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Desmethylimipramine attenuates cocaine withdrawal in rats

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Abstract. Depression and anhedonia are two major symptoms of cocaine withdrawal in humans. Hence, pharmacological treatments effective in depression might also alleviate the symptoms of cocaine withdrawal. In the present study, the effects of acute and repeated administration of a tricyclic antidepressant, desmethylimipramine (DMI), were investigated in naive and cocaine-withdrawing rats. An animal model of cocaine withdrawal was used that employs the elevation in intracranial self-stimulation (ICSS) thresholds following the termination of prolonged periods of cocaine self-administration as a measure of an animal's "anhedonic" state. The influence of chronic DMI treatment on β-adrenergic receptor binding and affinity was also correlated with the behavioral signs of cocaine withdrawal. Neither acute nor repeated DMI treatment influenced reward functions in rats that were not undergoing cocaine withdrawal. However, repeated DMI treatment significantly down-regulated β-adrenergic receptors, and shortened the duration of the post-cocaine "anhedonia" (elevation in thresholds). Furthermore, the magnitude of the β-adrenergic receptor down-regulation correlated significantly with the degree of effectiveness of DMI treatment in reversing the post-cocaine "anhedonia". However, chronic DMI treatment did reduce the amount of cocaine self-administered by the animals. The reversal of the post-cocaine anhedonia in this animal model of cocaine withdrawal by chronic DMI treatment demonstrates the potential usefulness of the model in identifying new pharmacotherapies for cocaine withdrawal. In addition, the results indicate that tricyclic antidepressants may be able to ameliorate some of the symptoms of cocaine withdrawal.

Key words: Intracranial self-stimulation – Brain stimulation reward – Thresholds – Self-administration – Cocaine – – Stimulants – Anhedonia – Depression – Withdrawal – DMI – Desipramine – Desmethylimipramine – Tricyclic antidepressants – Drug abuse – Beta-adrenergic receptors – Downregulation

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Depression and anhedonia are two major symptoms of cocaine withdrawal in humans (Ellinwood and Petrie 1977; Resnick and Schuyten-Resnick 1977; Gawin and Kleber 1986, 1988; American Psychiatric Association 1987; Gawin 1991). Hence, pharmacological treatments effective in depression might also alleviate the cocaine withdrawal symptoms (Gawin and Kleber 1984; Giannini and Billet 1987). Preliminary clinical trials indicated some success in the treatment of cocaine addiction with chronic treatment with a tricyclic antidepressant, desmethylimipramine (DMI) (Gawin and Kleber 1984; Gianninini and Billet 1987; Gawin et al. 1989), even when patients with a psychiatric diagnosis of major depression were excluded from the study (Gawin 1986).

An animal model of cocaine withdrawal has been developed recently using the elevation in intracranial self-stimulation (ICSS) thresholds after the termination of a prolonged period of cocaine self-administration as a measure of an animal's "anhedonic" state (Markou and Koob 1991). In this model, rats are allowed to self-administer cocaine at doses and rates that are presumably most reinforcing for them, similarly to human users of the drug, avoiding any potentially aversive, stressful or toxic effects of experimenter-administered injections of cocaine (Dworkin et al. 1987). Thus, the etiology of this model of cocaine withdrawal is homologous to that of the human condition; both are the result of cocaine self-administration.

The primary symptoms of cocaine withdrawal in humans, anhedonia and depression, are difficult to assess in animals. Nevertheless, intracranial self-stimulation (ICSS) thresholds have been shown to be a reliable measure of reward (Gallistel 1973; Olds and Fobes 1981; Stellar and Stellar 1985) that can reflect the entire continuum from "anhedonia to hedonia". Furthermore, ICSS is believed to directly activate some of the same neuronal substrates that mediate the rewarding effects of natural reinforcers (e.g., food, water) and of euphorigenic drugs of abuse (e.g., stimulants, opiates) (for a review, see Liebman and Cooper 1989). In addition, the discrete-trial ICSS procedure used in the present study provides a current threshold measure which is a reliable index of reward unconfounded by

performance effects (Markou and Koob 1991, 1992a). Thus, in addition to etiologic validity, the model also has construct validity because animals in the model exhibit behavior which can be interpreted as anhedonia. Finally, the ICSS model has high "overall validity" as a model of depression because it has predictive, etiologic and construct validity (Willner 1984).

The duration of the post-cocaine elevation of thresholds in rats, although dose-dependent, is short (maximum 5 days; Markou and Koob 1991). Thus, the ICSS animal model of post-cocaine anhedonia appears to be primarily a model of the "crash" phase of cocaine withdrawal in humans (Gawin and Kleber 1986). However, such time comparisons are always difficult to make because of differences in life span and pharmacokinetics between humans and rats.

The purpose of this study was to investigate whether a tricyclic antidepressant, desmethylimipramine (DMI), would prevent the elevation in ICSS thresholds ("anhedonia") observed during cocaine withdrawal in rats. Such a result would further establish the predictive validity of the model. Previous studies have shown that bromocriptine, a dopamine agonist with putative efficacy in the treatment of cocaine withdrawal (Giannini et al. 1989), prevents the post-cocaine elevation in thresholds (Markou and Koob 1992b). The reversal of the post-cocaine elevation of thresholds by two pharmacological agents which produce their effects through different neuronal mechanisms would provide converging evidence of the model's predictive validity.

β-Adrenergic receptor affinity and binding in the frontal cortex of rats during cocaine withdrawal were also measured to confirm the effectiveness of the repeated DMI treatment. Because β-receptor down-regulation is a well-established biochemical effect of chronic DMI treatment (Wolfe et al. 1978; Wolfe and Harden 1981; Manier et al 1984, 1987; Asakura et al. 1987), the degree of this down-regulation provides one with a measure of the efficacy of DMI treatment. The β-adrenergic receptor density was correlated with the post-cocaine ICSS thresholds to provide some evidence for dose-responsiveness.

