Pharmacology Biochemistry and Behavior, Vol. 43, pp. 697–704, 1992
Printed in the U.S.A.

0091-3057/92 $5.00 + .00

Anxiolytic-Like Effects of Alpha-2-Adrenoceptor Agonists on Conflict Behavior in the Rat: Pre- Versus Postsynaptic Receptor Mechanisms

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Received 20 November 1990

FONTANA, D. J. AND R. L. COMMISSARIS. *Anxiolytic-like effects of alpha-2-adrenoceptor agonists on conflict behavior in the rat: Pre- versus postsynaptic receptor mechanisms.* PHARMACOL BIOCHEM BEHAV 43(3) 697–704, 1992.—The effects of acute pretest administration and chronic posttest administration of clonidine or the selective α_2-adrenoceptor agonist UK-14,304 on conflict behavior were investigated. In daily 10-min sessions, water-deprived rats were trained to drink water from a tube that was occasionally electrified (0.25 mA); electrification was signaled by a tone. Prior to treatment, subjects accepted 25–30 shocks/session (punished responding) and consumed approximately 12–15 ml/session (unpunished responding). Acute pretest administration of clonidine or UK-14,304 did not increase punished responding. In contrast, chronic posttest clonidine administration (40 μg/kg, IP, twice daily for 8 weeks) resulted in a robust and time-dependent increase in punished responding (60–70 shocks/session) relative to saline-treated controls. Moreover, the selective α_2-adrenoceptor agonist UK-14,304 also increased punished responding when administered chronically (1.0 mg/kg, BID). Administration of the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine HCl (DSP4, 65 mg/kg, IP) significantly decreased punished responding in control conditioned suppression of drinking sessions. The anticonflict effect associated with chronic posttest clonidine treatment was not altered by DSP4 pretreatment. These findings suggest that chronic posttest α_2-adrenoceptor agonist treatment produces an anticonflict effect independent of its actions at presynaptic α_2-adrenoceptors.

Conflict behavior Clonidine DSP4 Norepinephrine α_2-Adrenoceptors UK-14,304 Anxiolytics

ALTHOUGH it is used more frequently in the treatment of hypertension (33), the α_2-adrenoceptor agonist clonidine does exhibit antianxiety effects in certain situations. Clonidine effectively reduces the anxiety associated with opiate (15,17) or ethanol (41) withdrawal. Clonidine also has been reported to be an effective antipanic agent (4,20,26,38,39).

In animal models for "anxiety," there is considerable disagreement regarding the effects of acute clonidine treatment, with many investigators reporting anxiolytic-like effects (2,7,19,21,23,37,42) and a nearly equal number reporting the lack of an anxiolytic-like effect of acute clonidine treatment (5,9,11,12,18,24,27,28). Some investigators have attributed these discrepant results to the conditions of the behavioral test used [level of behavioral suppression in conflict paradigms: Sepinwall and Cook (34)] and/or dose of clonidine administered [selective α_2- vs. nonselective α_1- and α_2-agonist effects: Soderpalm and Engel (35)]. Unfortunately, however, no single explanation offered completely explains these discrepancies regarding the effects of acute treatment with clonidine in animal models for anxiety.

It has been reported recently that chronic posttest treatment with clonidine results in a robust and reliable anxiolytic-like effect in the conditioned suppression of drinking (CSD) conflict paradigm (5,11), a hybrid of the Vogel acute conflict task (40) and the Geller-Seifter conditioned conflict task (13,14). In this paradigm, acute pretest treatment with clonidine does not exert an anxiolytic-like effect (5,11).

Clonidine acts as an agonist at both pre- and postsynaptic

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α_2-adrenoceptors in the brain. It has been proposed that the anxiolytic effects of clonidine are the result of the suppression of the activity of locus coeruleus noradrenergic neurons via actions at presynaptic α_2-adrenoceptors (4,30,31). The importance, if any, of postsynaptic α_2-adrenoceptors in the anxiolytic effects of clonidine presently is unknown.

Acute systemic treatment with the noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine HCl (DSP4) produces a relatively selective destruction of noradrenergic neurons in both the periphery and CNS (2,22,32). Recovery is quite dramatic in the periphery but far less dramatic in the CNS (8,22,32). Because DSP4 treatment would effectively destroy presynaptic α_2-adrenoceptors while leaving postsynaptic α_2-adrenoceptors intact, this agent is a useful tool for studying the importance of presynaptic vs. postsynaptic α_2-adrenoceptor function on a particular behavior.

The purpose of the present studies was to investigate the importance of pre- vs. postsynaptic α_2-adrenoceptors in the anxiolytic-like effects of chronic posttest treatment with the α_2-adrenoceptor agonists on conflict behavior. To this end, the effect of DSP4-induced lesions of brain noradrenergic neurons on the anticonflict effect of chronic clonidine treatment was determined. In addition, the effects on conflict behavior of acute pretest and chronic posttest treatment with the relatively more selective α_2-adrenoceptor agonist UK-14,304 (3) were determined.

