eight consecutive days, in 1-h sessions during the dark cycle. Each self-administration chamber (30 × 25 × 22 cm) had two levers, active and inactive, located 5 cm above the floor of the chamber. An active lever press generated a cocaine infusion (i.v., 0.13 ml/0.5 mg/kg/5 s) through the catheter, which was connected to an injection device. Throughout cocaine infusion intervals, a light located above the active lever was lit for 20 s, 15 s beyond the cocaine infusion period that lasted only 5 s. During the 15-s intervals, active lever presses were recorded, although no additional cocaine reinforcement was provided. Stable maintenance levels were attained when rats showed at least 3 days with < 20% variation in the number of active lever presses. Presses on inactive levers were recorded, although they did not activate the infusion pump and light. Rats were returned to home cages at the end of the daily session.

Extinction testing

After 8 days of cocaine self-administration training, rats were placed in the operant conditioning chambers for daily extinction sessions (days 9–17), for 1 h, with only the light cue, and no cocaine available.

Reinstatement

On day 18, 90 min after receiving their respective treatments (DHEA or vehicle injections), rats received a priming cocaine injection (10 mg/kg, i.p.) and were immediately placed in the self-administration chamber, with only the light cue and no cocaine available, for 1 h. The number of active lever presses was recorded.

At the conclusion of the session, rats were decapitated, blood was obtained and the level of CORT in plasma was examined.

CORT minipump

Minipumps (Alzet Osmatic Minipump, 200-μl reservoir; Durect Corp., Cupertino, CA, USA) were filled with either CORT (15 mg/kg, 200 μl in DMSO) or DMSO (200 μl) for controls. The concentration of CORT was based on the preliminary assay measuring decreased CORT levels after DHEA treatment. Rats were anaesthetised with 10% chloral hydrate for s.c. implantation of the minipump on the back. Pump rate was 0.5 μl/h, over 17 days.

Plasma preparation and determination of corticosterone levels

The blood was collected in ethylenediaminetetraacetic acid-containing tubes and plasma was separated by centrifugation at 1000 **g** for 10 min at room temperature. CORT was determined using the Corticosterone ImmuChem™ Double Antibody radioimmunoassay kit (MP Biomedicals LLC, Orangeburg, NY, USA). The minimal detectable dose according to the standard curve is 20 ng/ml. Cross-reactivity with similar steroids was negligible. Assay variability was 6.5–7.2% between runs and 4.4–10.3% within runs, according to CORT level.

Statistical analysis

One- or two-way ANOVA with or without repeated measures, followed by a Student–Newman–Keuls post-hoc or a two-tailed Student's unpaired t-test, was used as appropriate. $P < 0.05$ was considered statistically significant.

Results

Preliminary assays: CORT levels after DHEA treatment with or without CORT minipump

Corticosterone levels were determined after daily i.p. injections of DHEA (2 mg/kg) or vehicle, given for three consecutive days (values were 101.42 ± 14.32 ng/ml versus 192.33 ± 27.7 ng/ml, respectively; $P < 0.05$, Student's t-test). Next, rats received daily i.p. injections of either DHEA alone, DHEA concomitant with CORT minipump (s.c.; CORT concentration based on first assay as detailed in the Materials and methods), or vehicle as control, for three consecutive days. DHEA treatment alone caused a significant decrease in the level of CORT, which was reversed by the CORT minipump (in the DHEA + CORT group) (one-way ANOVA: $F_{2,17} = 4.856$; $P = 0.0215$; DHEA versus both control and DHEA + CORT; $P < 0.05$) (Fig. 2). It was found that 15 mg/kg of CORT administered by the minipump is sufficient to counteract the effect of DHEA (2 mg/kg, i.p.) on plasma CORT level, namely to restore it to the normal range.

