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Inhibition by Antibiotic Tetracyclines of Rat Cortical Noradrenergic Adenylate Cyclase and Amphetamine-Induced Hyperactivity

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KOFMAN, O., E. KLEIN, M. NEWMAN, R. HAMBURGER, O. KIMCHI, T. NIR, H. SHIMON AND R. H. BELMAKER. *Inhibition by antibiotic tetracyclines of rat cortical noradrenergic adenylate cyclase and amphetamine-induced hyperactivity.* PHARMACOL BIOCHEM BEHAV 37(3) 417–424, 1990.—Two antibiotic tetracyclines, demeclocycline (DMC) and minocycline, share several biochemical and behavioral properties with lithium (Li). DMC inhibited both noradrenaline- and chloradenosine-sensitive cyclic AMP accumulation in rat cerebral cortical slices both in vitro and ex vivo following two weeks of chronic dietary treatment. Minocycline, a lipophilic tetracycline, produced similar results in vitro. Both DMC and minocycline reduced open-field activity levels in rats following acute treatment, four hours prior to testing. Moreover, both drugs inhibited amphetamine-induced hyperactivity in the open field. Chronic treatment with 0.4% and 0.8% dietary DMC for two weeks attenuated amphetamine hyperactivity without affecting baseline activity levels in the open field. Neither DMC nor minocycline attenuated apomorphine-induced stereotypy at doses that attenuated amphetamine hyperactivity, a profile which is similar to that of lithium. Unlike lithium, however, DMC did not reverse reserpine-induced hypoactivity.

Lithium Adenylate cyclase Amphetamine Demeclocycline Minocycline Bipolar affective illness

AMONG Li's numerous biological effects, its inhibition of adenylate cyclase has been extensively studied (3). Li at near therapeutic concentrations inhibits noradrenaline-sensitive adenylate cyclase in rat cortical slices (15). Li at more clearly therapeutic concentrations inhibits noradrenaline-sensitive adenylate cyclase in slices of human cortex from the normal edges of surgically removed brain tumours (32). Li at therapeutic concentrations, in patients under chronic treatment, inhibits the epinephrine-induced rise in plasma cyclic AMP (13).

Recently, the G-protein component of the adenylate cyclase complex has been suggested as a molecular site of the Li effect on adenylate cyclase (1). Since these effects on adenylate cyclase are unlike those of other psychoactive drugs, compounds with similar properties should be screened to determine if they have the behavioral and clinical profile of Li.

Antibiotic tetracyclines have been shown to inhibit ADH-sensitive adenylate cyclase in the kidney at a site distal to the ADH receptor (12). This inhibition is similar to that involved in the pathogenesis of the Li side effect of nephrogenic diabetes insipidus (19, 39, 40). Since adenylate cyclase is similar in many tissues, tetracycline effects on rat cortical noradrenergic adenylate cyclase were examined in the present study. Since Li inhibits hyperactivity induced by low dose amphetamine in rats (7,31), we also tested the effects of tetracyclines on this behavior. Preliminary data in both the biochemical and behavioral tests have been previously published (4,5).

Neuroleptics, but not Li, inhibit apomorphine-induced stereotypy (14); therefore, the effects of tetracyclines on apomorphine-induced stereotypy were tested to define the specificity of their behavioral effects. Li has been reported to inhibit reserpine-induced hypoactivity (30) and so here too tetracyclines were studied. Demeclocycline (DMC) was chosen for most experiments, since it is the tetracycline with most marked effects on polyuria and adenylate cyclase in the kidney (18); minocycline was used in some confirmatory experiments since it is lipophilic and crosses the blood-brain barrier more readily than DMC (38).

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TABLE 1

THE EFFECT OF DEMECLOCYCLINE IN VITRO ON CYCLIC AMP ACCUMULATION INDUCED BY NORADRENALINE AND 2-CHLORO-ADENOSINE IN CHOPPED CEREBRAL CORTEX TISSUE

	cAMP, pmol/mg Protein Addition to Incubation Medium		
DMC μM	None	50 μM Noradrenaline	50 μM 2-Chloro-Adenosine
0	23.6 ± 2.0 (20)	65.4 ± 9.0 (8)	68.5 ± 8.3 (8)
1	26.3 ± 4.3 (7)	49.2 ± 4.9 (8)	63.1 ± 13.1 (8)
5	22.5 ± 7.6 (4)	40.7 ± 5.7 (4)*	
10	22.4 ± 4.6 (7)	38.8 ± 7.3 (9)*	64.7 ± 9.1 (3)
50	29.5 ± 4.4 (4)	30.4 ± 3.7 (4)*	
100	29.1 ± 5.9 (6)	44.1 ± 8.0 (10)*	52.8 ± 6.2 (7)

Slices were preincubated for 30 min and incubated for 20 min with additions as indicated before homogenization in ethanol and determination of cyclic AMP. Values are mean ± S.E.M. of the number of observations in parentheses.

