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Pharmacological validation of the chronic mild stress model of depression

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Abstract

Chronic exposure to mild unpredictable stress has previously been found to depress the consumption of palatable sweet solutions, and this effect was reversed by chronic treatment with a variety of antidepressant drugs. The present study reports three experiments examining the effects in this model of further antidepressant agents, a number of non-antidepressants, and some compounds of indeterminate clinical status. Male Wistar rats were exposed sequentially to a variety of mild stressors, which continued throught the experiments. Drug treatments commenced after 3 weeks of stress, by which time intake of a 1% sucrose solution (measured in a 1-h weekly test) was significantly depressed. No drug effects were seen after 1 week of treatment. Normal levels of sucrose drinking were seen following chronic (3–5 weeks) of treatment with the antidepressants imipramine (10 mg/kg per day), brofaromine (20 mg/kg per day), and buspirone (5 mg/kg per day). Positive effects were also seen following chronic treatment with atropine (1 mg/kg per day) and mepyramine (5 mg/kg per day). *d*-Amphetamine (1 and 3 mg/kg per day), the neuroleptics haloperidol and chlorprothixene (1 mg/kg per day), and morphine (administered at doses rising to 110 mg/kg per day) were ineffective; amphetamine (3 mg/kg) and morphine decreased sucrose intake in control animals. No inferences can be drawn from the effects of atropine and mepyramine, which are of indeterminate clinical status; data from the other seven agents tested support the hypothesis that the chronic mild stress model responds appropriately to antidepressant and non-antidepressant agents.

Keywords: Stress, chronic, mild; Depression, animal model; Pharmacological validation

1. Introduction

Chronic sequential exposure to a variety of mild stressors (chronic mild stress) has been found to decrease the consumption of and/or preference for a palatable weak sucrose solution in rats or mice. Chronic mild stress also causes an increase in the threshold current required to support intracranial self-stimulation (brain stimulation reward), and attenuates or abolishes the ability to associate rewards with a distinctive environment (place conditioning). The latter effect has been demonstrated with a variety of different natural or drug reinforcers, but does not extend to aversive place conditioning. These findings support the concept that chronic mild stress causes anhedonia, a generalized decrease in sensitivity to rewards (reviewed by Willner et al., 1992; Willner, 1995).

These behavioural deficits may be maintained for several months by continued application of the chronic mild stress procedure. However, normal behaviour is restored by chronic treatment with tricyclic or atypical antidepressants, at doses which have no effect on rewarded behaviours in non-stressed animals (reviewed by Willner et al., 1992; Willner, 1995). Drugs shown to be effective in reversing chronic mild stress-induced anhedonia include tricyclics, the specific monoamine uptake inhibitors fluoxetine and maprotiline, the monoamine oxidase-A inhibitor moclobemide, and the atypical antidepressant mianserin. Electroconvulsive shock (Moreau et al., 1994), and lithium (Sluzewska and Nowakowska, 1994) have also recently been shown to be effective in this model. The reversal of chronic

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mild stress-induced anhedonia typically requires 3–4 weeks of treatment, which closely resembles the clinical time course of antidepressant action.

Although previous work demonstrates that the chronic mild stress procedure responds appropriately to chronic treatment with antidepressant drugs, there is little information concerning the effects of other agents. The benzodiazepine anxiolytic chlordiazepoxide was ineffective (Muscat et al., 1992), but this is the only clinical non-antidepressant for which data have been reported. The purpose of the present study was therefore to examine the effects in the chronic mild stress model of a variety of non-antidepressants, including neuroleptics, amphetamine and morphine, as well as compounds of indeterminate status such as anticholinergics (atropine) and antihistamines (mepyramine). We also studied the effects of two further antidepressant drugs, the monoamine oxidase-A inhibitor brofaromine and the 5-HT_{1A} receptor agonist buspirone; imipramine was routinely included in all experiments as a positive control. All experiments involved chronic drug treatment, which places logistical constraints on a study designed to screen a wide range of compounds. Consequently, most drugs were tested at a single dose; the extent to which this limits the conclusions of the study is discussed below.

2. Materials and methods

2.1. Subjects

Male Wistar rats were brought into the laboratory 2 months before the start of each study. Except as described below the animals were singly housed in plastic cages ($40 \times 25 \times 15$ cm) with food and water freely available, and maintained on a 12-h light/dark cycle (08:00–20:00 light), at a temperature of 22 ± 2°C. Animals weighed 300–350 g at the time experiments commenced.