Finally, the influence of repeated DMI treatment on cocaine intake was determined. It is believed that the initiation of an abstinence period from cocaine use is a critical first step in recovery from cocaine dependence (Gawin and Kleber 1986; Gawin et al. 1989). Thus, it is important to evaluate the effects of a pharmacological agent, proposed as treatment for cocaine dependence, on cocaine intake.

Materials and methods

Subjects

Male albino Wistar rats (250–350 g) were obtained from Charles River, Kingston, NY, housed in groups of three and maintained in a temperature and light controlled environment. They had free access to food and water except during testing sessions that were less than 12 h in duration. They were maintained on a 12 h light/dark cycle, and tested during the light phase.

Apparatus

ICSS apparatus. ICSS training and testing took place in eight Plexiglas, sound attenuated operant chambers (30.5 cm × 30 cm × 17 cm). One wall contained a metal wheel manipulandum which required a 20 g force to rotate one quarter of a turn. Gold-contact swivel commutators and bipolar leads connected the animals to the stimulation circuit. Brain stimulation was administered by constant current stimulators using 60 Hz sine waves.

Self-administration apparatus. Training and testing took place in four metal and Plexiglas, sound-attenuated operant chambers (29 cm × 24 cm × 19.5 cm). One wall contained a metal retractable lever mounted 2.5 cm above the floor which required a 10 g force to be pressed. Plastic Products swivels (Plastic Products Inc., Roanoke, Vi) connected the animals to syringes which were operated by pumps (Razel, Stamford, Ct) that delivered the drug.

Surgeries

ICSS surgery. Rats were anesthetized with 50 mg/kg sodium pentobarbital (IP) supplemented with 0.06 mg atropine sulfate (SC) to alleviate respiratory congestion. A stainless-steel bipolar electrode (Plastic Products Co., Roanoke, VA, diameter = 0.25 mm), was implanted in the rats' lateral hypothalamus (AP − 0.5 mm from bregma, L ± 1.7 mm and 8.3 mm below dura with the incisor bar 5.0 mm above the interaural line). For half of the animals, the electrode was placed on the right side of the brain and for the other half on the left side to counterbalance any possible brain asymmetries (Glick et al. 1980, 1981; Markou and Frank 1987).

Self-administration surgery. Rats were anesthetized with halothane and a silastic catheter was implanted in the jugular vein as previously described (Roberts and Koob 1982). The catheter was passed subcutaneously to a polyethylene assembly mounted on the animal's back. This assembly consisted of a Plastic Products guide cannula (C313G) attached into a 3 cm^2 piece of marlex mesh with epoxy. The marlex mesh was positioned under the skin on the rat's back and the skin was sutured around the guide cannula. A stylet was inserted into the guide cannula which protruded from the rat's back.

Behavioral procedures

Discrete-trial current-threshold ICSS procedure. The procedure used was a modification of the Kornetsky and Esposito discrete-trial current-threshold procedure (Kornestksy and Esposito 1979) and has been described in detail previously (Markou and Koob 1992a). At the start of each trial, rats received a noncontingent sinusoidal electrical stimulus of 100 ms duration and 60 Hz frequency. Subjects had 7.5 s to turn the wheel manipulandum to obtain a contingent stimulus identical to the noncontingent stimulus (correct response). After the delivery of a contingent stimulus, there was an intertrial interval (ITI) averaging 10 s (7.5–12.5 s). If responding did not occur within the 7.5 s (incorrect response), the ITI followed and the trial was terminated. Any responding during the ITI (incorrect response) resulted in a 10 s delay before the start of the next trial. Stimulus intensities were varied according to the classical psychophysical method of limits (Fechner 1860) and presented in an alternating descending and ascending series with a step size of 5 μA. Each series' threshold value was defined as the midpoint in μA between the current intensity level at which the animal made two or more correct responses out of the three stimulus presentations and the level where the animal made less than two correct responses. Subjects completed four series (i.e., descending, ascending, descending, ascending) during each test session which was approximately 30 min in duration. The animal's estimated threshold for each test session was the mean of the four series thresholds.

Self-administration procedure. Each self-administration session began with two priming injections. The retractable lever was then

extended into the operant chamber. Rats were trained on a fixed ratio 5 (FR-5) schedule and reinforced with an intravenous injection of 0.1 ml saline solution of cocaine HCL (Sigma Chemical Co., St Louis, MO; 0.25 mg/injection, approximately 0.5 mg/kg/injection) administered over a period of 4 s. A signal light located above the lever indicated the onset of an injection and remained lit for 20 s during which time the lever was inactive. The session durations were 3 h during training and 12 h in the final testing session.

β_2 *Adrenergic receptor assay*

Membrane preparation. Rats were decapitated and the frontal cortex was rapidly dissected on ice. Cortical tissue was collected in 17 × 100 mm polypropylene tubes containing 5 ml isotonic saline. The tubes were placed immediately in liquid nitrogen and stored at − 70°C until β-adrenergic binding was measured.

β_2-Adrenergic receptors were measured using a modification of the methods of Wolfe and Harden (Wolfe and Harden 1981) and O'Donnell et al. (O'Donnell et al. 1984). A "saturation" method was used to generate the equilibrium binding data.

On the day the binding assay was performed, cortical tissue (34 mg wet weight/ml buffer) was thawed, weighed, and placed in an ice-cold buffer consisting of 50 mM TRIS-HCl, pH 7.4 with 5 mM $MgCl_2$ and 2 mM EGTA. The frontal cortex from individual rats was homogenized for 15 s with a tissue disrupter (Kinematica, setting 6) and centrifuged at 35 000 *g* for 30 min. The pellet "washing" was repeated by resuspending the tissue in 20 ml of the above buffer, homogenized at setting 6 for 15 s, and centrifuged again at 35 000 *g* for 30 min. Individual cortical particulate preparations received a total of three "washes". Because a linear correlation ($r = 0.9$, $n = 5$) was observed between specific binding and added membrane protein (2–289 µg/tube) (Hauger and Markou, unpublished data), the final resuspension of cortical tissue was 9 mg wet weight/ml buffer which gave a membrane protein concentration of 88.4 + / − 3.7 µg ($n = 31$) per 200 µl in the binding assay. This concentration was in the middle of the linear part of the protein curve.

