Pharmacology Biochemistry & Behavior, Vol. 16, pp. 979–982, 1982. Printed in the U.S.A.

Further Analysis of the Specificity of a Novel Animal Model of Depression—Effects of an Antihistaminic, Antipsychotic and Anxiolytic Compound

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Received 16 January 1981

KATZ, R. J. AND M. SIBEL. *Further analysis of the specificity of a novel animal model of depression—effects of an antihistaminic, antipsychotic, and anxiolytic compound.* PHARMAC. BIOCHEM. BEHAV. 16(6) 979–982, 1982.—In previous papers we reported that chronic stress elicited reductions in selected forms of open field activity resembled endogenomorphic depression upon behavioral, motivational, neuroendocrine, and neuropharmacological grounds. In particular, the loss of acute stress elicited activity proved to be exclusively reversible by antidepressant treatments. Insofar as clinically ineffective compounds were tested, the deficit proved refractory to treatment, further suggesting the model reflected just those processes which were disrupted in depression. A number of ineffective compounds are known to yield false positives upon other related tests, but have yet to be examined in the present model. Three such compounds, an antihistamine (tripelennamine), a neuroleptic (haloperidol), and an anxiolytic (oxazepam) were examined for their behavioral and neuroendocrine effects. Although other stress related phenomena were replicated, none of the above compounds was effective in restoring the activation deficit or in eliminating the endocrine abnormality. This suggests the depression model is relatively selective pharmacologically and not critically dependent upon receptor blocking properties of the above drugs.

Activity Antihistamines Anxiolytics Benzodiazepine Corticosterone Defecation Haloperidol Neuroleptics Open field Oxazepam Tripelennamine

AS we previously noted, all pharmacologically based models of clinical disorders must be judged jointly in terms of their selectivity and specificity, i.e., by their ability to accurately identify compounds of potential clinical utility and by their ability to reject ineffective compounds as such. The present paper in this series examines the specificity of a model with respect to 3 types of psychoactive compounds which have yielded falsely positive results in other preclinical tests or, alternately, which have been shown to affect initial open field behavior and endocrine responsiveness to stress.

The compounds chosen were tripelennamine, an antihistaminic; haloperidol, an antipsychotic; and oxazepam, an anxiolytic. At the doses employed one or more of these compounds reversed the effects of reserpine and acted synergistically with amphetamines, in previous studies. Both effects may be taken as signs that drugs are potentially antidepressant [6], although the drugs in fact lack clinical utility in treating depression. One or more of these drugs also have been shown to augment open field behavior and lower the release of corticosterone brought about by stress [5, 6, 8]. Since these are the dependent variables of interest, assessment of these compounds is particularly important.

METHOD

Subjects

A total of 144 (n=6/cell) adult male Sprague-Dawley rats (Charles River Farms) each 70 days at the start of testing were double housed in standard rack mounted cages with food (Teklad 4.0% fat rodent diet S-0836) and tap water continuously available and normal 12 hr/12 hr lighting cycles (lights on=0700–1900 hr).

Apparatus and Behavioral Procedure

Both apparatus and procedures are identical to those reported previously [3, 4, 7]. Rats were tested in a 1.22 m^2 × 45

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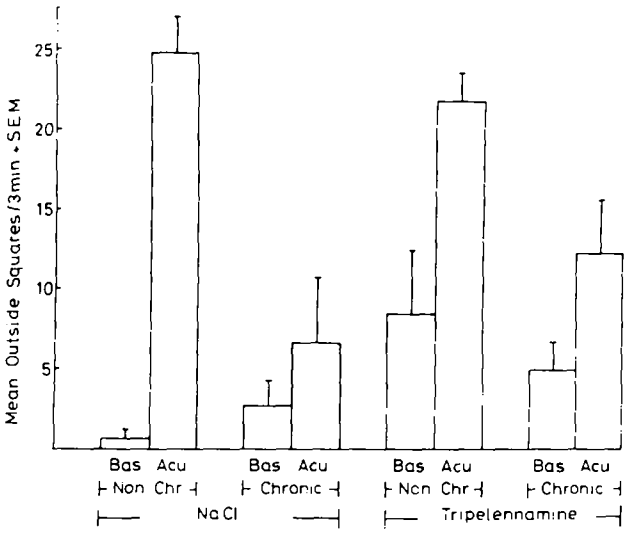