*Significantly different from values in the absence of DMC ($p<0.05$) by Student's *t*-test.

EXPERIMENT 1: INHIBITION OF NORADRENALINE AND CHLORADENOSINE-SENSITIVE CYCLIC AMP ACCUMULATION BY DMC AND MINOCYCLINE

Method

Male rats, Sabra strain, 150–200 g, were used for all experiments. The cortex was dissected as described (22), and slices were prepared by chopping in one direction with a McIlwain tissue chopper set at 0.35 mm and preincubated in Krebs-Ringer's bicarbonate buffer containing 10 mM glucose and 1.29 mM calcium chloride ($CaCl_2$), with constant shaking and gassing with 95% O_2/5% CO_2. After 30 min the slices were collected on a Buchner funnel and distributed between vials containing 5 ml Krebs-Ringer's with additions for a further 20-min incubation. At the end of this period, the slices were transferred to test tubes, centrifuged, the medium decanted and the pellets homogenized in 1 ml 95% ethanol. Aliquots of the supernatants were evaporated to dryness under N_2 and cyclic AMP determined by a protein binding method based on that of Brown *et al.* (8). Chronically treated animals were fed 0.8% DMC in powdered rat food for two weeks. They were then sacrificed and studied ex vivo as above.

Results

DMC alone had no effect on accumulation of cyclic AMP in cerebral cortical slices incubated with various concentrations of the drug from 0.1 to 100 μM (Table 1). When the slices were incubated with 50 μM noradrenaline, DMC produced a dose-dependent decrease in the stimulatory response to noradrenaline, which was significant at all concentrations of the drug tested above 5 μM. In a chronic ex vivo experiment, incubation of cortical slices from animals which had received DMC in their diet at a concentration of 0.8% showed an increase in basal cyclic AMP levels compared to slices from control animals, but a reduction in the response to both 100 μM noradrenaline and 100 μM 2-chloro-adenosine (Table 2). The lipophilic tetracycline minocycline (Table 3) produced effects similar to DMC when tested in vitro. The decrease in the stimulation of cyclic AMP production due to both 50 μM noradrenaline and 50 μM 2-chloro-adenosine reached statistical significance already at 0.1 μM concentration of the drug. Absolute cyclic AMP values in these experiments were

TABLE 2

THE EFFECT OF CHRONIC DEMECLOCYCLINE (0.8%) IN VIVO ON CYCLIC AMP RESPONSES TO NORADRENALINE AND 2-CHLORO-ADENOSINE IN CHOPPED CEREBRAL TISSUE

	cAMP, pmol/mg Protein	
Addition to Incubation	Control-Fed Rats	DMC-Fed Rats
None	10.3 ± 2.5 (5)	20.1 ± 6.6 (5)*
0.1 μM noradrenaline	27.9 ± 6.4 (5)	18.8 ± 4.9 (5)*
0.1 μM 2-Cl adenosine	18.2 ± 2.8 (3)	10.8 ± 2.4 (6)*

*Significantly different ($p<0.05$) from control-fed rats by *t*-test.

lower than those reported in Table 1 but similar in range to the values given in Table 2, and are therefore given as percentages of the stimulated value (in the absence of minocycline) in Table 3, where corresponding figures derived from Table 1 for DMC are also given for comparison. When expressed in this way the results showed no difference between the two antibiotics in the degree of inhibition of the two agents.

EXPERIMENT 2: EFFECT OF ACUTE DMC ON AMPHETAMINE-INDUCED HYPERACTIVITY

Method

Twenty male albino Sabra rats were housed in groups of 5 (randomly with respect to treatment group) with ad lib access to chow and water on a reversed 12-hour light cycle. Activity was manually recorded in an open-field box (1 × 1 meter) divided into 25 squares for half an hour by an observer who was naive to the treatment condition.