METHOD

Animals

Female Sprague-Dawley rats (Charles River Farms, Cambridge, MA; 225–250 g at the start of experiments) were housed in groups of four in a climate-controlled room with a 12 L : 12 D cycle (lights on 0700–1900 h). Initially, food and water were available continuously. Following a 2-week accommodation period and continuing throughout the period of behavioral assessment, all animals were maintained on a restricted water schedule (see below). Food continued to be available in the home cage.

Conflict Testing—Apparatus

Conditioned suppression testing was conducted in an apparatus similar to that described by Fontana et al. (10,11). The testing chamber was a rectangular box with Plexiglas sides and a metal floor and top. Protruding from one wall was a metal drinking tube, to which a calibrated (1.0-ml units) length of polyethylene tubing was attached for measuring the volume of water consumed. Programming for the test sessions was controlled by solid-state modular programming equipment (Coulbourn Instruments Co., Lehigh Valley, PA).

Conflict Testing—General Procedure

For the first few sessions, water-restricted (24 h deprivation) subjects were placed in the experimental chamber and allowed to consume water freely without the shock contingency. After 1 week of nonshock sessions, the tone/shock contingency was initiated. The 7-s tone periods were presented at regular (22-s ISI) intervals to subjects. During the last 5 s of these tone periods, contact between the floor and the metal drinking tube completed a circuit that resulted in the delivery of a shock (0.25 mA) to the rat. Shocks were delivered by a Coulbourn Instruments Shocker (Model E13-02).

In all experiments, subjects were tested individually at the same time of day. All subjects achieved stable baselines (day-to-day coefficients of variation of approximately 30% for individual rats) for punished and unpunished responding by the end of the second week of CSD sessions with the alternating tone : no tone periods. Baseline (i.e., nondrug) CSD testing was continued for 2 additional weeks before drug testing was initiated. CSD testing was conducted 4 days per week (Monday–Thursday) and free access to water was provided on nontest days (Thursday posttest until Sunday a.m.).

Mydriatic Response Testing

In some experiments, the neurotoxin DSP4 was administered to destroy brain noradrenergic neurons. As a functional assay for central noradrenergic depletion, the mydriatic response following an acute challenge with the norepinephrine reuptake blocker desipramine (DMI) was conducted using a modification of the procedure described by Pitts and Marwah (29). In these experiments, pupillary diameter was examined in conscious rats in a dimly lit room before and at 10, 20, 30, and 60 min after acute treatment with 10 mg/kg DMI. This dose was selected because in pilot studies it was found to exert a mydriatic response in over 98% of naive rats examined. The mydriatic response was rated to be present or absent as judged by a trained observer (D.J.F.) who was blinded to the pretreatment condition (DSP4 or vehicle).

Neurochemical Determinations

The effects of DSP4 treatment on dopamine and norepinephrine concentrations in various brain regions were determined in a separate group of subjects (i.e., not tested in the CSD conflict paradigm). These subjects were sacrificed eight weeks after DSP4 or vehicle treatment. Forty-eight hours prior to sacrifice, these subjects were tested for a mydriatic response to an acute DMI challenge using the procedure described above. Following sacrifice, brains were rapidly removed and dissected into frontal cortex, hippocampus, hypothalamus, and striatum. The concentrations of dopamine and norepinephrine were determined in these brain regions using a modification of the methods of Glowinski and Iverson (16). Briefly, brain regions were weighed and homogenized by sonification in 10 vol ice-cold 0.1 M HCl containing 1.0 M KCl and 2.3 mM ascorbic acid; the samples were then centrifuged at 40,000 $\times$ *g* for 7 min. Aliquots (20 μl) of the supernatant were assayed for dopamine and norepinephrine using high-performance liquid chromatography (HPLC) with electrochemical detection [BioAnalytical Systems LC-B4 (West Lafayette, IN)] at a voltage setting of 0.7 V. The mobile phase was delivered by a pump [LKB 2150] (Uppsala, Sweden) fitted to a C-18 reverse-phase Biophase column (250 $\times$ 2.6, 5-μm particle size). Chromatograms were registered on a dual pen chart recorder. The mobile phase consisted of 0.1 M NaH_2PO_4, 0.5 mM EDTA, 1.0 mM sodium octyl sulfate, and 6% acetonitrile (pH = 3.1) and was delivered at 1.5 ml/min. Standard curves, with concentrations ranging from 0.1–100 pM, were used to quantify each compound; 1.0 μM 3,4-dihydroxy-benzylamine (Sigma Chemical Co., St. Louis, MO) served as the internal standard for the catecholamine assays. Catecholamine concentrations are expressed as ng/g wet weight of tissue.