2.2. Chronic mild stress procedure

During the second month of adaptation to the laboratory, all animals were trained to consume a palatable sucrose solution. Training consisted of five to seven 1-h tests in which 1% sucrose in water was presented, in the home cage, following 14 h of food and water deprivation. Testing was carried out at 10:00 h; at the end of each test, sucrose intake was measured by weighing pre-weighed bottles. Subsequently, sucrose intake was measured, under similar conditions, at weekly intervals for the duration of the experiment. On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group of animals was subjected for 8 (experiment 1) or 9 (experiments 2 and 3) weeks to a chronic mild stress procedure, each week of which consisted of: two 14-h periods of food or water deprivation; two 14-h periods of 45° cage tilt; two periods of intermittent overnight illumination (lights on and off every 2 h); two 12-h periods in a soiled cage (200 ml water in sawdust bedding); two 12-h periods of paired housing; two 12-h periods of low intensity stroboscopic illumination (150 flashes/min). The stressors were scheduled randomly and applied continuously, day and night. Non-stressed control animals were housed in a separate room and had no contact with the stressed animals. Food and water were freely available to control animals, except for a 14-h period of food and water deprivation immediately preceding each sucrose intake test.

2.3. Drug administration

The study consisted of three consecutive experiments. In each experiment, stressed and control animals were further divided into subgroups, matched for sucrose intakes following 3 weeks of stress. In experiment 1, subgroups of control and stressed animals ($n = 10$) were administered vehicle (p.o., b.i.d.), imipramine (10 mg/kg p.o., b.i.d.), brofaromine (10 mg/kg p.o., b.i.d.), or *d*-amphetamine (0.5 mg/kg p.o., b.i.d.), for a total of 5 weeks. In experiment 2, subgroups of control and stressed animals ($n = 8$) received once-daily i.p. administration of vehicle, imipramine (10 mg/kg), or mepyramine (5 mg/kg), or twice-daily i.p. administration of a higher dose of *d*-amphetamine (1.5 mg/kg) for a total of 6 weeks. In experiment 3, subgroups of control and stressed animals ($n = 8$) received daily i.p. administration, for 6 weeks, of vehicle, imipramine (10 mg/kg), buspirone (2.5 mg/kg b.i.d.), atropine (1 mg/kg), haloperidol (1 mg/kg), chlorprothixene (1 mg/kg) or morphine, which was administered b.i.d., at an initial dose of 5 mg/kg and rising by 5 mg/kg every 4 days to a final dose of 55 mg/kg. 9 h after the final sucrose test, morphine-treated control and stressed animals were injected with naloxone (2 mg/kg i.p.) and observed for 15 min in transparent cages; all animals showed characteristic morphine-withdrawal signs (e.g. wet dog shakes, teeth chattering, flat body posture).

All drug injections were in a volume of 1 ml/kg body weight. Drugs were administered at 12:00 h, with a second administration in the case of b.i.d. regimes at 17:00 h. Sucrose intake tests were carried out 22 h following the last drug treatment; in the case of b.i.d. regimes the 17:00 h treatment immediately preceding the sucrose test was omitted.

2.4. Drugs

The following agents were tested: *d*-amphetamine sulphate (Smith Kline and French), atropine sulphate

Table 1
Results of analyses of variance

Source of variance	Experiment 1	Experiment 2	Experiment 3
Drugs	$P < 0.01$	$P < 0.001$	$P < 0.001$
Stress	$P < 0.001$	$P < 0.001$	$P < 0.001$
Weeks	$P < 0.001$	$P < 0.001$	NS ($P = 0.15$)
Drugs × Stress	$P < 0.02$	$P < 0.001$	$P < 0.01$
Drugs × Weeks	NS ($P = 0.22$)	$P < 0.001$	$P < 0.001$
Stress × Weeks	$P < 0.025$	$P < 0.001$	$P < 0.001$
Drugs × Stress × Weeks	NS ($P = 0.11$)	$P < 0.001$	$P < 0.001$

(Sigma), brofaromine HCl (Ciba-Geigy, buspirone (RBI), chlorprothixene HCl (Sigma), haloperidol (Richter), imipramine HCl (Polfa), mepyramine maleate (May and Baker), morphine (Polfa). All agents were dissolved in distilled water, which was used for vehicle administrations.