Both baseline open-field activity and postamphetamine hyperactivity were tested following acute DMC treatment. Animals were pretreated four hours prior to test with either physiological saline (3 cc) or DMC (166.8 mg/3 cc/rat) IP (pretreatment). Immediately prior to open-field observation, the animals were injected with either 0.8 cc saline or 0.8 cc of a 1 mg/ml solution of d-amphetamine IP (test). The animals were divided into four groups of five rats each using a 2 × 2 design for pretreatment and

TABLE 3

THE EFFECT OF MINOCYCLINE AND DEMECLOCYCLINE IN VITRO ON CYCLIC AMP ACCUMULATION INDUCED BY NORADRENALINE AND 2-CHLORO-ADENOSINE IN CHOPPED CEREBRAL CORTEX TISSUE

	Response to			
	Noradrenaline		2-Cl-Adenosine	
Conc. of Antibiotic (μM)	DMC	MIN	DMC	MIN
0.1	112 ± 26 (4)	83 ± 8 (6)*	91 ± 4 (4)	82 ± 4 (6)*
1	89 ± 6 (6)	73 ± 11 (6)*	83 ± 11 (6)	84 ± 8 (8)*
5	87 ± 8 (3)			
10	62 ± 5 (8)*	63 ± 11 (6)*	87 ± 5 (6)*	57 ± 8 (6)*
50	53 ± 3 (3)*			
100	52 ± 3 (8)*	45 ± 7 (6)*	71 ± 13 (5)*	48 ± 6 (4)*

Results are expressed as percentages of stimulated values in the absence of added antibiotic.

*Significantly different from 100% ($p<0.05$) by paired *t*-test.

TABLE 4

THE EFFECT OF ACUTE DMC (166.8 mg) ON BASELINE ACTIVITY AND ON AMPHETAMINE-INDUCED (1 mg/kg) HYPERACTIVITY

Period Ending (min)	SAL-SAL	SAL-DMC	SAL-AMPH	DMC-AMPH
5	59.6 ± 8.34	21.4 ± 7.06	72.8 ± 4.64	43.0 ± 12.99
10	30.8 ± 9.69	4.6 ± 3.31	71.6 ± 10.72	58.6 ± 7.85
15	32.8 ± 13.55	2.4 ± 2.40	76.0 ± 11.45	55.0 ± 12.84
20	16.0 ± 8.55	2.4 ± 2.40	57.0 ± 10.93	52.8 ± 7.51
25	13.8 ± 6.00	2.4 ± 2.40	45.8 ± 10.92	43.2 ± 9.15
30	25.0 ± 10.87	0	47.6 ± 17.14	55.0 ± 18.14

Data represent the number of squares crossed in an open field.

test injections: SALINE-SALINE, SALINE-AMPHETAMINE, DMC-SALINE, DMC-AMPHETAMINE.

Results

The data were analyzed using a three-way ANOVA for the effects of DMC and amphetamine over time. AMPHETAMINE increased motor activity significantly, $F(1,18)=22.407$, $p<0.001$. A main motor suppressant effect of DMC was found, $F(1,18)=4.441$, $p<0.05$, but there was no interaction between AMPHETAMINE and DMC. In addition, there was a main effect of TIME in the open field, $F(5,18)=8.088$, $p<0.001$, and significant interactions of AMPHETAMINE × TIME, $F(5,18)=3.817$, $p=0.004$, and DMC × TIME, $F(5,18)=3.449$, $p=0.007$ (Table 4).

Acute injections of DMC, like acute lithium, had a general suppressant effect on motor behavior. Since chronic lithium has more specific effects on stimulant-induced activity, the subsequent experiment examined the effects of chronic dietary DMC on activity before and after injections of amphetamine.

EXPERIMENT 3: THE EFFECT OF CHRONIC DMC ON AMPHETAMINE-INDUCED MOTOR ACTIVITY

Method

Forty-five rats, divided into 3 groups of 15, were fed a diet containing various concentrations of powdered DMC: 0%, 0.4%, or 0.8% DMC mixed with powdered commercial rat chow during two weeks. Then they were tested for baseline activity rates in a open field as described above. Following the baseline test, they were fed for one more week with the DMC diet and then tested again in an open field for 30 min following an IP injection of 0.5 mg/kg d-amphetamine.

