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Effects of Zometapine, A Structurally Novel Antidepressant, in an Animal Model of Depression[1]

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KATZ, R. J. *Effects of zometapine, a structurally novel antidepressant, in an animal model of depression.* PHARMACOL BIOCHEM BEHAV 21(4) 487–490, 1984.—Zometapine, a pyrazolodiazepine, bears a close structural resemblance to benzodiazepines. It possesses an unusual pharmacological profile, and is active in some, but not all, tests of antidepressant activity. In clinical tests it appears to be an extremely effective pharmacotherapeutic agent, and may represent a new class of antidepressant. Because the preclinical profile of zometapine is unusual, we examined its effects in a behavioral test of antidepressant potential. Following three weeks of treatment, the drug selectively reversed a behavioral depression following chronic stress. Drug-induced reversal was seen only in rats activated by acute noise exposure, and was dose related. Reversal was confirmed by a second measure, defecation, and partially confirmed by the normalization of an elevated basal corticosterone response.

Activity Corticosterone Defecation Open field Pyrazolodiazepine stress Zometapine

ZOMETAPINE is a structurally and pharmacologically unusual compound with antidepressant potential. Since it is a pyrazolodiazepine, it is closely related to anxiolytic compounds, particularly ripazepam (see Fig. 1). Despite this resemblance, zometapine lacks anxiolytic potency, based upon preclinical tests [9]. The drug also has few antihistaminic and anticholinergic effects. Based upon its ability to potentiate the effects of amphetamine and cocaine upon appetite, reinforcement, and motor performance, the drug may be antidepressant. Recent clinical reports support its efficacy, and suggest a relatively rapid onset of action with few side effects [2,11].

Zometapine has not proved positive in all preclinical tests of antidepressant activity, however. It appears inactive both in amine reuptake inhibition and in inhibition of monoamine oxidase. The compound also fails to antagonize the convulsant effects of electroshock and pentylenetetrazol. Thus, its mode of action is atypical, and may be related to a sensitization of a specific population of adrenergic receptors [9].

In the stress model of depression [5–7], exposure to chronic stress reduces the typical open field motor activation which follows acute noise exposure. Antidepressant potential is measured through the restoration of noise-stress reduced activation. The test appears sensitive to all typical and atypical antidepressant compounds evaluated to date (e.g., tricyclic and tetracyclic compounds, ECT, monoamine oxidase inhibitors, Wellbutrin, and Iprindole). Since zometapine is clinically active, structurally unusual, and has only a partial antidepressant profile in preclinical tests, it is of particular interest to evaluate its profile of activity in the stress test.

METHOD

Subjects

Seventy-two adult male Sprague-Dawley rats (Charles River Farms), each 70 days of age at the start of the experiment, were maintained with food (Teklad 4% fat rodent diet S-0836) and tap water continuously available, and normal 12 hr/12 hr lighting cycles (lights on = 0700–1900 hr).

Experimental Design

Zometapine was tested in a 2×2×3 factorial design. The first factor was the presence or absence of chronic stress (i.e., the depression induction procedure) and the second was presence vs. absence of acute stress (activation induction procedure). In addition, vehicle was compared to 2 doses of the compound. As noted, the chronic stress procedure consisted of 3 weeks of unpredictable stress administration. Stressors were unpredictable as to type, onset, and offset, and included: sixty minutes of unpredictable shock (average 1 mA, 1–10 sec duration) average 1 shock/minute (3 exposures); 40 hours food deprivation (2 times); cold swim at 4.0°C for 5 minutes (3 exposures); 40 hours water deprivation (2 times); 5 minutes exposure to heat stress at 40°C (2 times); 30 minutes shaker stress (2 times); reversal of day/night (2 times). These stresses were delivered in a semi-random fash-

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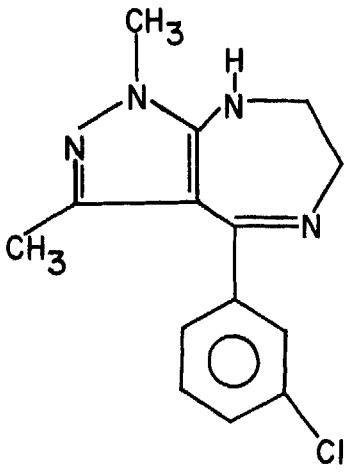

FIG. 1. The structural formula for zometapine: Technically the compound is 4-(m-chlorophenyl)-1,6,7,8-tetrahydrol-1,3-dimethyl-pyrazolo-[3,4-e], [1,4]diazepine.

ion every 2–3 days throughout the day/night cycle from 0800 through 2200 hr. The order of stress administration has been presented [5].