[^{125}I]-(−)Iodopindolol binding method. [^{125}I]-(−)Iodopindolol (SA 2200 Ci/mmol) was obtained from Dupont-NEN (Wilmington, DE) and used within one week of the iodination date. [^{125}I]-(−)Iodopindolol was chosen on evidence that it is currently the best ligand for measuring β-adrenergic receptors in brain tissue (Wolfe and Harden 1981; Stiles et al. 1984; O'Donnell et al. 1984). (−)Isoproterenol (Sigma Co., St Louis, Mo) at a final concentration of 100 µM was used to define nonspecific binding because it does not inhibit nonspecific binding of the radioligand (Leichtling et al. 1978).

Increasing concentrations of [^{125}I]-(−)iodopindolol (56.25, 112.5, 225, 450 and 900 pM) in a total assay volume of 500 µl 50 mM TRIS-HCl buffer, pH 7.4 with 5 mM MgC_2 and 2 mM EGTA were incubated in borosilicate glass tubes containing the frontal cortex membrane preparation in the presence and absence of 100 µM (−)-isoproterenol. The addition of the membrane preparation started the binding reaction. After incubation for 30 min at 37° C, 5 ml of ice-cold binding buffer was added to the incubation contents. Immediately afterward, the samples were filtered quickly over GF/C glass fibre filters using a Brandell cell harvester to separate bound from free (Model M-48R, Biomedical Research and Development Laboratories, Inc., Gaithersburg, MD). After the first filtration, the GF/C filters were washed twice with an additional 5 ml of ice-cold buffer.

The GF/C filters were dried and then counted for bound radioactivity in a gamma-spectrometer (70% efficiency). Protein concentrations of the cortical preparation were measured by Bicinchoninic protein assay (BCA from Pierce, Rockford, Il).

Experiments

Experiment 1: Effects of acute DMI treatment on ICSS current thresholds. Four days after surgery rats ($n = 8$) were trained in the ICSS procedure until their thresholds were stable (± 10% variation in 3 consecutive days). Pre-drug and post-drug baseline thresholds were assessed for 5 consecutive days after intraperitoneal (IP) saline injections (30 min pretreatment). Animals received IP injections (3 ml/kg in saline) of DMI HCl (1.25, 2.5, 5, 10, 20 or 40 mg/kg, Lilly Co.). The four lowest doses were administered in a counterbalanced order prior to the 20 and 40 mg/kg doses. Three days elapsed between injections, during which animals were tested after saline injections.

Experiment 2: Effects of repeated DMI treatment on ICSS current thresholds. Seven of the subjects used in expt 1 were also used in expt 2. During the post-drug baseline phase of expt 1 and before the initiation of expt 2, animals' thresholds returned to pre-drug baseline levels (see Results section for expt 1). In expt 2, animals were tested twice daily, with 30 min between the two sessions (pre-injection and post-injection session). Immediately after the pre-injection session, rats received their daily injection of saline or DMI. Pre-drug and post-drug baseline thresholds were assessed for 3 consecutive days during which saline injections were administered. Animals received 10 mg/kg DMI HCl for 11 consecutive days.

Experiment 3: Effects of acute DMI treatment on cocaine self-administration rates. Catheterized animals ($n = 4$) were trained to self-administer cocaine on a FR-5 reinforcement schedule during 3 h daily sessions until responding stabilized. After baseline cocaine intake was assessed for 3 consecutive days, animals received four doses of DMI HCl (0, 2.5, 5 and 10 mg/kg) in a random order. Three days elapsed between drug injections. The injections were administered 1 h into the 3 h self-administration session.

Experiment 4: Effects of repeated DMI treatment on β-adrenergic down-regulation, and cocaine intake and withdrawal. Baseline thresholds were assessed for 5 consecutive days. Then, rats ($n = 31$) were assigned randomly to two groups, COC (cocaine) and SAL (saline) groups. The rats in the COC group were prepared with intravenous catheters and trained to self-administer cocaine. During the self-administration training period, both SAL and COC animals were tested on the ICSS procedure three times a week to ensure that the animals were still able to perform the ICSS task and their thresholds were stable. Subsequently, half the animals in the SAL (group 1, SAL-SAL) and COC groups (group 3, COC-SAL) received saline, twice daily for 5 days. The rest of the subjects (group 2, SAL-DMI; group 4, COC-DMI) received 10 mg/kg DMI HCl (5 ml/kg in saline), twice daily for 5 days (see Fig. 1).

This injection regimen produces down-regulation (30–40% below controls) of β-adrenergic receptors in the frontal cortex of rats (Wolfe et al. 1978; Wolfe and Harden 1981; Manier et al. 1984; Asakura et al. 1987), which might be one of the mechanisms mediating the antidepressant effects of tricyclics in humans (Vetulani et al. 1976; Green 1987; however see Riva and Creese 1989b; Willner et al. 1991). On the fourth injection day, approximately 12 h after the sixth injection, rats in the two COC groups (COC-SAL and COC-DMI) were allowed to self-administer cocaine for 3 h. The purpose of this session was to maintain the rats trained on the self-administration procedure and test the effects of chronic DMI treatment on cocaine self-administration rates. Immediately after this session, rats received saline or DMI according to their group assignment. On the fifth injection day, rats in the two COC groups (COC-SAL and COC-DMI) were allowed to self-administer cocaine for 12 h (9 p.m.–9 a.m.) continuously (cocaine "binge"). Five hours elapsed between the last injection and the beginning of the self-administration session to minimize any acute effects of the last DMI injection on cocaine intake. However, it was also necessary for the animals to be in cocaine withdrawal within 24 h after the last injection because there is rapid recovery of β-adrenergic receptor down-regulation after the cessation of DMI treatment (Wolfe et al. 1978). All subjects were tested on the ICSS procedure at 1, 3 and 6 h after the termination of the self-administration session for the COC animals (see Fig. 1). A previous study indicated that after 12 h of cocaine self-administration, ICSS thresholds were elevated at 1, 3 and 6 h post-cocaine (Markou and Koob 1991). Immediately after the last ICSS session (6.5–7 h post-cocaine and 23.5–24 h after the tenth injection),