2.5. Statistical analysis

Results were analysed by analysis of variance, supplemented by tests of simple main effects and F-tests for contrasts using the appropriate analysis of variance error term (Winer, 1971). The analyses involved two between-subject factors (stress/control and drug treatment) and one within-subject factor (successive tests).

3. Results

The results of the data analyses are summarized in Table 1. Data from all groups of subjects at the start of drug treatment and following 1 and 5 weeks of treatment are shown in Table 2.

Table 2
Drug effects on sucrose intake after 1 or 5 weeks of treatment *

Drug (dose)		Week 0	Week 1	Week 5	Stress × Weeks interaction
Experiment 1					
Vehicle	CON	14.2 (±0.9) [c]	12.0 (±1.4) [c]	13.9 (±0.8) [c]	NS
(1 ml/kg b.i.d. p.o.)	STR	8.3 (±0.8)	7.8 (±0.5)	7.4 (±0.4)	
Imipramine	CON	14.3 (±0.9) [c]	11.9 (±0.7) [a]	13.9 (±0.8)	$P < 0.025$
(10 mg/kg b.i.d. p.o.)	STR	8.1 (±0.7)	8.5 (±0.9)	12.6 (±1.1) [e]	
Brofaromine	CON	14.3 (±0.9) [c]	11.9 (±0.7) [a]	13.4 (±0.9)	$P < 0.025$
(10 mg/kg b.i.d. p.o.)	STR	8.2 (±0.7)	8.9 (±0.9)	13.0 (±0.9) [e]	
Amphetamine	CON	14.2 (±0.9) [c]	11.6 (±1.4) [a]	13.6 (±1.3) [c]	NS
(0.5 mg/kg b.i.d. i.p.)	STR	8.2 (±0.7)	8.6 (±1.3)	7.9 (±0.5)	
Experiment 2					
Vehicle	CON	13.1 (±0.8) [c]	12.4 (±0.7) [c]	12.8 (±0.5) [c]	NS
(1 ml/kg i.p.)	STR	8.2 (±0.8)	7.9 (±1.1)	6.6 (±0.5)	
Imipramine	CON	13.2 (±0.5) [c]	12.1 (±0.4) [c]	12.5 (±0.4)	$P < 0.001$
(10 mg/kg i.p.)	STR	8.1 (±0.9)	7.2 (±0.8)	13.4 (±0.7) [e]	
Mepyramine	CON	13.2 (±0.9) [c]	11.7 (±1.4) [b]	13.1 (±0.6) [a]	$P < 0.001$
(5 mg/kg i.p.)	STR	8.1 (±0.6)	8.7 (±0.6)	10.9 (±0.9) [e]	
Amphetamine	CON	13.2 (±1.2) [b]	12.4 (±0.9) [b]	8.2 (±1.1) [e]	$P < 0.05$
(1.5 mg/kg b.i.d. i.p.)	STR	8.9 (±1.1)	8.0 (±1.1)	7.4 (±1.9)	
Experiment 3					
Vehicle	CON	13.3 (±0.5) [c]	12.9 (±0.5) [c]	11.9 (±1.1) [b]	NS
(1 ml/i.p.)	STR	7.6 (±0.8)	6.7 (±0.4)	7.7 (±0.7)	
Imipramine	CON	13.3 (±0.4) [c]	12.1 (±0.5) [a]	11.2 (±1.6)	$P < 0.001$
(10 mg/kg i.p.)	STR	7.8 (±0.6)	8.6 (±0.5)	12.0 (±0.8) [d]	
Buspirone [†]	CON	13.3 (±0.4) [c]	12.6 (±0.4) [c]	11.9 (±1.3)	$P < 0.001$
(2.5 mg/kg b.i.d. i.p.)	STR	7.7 (±0.5)	6.9 (±1.1)	13.8 (±1.1) [e]	
Atropine	CON	13.3 (±0.4) [c]	13.6 (±0.7) [c]	11.6 (±1.3)	$P < 0.001$
(1 mg/kg i.p.)	STR	7.4 (±0.5)	8.5 (±0.7)	12.4 (±1.5) [e]	
Haloperidol	CON	13.3 (±0.5) [c]	12.7 (±1.0) [c]	11.3 (±1.1) [c]	NS
(1 mg/kg i.p.)	STR	7.7 (±0.6)	7.8 (±0.8)	6.4 (±1.0)	
Chloprothixene	CON	13.4 (±0.4) [c]	12.8 (±0.7) [c]	13.8 (±1.9) [c]	NS
(1 mg/kg i.p.)	STR	7.7 (±0.6)	8.2 (±0.5)	4.9 (±1.1)	
Morphine	CON	13.5 (±0.4) [c]	10.6 (±0.8) [a]	6.0 (±0.8) [e]	$P < 0.01$
(see text)	STR	7.6 (±0.8)	7.0 (±0.5)	5.9 (±0.9)	