FIG. 1. Effects of acute and chronic stress and tripelennamine upon initial open field motor activity in the rat. Mean values and standard errors are preserved and a single placement is employed. Bas=basal (no acute stress). Acu=acute stress, consisting of 1 hr exposure to 95 dB of white noise. Non chronic=no chronic stress (cage rest). Chr=3 weeks of chronic intermittent stress exposure involving various stimulus modalities (see text). NaCl=vehicle (0.9% sodium chlorine) injected for 3 weeks. Tripelennamine=drug (5 mg/kg) treated for 3 weeks.

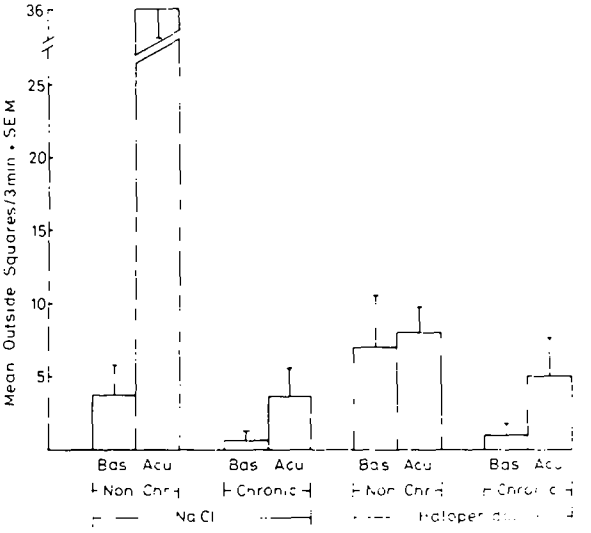

FIG. 2. Effects of acute and chronic stress and haloperidol upon initial open field motor activity in the rat. Mean values and standard errors are presented and a single placement is employed. For abbreviations see Fig. 1. Note a tartaric acid vehicle is used for control haloperidol injections.

cm height white Plexiglas open field which was divided into 16 squares for the assessment of motor activity. A 2×2×2 experimental design was used for each compound. The factors were (1) drug vs vehicle; (2) 1 hour's exposure to 95 dB white noise stress immediately prior to a single 6 min placement in the open field vs no such pre-exposure (i.e., presence vs absence of acute stress); and (3) chronic intermittent stress for 3 weeks prior to testing vs undisturbed home cage accommodation for the same period (presence vs absence of chronic stress). It should be noted that all rats including controls received daily injections and regular replacement of food, water, and bedding materials throughout the experimental period. The chronic stress regimen consisted of the following over a 21 day period: exposure to 60 minutes of unpredictable shock (average 1 mA, 1–10 sec duration; average 1 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 hr through 2200 hr. Forty-eight to 72 hours intervened between the final stress and behavioral testing. The precise order of presentation of these stressors has been presented elsewhere [3,4]. Four behavioral measures [outside squares activity (min 0–3); defecation score (bolus count); latencies (in sec) for motor activity and defecation; and an endocrine measure (circulating corticosterone)] were obtained for each rat. As was the case previously, in the interests of brevity, the motor activity measure graphically and remaining data are included in a single table in the appendix.

Drugs

Tripelennamine, haloperidol, and oxazepam were administered 5, 0.5, and 5 mg/kg once daily for the course of the chronic stress period. Vehicle solutions consisted of 0.9% saline, 0.3% tartaric acid in saline; and 1% Tween-80 in saline, respectively. All injections were administered intraperitoneally at 1 ml/kg of body weight.

Statistical Analysis

All results are presented as means and standard errors. Analysis was by univariate analysis of variance using Sheffe limits for post-hoc comparisons [1].