Drugs

Based upon initial tests, published data, and consultation with Dr. Paul Poschel of Warner-Lambert Inc., doses of 2.5 and 5.0 mg/kg of CI 781 (zometapine) were compared with placebo. Drugs were injected intraperitoneally 1 ml/kg in sterile 0.9% sodium chloride with pH adjusted to maintain compounds in solution. For all vehicle and drug injections pH was adjusted by adding 1 MEQ of hydrochloric acid and adjusting to a final value of 7.6. Prior to the reported experiments, subsamples of 5 rats per dose per drug were tested for three weeks to determine what (if any) ill effects followed daily IP injection. Since the pilot rats appeared normal after this period, and since no obvious deleterious effects were noted in the initial sample upon sacrifice, doses and injection procedures were employed.

Behavioral Procedures

Experimental testing took place between 0900 and 1300 hr. Rats were individually removed from their cages and either immediately (i.e., less than 15 sec) placed in a 1.22 m^2 × 45 cm height white Plexiglas open field containing a 4×4 grid for observation of motor activity for six minutes of behavioral observation (control condition), or similarly removed and subjected to 95 dB of white noise for 1 hr, and then tested (acute stress condition). For all subjects, motor activity in the initial three minutes of open field exposure was taken as primary behavioral measure. For all motor measures a count of 1 was based upon all 4 feet of a rat crossing a grid mark. Total counts indicated total number of complete grid crossings by the subject. Defecation score (bolus count/session) was taken as a supplementary measure. The rationale for using these particular measures has been presented elsewhere [10,12]. Moreover, in previous studies we have found these two measures of particular value in discriminating antidepressant compounds. Behavioral ratings were carried out by an observer blind to the group and drug assignments of individual subjects.

At the close of testing, rats were removed from the testing apparatus and immediately (less than 20 sec) sacrificed by decapitation. Trunk blood was collected in chilled heparinized tubes and centrifuged at 2400 rpm for 30 min. Plasma was collected and frozen at −40°C for later corticosterone assay by competitive protein binding assay [4]. All determinations were made in triplicate with rat corticosterone as the assay standard. Within assay coefficient of variation was less than 5%. As with behavioral observations, all samples were numerically coded and run blind, with the code broken only after the completion of the analyses.

Unless specified to the contrary, all results are routinely presented as means and standard deviations. Statistical analysis was by factorial analysis of variance. Post-hoc comparisons utilized Sheffe limits for the assessment of statistical reliability. To simplify presentation of the data, all comparisons reported in the text may be assumed statistically significant at $p<0.05$, unless specifically stated to the contrary.

RESULTS

Profiles of behavioral responses were consistent with the presence of antidepressant activity for zometapine. Although behavioral findings were relatively clear cut, endocrine findings were only partially so. Results are presented below and in the accompanying figures.

Results for CI-781-Motor Activity

An overall difference across cells was present ($F,11.60)=8.2$). Specific factorial comparisons follow. For the three main factors of chronic stress, acute stress, and drug dose, respectively, the first and last factors were significant, while the second was not (respectively, F ratios=5.3, 0.9, 4.6: $df=1,60$; 1,60; 2,60). For two way interactions of the stress factors, and chronic and acute stress with the dose factor respectively, the first interaction was significant, but the other interactions were not (F's, respectively, =6.9, 3.0, 1.8: $df=1,60$; 2,60; 2,60). A three way interaction was present ($F=5.5$, $df=2,60$). Individual comparisons based upon Sheffe ratios follow. As has been found previously, acute stress was activating for control rats (basal vs. acute stress, non-drugged, $F=16.0$, $p<0.0002$) but not following chronic stress (chronic vs. chronic and acute, non-drugged, $F=0.1$; $p\sim0.5$). This confirms the presence of behavioral depression following chronic stress.

The compound reversed this chronic stress related behavioral depression in a dose related fashion (for the chronic × acute dose response curve, $F=21.8$; $p<0.0001$). This did not appear to be related to the effects of the drug upon basal open field activity which were not dose related, and which were significantly above placebo levels for the lower dose only. Therefore, a specific antidepressant activity was identified. These findings are presented in Fig. 2.