Specific Experiments Conducted

Experiment 1: Effects of acute pretest administration of UK-14,304 on conflict behavior. Acute pretest treatment with clonidine has been reported not to exert anxiolytic-like effects

on CSD conflict behavior (5,11). The effects of the selective postsynaptic α_2-adrenoceptor agonist UK-14,304 (3) have not been examined. A group of subjects, trained for CSD testing as described above, was used to determine the effects of various doses (0.015–1 mg/kg) of UK-14,304 at a 10-min pretreatment. All drug tests were conducted on Wednesdays and Thursdays each week using a standard "crossover" procedure. On the Wednesday drug tests, half the subjects received a given dose of UK-14,304 and half received vehicle (saline). These treatments were reversed on the Thursday drug tests. Thus, each animal served as its own control. In this experiment, the order of UK-14,304 doses examined was randomized for each subject.

Experiment 2: Antagonism by acute pretest yohimbine administration. Because antagonism by yohimbine is important to establish the involvement of α_2-adrenoceptors, the effects of yohimbine pretreatment on the effects of a single dose of clonidine or UK-14,304 were determined using a modification of the procedure described by Commissaris et al. (6). The agonist doses (20 μg/kg clonidine, 61.25 μg/kg UK-14,304) were selected because they produced approximately 50% reductions in both shocks received and water intake when administered alone. In these studies, subjects were pretreated (IP; 15 min prior to testing) with yohimbine (1.25 mg/kg) or saline on both the Thursday and Friday test days of a given test week, while the α_2-adrenoceptor agonist and its vehicle (saline) were administered on alternate days (IP; 10 min pretreatment). Thus, for a given test week the "net" effect of clonidine or UK-14,304 ("net" effect = agonist − saline) was determined in animals pretreated with either yohimbine or saline. The interactions of yohimbine with clonidine and UK-14,304 were determined in four groups (n = 8 subjects/group) of subjects.

Experiment 3: Chronic posttest UK-14,304 treatment effects on conflict behavior. A third group of subjects (n = 16), trained for CSD testing as described above, was used to determine the effects of chronic posttest treatment with UK-14,304 on CSD behavior. Following 4 weeks of nondrug CSD sessions, subjects were assigned to two treatment conditions with comparable levels of punished responding over the last 2 weeks of these control CSD sessions (baseline). Subjects in one treatment condition received chronic posttest treatment with UK-14,304 (1.0 mg/kg, twice daily); controls received saline injections. CSD testing (4 days/week) was continued throughout the period of chronic drug treatment (4 weeks). Chronic drug or vehicle treatments were administered immediately posttest and again 12 h later so that the minimum interval between any drug injection and CSD testing in this experiment was 12 h. UK-14,304 and saline treatments were administered twice daily 7 days/week.

Experiment 4: Chronic posttest clonidine treatment effects on conflict behavior—effects of DSP4 pretreatment. A fourth group of subjects (n = 24) was trained for CSD testing as described above. Following 4 weeks of nondrug CSD sessions, subjects were assigned to two pretreatment conditions with comparable levels of punished responding over the last 2 weeks of these control CSD sessions (pre-DSP4/saline baseline). Subjects in one treatment condition received a single injection of DSP4 (65 mg/kg, IP); controls received comparable saline injections.

Subjects were allowed to recover for 24 h prior to the reinstatement of CSD behavioral testing (4 days/week). Subjects were then tested in the CSD paradigm for 2 additional weeks. At this time, the mydriatic response to an acute challenge with 10 mg/kg DMI was conducted as described above. All vehicle-treated subjects exhibited a mydriatic response, whereas none of the DSP4-treated subjects did.

The average number of shocks accepted in conflict sessions over the 2 weeks following DSP4 or saline treatments (post-DSP4/saline baselines) was used to divide rats into chronic treatment groups (clonidine or saline) with comparable levels of punished responding. Half the subjects in each pretreatment condition (DSP4 vs. saline) received chronic posttest treatment with clonidine (40 μg/kg, IP, twice daily); controls received saline injections. CSD testing (4 days/week) was continued throughout the period of chronic drug treatment (8 weeks). Chronic drug or vehicle treatments were administered immediately posttest and again 12 h later. Thus, the minimum interval between any drug injection and CSD testing in this experiment was 12 h. Clonidine and saline treatments were administered twice daily 7 days/week.

Drugs

Clonidine hydrochloride and yohimbine hydrochloride were obtained from Sigma and were dissolved in distilled water. UK-14,304 HCl was received as a gift from Pfizer Co. (Groton, CT) and was dissolved in saline. All drug doses refer to the salt; drugs were administered intraperitoneally in a volume of 1 ml/kg body weight.