Behavioural experiments

Effect of treatment with DHEA or DHEA + CORT throughout phases

Rats received DHEA treatment, with or without CORT, with 3 days pretreatment and throughout self-administration training, extinction sessions and before reinstatement. Two-way ANOVA with repeated

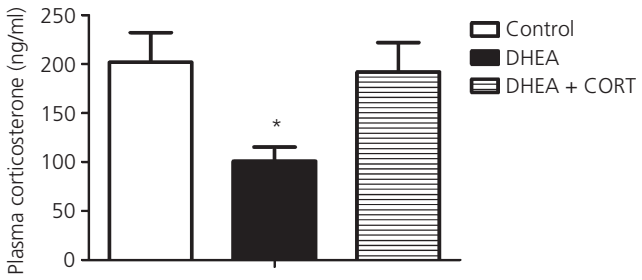

Fig. 2. Determination of corticosterone (CORT) levels after dehydroepiandrosterone (DHEA) treatment. In a preliminary assay, DHEA treatment caused a significant decrease in the level of CORT, which was reversed by addition of treatment with a CORT minipump (DHEA + CORT group) (*$P < 0.05$ versus control and DHEA + CORT).

measures for training and extinction sessions showed main effect of group ($F_{2,288} = 116.55$, $P < 0.0001$), main effect of time ($F_{16,288} = 23.42$, $P < 0.0001$) and an interaction between group and time ($F_{232,288} = 5.35$, $P < 0.0001$). A Student–Newman–Keuls post-hoc test showed that DHEA-treated and DHEA + CORT groups demonstrated a significantly lower number of active lever presses during acquisition and maintenance compared to controls ($P < 0.001$ days 1–5, $P < 0.05$ days 6–8 for control versus DHEA; day 1 and 7 $P < 0.01$; days 2–6 $P < 0.001$; day 8 $P < 0.05$ control versus DHEA + CORT) (Fig. 3A). This finding implies that the effect of DHEA during acquisition and maintenance is independent of CORT because the level of CORT was maintained throughout self-administration training. Additionally, during extinction sessions, rats treated with DHEA or DHEA + CORT showed a significantly lower number of active lever presses compared to control rats ($P < 0.01$ for DHEA and DHEA + CORT versus control on days 9–12, $P < 0.05$ for DHEA versus control on day 13) (Fig. 3A) (note that the pump ceased CORT infusion on day 14). It is notable that there is a significant increase in the number of active lever presses in all groups compared to their respective results on the last day of maintenance (Student's t-test, $P < 0.05$).

One day after conclusion of the extinction sessions, rats received a priming injection of cocaine (10 mg/kg, i.p.), and were immediately placed in the self-administration chambers, with no cocaine available, for 1 h. DHEA-treated rats and DHEA + CORT demonstrated a significantly lower number of active lever presses compared to vehicle-treated controls (one-way ANOVA, $F_{2,17} = 19.985$, $P < 0.0001$; Student-Neuman-Keuls $P < 0.001$) (Fig. 3B).

Inactive lever presses for all groups (on maintenance day 8, extinction day 1, and reinstatement) are presented in Table 1, and showed no sedative effect of DHEA or DHEA + CORT on motor activity ($P > 0.05$ control versus treatment groups).

Effect of DHEA treatment given upon extinction

To examine the effect of DHEA on extinction of cocaine seeking, a separate group of cocaine-trained rats were divided into two subgroups: one received DHEA 90 min before day 1 extinction testing and throughout extinction sessions, up to reinstatement (day 18) and the other continued to receive vehicle as control (Fig. 1). We found that the first DHEA injection given before the first extinction session blocked the increase in active lever pressing compared to controls (Student's t-test, $P < 0.0001$) (Fig. 3C). Responding continued to be low throughout extinction sessions. One day after conclusion of the extinction sessions, rats received a priming injection of cocaine (10 mg/kg, i.p.), and were immediately placed in the self-administration chambers, with no cocaine available, for 1 h. DHEA-treated rats demonstrated a significantly lower number of active lever presses compared to controls (Student's t-test, $P < 0.0001$) (Fig. 3D). Inactive lever presses for extinction and reinstatement are shown in Table 1 ($P > 0.05$ control versus treatment).

Effect of acute DHEA injection on reinstatement

Next, we examined the effect of a single DHEA injection on reinstatement of cocaine seeking. A separate group of cocaine-trained rats that underwent extinction were divided into two subgroups: one received a single DHEA injection 90 min prior to reinstatement and the other received vehicle as control (Fig. 1); both received a priming injection of cocaine (10 mg/kg, i.p.), and were immediately placed in the self-administration chamber. The DHEA-treated group showed a significantly lower amount of active lever presses compared to vehicle-treated controls (Student's t-test, $P < 0.01$) (Fig. 3E). Inactive lever presses during reinstatement are shown in Table 1.

