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Animal Model of Depression: Tests of Three Structurally and Pharmacologically Novel Antidepressant Compounds

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KATZ, R. J. AND M. SIBEL. *Animal model of depression: Tests of three structurally and pharmacologically novel antidepressant compounds.* PHARMAC. BIOCHEM. BEHAV. **16**(6) 973–977, 1982.—Previous studies have identified behavioral and neuroendocrine abnormalities in chronically stressed rats which resemble some of the more prominent features of clinical depression. These abnormalities have proved responsive to pharmacotherapy by standard antidepressant drugs and related somatic treatments. Several structurally and pharmacologically atypical compounds, resembling neither standard agents, nor each other, have recently been identified as clinically effective antidepressants. These drugs do not show typical preclinical response profiles in other drug screening tests and, therefore, represent critical instances for evaluating the selectivity of the chronic stress model. Three drugs were tested, these being iprindole, bupropion, and mianserine; a tricyclic indole, propriophenone, and tetracyclic compound respectively. Four behavioral measures, which previously proved most useful in discriminating antidepressant potential, and a measure of circulating corticosterone were obtained for subjects examined factorially in a 2×2×2 experimental design (chronic stress vs none, acute stress vs none, and drugs vs control). All compounds proved capable of reversing chronic stress induced behavioral deficits, and all but one compound reversed the attendant basal hypersecretion of corticosterone. These findings argue that the chronic stress model provides an accurate and selective assessment of the therapeutic potential of both standard and structurally novel compounds.

Activity Antidepressant Bupropion Corticosterone Iprindole Mianserine Stress

STUDIES by Seligman and co-workers (e.g., [5]), Porsolt *et al.* [14], Weiss *et al.* [18,19], and by ourselves [7–11] have identified a number of changes in patterns of activation and coping in rats and other species following chronic stress. In previous papers we identified a stress-induced model state in the rat similar to learned helplessness (and related designs) [11,12]. At a procedural level all employed some form of intermittent unpredictable stress (e.g., shock or loud noise for helplessness, cold water swimming stress for the Weiss and Porsolt procedures [14, 18, 19]; the above, and several unrelated aversive stimuli for the chronic stress procedure to be reported).

Although procedurally related, the models differ as to specific stress types and chronicity of stress exposure. They also differ conceptually, since helplessness assumes a cognitive deficit not presupposed by other procedures [7]. Finally the models possess unique behavioral endpoints, e.g., impaired avoidance learning vs swimming or open field behaviors. It appears that these factors produce markedly different degrees of impairment and pharmacological sensitivity. For example, striking differences exist in pharmacological responses to anticholinergic treatments across models, with greater stress and unpredictability causing greater resistance to treatment (compare for example [2, 9, 15]). Likewise long-lasting and behaviorally significant changes in epinephrine disposition and turnover require a more protracted course of treatment [11]. These findings suggest that under certain conditions a more longstanding stress exposure is necessary

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for the production of neural and neuroendocrine disorders resembling the clinical condition of depression. Other motivational differences have been reviewed elsewhere [7].

The stress model we employed used multiple stressors to produce a model of endogenous depression behaviorally, physiologically, and also psychopharmacologically similar to the clinical disorder. In particular, only clinically effective antidepressant treatments reversed the behavioral and physiological changes brought about by the chronic stress treatment. These effects included, but were not limited to, activation in an open field and pituitary-adrenal hyperactivity [7–11].

To date, the pharmacological selectivity of the chronic stress model has been assessed solely with respect to standard clinically established agents and techniques including tricyclic antidepressants, monoamine oxidase inhibitors, and electroconvulsive shock therapy (e.g., [7–10]). In all cases these compounds, when given chronically, restored an acute activation response to stress which was otherwise blunted by the chronic treatment. In a series of studies we demonstrated that antidepressants alone had the capacity to restore this particular acute coping response, independently of their actions upon baseline activity. Thus, the response to acute stress was the most accurate behavioral discriminator of antidepressant potential.

Recently several structurally and pharmacologically distinctive, but nonetheless clinically effective, compounds have been identified. These compounds do not necessarily resemble antidepressants either based upon their preclinical profiles or their hypothesized modes of action. Indeed, at least one drug of this class (mianserine) which is now in wide clinical use appeared ineffective based upon traditional drug screening procedures [6], and a second (iprindole) was relatively ineffective in reversing reserpine effects [5]. Clearly, such compounds both challenge traditional assumptions about the central mechanisms underlying depression [1,4] and the mode of action of antidepressants and offer a critical benchmark for the evaluation of the selectivity of the present model.