Statistical Analyses

The effects of the acute pretest challenges with clonidine or UK-14,304 on shocks received and water intake were analyzed using paired t-tests. Similarly, the effects of DSP4 or its vehicle on shocks received and water intake were analyzed using paired t-tests. The effects of DSP4 or its vehicle on brain monoamine/metabolite concentrations were analyzed using t-tests for unpaired values. The effects of yohimbine pretreatment on the response to clonidine or UK-14,304 were analyzed using 2 × 2 factorial analyses of variance (ANOVAS) with repeated measures (main effects: yohimbine/saline pretreatment; α_2-adrenoceptor agonist/saline treatment). For the chronic treatment studies, pretreatment (i.e., baseline) water intake and punished responding were compared using t-tests for unpaired values. The effects of chronic posttest clonidine or saline treatment on weekly averages for these parameters were compared in DSP4- and vehicle-treated subjects using 2 × 2 × 9 factorial ANOVAs with repeated measures [main effects: DSP4/vehicle pretreatment; clonidine/saline treatments; test weeks (baseline + 8 weeks)]. The effects of chronic UK-14,304 or saline treatment on weekly averages for these parameters were compared using 2 × 5 factorial ANOVAs with repeated measures [main effects: UK-14,304/saline treatment; test weeks (baseline + 4)]. Posthoc comparisons were made using the least significant differences (LSD) test. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance (36).

RESULTS

Initially, the shock inhibited fluid consumption in the test chamber. After several days, however, all subjects learned to consume stable volumes of water during the silent periods and made relatively few and brief contacts with the tube during the tone. The duration of the shock received was equal to the duration of the tube contact (less than 200 ms). The average baseline (i.e., nondrug) responding for all subjects in the CSD paradigm at the 0.25-mA shock intensity in the present study was 27 ± 4 (mean ± SEM) shocks/session and 13.1 ± 0.6

TABLE 1

EFFECTS OF ACUTE PRETEST ADMINISTRATION OF UK-14, 304 ON CSD CONFLICT BEHAVIOR

UK-14,304 (mg/kg; 10-min pretreatment)	Change in Shocks Received*	Change in Water Intake (ml)†
0.015	+1 ± 7	−1..3 ± 0.7
0.031	−20 ± 17	−1.9 ± 1.2
0.0625	−17 ± 8‡	−6.1 ± 1.0‡
0.125	−37 ± 9‡	−7.3 ± 1.1‡
0.25	−31 ± 6‡	−7.4 ± 1.0‡
0.5	−40 ± 7‡	−12.5 ± 2.1‡
1.0	−42 ± 9‡	−14.5 ± 0.9‡

See the text for baseline values for shocks received and water intake.

*Values represent the absolute mean ± SEM change from baseline (UK-14,304 − saline) shocks received; $n = 8$.

†Values represent the absolute mean ± SEM change from baseline (UK-14,304 − saline) water consumed (ml); $n = 8$.

‡$p < 0.05$, t-test for paired values.

ml water/session. It should be noted that the number of tube contacts during the shock component was insignificant when compared to the number of tube contacts during the unpunished component (2,000–3,000 per session). Thus, the volume of water consumed accurately reflects unpunished responding in the CSD conflict paradigm.

Experiment 1: Effects of Acute Pretest Administration of UK-14,304 on Conflict Behavior

Table 1 illustrates the effects of acute pretest administration of UK-14,304 on CSD behavior. As can be seen, acute pretest administration of UK-14,304 decreased both punished responding (shocks received) and unpunished responding (water intake) at doses of 0.06125–1 mg/kg. At no dose did acute pretest administration of UK-14,304 significantly increase punished responding.

Experiment 2: Antagonism by Acute Pretest Yohimbine Administration

Table 2 illustrates the effects of acute pretest administration of either clonidine or UK-14,304 on CSD conflict behavior in saline- or yohimbine-pretreated subjects. With respect to punished responding, there was a significant main effect for yohimbine/saline pretreatment in both the clonidine, $F(1, 14) = 13.86$, $p < 0.05$, and UK-14,304, $F(1, 14) = 5.98$, $p < 0.05$, studies. The main effect for agonist/vehicle treatment was significant in the clonidine study, $F(1, 14) = 7.08$, $p < 0.05$, but not in the UK-14,304 study, $F(1, 14) = 2.52$, n.s. The antagonist × agonist interaction was not significant in either study [clonidine, $F(1, 14) = 1.23$, n.s.; UK-14,304, $F(1, 14) < 1.0$, n.s.]. Thus, although yohimbine administered alone significantly increased punished responding there was only a tendency for yohimbine pretreatment to antagonize the decrease in punished responding produced by clonidine or UK-14,304. With respect to water intake, there was a significant main effect for yohimbine/saline pretreatment in both the clonidine, $F(1, 14) = 22.04$, $p < 0.05$, and UK-14,304, $F(1, 14) = 10.46$, $p < 0.05$, studies. Moreover, significant main effects for the agonists were also observed [clonidine, $F(1, 14) = 33.90$, $p < 0.05$; UK-14, 304, $F(1, 14) = 16.70$, $p < 0.05$]. Finally, yohimbine pretreatment significantly antagonized the decrease in water intake produced by both clonidine and UK-14,304, as evidenced by significant antagonist × agonist interaction terms [clonidine, $F(1, 14) = 15.29$, $p < 0.05$; UK-14,304; $F(1,14) = 11.09$, $p < 0.05$].