Results

Activity levels prior to and following amphetamine injection varied greatly between animals. A three-way ANOVA for the effects of AMPHETAMINE, DMC with TIME as a repeated measure was conducted. Amphetamine significantly increased activity, $F(1,42)=16.995$, $p<0.001$. There was a significant decline in hyperactivity over the half-hour test, i.e., a main effect of time, $F(5,210)=63.652$, $p<0.001$, and a significant interaction between TIME and AMPHETAMINE, $F(5,210)=8.201$, $p<0.001$.

ANOVA was then repeated for the total activity scores for half an hour, as shown in Fig. 1. Two-way ANOVA between DMC and AMPHETAMINE, with AMPHETAMINE as a repeated

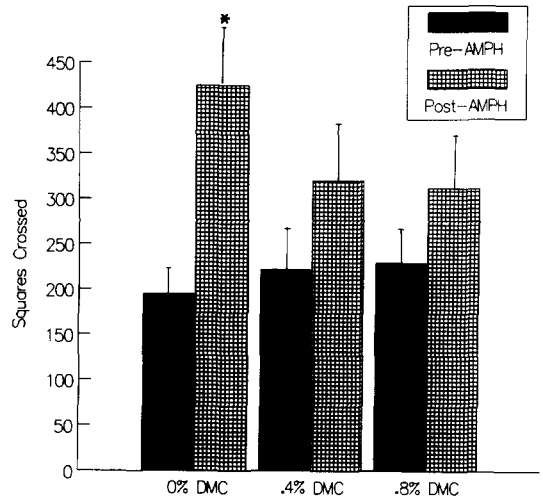

FIG. 1. Effect of chronic dietary DMC (0%, 0.4%, 0.8%) on baseline open-field activity and amphetamine-induced hyperactivity. The asterisk indicates a significant difference between amphetamine and baseline activity ($p<0.01$). The ordinate represents the number of squares crossed in an open field in 30 minutes.

measure was conducted. There was a significant effect of amphetamine, $F(1,42)=16.2067$, $p=0.00045$, but the effect of DMC, $F(2,42)=0.34556$, and the DMC × AMPHETAMINE interaction, $F(2,42)=1.87$, $p=0.164$, did not reach statistical significance. Simple main effects were examined using the error term and degrees of freedom for the general ANOVA (29). A simple main effect of amphetamine was found for the group that received no dietary DMC (0% DMC), $F(1,42)=15.196$, $p<0.01$, but not for the groups that received 0.4% DMC, $F(1,42)=2.79$, or 0.8% DMC, $F(1,42)=1.97$. Thus, amphetamine significantly increased activity only for the group which was not administered dietary DMC (Fig. 1). These data suggest that chronic dietary DMC attenuates amphetamine-induced hyperactivity.

EXPERIMENT 4: THE EFFECT OF CHRONIC DMC ON APOMORPHINE-INDUCED STEREOTYPY

Method

The effect of DMC on apomorphine-induced stereotypy was measured following two weeks on a diet of powdered rat chow mixed with 0.4% DMC (n=14), or regular powdered chow (n=13). All rats were injected with 1 mg/kg apomorphine IP and scored for stereotypic behavior during 50 minutes by an observer who was blind to the treatment condition.

Stereotypy was rated according to the scale of Kelly and Iversen (28), as modified (21): 0—asleep or stationary, 1—active without stereotypy, 2—bursts of stereotyped sniffing, head movements or rearing, 3—intense stereotyped sniffing, head movements or rearing confined to one area, 4—stereotyped sniffing, head movements or rearing accompanied by bursts of licking or gnawing including self-licking or biting, 5—stereotyped licking or gnawing, 6—stereotyped licking or gnawing confined to one small area of cage. Rats were placed in clear plastic cages resembling their home cages. Each rat was observed once every 5 minutes for one minute and assigned a score.

Results

There was no significant difference between the DMC and

TABLE 5

EFFECT OF CHRONIC DIETARY DMC (0.4%) ON APOMORPHINE-INDUCED STEREOTYPY (1 mg/kg)

Period Ending (min)	Saline	DMC
5	1.62 ± 0.24	1.29 ± 0.19
10	3.08 ± 0.44	2.50 ± 0.45
15	2.62 ± 0.51	2.50 ± 0.51
20	3.23 ± 0.46	3.07 ± 0.46
25	3.08 ± 0.55	2.86 ± 0.54
30	2.92 ± 0.61	2.71 ± 0.53
35	3.31 ± 0.50	2.50 ± 0.54
40	2.85 ± 0.41	2.57 ± 0.58
45	2.77 ± 0.47	2.86 ± 0.47
50	2.54 ± 0.46	2.43 ± 0.54

The data represent scores for apomorphine-induced stereotypy (mean ± SEM) as described in Experiment 4.

saline controls on apomorphine-induced stereotypy [DMC main effect, $F(1,25)=0.282$] (Table 5).