* Data are means (±standard error). [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, for differences between control and stressed groups. [d] $P < 0.01$, [e] $P < 0.001$, for differences between drug- and vehicle-treated groups. [†] Data for buspirone shown under 'week 5' were actually collected in week 6; the effects of buspirone were not significant in week 5 (see text).

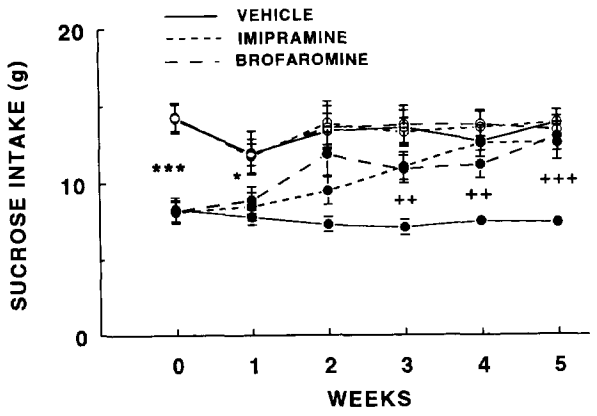

Fig. 1. Sucrose intake in control (open circles) and stressed animals (filled circles) treated for 5 weeks with vehicle (distilled water), imipramine or brofaromine (10 mg/kg p.o., b.i.d.). Stress commenced 3 weeks prior to the start of drug treatment and continued throughout the experiment. Values are means ± standard error; where no error bar is shown the standard error falls within the symbol. Statistical comparisons refer to both drugs; for clarity, comparisons significant for one drug but not the other are not shown: $^{*}P < 0.05$, $^{***}P < 0.001$ for differences between drug-treated control and stressed groups; $^{++}P < 0.01$, $^{+++}P < 0.001$, for differences between drug- and saline-treated stressed groups.

3.1. Negative (vehicle-treated) control data

In all experiments, stress caused significant decreases in sucrose intake ($P < 0.001$); and in vehicle-treated animals, these changes were maintained throught the experiment. The results of experiment 1 are shown in Fig. 1, and representative data from experiments 2 and 3 are summarized in Table 2.

3.2. Positive (antidepressant-treated) control data

In all experiments, imipramine treatment had no effect in control animals, but caused a complete restoration of sucrose drinking in stressed animals, which is reflected in significant main effects of Drug treatment and significant Drug × Stress interactions (Table 1). The data in Table 2 show that whereas 5 weeks of imipramine treatment restored normal behaviour in stressed animals, treatment for only 1 week was without effect. These time-dependent effects give rise to significant main effects of Weeks, and signficant 2- and 3-way interactions of Stress and Drugs with Weeks (Table 1).

The gradual onset of action of imipramine is illustrated in Fig. 1, which shows data from experiment 1 (imipramine 10 mg/kg p.o., b.i.d.). Also shown are the effects of brofaromine (10 mg/kg p.o., b.i.d.) in the same study. No significant drug effects were seen with either drug after 1 week of treatment, and in both cases full effects were apparent after 4 weeks of treatment.

3.3. Other active agents

In addition to imipramine and brofaromine, antidepressant-like effects were also shown by buspirone, atropine and mepyramine (Table 2). All showed a delayed onset of action (no effects at 1 week). The effects of atropine (1 mg/kg i.p.) were comparable to those of imipramine. Buspirone (2.5 mg/kg i.p., b.i.d.) appeared to act more slowly than imipramine, in that significant increases in sucrose intake relative to vehicle-treated stressed animals were seen only after 6 weeks of treatment (these are the data shown under 'Week 5' in Table 2; see note 2 to Table 2). After 5 weeks of treatment, intakes in buspirone-treated stressed animals (9.8 ± 0.9) were midway between (and not significantly different from) those seen in vehicle-treated stressed animals (7.7 ± 0.7) and in buspirone-treated controls (11.7 ± 1.2). Mepyramine (5 mg/kg i.p.) appeared to be less effective than imipramine: although mepyramine significantly increased sucrose intake, relative to vehicle-treated stressed animals, intake remained suppressed relative to mepyramine-treated controls (Table 2).