Experiment 3: Chronic Posttest UK-14,340 Treatment Effects on Conflict Behavior

The upper panel of Fig. 1 illustrates the effects of chronic posttest treatment with UK-14,304 on punished responding in the CSD. The pretreatment baselines for punished responding in the two groups were not statistically different [saline, 26 ± 5; UK-14,304, 27 ± 5; $t(14) = 0.14$, n.s.]. Chronic saline treatment did not affect punished responding over the course of 4 weeks of chronic treatment. In contrast, chronic treatment with UK-14,304 was associated with a gradual increase

TABLE 2

YOHIMBINE ANTAGONISM OF THE EFFECTS OF ACUTE PRETEST ADMINISTRATION OF CLONIDINE OR UK-14,304 ON CSD CONFLICT BEHAVIOR

	Saline Pretreatment		1.25 mg/kg Yohimbine Pretreatment	
	Saline	0.02 mg/kg Clonidine	Saline	0.02 mg/kg Clonidine
Shocks received*	35 ± 5	13 ± 2†	73 ± 10	65 ± 15
Water intake (ml)‡	12.4 ± 0.6	5.1 ± 1.3†	15.4 ± 0.9	14.4 ± 0.7§
	Saline Pretreatment		1.25 mg/kg Yohimbine Pretreatment	
	Saline	0.06 mg/kg UK-14,304	Saline	0.06 mg/kg UK-14,304
Shocks received*	45 ± 7	22 ± 5†	62 ± 10	56 ± 10
Water intake (ml)‡	14.0 ± 0.6	7.5 ± 3.7†	14.8 ± 1.3	14.2 ± 1.3§

*Values represent the mean ± SEM number of shocks received; $n = 8$.

†$p < 0.05$, clonidine or UK-14,304 treatment significantly different from saline controls, t-test for paired values.

‡Values represent the mean ± SEM volume of water consumed; $n = 8$.

§Yohimbine pretreatment significantly antagonized the effects of clonidine or UK-14,304, as evidenced by a significant yohimbine × agonist interaction.

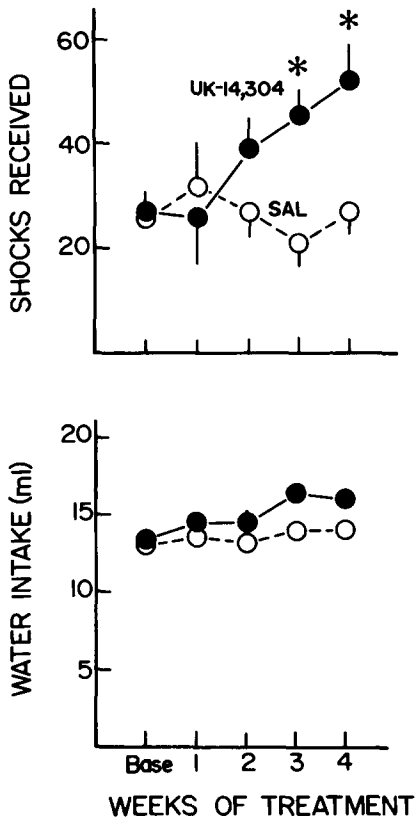

FIG. 1. Effects of chronic posttest UK-14,304 or saline administration in the CSD conflict paradigm. Plotted are the number of shocks received (upper panel) and volume of water consumed (lower panel) associated with chronic posttest saline (○) or chronic posttest UK-14,304 (●; 1 mg/kg, BID) administration. Each symbol and vertical bar represents the mean ± SEM obtained from eight subjects. *$p <$ 0.05, UK-14,304-induced change from baseline is significantly different from saline-induced change at the indicated test week, posthoc LSD test following factorial ANOVA.

in punished responding. Statistically, there was no main effect for UK-14,304/saline treatment, $F(1, 14) = 1.75$, n.s. However, a significant main effect for test weeks, $F(4, 56) = 4.75$, $p < 0.05$, as well as a significant UK-14,304/saline × test weeks interaction, $F(4, 56) = 8.47$, $p < 0.05$, was observed. Posthoc LSD comparisons revealed that the chronic UK-14,304-treated subjects accepted significantly more shocks than did saline-treated controls at test weeks 3 and 4 of chronic treatment.