Saline	Group 1: SAL-SAL (n=8)	ICSS baseline (5 days)		Saline injections (5 days)		ICSS testing 1, 3 and 6 h
Saline	Group 2: SAL-DMI (n=8)	ICSS baseline (5 days)		DMI injections (5 days)		ICSS testing 1, 3 and 6 h
Cocaine	Group 3: COC-SAL (n=7)	ICSS baseline (5 days)	Training on COC SA 3 h COC SA on day 4	Saline injections (5 days)	COC SA (12 h)	ICSS testing 1, 3 and 6 h post-coc
Cocaine	Group 4: COC-DMI (n=8)	ICSS baseline (5 days)	Training on COC SA 3 h COC SA on day 4	DMI injections (5 days)	COC SA (12 h)	ICSS testing 1, 3 and 6 h post-coc

Fig. 1. Time sequence of behavioral testing for the four groups in expt 4. Protocol for testing the efficacy of DMI in reversing cocaine withdrawal

rats were decapitated, their brains were removed, and the cortex was dissected and stored in saline at $-70°$ C, until the β-adrenergic receptor-binding assay was performed.

Data analyses and statistics

The BMDP (Dixon 1988) and SYSTAT (Wilkinson 1986) statistical packages were used for all statistical analyses. The level of significance was set at $p < 0.05$. The Greenhouse-Geisser (Geisser and Greenhouse 1959) degrees of freedom were used in repeated-measures analyses of variance (ANOVAs) when the sphericity test indicated non-homogeneity of variance. Significant ANOVAs were followed with Newman-Keuls posthoc comparisons (Winer 1971).

Experiment 1. A paired *t*-test was performed on the pre- and post-drug threshold baseline data. When this test showed the absence of significant differences, the mean of the pre- and post-drug baseline data was used as a saline baseline. A repeated-measures ANOVA was performed on the threshold data (four levels corresponding to doses 1.25, 2.5, 5 and 10 mg/kg DMI) with order of injection as the independent variable to test for any confounding effects of the order of presentation of the drug doses. Given no significant order effect, a repeated-measures ANOVA was performed on the data with DMI dose as the independent variable (seven levels: 0, 1.25, 2.5, 5, 10, 20 and 40 mg/kg DMI).

Experiment 2. Paired *t*-tests compared the pre-and the post-injection thresholds before and after the drug treatment. In the absence of significant changes, the means were used as baseline. Repeated-measures ANOVAs with day as the independent variable (12 levels; baseline, 1–11 DMI days) were performed on the pre- and post-injection threshold data.

Experiment 3. A repeated-measures ANOVA with DMI dose as the independent variable (four levels: 0, 2.5, 5 and 10 mg/kg DMI) was performed on the number of injections self-administered during the last 2 h of the cocaine session.

Experiment 4. Because there can be large individual differences among rats' baseline thresholds, and because the most critical comparisons for this experiment were between-group comparisons, all thresholds were converted to percent change from each animal's pre-drug baseline threshold. In the few cases when an animal completely failed to respond following the 12 h cocaine self-administration session (see Results section), the arbitrary threshold value of 270% of baseline was assigned. This value was selected because it was slightly larger than the highest threshold that any animal exhibited at any point during the experiment. The assumption that failure to respond is due to higher thresholds and not to motor effects is supported by the fact that subjects that responded after the same manipulations exhibited higher thresholds compared to their baseline thresholds. The threshold data were analyzed with a three-factor ANOVA with repeated-measures on one of the three factors. The between factors were Drug (two levels: saline and DMI) and Cocaine Withdrawal (two levels: control and COC), and the repeated-measures factor was Hours post-cocaine (three levels: 1, 3 and 6 h).

The number of cocaine injections self-administered by the two COC groups (COC-SAL and COC-DMI) during the pre-DMI baseline days (mean of 3 days), during the 3 h cocaine self-administration session on the fourth day of experimenter-administered injections, and during the final 12 h cocaine session were transformed to mg/kg. A two-factor ANOVA with one-repeated measures factor was performed on the cocaine intake data. The between-factor was Group (two levels: COC-SAL and COC-DMI) and the repeated-measures factor was Condition (two levels: pre-DMI baseline and 3 h session). A repeated-measures ANOVA was also performed on data from the 12 h session with Group as the independent variable (two levels: COC-SAL and COC-DMI).

The nonlinear, least squares curve-fitting program LIGAND (Munson and Rodbard 1980) was used for the estimation of the β_2-adrenergic receptor affinity (K_d) and concentrations. (B_{max}). The B_{max} value for each subject was adjusted for protein concentration by dividing the calculated B_{max} value by the protein concentration in the 200 µl membrane preparation. Then, the K_d and B_{max} values were analyzed with two-factor ANOVAs. The two-between factors in the ANOVAs were Drug (two levels: saline and DMI) and Cocaine Withdrawal (two levels: SAL and COC).

Finally, regression analyses were performed, with B_{max} and cocaine dose as the predictor variables, and the animal's post-cocaine thresholds as the dependent variable. Amount of cocaine consumed during a binge determines the duration and severity of the post-cocaine depression in both rats (Markou and Koob 1991) and humans (Gawin and Kleber 1986). Because in this experiment, animals were allowed to self-administer cocaine but the main interest was in the correlation between B_{max} and thresholds, it was important to statistically separate the influence of cocaine dose and B_{max} on thresholds. This separation is achieved by estimating partial correlations that allow the assessment of a predictor variable's effect independent of the effects of another variable (Cohen and Cohen 1975; Pedhazur 1982). Only data from the COC-SAL and COC-DMI groups were used in the regression analyses. Three correlation coefficients were calculated corresponding to the three time-points that the subjects were tested following the 12 h cocaine session.