With this in mind three structurally atypical, but clinically effective compounds, iprindole [5,20], bupropion [13,16], and mianserine [6, 17, 20], were evaluated for their ability to restore an otherwise present chronic stress induced loss in activation to acute noise stress and also were examined for their abilities to reduce heightened resting plasma corticosterone levels. Both behavioral restoration and (to a lesser degree) the reversal of pituitary-adrenal hyperactivity have heretofore been successful in discriminating antidepressant efficacy.

METHOD

Subjects

Adult male Sprague Dawley rats (n=144; 6 rats/experimental cell) (Charles River), each 70 days at the start of testing, were double housed in standard 25×18×17 cm rack mounted cages with food (Teklad 4.0% fat rodent diet S=0836) and tap water continuously available and automatically programmed 12 hr/12 hr lighting cycles (lights on= 0700–1900).

Apparatus and Behavioral Procedure

Apparatus and behavioral procedures are essentially identical to previously published descriptions. Rats were tested in a standard 1.22 m^2 × 45 cm height white Plexiglas open field, as previously described [7, 8, 9]. Each subject received a single six minute placement in the field for behavioral observation prior to sacrifice. A complete (2×2×2) factorial design was employed, with the factors being: acute stress vs no acute stress, chronic stress vs no chronic stress, and drug vs control treatment. Acute stress consisted of removal from double housing and 1 hour's exposure to 95 dB of white noise, as opposed to immediate open field testing under basal conditions. The acute stress exposure occurred 48–72 hours after the final stress of the chronic series. Chronic stress consisted of a regimen of unpredictable aversive stimulation, involving over a 21 day period: exposure to 60 minutes of unpredictable shock (average 1 mA, 1–10 sec duration; average one shock/min (3 exposures)), 40 hr food deprivation (2 times), cold swim at 4°C for five min (3 times), 40 hr water deprivation (2 times), 5 min exposure to the heat stress at 40°C (2 times), 30 minutes shaker stress (2 times), reversal of day/night cycle (2 times). Stressors were delivered in a semirandom fashion every 2–3 days throughout the lighting cycle from 0800 through 2200 hr. Order of stress administration was identical to previous descriptions [7, 8, 9, 11] and is presented in Table 1.

The following behavioral measures were taken for open field testing: a primary measure of outside squares (min 0–3) and secondary measures of motor latency (i.e., latency to initial movement, in sec), defecation score (bolus count/6 min), and defecation latency (in sec). At the close of testing rats were removed for immediate (<20 sec) sacrifice by decapitation, with trunk blood collected in heparinized tubes. Blood was centrifuged at 2400 rpm for 40 min, and plasma was removed and frozen at −40°C for subsequent determination of corticosterone by competitive protein binding assay, using rat corticosterone as the assay standard [12]. The field was cleared thoroughly between tests.

Daily treatments involved once-daily injections of the HCl salts of each of the compounds iprindole, bupropion, and mianserine, at 5, 10, and 5 mg/kg respectively. Drugs were freshly prepared in 0.9% sodium chloride vehicle and were administered daily throughout the 21 day stress period, and all dosages were based upon the established preclinical pharmacology of these compounds, each dose representing approximately behavioral ED 50 in affecting motor behavior.

Statistical Analysis

Statistical analysis was by univariate analysis of variance, with post hoc comparisons based upon Sheffe allowances. In the interests of brevity only the primary behavioral (open field activity) measure will be presented in graphic form. Defecation scores were variable, and are not reported separately. Remaining data are included in Table 1.

RESULTS

For all three compounds, acute noise stress was behaviorally activating in otherwise untreated subjects. This effect was reduced or eliminated by prior treatment with chronic stress but was restored by concomitant pharmacotherapy. Drugs had few effects upon control performance.

Findings for individual compounds and measures are presented below. Activity scores are presented graphically (Figs. 1–3) while remaining data including circulating steroid levels are presented in Table 1.