The lower panel of Fig. 1 illustrates the effects of chronic posttest treatment with UK-14,304 on water intake in the CSD. Again, the pretreatment baselines for water intake in the two groups were not statistically different [saline, 13.2 ± 0.5; UK-14,304, 13.3 ± 0.7; $t(14) = 0.06$, n.s.]. Both treatment groups exhibited an increase in water intake across 4 weeks of chronic treatment. This was supported statistically by a significant main effect for test weeks, $F(4, 56) = 4.52$, $p < 0.05$. However, no main effect for UK-14,304/saline treatment was observed, $F(1, 14) = 3.61$, n.s. The UK-14,304/saline × test weeks interaction also was not significant, $F(4, 56) = 2.31$, n.s. Thus, chronic posttest administra-

TABLE 3

EFFECTS OF A SINGLE TREATMENT WITH DSP4 OR SALINE ON CSD CONFLICT BEHAVIOR

Treatment	Baseline Shocks Received	Baseline Water Intake
Saline		
Pretreatment*	31 ± 8†	11.6 ± 0.7‡
Posttreatment§	28 ± 7	12.0 ± 0.7
DSP4		
Pretreatment	30 ± 5	11.1 ± 0.9
Posttreatment	22 ± 4¶	12.0 ± 0.6

*Pretreatment defined as the average for the 2 weeks prior to DSP4/SALINE administration.

†Values represent the mean ± SEM ($n = 12$) number of shocks received during punished periods.

‡Values represent the mean ± SEM ($n = 12$) water intake (ml).

§Posttreatment defined as the average for the 2 weeks following DSP4/SALINE administration.

¶$p < 0.05$, posttreatment value significantly different from pretreatment value, paired t-test.

TABLE 4

MYDRIATIC RESPONSE TO DESIPRAMINE CHALLENGE AND REGIONAL BRAIN MONOAMINE CONCENTRATIONS IS DSP4- OR SALINE-TREATED RATS

	Mydriatic Response	
	Vehicle (saline)	DSP4 (65 mg/kg)
	7/7*	0/7†
	Brain Monoamine Concentrations	
	Vehicle (saline)	DSP4 (65 mg/kg)
Frontal cortex		
NE	337 ± 45‡	64 ± 14§
DA	756 ± 70	817 ± 89
Hippocampus		
NE	556 ± 52	67 ± 10§
DA	9.1 ± 1.4	8.1 ± 1.5
Hypothalamus		
NE	2,513 ± 211	892 ± 102§
DA	379 ± 56	364 ± 71
Striatum		
NE	ND	ND
DA	8,321 ± 776	8,632 ± 523

NE, norepinephrine; DA, dopamine; ND, not determined. Brain monoamine concentrations were determined in subjects not used for conflict testing.

*Values represent the number of subjects exhibiting a mydriatic response/number of subjects tested.

†$p < 0.05$, χ_2 for independence of proportions.

‡Values represent the mean ± SEM ($n = 7$) expressed as ng/g wet weight of tissue.

§$p < 0.05$, t-test for unpaired values.

tion of the selective α_2-adrenoceptor agonist UK-14,304 resulted in a time-dependent anticonflict effect.

Experiment 4: Chronic Posttest Clonidine Treatment Effects on Conflict Behavior: Effects of DSP4 Pretreatment

Table 3 illustrates the effects of DSP4 or saline treatment on CSD conflict behavior. As can be seen, acute saline administration did not affect punished or unpunished responding in the CSD paradigm. In contrast, a single treatment with DSP4 significantly decreased punished responding, $t(11) = 2.35$, $p < 0.05$. DSP4 treatment did not affect water intake in the CSD paradigm. Thus, DSP4 treatment resulted in a modest but selective proconflict effect in the CSD paradigm. This modest proconflict effect was apparent on the first day following DSP4 treatment and did not change appreciably over the 2-week period preceding chronic clonidine or saline treatment.

Table 4 illustrates the effects of DSP4 or vehicle treatment on the DMI-induced mydriatic response and on monoamine concentrations in various brain regions. As can be seen, all saline-treated subjects exhibited a mydriatic response following acute DMI challenge, whereas none of the DSP4-treated subjects did. DSP4 treatment also resulted in a selective and dramatic depletion of norepinephrine in all brain regions examined.

The upper half of Fig. 2 illustrates the time course for the effects of chronic posttest clonidine or saline treatment on punished responding in subjects pretreated with vehicle (sham: left panel) or DSP4 (lesion: right panel). As can be seen, in both vehicle- and DSP4-treated subjects, chronic administration of saline had no effect on punished responding over the course of the study. In contrast, in both vehicle- and DSP4-treated subjects, chronic administration of clonidine resulted in a gradual and time-dependent increase in the number of