EXPERIMENT 5: EFFECT OF CHRONIC DMC ON RESERPINE-INDUCED HYPOACTIVITY

Method

The effect of DMC on reserpine-induced hypoactivity was measured following three weeks on a diet of powdered rat chow mixed with 0.4% DMC ($n=12$), or regular powdered chow ($n=12$). The rats were placed in individual plastic cages and rated on a behavioral activity scale as follows: 1—immobility, 2—minor movements, 3—crawling, 4—walking, 5—rearing or jumping, 6—grooming.

Each rat was observed 20 times for a duration of 30 sec each observation, over a period of 30 minutes. The observer was blind to the treatment condition. The number of times that each particular behavior was observed was recorded for each animal. Then the total occurrences of each behavior were averaged for each of the two groups to provide a baseline measure for each behavior.

The rats were then fed another week with either the DMC or regular chow diet and treated with an IP injection of reserpine (2.5 mg/kg). One day later all rats received a second injection of reserpine (1 mg/kg) (9) and were rated for each of the six behavioral categories as described above (5). Two-way ANOVA with repeated measures was conducted on each of the behavioral categories (Table 6).

Results

Immobility and grooming were significantly increased, and walking and rearing/jumping significantly decreased by reserpine. Crawling and minor movements were unaffected. There was a main effect of DMC to increase walking only. There were no significant interactions between DMC and reserpine, indicating that DMC did not prevent or enhance any of the reserpine effects. These data suggest that, unlike Li, DMC does not prevent reserpine-induced hypoactivity.

EXPERIMENT 6: EFFECT OF ACUTE MINOCYCLINE ON AMPHETAMINE-INDUCED HYPERACTIVITY

Method

The interaction between minocycline and amphetamine on open-field activity was tested using a 2×2 design. Rats were divided into four groups of ten according to pretreatment and test injections: SALINE-SALINE, SALINE-AMPHETAMINE, MINOCYCLINE-SALINE, MINOCYCLINE-AMPHETAMINE. Minocycline (100 mg/kg) (Lederle) or physiological saline was administered subcutaneously four hours prior to the open-field test (pretreatment). Immediately prior to the test, animals received either physiological saline or d-amphetamine (0.5 mg/kg) IP (test). All drugs were injected in a volume of 1 ml/kg.

Results

After the second injection, the animals were placed in an open field and observed for 30 minutes as described in Experiment 2. The number of peripheral squares and central squares crossed, and number of rears were manually counted for each five minute period. Since there is variability in the peak of the amphetamine effect over time and the main goal was to analyze the interaction of minocycline and amphetamine hyperactivity, the analysis of

TABLE 6

EFFECT OF CHRONIC DMC (0.8%) ON RESERPINE HYPOACTIVITY (MEAN ± SEM)

	Saline		DMC		
Behavior	Baseline	Post-Reserpine	Baseline	Post-Reserpine	ANOVA
Immobility	6.00 ± 1.16	8.92 ± 1.20	3.75 ± 1.17	9.33 ± 1.47	*$p=0.003$
Minor movements	5.08 ± 0.45	3.75 ± 0.71	6.08 ± 0.70	4.83 ± 0.74	n.s.
Crawling	0.33 ± 0.14	0.67 ± 0.26	0.50 ± 0.15	0.58 ± 0.17	n.s.
Walking	1.00 ± 0.21	0.0	1.75 ± 0.43	0.25 ± 0.18	†$p=0.027$ *$p=0.001$
Rearing and jumping	6.17 ± 1.49	2.75 ± 0.73	6.33 ± 1.39	2.17 ± 0.74	*$p=0.004$
Grooming	1.42 ± 0.31	3.92 ± 0.69	1.58 ± 0.61	2.83 ± 0.64	*$p=0.006$

*Main effect, reserpine.
†Main effect, DMC.