Results

Acute DMI treatment and ICSS thresholds (experiment 1)

The mean of the pre and post-drug baseline thresholds was used in the ANOVAs as the saline baseline, since they did not differ [$t(7) = 0.025$, n.s.]. The ANOVA with order of injections as the independent variable confirmed the absence of an order effect [$F(3,21) = 0.939$, n.s.]. Finally, the ANOVA with DMI dose as the independent variable demonstrated no significant effect of acute DMI treatment on ICSS current thresholds [$F(6,24) = 3.17$, n.s. with Greenhouse-Geisser degrees of freedom adjustment (epsilon factor for adjustment = 0.4176)] (see Fig. 2).

Repeated DMI treatment and ICSS current thresholds (experiment 2)

The means of the pre- and post-drug pre-injection, and the pre- and post-drug post-injection thresholds were used in subsequent analyses as saline baseline, since they did not differ [$t(6) = 0.703$, 1.517, n.s.]. The ANOVAs on the pre- [$F(11,66) = 0.82$, n.s.] and post-injection [$F(11,66) = 1.91$, n.s.] data indicated no significant effect of chronic DMI treatment on thresholds (see Fig. 3).

Acute DMI treatment and cocaine intake (experiment 3)

The repeated-measures ANOVA indicated that acute DMI treatment did not alter cocaine self-administration rates [$F(3,9) = 0.728$, n.s.] (see Table 1).

Repeated DMI treatment and cocaine withdrawal (experiment 4)

The three-factor ANOVA on thresholds gave a significant main effect for Cocaine Withdrawal [$F(1,27) = 26.94$, $P < 0.01$], indicating that rats in the two cocaine groups had higher ICSS thresholds than controls. The ANOVA also indicated no main effects for Drug [$F(1,27) = 0.85$, n.s.] and Hours [$F(2,54) = 1.13$, n.s.]. Most importantly, there was a significant interaction between Withdrawal and Hours [$F(3.61) = 3.61$, $P < 0.05$]. None of the other interactions were statistically significant [$F(1,27) = 2.41$, $F(2,54) = 0.88$, $F(2,54) = 0.68$, n.s.]. To more fully explore the source of the significant main and interaction effects, Newman-Keuls post-hoc tests were performed (see Fig. 4). There were no significant differences between the SAL-SAL and SAL-DMI groups at any time-point ($P > 0.05$). Thresholds for groups COC-SAL and COC-DMI were significantly higher than SAL groups thresholds at 1 and 3 h post-cocaine ($P < 0.05$). At 6 h post-cocaine, the COC-DMI group thresholds were not different from either of the two SAL group thresholds but were lower than the COC-SAL group thresholds ($P < 0.05$).

As described above, two out of eight rats in the COC-SAL group and two out of seven rats in the COC-DMI group failed to respond on some occasions after the 12 h cocaine session. In these cases, the threshold value of 270% of baseline was assigned. None of the rats in the two SAL groups failed to respond at any time. Analyses of the data, excluding these four subjects, gave identical overall results as the previous analyses.

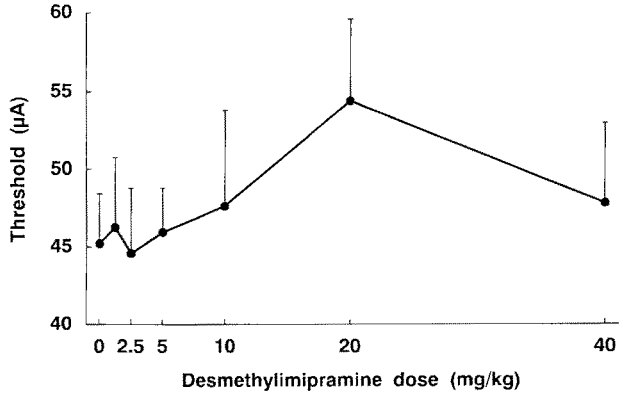

Fig. 2. Acute DMI treatment, administered 30 min before the start of the ICSS session, did not alter ICSS thresholds ($n = 8$). The means and standard errors of the means for eight subjects are shown

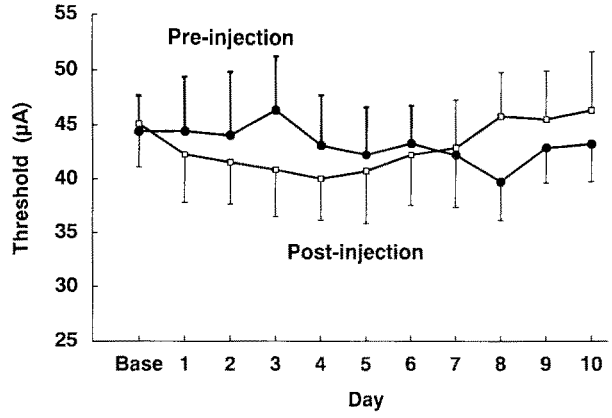

Fig. 3. Chronic DMI treatment (10 mg/kg for 11 consecutive days) did not alter ICSS thresholds ($n = 7$). Subjects' thresholds were assessed twice daily. Immediately after the first ICSS session (pre-injection session), an injections was administered. Thirty minutes post-injection, the second daily ICSS session was initiated (post-injection session). The means and standard errors of the means for seven subjects are shown

Table 1. Mean ± sem number of cocaine injections (0.25 mg/kg injection) self-administered during the last 2 h of a 3-h cocaine self-administration session immediately after an acute dose of DMI ($n = 4$). Injections were administered 1 h into the self-administration session

DMI dose (mg/kg)	Number of COC injections
0 (saline)	21.8 ± 1.6
2.5	20.8 ± 2.7
5	20.3 ± 0.9
10	17.5 ± 3.5