3.4. Inactive agents

No increase in sucrose intake was seen following chronic treatment of stressed animals with amphetamine (0.5 mg/kg i.p., b.i.d.), haloperidol (1 mg/kg i.p.) or chlorprothixene (1 mg/kg i.p.) (Table 2). Following chronic treatment with a higher dose of amphetamine (1.5 mg/kg b.i.d.) or with morphine, at escalating doses, the difference between control and stressed animals disappeared (giving rise to a significant Stress × Weeks interaction). However, this was entirely attributable to a decrease in sucrose intake in amphetamine- or morphine-treated control animals, rather than to any increase in intake by stressed animals.

4. Discussion

The chronic mild stress model has been proposed as a relatively valid animal model of depression, which meets criteria for construct, face and predictive validity (Willner et al., 1992). The construct validity of the model derives from the evidence that chronic mild stress causes a generalized decrease in responsiveness to rewards, which models the core symptom of major depressive disorder, anhedonia. Thus, decreases in sucrose drinking cannot be explained by non-specific changes (e.g. decreased thirst), since the intake of plain water is unaffected by chronic mild stress; and in addition to decreasing the intake of sweet solutions, chronic mild stress also impairs place preference (but

not place aversion) conditioning, and increases the threshold for brain-stimulation reward. All of these effects are reversed by chronic antidepressant treatment (reviewed by Willner et al., 1992; Willner, 1995). In addition to inducing a state of anhedonia, chronic mild stress also causes the appearance of a variety of other symptoms of major depressive disorder. These include decreases in sexual and aggressive behaviour; loss of body weight; adrenal hypertrophy and corticosterone hypersecretion; and a variety of sleep disorders characteristic of depression, including decreased rapid eye movement (REM) sleep latency. In contrast, chronic mild stress did not cause the appearance of an 'anxious' profile in two animal models of anxiety, the elevated plus-maze and the social interaction test (reviewed by Willner, 1995). These data speak to the face validity of the chronic mild stress model.

The purpose of the present study was to investigate further the predictive validity of the chronic mild stress model. In previous studies, reversal of the effects of chronic mild stress on rewarded behaviours has been demonstrated following chronic treatment with tricyclic antidepressants, the specific monoamine uptake inhibitors fluoxetine and maprotiline, the monoamine oxidase-A inhibitor moclobemide, the atypical antidepressant mianserin, electroconvulsive shock, and lithium (reviewed by Willner et al., 1992; Willner, 1995). The present study confirmed the anti-anhedonic effect of imipramine, which was included as a positive control in all three experiments, and extends the list of antidepressants effective in the chronic mild stress model to a second monoamine oxidase-A inhibitor, brofaromine, which has been shown in controlled trials to be equivalent in antidepressant efficacy to tricyclics (Moller and Volz, 1992; Chouinard et al., 1993). The 5-HT_{1A} receptor agonist buspirone was also effective in the chronic mild stress model. Although marketed primarily as an anxiolytic agent (Gelenberg, 1994), controlled trials in major depression have demonstrated that buspirone is also an effective antidepressant (Fabre, 1990; Rickels et al., 1991). In the present study, the onset of action of buspirone was slower than that of imipramine. This finding is difficult to interpret given that the comparison is between single doses of both drugs, and more extensive dose-response data would obviously be desirable. Nevertheless, it is of interest that a similar outcome was reported in a recent clinical study comparing buspirone and imipramine (Rickels et al., reported by Gelenberg, 1994).