RESULTS

In all cases to be presented both acute stress induced behavioral activation and chronic stress induced reductions in this response were found. None of the 3 compounds restored the stress related reduction, however.

Tripelennamine's effects upon initial activity are presented in Fig. 1. An overall effect of groups was present, $F(7,40)=10.5$; $p<0.00001$. A significant activating effect due to acute stress was present, $F=37.8$, $p<0.00001$, in all cases to be reported a post-hoc significance criterion of $p<0.05$ is employed, all F ratios are significant at this level unless specified to the contrary). The activation effect was reduced 75% by chronic stress, subsequent $F=1.0$, $p=0.4$, and was marginally (50%), but not significantly, restored by drug treatment. $F=3.1$, $p=0.1$. In further support of the ineffectiveness of chronic drug treatment the chronic stress groups with and without drug did not differ from each other, $F=3.0$, $p=0.1$. Other measures may be found in Table 1. In no case was evidence of drug related recovery found.

Effects of haloperidol upon activity are presented in Fig. 2. It may be seen that an overall effect of groups was present,

TABLE 1

EFFECTS OF ACUTE AND CHRONIC STRESS AND THREE COMPOUNDS UPON OPEN FIELD AND PITUITARY-ADRENAL ACTIVITY IN THE RAT (MEAN ± SEM)

	Vehicle				Tripelennamine			
	Non Chronic		Chronic		Non Chronic		Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Defecation Score (Bolus Count)	6.2± 0.5	1.3± 0.4†	7.0± 0.5	3.5± 0.5	4.5± 0.8	0.6± 0.2	5.2± 0.3	4.0± 0.6
Activity Latency (sec)	280 ±30	94 ±49†	260 ±33	107 ±40	215 ±43	41 ±27	223 ±49	77 ±27
Defecation Latency (sec)	35 ±11	273 ±43‡	20 ± 3	46 ±11	88 ±28	286 ±35	26 ± 6	42 ± 3
Plasma Corticosterone (μg/dl)	14 ± 2	59 ± 2*	30 ± 3*	60 ± 2	15 ± 1	64 ± 4	27 ± 2	63 ± 3

	Vehicle				Haloperidol			
	Non Chronic		Chronic		Non Chronic		Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Defecation Score (Bolus Count)	3.2± 0.8	1.1± 0.3†	5.5± 0.6	4.0± 0.6	4.6± 1.0	1.8± 0.6	7.3± 0.2	2.0± 0.7
Activity Latency (sec)	244 ±43	39 ± 9†	318 ±21	135 ±36	159 ±48	16 ± 5	302 ±27	208 ±51
Defecation (sec)	77 ±44	245 ±45*	9 ± 6	36 ±10	73 ±31	150 ±31	25 ± 7	152 ±50
Plasma Corticosterone (μg/dl)	15 ± 1	49 ± 4*	29 ± 3*	63 ± 3	22 ± 1	63 ± 3	28 ± 2	57 ± 4

	Vehicle				Oxazepam			
	Non Chronic		Chronic		Non Chronic		Chronic	
Category	Basal	Acute	Basal	Acute	Basal	Acute	Basal	Acute
Defecation Score (Bolus Count)	5.5± 0.8	0.8± 0.3	6.3± 0.2	2.9± 0.6	4.8± 0.9	0.3± 0.2	5.2± 1.0	4.0± 0.3
Activity Latency (sec)	360 ± 0	26 ± 9†	158 ±48	87 ±41	259 ±49	20 ± 9	360 ± 0	99 ±39
Defecation Latency (sec)	40 ±12	205 ±55*	17 ± 5	60 ±19	99 ±40	248 ±51	75 ±44	18 ± 3
Plasma Corticosterone (μg/dl)	17 ± 2	49 ± 4*	30 ± 1	59 ± 4	20 ± 2	52 ± 3	30 ± 2	43 ± 7

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

F=23.1; *df* as above, $p<0.00001$, representing an acute stress induced increase in activity in otherwise basal subjects, F=90.1. The activation effect was significantly reduced (88%) following chronic stress, F=91.6, to an overall level not differing from chance, F=0.3, $p=0.4$, and this was not reversed by drug treatment (improvement was 4%, F=0.4, $p=5$). As might be expected, the effect of haloperidol was not significantly different from chance, F=1.9, $p=0.2$. The remaining data (in Table 1) also fail to support any drug related therapeutic effects.