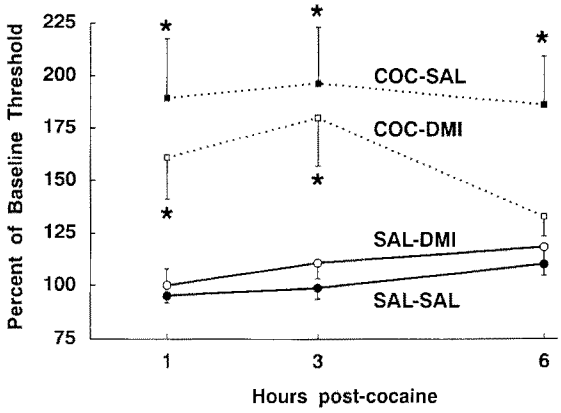

Fig. 4. Mean ICSS thresholds ± standard error of the mean at 1, 3 and 6 h after the termination of a 12 h cocaine self-administration session. The results are expressed as percent change from baseline threshold levels. The mean ± SEM baseline threshold for the SAL-SAL group ($n = 8$) was 42.105 ± 3.654 μA, for the SAL-DMI ($n = 8$) 41.786 ± 3.38 μA, for the COC-SAL ($n = 8$) 34.464 ± 2.043 μA and for the COC-DMI ($n = 7$) 36.041 ± 3.839 μA. The *asterisks* indicate significantly higher thresholds ($P < 0.05$) than the thresholds of groups SAL-SAL and SAL-DMI with Newman-Keuls post-hoc comparisons, following a significant Withdrawal × Hours interaction in the ANOVA (see Results section for detailed description of post-hoc comparisons)

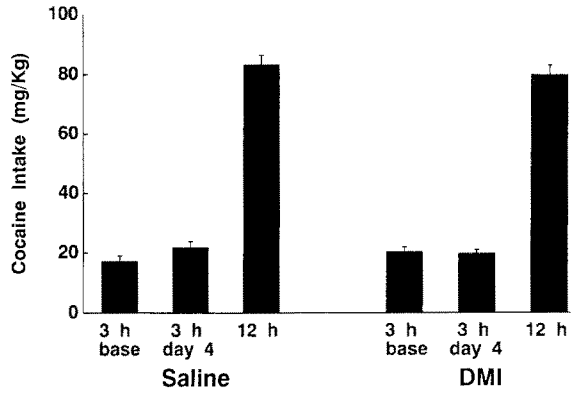

Fig. 5. The mean cocaine intake in mg/kg ± the standard error of the means for the COC-SAL and COC-DMI groups during baseline, during the fourth injection day (after 3 days of chronic DMI treatment – 10 mg/kg/12 h) and during the 12 h self-administration session (after 5 days of chronic DMI treatment — 10 mg/kg/12 h). There were no statistically significant differences between the two groups on any of these three self-administration sessions

Repeated DMI treatment and cocaine intake (experiment 4)

The ANOVA on cocaine intake for the two COC groups during baseline (mean of 3 days before the initiation of the chronic injection regimen) and on the fourth injection day revealed no significant main effect for Group [$F(1,12) = 0.02$, n.s.], but a significant main effect for Day [$F(1, 12) = 4.78$, $P < 0.05$] and a significant Group X Day interaction [$F(1,12) = 8.12$, $P < 0.05$]. Newman-Keuls comparisons attributed these effects to a significant increase in cocaine intake for the COC-SAL group on the fourth injection day ($P < 0.05$) and the absence of such an increase for the COC-DMI group ($P > 0.05$). Nevertheless, there were no significant differences between the two groups on cocaine intake on baseline days ($P > 0.05$) or on the fourth injection day ($P > 0.05$). Furthermore, the ANOVA on cocaine intake during the 12 h self-administration session indicated no difference between the two groups [$F(1,12) = 0.43$, n.s.] (see Fig. 5).

Repeated DMI treatment, and the equilibrium dissociation constant (K_d) and maximal number of β-adrenergic binding sites (B_{max}) in the frontal cortex (experiment 4)

Using a "saturation" method, we measured a high affinity (107 ± 4 pM, $n = 31$) population of β-adrenergic receptor in the frontal cortex, in good agreement with K_d values reported by other investigators (Wolfe et al. 1978; O'Donnell et al. 1984; Stiles et al. 1984). The ANOVA on K_d indicated the absence of a significant effect of Drug (SAL versus DMI) [$F(1,27) = 1.57$, n.s.], or Withdrawal (SAL versus COC) [$F(1,27) = 0.47$, n.s.]. There was no interaction effect either [$F(1,27) = 0.0$, n.s.] (see Table 2).

Table 2. Mean ± sem equilibrium dissociation constants (K_d) and maximal number of β-adrenergic binding sites (B_{max}) in the frontal cortex for the four groups in experiment 4. Asterisks indicate significantly lower B_{max} values compared to SAL-SAL (Newman-Keuls tests following a significant ANOVA, see Results section)

Group	K_d (pM)	B_{max} (fmol/mg)
SAL-SAL	109.6 ± 5.6	69.1 ± 3.4
SAL-DMI	100.0 ± 6.8	53.3 ± 2.8*
COC-SAL	114'7 ± 12.1	69.1 ± 5.8
COC-DMI	105.2 ± 5.4	52.0 ± 2.5*

The ANOVA on B_{max} indicated a significant effect for Drug (SAL versus DMI) [$F(1,27) = 20.04$, $P < 0.05$]. There was no Withdrawal (SAL versus COC) [$F(1,27) = 0.03$, n.s.], or interaction effects [$F(1,27) = 0.03$, n.s.]. Newman-Keuls comparisons indicated significant lower B_{max} for the groups SAL-DMI and COC-DMI compared to groups SAL-SAL and COC-SAL ($P < 0.05$). However, there were no differences between groups SAL-SAL and COC-SAL, and SAL-DMI and COC-DMI ($P > 0.05$). This pattern of results indicates that the significant main effect in the ANOVA is due to lower B_{max} values for the groups that received DMI compared to the groups that received SAL (see Table 2).