Positive ('antidepressant-like') effects in the present study were also shown by the anticholinergic agent atropine. The possibility that anticholinergic drugs possess antidepressant properties, which would be consistent with cholinergic supersensitivity hypotheses of depression (Janowsky and Overstreet, 1990; Fritze, 1993), was first suggested in the 1930s (Hoch and Mauss, 1932), and has never been satisfactorily resolved. However, small, but significant, and rapid antidepressant effects have been reported in several studies of biperiden and scopolamine (Janowsky and Risch, 1984; Beckman and Moise, 1982; Gillin et al., 1991). A further, little known, but potentially important, observation is that out of seven early controlled trials of tricyclic antidepressants in which a low dose of atropine was employed as an 'active placebo', only one study found that imipramine was superior to atropine (Thomson, 1982). The effectiveness of atropine in the present study highlights the need for controlled trials of anticholinergics in depression: the outcome of such trials would be unlikely to lead to significant therapeutic developments, but would be of theoretical importance.

The histamine H_1 receptor antagonist, mepyramine, also showed positive ('antidepressant-like') effects on sucrose intake in stressed rats. Tricyclic antidepressants are histamine H_1 receptor antagonists, but this is not true of many newer antidepressants (Hall and Ögren, 1984). Histamine H_1 receptor antagonists can have reinforcing effects (White and Rumbold, 1988), and may have some abuse liability in people (Giannini et al., 1984); they have been found to exert antidepressant-like effects in some (Noguchi et al., 1992a, b; Delina-Stula et al., 1988) but not all (Katz and Sibel, 1982; Nath et al., 1988) studies in animal models of depression. One uncontrolled study reported antidepressant effects in a majority of patients treated with diphenhydramine (Hankoff et al., 1964); however, a second, controlled study found that promethazine was significantly inferior to amitriptyline in the treatment of depression (Beckman and Schmauss, 1983). A conservative conclusion would be that mepyramine represents a false negative for the chronic mild stress model. However, some limitations on this conclusion should be noted. First, the effect of mepyramine in the present study was partial: significant improvements were observed in mepyramine-treated stressed animals, but the effects were significantly smaller than those of imipramine. Again, the interpretation of this difference is limited by the single-dose design. However, while it is possible that a higher dose of mepyramine might produce a greater effect, there is no precedent in our previous studies for a partial antidepressant response, which may indicate that mepyramine genuinely differs from confirmed antidepressants. Second, mepyramine binds potently to a site distinct from the histamine H_1 receptor (Lui et al., 1994), which raises the question of how reliably the effect of mepyramine in this study can be ascribed to blockade of histamine H_1 receptors. Indeed, some antihistamines are potent monoamine uptake inihibitors, including the close congener of mepyramine, tripellenamine (Carlsson and Lindqvist,

1969). Finally, in an earlier study in the chronic mild stress model, no antidepressant effect was seen with (−)-mianserin (Cheeta et al., 1994), which is only slightly less potent as an antihistamine than mepyramine (Nickolson et al., 1982). Together, these considerations suggest that more work is needed, both in the chronic mild stress model and in the clinic, to establish definitively whether mepyramine has antidepressant properties.

We have previously reported that chronic treatment with chlordiazepoxide was ineffective in the chronic mild stress model (Muscat et al., 1992). Also ineffective, in the present study, were amphetamine, two neuroleptic agents, and morphine. Amphetamine was administered chronically at 0.5 or 1.5 mg/kg b.i.d., which represent the lower and higher margins of the behaviourally active but non-stereotypy-inducing range. Neither dose increased sucrose intake in stressed animals, and the higher dose decreased sucrose intake in controls. The latter effect probably reflects a withdrawal anhedonia (testing was carried out 22 h following the final drug administration), comparable to the increases in brain-stimulation reward threshold that have frequently been reported following prolonged treatment with psychostimulant drugs (see Markou and Koob, 1991; Borowski and Kokkinidis, 1992). Amphetamine is quite widely used in the management of refractory depressions (Ayd and Zohar, 1987), and some controlled trials indicate that amphetamine may have antidepressant effects (usually partial) in some atypical depressive syndromes: specifically, geriatric depression and depression secondary to physical illness (Satel and Nelson, 1988). However, controlled trials of psychostimulants (amphetamine or methylphenidate) in major depression have consistently failed to demonstrate antidepressant efficacy for stimulants, relative to placebo treatment (Satel and Nelson, 1988).