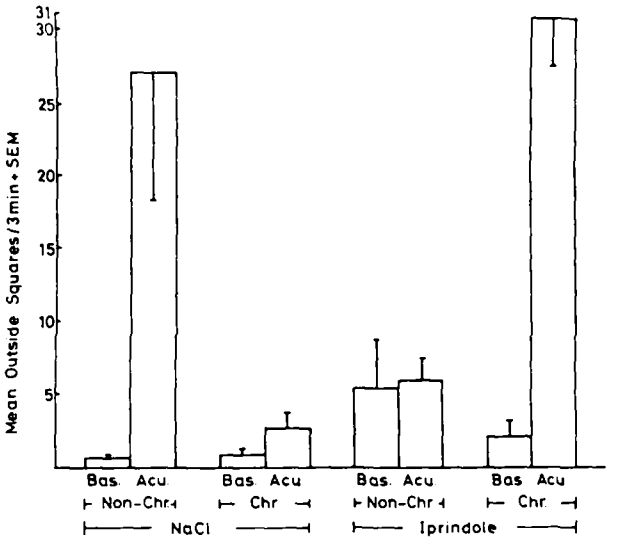

FIG. 1. Reversal of chronic stress produced activation deficit by the experimental antidepressant iprindole HCl. Mean outside squares, based upon the initial 3 minutes of a single test in a novel open field (plus standard errors of the mean), are presented. Bas=basal—no acute stress; rats were tested immediately upon cage removal: Acu= acute stress; rats were removed from double housing and individually subjected to 60 min of white noise at 95 dB prior to testing: Non chr=nonchronic; rats were tested after 3 weeks of undisturbed accommodation to laboratory surroundings in standard cages: Chr= chronically stressed; rats were subjected to periodic stressors for 3 weeks, as further described in the text: NaCl=daily injections of vehicle: Iprindole=daily injections of drug at 5 mg/kg for 3 weeks. It may be that iprindole has no intrinsic activating effects, but restores normal acute-stress elicited activity.

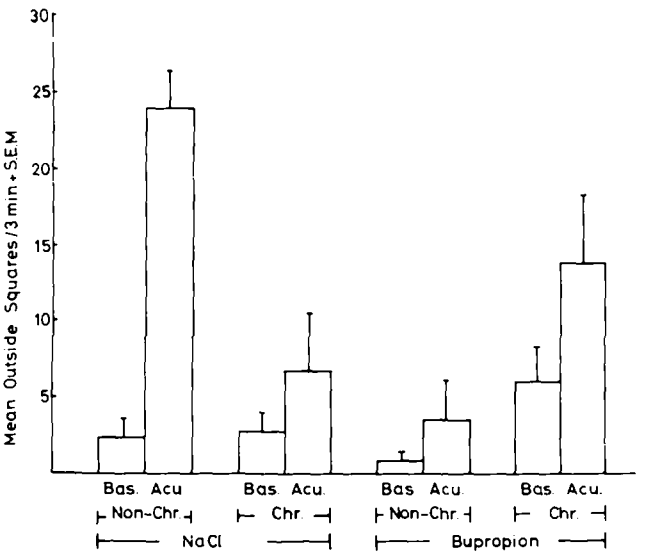

FIG. 2. Reversal of chronic stress produced activation deficit by the experimental antidepressant bupropion. Mean outside squares (plus standard errors of the mean), based upon the initial 3 minutes of a single exposure to a novel open field, are presented. Abbreviations are identical to those in Fig. 1. Bupropion=10 mg/kg of bupropion HCl per day for 3 weeks. It may be that bupropion has no intrinsic activating effects, but restores normal acute-stress elicited activity.

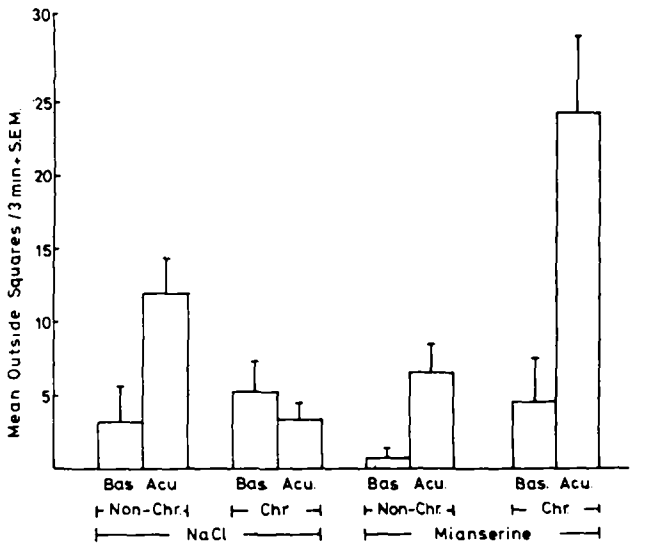

FIG. 3. Reversal of chronic stress elicited activation deficit by the experimental antidepressant mianserine. Mean outside squares (plus standard errors of the mean), based upon the initial 3 minutes of a single exposure to a novel open field, are presented. Abbreviations are identical to those in Fig. 1. Mianserine=5 mg/kg of mianserine HCl per day for 3 weeks. It may be that mianserine has no intrinsic activating effects, but restores normal acute-stress elicited activity.