Correlational analysis of the maximal number of β-adrenergic receptors (B_{max}) and ICSS thresholds during cocaine withdrawal (experiment 4)

Regression analyses were performed to investigate the relationship between the number of β-adrenergic receptors and the post-cocaine elevation in ICSS thresholds. Data from one animal in the COC-SAL group were excluded because this animal was an outlier (two standard deviations different) on one of the predictor variables (i.e.,

B_{max}). When both cocaine dose and B_{max} were entered into the regression equation, the multiple *R*s were 0.41 ($P > 0.05$), 0.169 ($P > 0.05$) and 0.744 ($P < 0.01$), corresponding to 1, 3 and 6 h post-cocaine, respectively. Most importantly, the partial correlation of thresholds at 6 h post-cocaine with B_{max}, when the effects of cocaine dose were partialled out, was 0.79 ($P < 0.01$). This small increase in the correlation of thresholds with B_{max}, when the cocaine effects are partialled out, indicates that cocaine dose is a suppressor variable (Cohen and Cohen 1975; Pedhazur 1982).

Discussion

The present study investigated the effects of acute and chronic DMI treatment on reward functions and β-adrenergic receptors in naive and cocaine-withdrawing rats as measured by ICSS thresholds and cocaine self-administration. The results indicated that acute or chronic treatment with a tricyclic antidepressant, DMI, did not alter the threshold for brain stimulation reward in non-cocaine withdrawing rats. However, chronic DMI treatment shortened the duration of the cocaine withdrawal (elevation in ICSS thresholds, anhedonia) with only a small and non-significant effect on the severity of the withdrawal during the first 3 h post-cocaine. Furthermore, DMI's attenuation of cocaine withdrawal was positively correlated with its ability to down-regulate β-adrenergic receptors in the frontal cortex, a proposed mechanism for antidepressant action. Finally, neither acute nor chronic DMI treatment altered the amount of cocaine consumed by the rats.

Acute DMI treatment did not influence reward functions in non-cocaine withdrawing rats, as indicated by the absence of change in ICSS thresholds (expt 1, Fig. 2). These results are in agreement with other reports of lack of effect of acute treatment with DMI or imipramine (a tricyclic antidepressant metabolized into DMI) on ICSS reward as measured by response rates (Binks et al. 1979), and frequency (Hall et al. 1990) and current thresholds (Fibiger and Phillips 1981).

Similarly, chronic DMI treatment did not alter the reward value of ICSS in non-withdrawing rats as demonstrated in two occassions in this report: a) in experiment 2, ICSS thresholds did not change after 11 days of 10 mg/kg/24 h DMI (see Fig. 3), and b) in experiment 4, Group SAL-DMI's thresholds (10 mg/kg/12 h for 5 days) did not differ from pre-drug baseline levels or from thresholds of controls (see Fig. 4). Most previous investigations of chronic DMI treatment on a variety of ICSS measures indicated no effect on ICSS reward from several brain sites (Simpson and Annau 1977; Aulakh et al. 1985; Hall et al. 1990). Two studies showed a lowering of ICSS current thresholds (reward increment) with repeated DMI treatment (Fibiger and Phillips 1981; McCarter and Kokkinidis 1989). However, one of these studies (Fibiger and Phillips 1981) demonstrated a small increase in reward only in the ascending current series, an effect attributed to positive contrast effects (Liebman 1983; Hall et al. 1990); and in the second study (McCarter and Kokkinidis 1989), the magnitude of the reward increment was small compared to reward effects usually seen with ICSS threshold procedures (Stellar and Rice 1989; Hall et al. 1990).

Most importantly for the interpretation of the effects of DMI administration on cocaine withdrawal in the present study, chronic DMI treatment did not influence reward functions in naive rats, as measured with current thresholds provided by the discrete-trial ICSS procedure. Thus, the effect of DMI administration on cocaine withdrawal cannot be attributed to an additive effect of the two manipulations (cocaine withdrawal and DMI).

The results of the present study (expt 4) indicated that chronic DMI treatment significantly shortened the duration of the post-cocaine anhedonia (elevation in ICSS thresholds) with no effect on the severity of the withdrawal syndrome during the first 3 h post-cocaine (see Fig. 4). Group COC-SAL, which was pretreated with saline before the 12 h cocaine session, showed an elevation in ICSS threshold levels (approximately 190%) at 1, 3 and 6 h post-cocaine, compared to control group SAL-SAL. This post-cocaine elevation in thresholds is interpreted as a symptom of cocaine withdrawal (post-cocaine anhedonia) and replicates previous work (Markou and Koob 1991, 1992b). In addition, the elevation in current-thresholds post-cocaine corroborates the findings of another cocaine withdrawal study in which cocaine was experimenter-administered (Kokkinidis and McCarter 1990). In contrast to the COC-SAL group, the COC-DMI group, which was pretreated with DMI before the 12 h cocaine session, had significantly elevated thresholds at 1 and 3, but not at 6 h post-cocaine compared to the SAL-DMI group. Thus, chronic DMI treatment had no effect on the severity of the withdrawal syndrome early during the withdrawal phase but shortened the duration of the withdrawal.

Chronic DMI administration has also been shown to reverse the attenuation of ICSS behavior observed following chronic treatment with another psychomotor stimulant, amphetamine (Kokkinidis et al. 1980). Thus, DMI could be a useful pharmacological treatment not only for cocaine withdrawal but for other stimulant withdrawal syndromes.

DMI and other tricyclics are effective antidepressants (Green 1987) and depression is a major symptom of cocaine withdrawal (Gawin and Kleber 1986). Preliminary clinical studies indicated that chronic DMI treatment decreases cocaine withdrawal symptoms in humans (Giannini et al. 1986; Baxter 1983; Gawin and Kleber 1984).

In the present study, chronic DMI treatment ameliorated the post-cocaine withdrawal symptom at a dose that produced a significant down-regulation of β_2-adrenergic receptors ($24.7 \pm 3.4\%$ decrease in B_{max}) in the frontal cortex, with no effect on receptor affinity (K_d) (expt 4, Table 2). The magnitude of this down-regulation is similar to the decreases seen in other studies (Wolfe et al. 1978; Wolfe and Harden 1981; Manier et al. 1984; Asakura et al. 1987). Finally, there was no effect of chronic cocaine administration on B_{max} or K_d and no interaction effect of cocaine and DMI administration on B_{max} or K_d for β_2-adrenergic receptors.