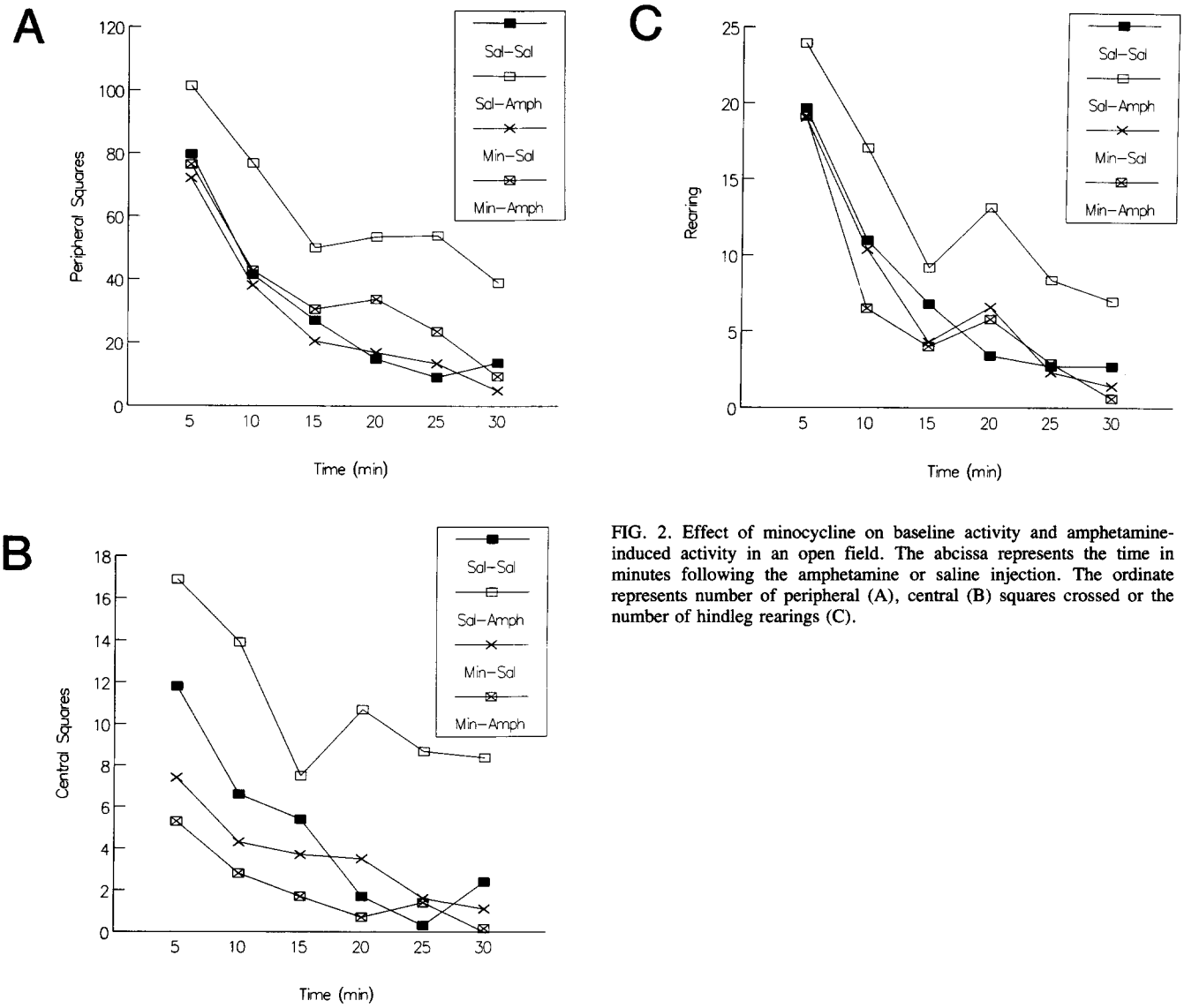

FIG. 2. Effect of minocycline on baseline activity and amphetamine-induced activity in an open field. The abcissa represents the time in minutes following the amphetamine or saline injection. The ordinate represents number of peripheral (A), central (B) squares crossed or the number of hindleg rearings (C).

variance for each measure was conducted on the total scores for half an hour. The time course of the activity is illustrated in Fig. 2A–C. Amphetamine significantly increased, $F(1,38) = 19.105$, $p<0.001$, while minocycline decreased peripheral square crossings, $F(1,38) = 10.417$, $p<0.002$ (Fig. 2A). There was a significant interaction between the two drugs which suggests that minocycline countered the hyperactive effect of amphetamine, MINOCYCLINE × AMPHETAMINE, $F(1,36) = 6.415$, $p = 0.015$. To confirm this, post hoc (Scheffe) tests were conducted for the two amphetamine-treated groups. Amphetamine increased the activity in saline-treated rats ($p<0.001$), but not in minocycline-treated rats (n.s).