Biochemical assays

CORT levels during the stages of cocaine self-administration

Effect of self-administration training on CORT levels and diurnal variation in untreated rats

To examine the effect of cocaine self-administration on CORT levels, an additional group of untreated rats was trained to self-administer cocaine. Blood was drawn from the jugular vein at baseline ($n = 9$), acquisition [day 1 ($n = 6$) and day 5 ($n = 8$)] and maintenance (day 8; $n = 9$) of self-administration.

On day 1, we found a significant increase in the level of CORT compared to baseline, which is in accordance with previous studies (4,8); over time, the level reverted almost to the basal level (one-way ANOVA: $F_{3,27} = 4.890$; $P = 0.0077$; $P < 0.01$ day 1 versus baseline, and $P < 0.05$ day 1 versus day 5 and maintenance) (Fig. 4A), which is consistent with findings of Galici *et al.* (32). Baseline plasma CORT levels are consistent with our previous data for control rats (33). Other studies have also shown similar basal CORT values measured in unstressed control male rats (34) and in control (chronically cannulated) male rats at night-time (35).

Diurnal variation in CORT levels was also examined in untreated rats during maintenance, at midday and midnight. We found that CORT levels during the light cycle (rest phase) were approximately 50% lower than those measured during the dark cycle (wake phase) (76.7 ± 16.7 ng/ml versus 150 ± 14.4 ng/ml, respectively; Student's t-test, $P < 0.004$), as expected for untreated rats.