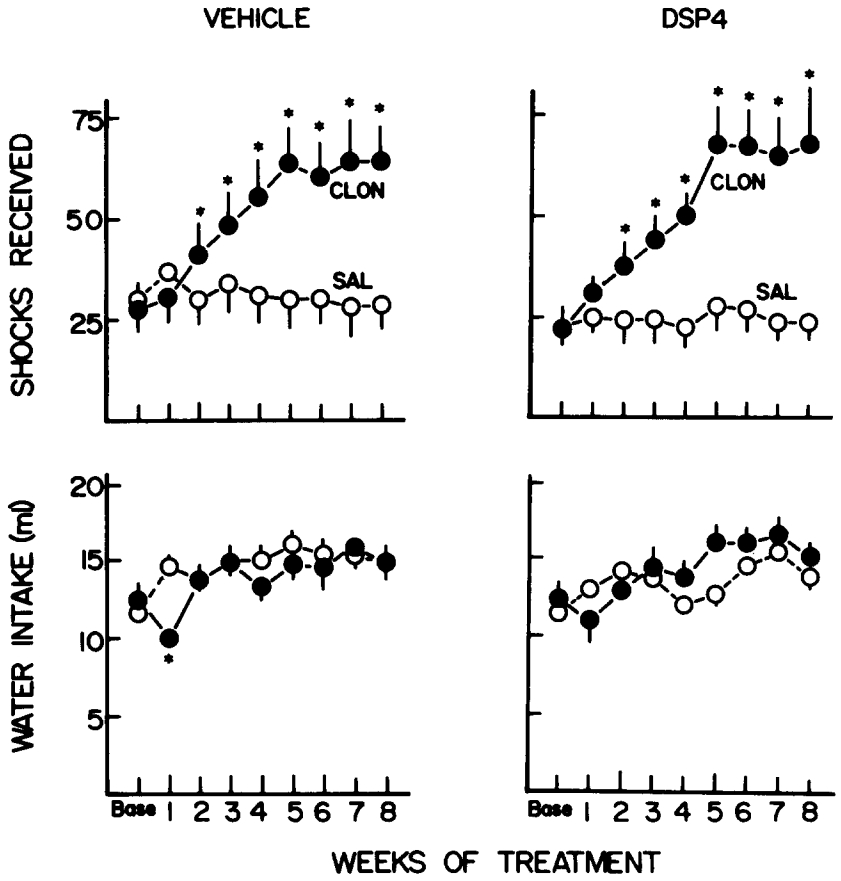

FIG. 2. Effects of DSP4 pretreatment on the effects of chronic posttest clonidine administration in the CSD conflict paradigm. Plotted are the number of shocks received (upper panel) and volume of water consumed (lower panel) associated with chronic posttest saline (○) or chronic posttest clonidine (●; 40 μg/kg, BID) administration. Depicted in the left panels are data from subjects pretreated with vehicle (saline); depicted in the right panels are data from subjects pretreated with DSP4 (65 mg/kg, IP). DSP4 or its vehicle was administered 2 weeks prior to initiation of chronic clonidine or saline treatment. Each symbol and vertical bar represents the mean ± SEM obtained from six subjects. *$p < 0.05$, clonidine-induced change from baseline is significantly different from saline-induced change at the indicated test week, posthoc LSD test following factorial ANOVA. DSP4 treatment did not alter the effects of chronic posttest clonidine administration on CSD conflict behavior.

shocks received over the course of 8 weeks of treatment. Statistically, there was a significant main effect for clonidine/saline treatment, $F(1, 20) = 34.32$, $p < 0.05$, as well as a significant main effect for test weeks, $F(7, 140) = 16.21$, $p < 0.05$. There was also a significant clonidine/saline × test weeks interaction, $F(7, 140) = 23.36$, $p < 0.05$. There was no influence of DSP4/vehicle treatment on shocks received, as evidenced by the lack of a significant DSP4/vehicle main effect, $F(1, 20)$,1.0, n.s., the lack of a significant DSP4/vehicle × clonidine/saline interaction, $F(1,20) < 1.0$, n.s., the lack of a significant DSP4/vehicle × test weeks interaction, $F(7, 140) = 1.42$, n.s., and the lack of a significant DSP4/vehicle × clonidine/saline × test weeks interaction, $F(7, 140) < 1.0$, n.s. Posthoc LSD comparisons revealed that, for both vehicle and DSP4 pretreatment conditions, clonidine-treated rats accepted significantly more shocks than did saline-treated controls at test weeks 2–8 of chronic treatment. Thus, the effect of chronic posttest clonidine treatment to increase punished responding was not affected by DSP4 lesions.