Most importantly, there was a significant positive correlation ($r = 0.79$) between the number of β_2-adrenegic

receptors and thresholds at 6 h post-cocaine. This correlation provides some indication of a dose-response relationship and further emphasizes the relationship between DMI administration and reversal of the threshold elevation. However, it should be emphasized that this correlational relationship does not necessarily imply a critical role for the noradrenergic system in cocaine withdrawal.

Nevertheless, it is possible that the neuronal mechanisms mediating the therapeutic effects of tricyclics on affective disorders and cocaine withdrawl are similar given the similarity of some of the symptoms for the two conditions. Several studies measured chronic tricyclic-induced down regulation of β-adrenergic receptors in both the frontal cortex (Wolfe et al. 1978; Wolfe and Harden 1981; Manier et al. 1984, 1987; Asakura et al. 1987) and the hippocampus (Brunello et al. 1982) of rats. In addition, the time-course for the therapeutic action of antidepressants in depressed humans occurs within the 2–3 week period when β-adrenergic receptor down regulation occurs. The parallel time-courses of the therapeutic and β-receptor effects, and the fact that other antidepressant treatments (e.g. atypical antidepressants, electroconvulsive therapy) (Asakura et al. 1987; Sethy et al. 1988) also result in a reduction in the number of β-adrenergic receptors have provided support for the catecholamine hypothesis of affective disorders (Schildkraut 1965; Vetulani et al. 1976), and led to the β-adrenergic hypothesis for the mechanism of action of antidepressants (Vetulani et al. 1976; Green 1987; however see Riva and Creese 1989a, b; Willner et al. 1991). A recent formulation of this hypothesis states that in affective disorders the noradrenergic system is dysregulated, which diminishes its ability to appropriately modulate neuronal firing of other neurotransmitter systems (Potter et al. 1988; Hauger et al. 1991). Thus, in individuals with affective disorders, the noradrenergic system may have lost its "flexibility" and consequently the individual's ability to adapt to environmental perturbations may be decreased. This hypothesis is consistent with suggestions that the noradrenergic system in the normal brain enhances the "signal to noise" ratio and thereby modulates responses to environmental stimuli (Flicker and Geyer 1982; Foote et al. 1983). Thus, tricyclic antidepressants and other antidepressant treatments might produce their therapeutic effect by restoring the noradrenergic synaptic efficiency (Potter et al. 1988).

However, it should be emphasized that there are complex neurotransmitter interactions that are likely to be important in these phenomena. For example, serotonergic pathways to the cortex appear to play a permissive role in the regulation of β-adrenergic receptors by tricyclics (Manier et al. 1984, 1987; Green 1987). In addition, chronic treatment with antidepressants induces changes not only in β-adrenergic receptor function but also in α_2-adrenergic, 5-HT_2, 5-HT_{1A}, and $GABA_B$ receptors (Green 1987; Riva and Creese 1989a, b). Finally, there is some behavioral evidence for an action of tricyclic antidepressants on dopaminergic function (Spyraki and Fibiger 1981; Martin-Iverson et al. 1983; Maj et al. 1989; De Montis et al. 1990; Muscat et al. 1990). This effect has been attributed to anticholinergic effects of tricyclics and is consistent with a dopaminergic-cholinergic interaction in affective disorders (Janowsky et al. 1972; Risch et al. 1980; Martin-Iverson et al. 1983). Given that both bromocriptine, a D_2 dopamine agonist (Markou and Koob 1992b), and DMI, a tricyclic antidpressant with possible indirect effects on dopaminergic function, reverse the post-cocaine elevation in thresholds, then it is possible that both bromocriptine and DMI produce their effect through direct or indirect actions on the dopaminergic system. However, the evidence for the role of the dopaminergic system in depression is far from conclusive (Willner et al. 1991).

Clinical trials indicated that chronic DMI treatment decreased cocaine use and craving in humans which led to more frequent and longer abstinence periods (Gawin and Kleber 1984; Gawin 1986; Gawin et al. 1989; however see Frank et al. 1988). However, neither acute (expt 3) nor chronic (expt 4) DMI treatment altered the amount of cocaine self-administered by animals in the present study. These results are in agreement with other experimental studies that also failed to show a reduction in cocaine consumption in rats (Tang and Falk 1990), monkeys (Kleven and Woolverton 1990), and humans (Fischman et al. 1990) with chronic DMI treatment. This discrepancy may be due to the differences between the experimental and clinical situations. In the experimental studies, subjects had access to the drug only for a limited period of time. In addition, human experimental subjects, similarly to self-administering rats, were not trying to abstain from cocaine (Fischman et al. 1990), unlike subjects in clinical trials (Gawin and Kleber 1984; Gawin 1986; Gawin et al. 1989). It is possible that DMI administration could assist in cocaine abstinence when other factors (e.g., desire to abstain, psychotherapy) are also operating.

In conclusion, in non-cocaine withdrawing rats neither acute nor repeated DMI treatment influenced reward functions, as measured by ICSS thresholds and cocaine self-administration. However, repeated DMI treatment significantly down-regulated β-adrenergic receptors and reversed the post-cocaine "anhedonia" (elevation in thresholds). Furthermore, the magnitude of the β-adrenergic receptor down-regulation was correlated with the degree of effectiveness of DMI administration in reversing the post-cocaine "anhedonia". The reversal of the post-cocaine anhedonia by chronic DMI treatment indicates that tricyclic antidepressants may ameliorate some symptoms of cocaine withdrawal and further establishes the predictive validity of this rat model of cocaine withdrawal. Two pharmocological treatments having some efficacy in treating cocaine withdrawal in humans, DMI (Gawin et al. 1989) and bromocriptine (Giannini et al. 1989) were also effective in this animal model of cocaine withdrawal (present study; Markou and Koob 1992b). Thus, this animal model could be useful in the identification of new pharmacotherapies for cocaine withdrawal.

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