The two neuroleptic drugs tested in the present study, haloperidol and chlorprothixene, also failed to increase sucrose intake in stressed animals. Although these drugs were tested at a single dose (1 mg/kg), this is the dose typically used in behavioural studies (e.g. Borowski and Kokkinidis, 1992). Like stimulant drugs, neuroleptics are frequently used in the management of depression, but their efficacy as antidepressants is questionable. Indeed, depression as a side effect of neuroleptic therapy (Randrup et al., 1975; Siris, 1991) and antidepressant effects on withdrawal of neuroleptics (Randrup et al., 1975; Del Zompo et al., 1990) are both well documented. The antidepressant potential of neuroleptics is most firmly established in delusional depression, which responds well to combined therapy with a neuroleptic/tricyclic mixture, but responds poorly if at all to tricyclics alone. However, neuroleptics alone are also ineffective in delusional depression (Nelson, 1987): they produce a substantial global improvement, but this arises almost entirely from a decrease in agitation and delusional thinking; motor retardation, lack of energy and anhedonia do not respond to neuroleptic treatment, and in fact, may become worse (Nelson, 1987). On the basis of these findings, it seems likely that the global improvement sometimes seen in endogenous depressives treated with neuroleptics results from the preponderance in these studies of agitated and delusional patients (Nelson, 1987; Robertson and Trimble, 1981). In contrast to the equivocal status of the majority of neuroleptics, one drug, sulpiride, does show clear antidepressant efficacy, comparable to that of tricyclics. However, these effects are seen in a dose range substantially lower than that used in the treatment of schizophrenia (Del Zompo et al., 1990; Maier and Benkert, 1994), and probably reflect a selective blockade, at low doses, of presynaptic dopamine receptors (Serra et al., 1990). In a recent study in the chronic mild stress model, amisulpride, which has a similar pharmacological profile to sulpiride but shows greater presynaptic selectivity (Scatton et al., 1994), had antidepressant-like effects at the lowest dose tested, which were lost at higher doses (Willner et al., 1996). These effects are exactly comparable to those seen in clinical studies of sulpiride.

Chlorprothixene is sometimes claimed to have antidepressant properties (Ravn et al., 1980; Antkiewicz-Michaluk, 1986). However, this claim is based primarily on open studies, not all of which were supportive of an antidepressant action (Denber et al., 1960), and which indicated that chlorprothixene was useful primarily in the control of agitation (Poldinger, 1960; Kielholz, 1963). A single double-blind trial in depression reported no significant difference between chlorprothixene and imipramine. However, imipramine was clearly superior to chlorprothixene on all measures: for example, imipramine caused significant improvement in the second phase of the cross-over design, while chlorprothixene did not (Patch et al., 1967). The dose of chlorprothixene used in this study (135 mg) was well below the dose range used in the treatment of schizophrenia (up to 1600 mg), suggesting that if chlorprothixene does possess some antidepressant efficacy, this probably results from a selective presynaptic action at low doses, comparable to that observed with sulpiride and amisulpride. A dose-response study of chlorprothixene in the chronic mild stress model, could be informative.

Finally, morphine was ineffective in the present study. Morphine failed to increase sucrose intake in stressed animals, and decreased sucrose intake in controls, following an escalating dose regime which led to dependence, as indicated by the presence of morphine-withdrawal signs following a naloxone challenge. A similar decrease in saccharin intake has been previously reported, during withdrawal from an escalating morphine dose regime (Lieblich et al., 1991). Al-

though, prior to the introduction of the tricyclic antidepressants, the 'opium cure' (Kraepelin, 1901) was the most frequently used treatment of depression, there is little evidence to support this practice. Some efficacy has been reported in controlled studies with the opioid partial agonist buprenorphine (Emrich, 1987), or following acute administration of β-endorphin (Gerner et al., 1980) or fentanyl (Matussek and Hoehe, 1989). On the other hand, negative results have also been reported following acute administration of β-endorphin (Pickar et al., 1981) or methadone (Extein et al., 1981). To the best of our knowledge, the antidepressant potential of morphine has not been evaluated; however, there is little in the literature to suggest that morphine would prove efficaceous in the treatment of major depression.

In conclusion, the present data from the chronic mild stress model are consistent with the clinical literature in demonstrating antidepressant-like effects of brofaromine, buspirone and (probably) atropine, and in failing to demonstrate antidepressant-like effects with amphetamine, neuroleptic drugs and morphine; the position of mepyramine is equivocal and requires further research. Together with the data from earlier studies demonstrating the efficacy in the chronic mild stress model of a wide variety of confirmed antidepressant drugs, the present data provide further support for the predictive validity of the chronic mild stress procedure as an animal model of depression.

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