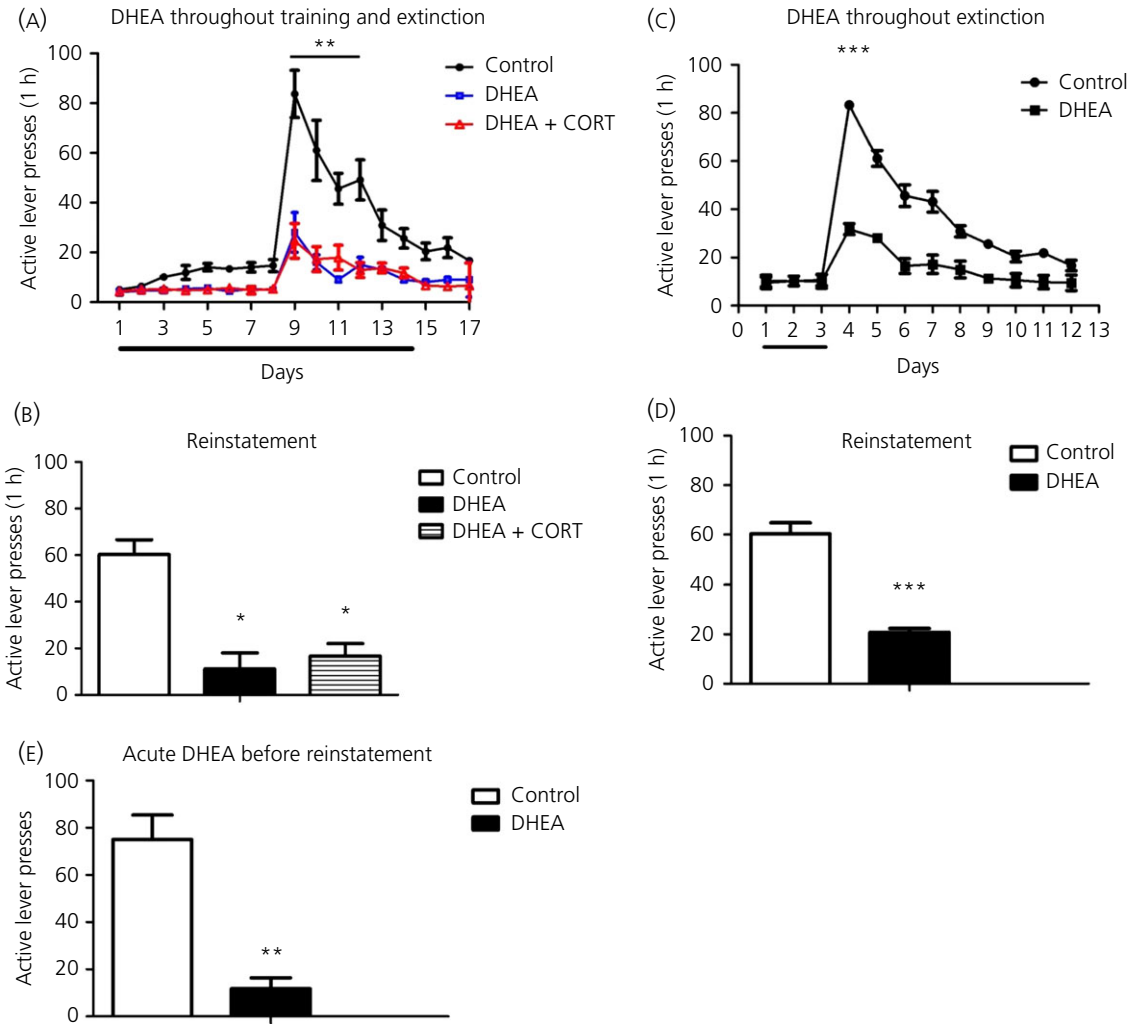

Fig. 3. Cocaine-seeking behaviour in rats treated with dehydroepiandrosterone (DHEA) or DHEA + corticosterone (CORT). (A) DHEA, DHEA + CORT or vehicle given throughout self-administration training and extinction sessions. Both DHEA-treated and DHEA + CORT rats showed significantly lower responding during training and lower cocaine seeking during extinction compared to controls (**P < 0.01 control versus both DHEA and DHEA + CORT groups, days 9–12). The line indicates the days that CORT was released from pump over training and extinction sessions. (B) At cocaine-primed reinstatement, both DHEA and DHEA + CORT treatments significantly decreased cocaine seeking, measured by active lever pressing, as compared to control (*P < 0.05; pump ceased releasing CORT prior to this stage). (C) DHEA treatment throughout extinction: Cocaine-trained rats treated with DHEA during extinction showed significantly attenuated cocaine-seeking behaviour on the first day of extinction compared to controls (***P < 0.0001). The line indicates the last 3 days of cocaine maintenance (during self-administration training) before DHEA treatment. (D) DHEA treatment caused a significant reduction in cocaine-seeking behaviour compared to controls (Student's t-test, ***P < 0.0001). (E) Acute DHEA was given to vehicle-treated cocaine-trained rats 90 min before reinstatement. These rats showed a significant reduction in cocaine-seeking behaviour (Student's t-test, **P < 0.01).

Table 1. Inactive Lever Presses.

	DHEA or DHEA + CORT throughout phases			DHEA upon extinction		DHEA upon reinstatement	
	Control	DHEA	DHEA + CORT	Control	DHEA	Control	DHEA
Maintenance (day 8)	2 ± 0.75	1.83 ± 0.6	2.125 ± 0.54	–	–	–	–
Extinction (day 1)	19.14 ± 3.6	12.8 ± 3.2	10.14 ± 4.1	22.5 ± 2.9	16.11 ± 1.8	–	–
Reinstatement	9.8 ± 2.72	6.33 ± 4.22	12.28 ± 8.95	27.85 ± 2.78	20.9 ± 3.9	19.83 ± 1.72	12.5 ± 1.12

DHEA, dehydroepiandrosterone; CORT, corticosterone.