Results for Iprindole

An overall effect of manipulations was present, $F(7,40)=12.1$, $p \ll 001$, which represented, as noted above, an elevation of activity due to acute stress, $F=27.8$, $p \ll 001$; in all cases to be reported a $p<0.05$ based upon post hoc analysis is considered statistically significant beyond chance (Fig. 1). All values to be reported are significant at this level unless otherwise specified). The acute elevation suffered an 88% reduction, thus it was not present to a significant degree after chronic stress, $F=0.1$, $p \sim 0.5$, nor was it found in unstressed drug treated rats, $F=0.5$, $p \sim 0.5$. However activity was significantly restored (~118%) by drug treatment in the experimental group, $F=33.7$.

Results for Bupropion

A groups effect was present, $F(7,40)=8.6$, $p \ll 001$, representing an acute stress induced increase in activity, $F=33.7$, which was reduced 75% following chronic stress, $F=1.1$, $p \sim 0.5$ (Fig. 2). Additionally, no acute effect was observed in drug treated rats, $F=0.5$, $p \sim 0.5$. However, the activation effect was restored 60% by drug treatment in chronically stressed rats, $F=4.1$.

Effects of Mianserine

A groups effect was present, $F(7,40)=8.0$, $p \ll 0.001$, representing an acute stress elicited elevation in activity, $F=3.8$, which was reduced 75% in chronically rats stressed, $F=0.1$, $p \sim 0.5$ (Fig. 3). No effect was present in drug treated rats, $F=2.6$, $p \sim 0.1$, but a significant (200%) restoration was present in chronically stressed drug treated rats, $F=16.5$.

TABLE 1

EFFECTS OF THREE NOVEL ANTIDEPRESSANTS UPON ACUTE AND CHRONIC STRESS ELICITED BEHAVIORS (MEAN ± S.E.M.)

	NaCl				Iprindole			
			Chronic				Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Movement Latency (sec)	311.0 ± 26.1	58.0 ± 20.9†	290.0 ± 53.1	145.8 ± 52.8†	321.8 ± 86.1	57.5 ± 14.2	149.7 ± 69.3	5.5 ± 1.0‡
Mean Plasma Corticosterone (μg/dl)	17 ± 2	64 ± 2*	31 ± 4*	42 ± 8	27 ± 2*	61 ± 5	31 ± 3	59 ± 5
	NaCl				Bupropion			
			Chronic				Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Movement Latency (sec)	251 ± 59	43 ± 14†	242 ± 68	230 ± 69	245 ± 67	208 ± 55	178 ± 63	45 ± 21‡
Plasma Corticosterone (μg/dl)	15 ± 1	43 ± 4*	25 ± 1*	41 ± 4	15 ± 3	50 ± 7	18 ± 2‡	49 ± 5
	NaCl				Mianserine			
			Chronic				Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Movement Latency (sec)	263 ± 65.2	90 ± 63.1†	213 ± 74.4	219 ± 67.9	270 ± 41.7	91 ± 5.3	74 ± 67.8	23 ± 12.0‡
Plasma Corticosterone (μg/dl)	166 ± 1	49 ± 3*	26 ± 1*	53 ± 4	25 ± 2	54 ± 4	20 ± 1‡	46 ± 8

*Significantly increased from control.
†Significantly decreased from control.
‡Significantly restored towards acutely stressed control value.

DISCUSSION

Three clinically effective drugs resembling neither standard clinically employed agents nor each other were tested for their effectiveness in a novel preclinical model of depression. Despite the fact that these drugs provided false negatives on other tests they were positively identified by the current procedure.

The above findings suggest that the depression procedure may be extended to the evaluation of potentially therapeutic structurally novel compounds. Selectivity in the identification of novel compounds clearly is a necessary, although hardly a sufficient requirement of a testing procedure.

An antidepressant screening procedure must also distinguish negative cases, i.e., it must be specific for just those agents which in fact are therapeutic. Thus we also note that the stress model accurately identifies tripelennamine, haloperidol, and oxazepam as inert for the reversal of the stress syndrome. Since the model can accurately discriminate both positive and negative instances of therapeutic effects it is, at least within the species tested, a specific and selective procedure.

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