The effects of oxazepam upon activity are presented in Fig. 3. An overall effect of groups was present, F=5.7; *df* as above; $p<0.0001$. Acute stress increased activity in otherwise untreated subjects, F=20.1. This effect was significantly (62%) reduced by chronic stress pretreatment, F=4.2, and yet further reduced (by an additional 14%) by drug treatment, F=4.5. Further experimental effects involving oxazepam are presented in Table 1.

DISCUSSION

Despite the replication of main and interaction effects for acute and chronic stress upon several measures of open field

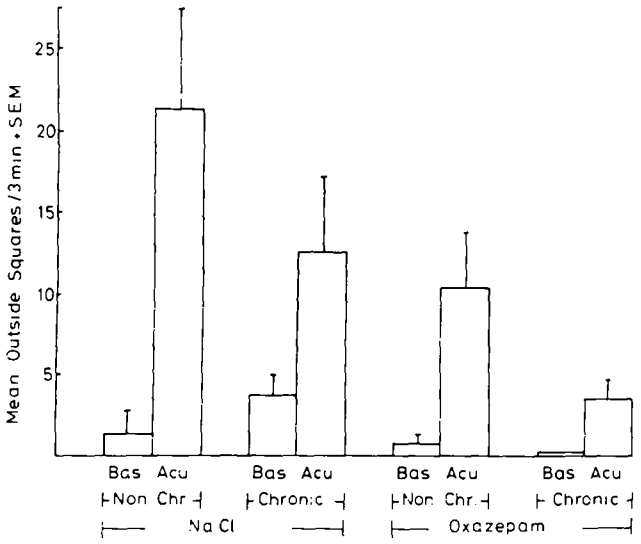

FIG. 3. Effects of acute and chronic stress and oxazepam upon initial open field activity in the rat. Mean values and standard errors are presented, and a single placement is employed. Abbreviations as in Fig. 1. Note a 1.0% Tween-80 in saline vehicle is used for control and oxazepam injections.

activity, no evidence for a pharmacological reversal of the chronic stress syndrome was obtained. In those few cases where drugs did affect chronic stress related performance the effect was opposite in direction to that obtained for antidepressants. It must be emphasized that the doses of drugs used in the present design are known to affect endocrine parameters and behavior in other circumstances (e.g., [2, 5, 8]).

The absence of any evidence for reversal implies that the underlying processes affected by these compounds are not normally involved in the recovery effect. This does not necessarily rule out all involvement by the systems affected by these drugs. Two compounds have established receptor-blocking activity. The third, oxazepam may have agonist or antagonist properties within several systems. Establishing an absence of relationship requires testing both agonists and antagonists of critical receptors. Nonetheless, these compounds were not effective.

Finally the absence of behavioral and neuroendocrine effects of both a neuroleptic and benzodiazepine in the present model is notable. Although haloperidol is not used to treat depression it is sucessfully used as an acute intervention in mania. Other neuroleptics have been used to treat depression (e.g., chlorprothixene, levropramazine), however, their use is both infrequent and of only limited therapeutic benefit [9]. As noted, benzodiazepines may affect open field behavior in other circumstances [2,8], and also may reduce pituitary adrenal responsiveness to stress [5]. The ineffectiveness of oxazepam in the present circumstances underscores the specificity of the present model.

ACKNOWLEDGEMENTS

Supported in part by grant MH31588 from the National Institute of Mental Health to Dr. B. J. Carroll. Dr. Katz is currently an Alfred P. Sloan foundation fellow in Neuroscience. The editorial assistance of Anne Marie Skepenaitis, technical assistance of Giulio Baldrighi, and statistical assistance of R. C. Shea are acknowledged with gratitude. Tripelennamine and oxazepam were generously donated by Ciba-Geigy and Wyeth Laboratories, respectively.

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