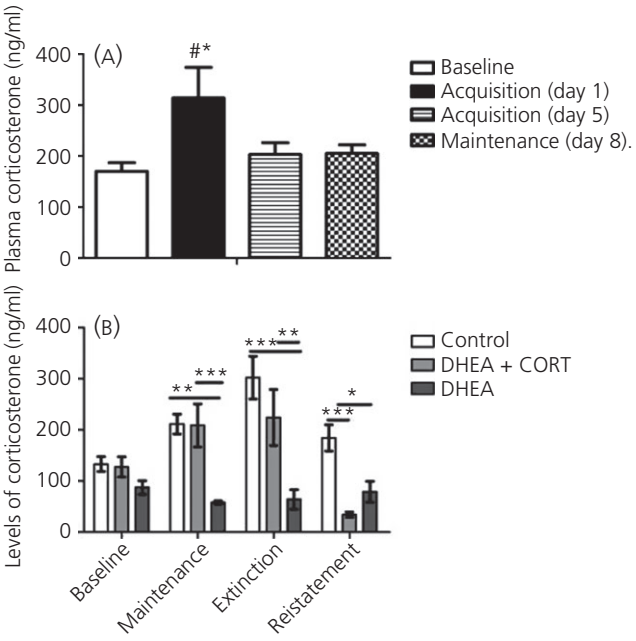

Fig. 4. Plasma corticosterone levels. (A) Corticosterone (CORT) level in untreated rats during cocaine self-administration training. A significant elevation in CORT level was seen on acquisition day 1 (training) compared to baseline (#$P < 0.01$); this increase reverted almost to the basal level on day 5 of acquisition and at maintenance (day 8; *$P < 0.05$). (B) CORT level of the dehydroepiandrosterone (DHEA) group was significantly lower than control at maintenance (day 8), extinction (day 9) and reinstatement (day 18), and lower than DHEA + CORT at maintenance and extinction (two-way ANOVA followed by Student–Neuman–Keuls test; maintenance: **$P < 0.01$ DHEA versus control and ***$P < 0.001$ DHEA versus DHEA + CORT; extinction: ***$P < 0.001$ DHEA versus control and **$P < 0.01$ DHEA versus DHEA + CORT; reinstatement, *$P < 0.05$ DHEA versus control; CORT level for DHEA + CORT rats was similar to controls at maintenece and extinction ($P > 0.05$) but not reinstatement (because no CORT pumped at this stage; ***$P < 0.001$ versus control).

Effect of treatment on CORT levels during maintenance, extinction and reinstatement

CORT levels were further assessed in DHEA-treated, DHEA + CORT and vehicle-treated rats at baseline, maintenance (day 8 of self-administration training), extinction (day 9) and reinstatement (day 18).

The two-way ANOVA for CORT plasma levels revealed a main effect of group ($F_{2,45} = 30.79$; $P < 0.001$), a main effect of time ($F_{3,45} = 8.13$; $P = 0.002$) and an interaction between group and time ($F_{6,45} = 5.16$, $P < 0.0001$). A Student–Newman–Keuls post-hoc test revealed that the DHEA-treated group had significantly lower CORT levels compared to controls at maintenance (day 8; $P < 0.01$), extinction (day 9; $P < 0.001$) and reinstatement (day 18; $P < 0.05$), and lower levels compared to DHEA + CORT at maintenance ($P < 0.001$) and extinction ($P < 0.01$; the pump stopped administering CORT at day 14, before reinstatement) (Fig. 4B). The CORT level of DHEA + CORT rats was similar to controls at maintenance and extinction ($P > 0.05$) but not at reinstatement (because no CORT was pumped at this stage; $P < 0.001$ versus control).

Next, we examined CORT levels in the cocaine-trained rats that received DHEA upon extinction. We found no decrease in CORT levels compared to controls (220.3 ± 17.5 ng/ml versus 198.3 ± 19.7 ng/ml, respectively; Student's t-test, $P > 0.05$, measured on the first day of extinction).

Discussion

Dehydroepiandrosterone decreases CORT levels by attenuating the activity of the HPA axis. The ratio of DHEA (or DHEAS, its sulphate ester)/cortisol was shown to be associated with states of anxiety and stress (36,37). We previously demonstrated that DHEA treatment attenuates cocaine acquisition, extinction and reinstatement (27–29). In the present study, we examined whether this effect is caused indirectly, through decreasing CORT levels (30), or by DHEA itself.