Central square crossings were also significantly reduced by minocycline treatment, $F(1,38) = 14.339$, $p<0.001$. The interaction between minocycline and amphetamine was significant, $F(1,36) = 9.331$, $p = 0.004$, indicating that minocycline antagonized amphetamine-hyperactivity. Post hoc comparisons (Scheffe) indicated that amphetamine increased the activity in the saline-treated ($p = 0.002$), but not in the minocycline-treated (n.s.) rats (Fig. 2B).

The number of rearings was also significantly reduced by minocycline, $F(1,38) = 7.871$, $p = 0.008$, and there was a significant interaction between MINOCYCLINE and AMPHETAMINE, $F(1,36) = 6.346$, $p = 0.015$) (Fig. 2C). Amphetamine increased rearing in saline-treated rats (Scheffe post hoc test, $p = 0.005$), and not in minocycline-treated rats.

EXPERIMENT 7: THE EFFECT OF ACUTE MINOCYCLINE ON APOMORPHINE-INDUCED STEREOTYPY

Method

Thirty male albino Sprague-Dawley rats were divided into two groups of 15 subjects each. One group received 100 mg/kg minocycline SC 4 hours prior to the test, and the other group received an equal volume (1 ml/kg) of physiological saline. Four hours after the first injection, all the animals were injected with apomorphine (1 mg/kg, IP) and placed in individual plastic cages containing a twig of wood. The rats were observed for one minute every five minutes during 40 or 60 minutes and rated for stereotypic

TABLE 7

EFFECT OF ACUTE MINOCYCLINE (100 mg/kg) ON APOMORPHINE (1 mg/kg) STEREOTYPY (MEAN ± SEM)

Group/Time	Saline	Minocycline
5	3.16 ± 0.17	3.00 ± 0.25
10	3.60 ± 0.20	3.27 ± 0.33
15	3.47 ± 0.25	3.57 ± 0.22
20	3.47 ± 0.29	3.57 ± 0.24
25	3.70 ± 0.23	3.47 ± 0.23
30	3.53 ± 0.25	3.30 ± 0.25
35	3.10 ± 0.28	3.30 ± 0.19
40	2.80 ± 0.25	2.63 ± 0.25
45	2.03 ± 0.25	2.13 ± 0.21
50	1.40 ± 0.29	1.27 ± 0.27
55	0.93 ± 0.27	1.17 ± 0.27
60	0.57 ± 0.17	0.80 ± 0.30
	Effect of minocycline (100 mg/kg) on apormorphine (0.5 mg/kg) stereotypy (mean ± SEM)	
5	1.53 ± 0.17	1.97 ± 0.35
10	2.30 ± 0.13	2.43 ± 0.36
15	2.97 ± 0.17	2.97 ± 0.44
20	3.37 ± 0.19	3.47 ± 0.25
25	3.57 ± 0.17	3.50 ± 0.26
30	3.67 ± 0.23	3.63 ± 0.27
35	3.60 ± 0.15	3.63 ± 0.20
40	3.40 ± 0.27	3.20 ± 0.20

behavior by two observers who were blind to the experimental condition, as in Experiment 4.

One week later this procedure was repeated using a lower dose of apomorphine (0.5 mg/kg). Since there was a high correlation between the ratings of the two observers ($r = .91$ for minocycline-treated rats and $r = .89$ for saline-treated rats, $p < 0.01$), the average score of the two observers for each animal was calculated for each of the 5-minute intervals.

Results

Two-way ANOVA for the effects of MINOCYCLINE and TIME was conducted for each dose of apomorphine. Minocycline had no effect on apomorphine induced stereotypy following 1 mg/kg apomorphine, $F(1,28) = 0.021$, or after 0.5 mg/kg apomorphine, $F(1,28) = 0.028$. In both cases there was a significant effect of time, reflecting the time course of the development and decline of stereotypy, but no interaction between minocycline treatment and time (Table 7).

GENERAL DISCUSSION

The present results confirm preliminary reports that DMC inhibits noradrenaline-sensitive cyclic AMP accumulation and amphetamine-induced hyperactivity (4,5). Similarly, minocycline was found to inhibit noradrenaline-sensitive cyclic AMP accumulation and amphetamine-induced hyperactivity.