The lower half of Fig. 2 illustrates the time course for the effects of chronic posttest clonidine or saline treatment on unpunished responding (water intake) in subjects pretreated with vehicle (sham: left panel) or DSP4 (lesion: right panel). In both DSP4- and vehicle-pretreated subjects, chronic posttest administration of saline was associated with a gradual increase in water intake across the 8 weeks of treatment. In vehicle-pretreated subjects, chronic clonidine treatment resulted in a decrease in water intake in test week 1, followed by a return to chronic saline treatment values over test weeks 2–8. A similar, although not statistically significant, depression of water intake was observed in test week 1 of DSP4-pretreated subjects, followed by a return to values comparable to or greater than saline-treated controls across test weeks 2–8. Statistically, there was a significant main effect for test weeks, $F(7, 140) = 12.91$, $p < 0.05$. The main effect for clonidine/saline treatment, $F(1, 20) = 2.81$, $p < 0.05$, and the test weeks × clonidine/saline interaction, $F(7, 140) = 5.21$, $p < 0.05$, also were significant. There was no main effect for DSP4/vehicle, $F(1, 20) < 1.0$, n.s. Finally, the clonidine/saline × DSP4/vehicle interaction, $F(1, 20) = 1.75$, n.s., the test weeks × DSP4/saline interaction, $F(7, 140) < 1.0$, n.s. and the clonidine/saline × DSP4/vehicle × test weeks interaction, $F(7, 140) = 2.00$, n.s., were not significant. Posthoc LSD comparisons revealed that chronic posttest clonidine treatment reduced water intake relative to chronic posttest saline-treated rats only in test week 1 in vehicle-pretreated subjects.

DISCUSSION

Across a wide range of doses, acute pretest treatment with UK-14,304 did not increase punished responding in the CSD conflict paradigm. Indeed, both clonidine (20 μg/kg) and UK-14,304 reduced punished and unpunished responding when administered pretest; these effects were effectively antagonized by the α_2-antagonist yohimbine, indicating they were the result of α_2-adrenoceptor activation at the time of CSD testing. This effect of acute clonidine or UK-14,304 administration to decrease punished responding cannot be described as proconflict because unpunished responding (water intake) also was decreased by these acute treatments. The observation that clonidine did not exhibit anxiolytic-like effects when administered acutely is consistent with previous reports on the acute effects of clonidine in the CSD and several other animal models for anxiety (5,9,11,12,18,24,27,28). It should be noted, however, that acute clonidine treatment has been shown to exert anxiolytic-like effects in some animal models for anxiety (2,7,19,21,23,37,42).

In contrast to their lack of anticonflict effect when administered acutely, chronic posttest treatment with clonidine or UK-14,304 resulted in a time-dependent increase in punished responding. Consistent with previous reports with clonidine (5,11), the anticonflict effects of chronic posttest clonidine or UK-14,304 treatment exhibited a delay to onset of approximately 3–5 weeks.

Systemic administration of DSP4 effectively decreased the concentrations of norepinephrine but not dopamine in several brain regions examined. The DSP4 depletion of brain norepinephrine was detected directly by neurochemistry and indirectly by the loss of the DMI-induced mydriatic response. Thus, the mydriatic response following DMI challenge may be a useful tool for achieving a relatively effective yet noninvasive assessment of noradrenergic neuronal integrity following lesioning procedures.

DSP4 treatment produced a weak anxiogenic-like effect (i.e., DSP4 treatment decreased punished responding relative to saline-treated controls). This finding is *not* consistent with the hypothesis that decreasing brain noradrenergic neuronal activity is related to decreased anxiety (4,30,31). Rather, the observation that DSP4 treatment decreases punished responding is consistent with the hypothesis, proposed by Amaral and Sinnamon (1), that the activation of noradrenergic locus coeruleus neurons normally serves an anxiety-rectifying role in the brain.

Pretreatment with DSP4 did not alter the anticonflict effect of chronic posttest clonidine treatment. Because DSP4 treatment spares postsynaptic α_2-adrenoceptors, the anticonflict effect of chronic clonidine treatment may be the result of repeated interaction of clonidine with postsynaptic α_2-adrenoceptors. Moreover, although yohimbine antagonism was not attempted in the present studies, Fontana et al. (11) reported that acute pretest administration of yohimbine does *not* reverse the anticonflict effect associated with chronic posttest administration of clonidine. Thus, it is unlikely that the anticonflict effect of chronic posttest clonidine treatment is the result α_2-adrenoceptor activation at the time of CSD testing. It is possible that chronic clonidine treatment is producing a change in the function of postsynaptic α_2-adrenoceptors and/or second messenger events and that one or more of these changes is influencing punished responding.

Finally, it should be noted that both clonidine and UK-14,304 exhibit high affinity for the imidazoline-preferring receptor (25). This receptor has been identified in the rat brain and has been found to be localized in various regions (dorsal septum, cortical amygdala, entorhinal cortex) that may be involved in the manifestation of anxiety-like behaviors (25). Thus, it is possible that the anxiolytic-like effects of chronic clonidine and UK-14,304 result from interactions with these imidazoline-preferring receptors. Given the limited information on the behavioral effects associated with activation of the imidazoline receptors, however, such a mechanism would be highly speculative.

ACKNOWLEDGEMENTS

This work was supported in part by MH 47181 to R.L.C. and by the Roland T. Lakey Research Fund at WSU. D.J.F. was supported by a Graduate Assistantship from the Department of Pharmaceutical Sciences and the Interdisciplinary Neuroscience Program (INSP), WSU. The authors acknowledge the excellent technical assistance of Love V. McMiller, Jr. and Jewell Akins in these studies.

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