We found that rats treated with DHEA or DHEA + CORT showed a significantly lower response to cocaine compared to control rats throughout all phases of the study; namely, acquisition, maintenance, extinction and reinstatement. However, the plasma level of CORT in DHEA-treated rats was low, whereas its level in DHEA + CORT rats was maintained at control levels. DHEA given only throughout extinction, although having no effect on CORT levels, significantly attenuated cocaine-seeking behaviour. Thus, our results suggest that DHEA attenuates cocaine-seeking behaviour possibly because of its central properties as a neurosteroid, independently of fluctuations in CORT levels. It is notable that the CORT dose given through the minipump was based on the difference between CORT levels at baseline and after DHEA treatment (also taking into account volume of distribution and bioavailability). Therefore, the CORT pump most likely could not increase endogenous CORT levels above the basal level at any timepoint, and could not suppress the HPA axis but rather normalised the DHEA-induced decrease in CORT levels throughout the entire day. Indeed, in the preliminary study, we not only found that this dose reversed the DHEA-induced deficiency in basal CORT levels, but also that, in CORT + DHEA rats, CORT levels were normalised as long as the pump released CORT (i.e. at baseline, at cocaine self-administration maintenance and at extinction). In addition, DHEA and DHEA + CORT groups show low cocaine self-administration during acquisition, although cocaine seeking increased on the first day of extinction compared to maintenance (yet cocaine seeking in these groups was significantly lower than controls). Therefore, in treatment groups, there may have been an underlying effect of cocaine exposure during training, which affected extinction response, although this response was significantly suppressed by DHEA treatment.

Moreover, recent work by Guerin *et al.* (7) showed that adrenalectomy did not significantly alter the maintenance phase of cocaine self-administration. Because earlier work found that adrenalectomy blunted the acquisition of cocaine self-administration (6), CORT levels may exert a greater influence on drug self-administration during the acquisition phase, rather than the maintenance phase.

There are several processes involved in drug-seeking behaviour, such as reward, stress and short- and long-term memory; DHEA may be involved in one or more of these phases. Neurosteroids affect the brain via transcriptional regulation through interaction with nuclear receptors, in addition to rapid, nongenomic and stereospecific steroid actions via specific membrane receptors, such as $GABA_A$, ionotrophic glutamate and sigma receptors (38,39). We have recently found that long-term, subthreshold activation of the sigma-1 receptor with a selective agonist can decrease cocaine self-administration (Ben-Moshe H, Maayan R, Gispan I, Weizman A, Yadid G, unpublished data), suggesting that this receptor may also mediate the effect of DHEA on cocaine-seeking behaviour. Future studies should further examine whether one or more of these receptor systems mediate the effect of DHEA.

Prolonged abstinence from substances of abuse is characterised by dysphoria, depression and anxiety, combined with heightened stress and craving, which increases the propensity for relapse to drug use (29,40). Studies indicate that DHEA administration improves memory and cognitive processing, induces neurogenesis and neural survival, and controls levels of the stress hormone cortisol (21,41,42). In healthy men and women, DHEA supplementation improved mood and sense of well-being, induced relaxation and a higher capability of handling stressful situations (43), and relieved depressive-like symptoms (44). We have previously shown that a persistent decease in brain DHEA levels may be associated with the decreased ability to cope with mood fluctuations and stress that accompany heightened drug craving (21,29). Moreover, we recently found that a DHEA add-on in human polydrug users improves mood and decision-making, and substantially decreases relapse (45). Because DHEA functions as an antidepressant in both humans and animals (30,46), it may lower the distress associated with cocaine withdrawal (47), similar to the β-endorphin-induced decrease in frustration during extinction (48,49). Thus, exogenous, long-term administration of DHEA may also have a beneficial effect on stress and anxiety, which increase particularly during heightened drug craving at extinction and reinstatement (21,29). This may contribute to the attenuating effect of DHEA on cocaine-seeking behaviour.

Another possible factor that may be involved in the effect of DHEA is dopamine. The reinforcing effect of cocaine is partly mediated by blockade of the presynaptic dopamine transporter, which increases dopamine availability in the reward system. We have previously shown that DHEA treatment increased dopamine content in the ventral tegmental area and nucleus accumbens (27,50). Thus, DHEA may increase dopamine across all stages (i.e. acquisition, maintenance, extinction and reinstatement), which may also contribute to attenuated cocaine self-administration and cocaine seeking over these stages.

In summary, we show that DHEA treatment attenuated cocaine self-administration and cocaine seeking even when CORT levels were constantly maintained in the normal range. Therefore, the effect of DHEA on cocaine taking and seeking is likely independent of fluctuations in CORT levels. Moreover, DHEA attenuated self-administration when given either as pretreatment, at extinction or at reinstatement, emphasising its potential as an effective anti-craving agent.

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