The two tetracycline derivatives, DMC and minocycline, attenuated amphetamine-induced hyperactivity in rats, but had no effect on apomorphine stereotypy. In this respect, the behavioral profile of the tetracyclines was similar to that of Li, which also attenuates amphetamine-induced hyperactivity but not apomorphine stereotypy (7,14).

The acute motor depressant effects of both DMC and minocycline were pronounced in this study. This is similar to previous findings with Li (26), although not all others have found similar results (2, 9, 43). Smith (41) has suggested that the acute effects of Li on behavior may be secondary to pain or general malaise experienced by the animals following intraperitoneal injections. We cannot rule out the possibility that the tetracycline injections caused the animals discomfort which subsequently affected their activity. It is known that parenteral tetracycline injection causes pain, and our animals showed signs of pain immediately after injection. However, the tests were conducted four hours after the tetracycline injections. Moreover, it is significant that only open-field activity and not stereotypy was inhibited by the tetracyclines. In order to ascertain if the acute motor suppressant effects were a consequence of peripheral discomfort, future research will include intracranial injections of the tetracycline derivatives.

Chronic dietary DMC cannot cause the pain of parenteral injection, and thus the chronic dietary DMC provides perhaps the most convincing behavioral evidence of tetracycline effects. Due to the large variance between animals, however, only the simple main effect of the within group factor (amphetamine) was statistically significant. The effects of chronic tetracycline treatment, especially that of the lipophilic derivative, minocycline should be tested further. The chronic dietary ex vivo study also confirmed the finding that DMC inhibits rat brain noradrenergic cyclic AMP accumulation. Ex vivo study of brain from rats on dietary DMC suggested that the inhibition is not an acute biochemical effect to which tolerance develops in vivo. These results are very similar to those found with Li (15). Inhibition by DMC of adenosine-analog-induced accumulation of cyclic AMP accumulation also parallels results reported for Li (16,34), and would be expected of a compound whose effect is distal to the specific receptor component of the adenylate cyclase-receptor complex.

DMC, in contrast to lithium (7,30), did not attenutate reserpine-induced hypoactivity. However, the effect of lithium on reserpine hypoactivity is probably due to competition at the site of reserpine binding on presynaptic vesicles and is not directly related to its action on adenylate cyclase (33, 35, 37). In order to further explore the possibility that DMC and minocycline have antidepressant as well as antimanic properties, a more adequate behavioral model of depression is needed. Lithium has been reported to significantly attenuate clenbuterol-induced hypoactivity (20), and this model could be used to test the effects of chronic DMC and minocycline on hypoactivity.

The significant increase in walking observed in DMC-treated rats (Experiment 5), at first glance, seem incompatible with the open-field activity data (Experiment 3). However, in the former paradigm, animals were observed in cages resembling their home cages, rather than in an open field. Reports on activity levels of lithium-treated mice indicated that lithium did not reduce activity in photocell cage that resembled the home cages (11). The possibility that DMC, like lithium, has a sedative effect in a novel environment and a slightly stimulatory effect in a familiar environment warrants further investigation.

DMC and minocycline are used clinically as antibiotics. Because of its ability to block ADH-stimulated increases in cyclic AMP activity, DMC has been used successfully in cases of SIADH (syndrome of inappropriate secretion of antidiuretic hormone) and in psychogenic drinking in psychotic patients (19, 23, 25, 36, 44). There is a report of effects on memory and sleep of a single dose of DMC in normal volunteers (24). Aside from vestibular side effects (17,46), few central effects of DMC and minocycline have been reported. With Li, as well, relatively few behavioral effects are noted in nonpsychiatrically ill humans (27). Since blockade of cyclic AMP accumulation is proposed as one of

the mechanisms by which lithium exerts its antimanic effects (3,45), DMC and minocycline are candidates as alternative antimanic therapies. Indeed, Belmaker and Roitman (6) recently reported that DMC attenuated the symptoms of acute manic patients.

Although the tetracycline derivatives had effects similar to those of lithium on both noradrenaline-stimulated cyclic AMP and on amphetamine-induced hyperactivity, hyperactivity is well-attenuated by dopamine antagonists (42). Hence, it is not certain that inhibition of noradrenaline-stimulated cyclic AMP accumulation is responsible for the ability of the tetracyclines to attenuate the behavioural effect of amphetamine.

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