Results for CI-781-Defecation Scores

Examination of drug interactions with acute and chronic stress based upon a second measure, open field defecation, also proved positive. A pattern reflecting antidepressant potential again was found to be present. Overall, across cells differences were present ($F=5.8$; $p<0.0001$, df as above). For the main factors of chronic stress, acute stress, and drug dose, only the factor was significant (F ratios=1.7, 2.5, 8.1;

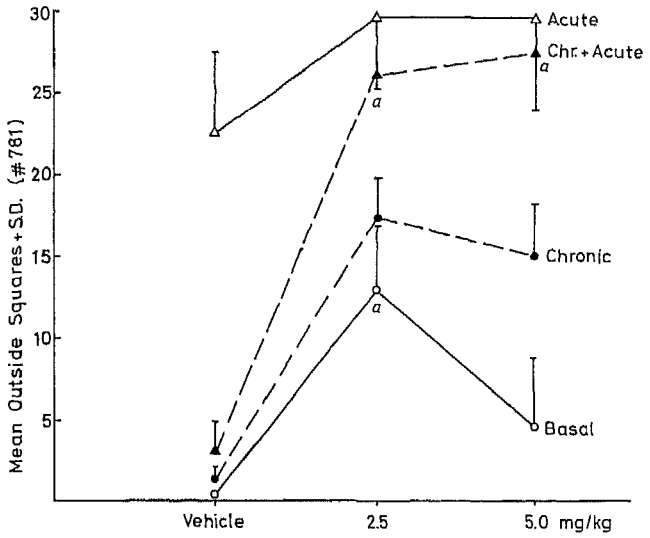

FIG. 2. Effects of acute and chronic stress, and zometapine upon initial open field activity in the rat. A single placement is employed. Acutely stressed rats are presented as triangles, and chronically stressed rats are presented with broken lines (respective control conditions are circles and solid lines). Mean outside squares indicate the 12 squares adjacent to the chamber wall (i.e., all grid crossings except those involving the 4 inner squares). *a* Indicates $p<0.05$ for vehicle vs. drug for the Chr+acute group.

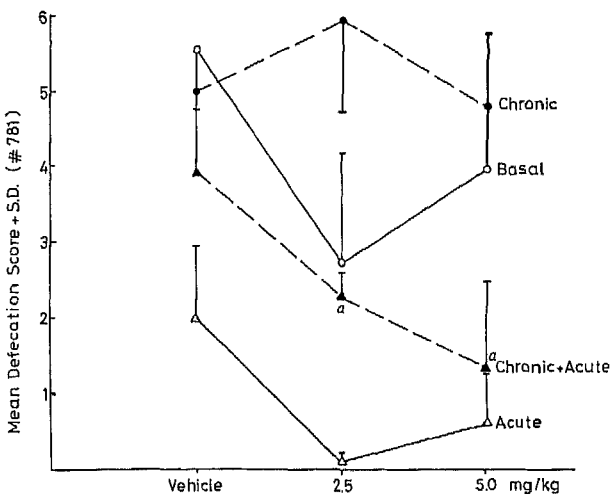

FIG. 3. Effects of acute and chronic stress and zometapine upon initial open field defecation in the rat. A single placement is employed. Acutely stressed rats are presented as triangles, and chronically stressed rats are presented with broken lines (respective control conditions are circles and solid lines). Symbols as in Fig. 2.

df as above). A two way interaction of chronic and acute stress was present, but remaining interactions of chronic or acute stress with drug dose were absent (F's=3.9, 3.0, 0.3; *df* as above). A three way interaction was present (F=7.3; *df*=2,60). Acute stress inhibited normally high levels of defecation (Basal vs. Basal-acute, non-drugged, F=13.8; $p<0.0005$). Chronic stress reduced this effect to nonsignificant levels (F=2.2, n.s.), but the acute effect was restored in chronic animals receiving drug treatment (for dose response, F=8.3; $p<0.005$). It may be seen (Fig. 3) that the therapeutic effect again was dose related, and was not present for control rats.

Results for Pituitary-Adrenal Activity (Fig. 4)

Although major trends consistent with previous findings were demonstrated at a significant overall level (F=18.2; *df*=11,60; $p<0.00001$). Main effects of chronic and acute stress were present, but drug dose was not significant (F=4.3, 7.0, 0.0, respectively; *df* as above). All interactions were nonsignificant (all F ratios were <1.4; *df* as above). Although overall trends were consistent with an antidepressant profile, not all individual comparisons were significant beyond chance. On the one hand acute stress provoked a significant corticosterone rise (Basal vs. Basal-acute, non-drugged, F=31.6; $p<0.0001$), and chronic stress produced a resting elevation in plasma steroid levels (Chronic vs. Basal, non-drugged, F=5.3; $p<0.02$). Although chronic drug treatment reduced these last levels, it did not produce a significant return. In somewhat less direct support of this trend towards reversal, no significant difference existed between basal corticosterone levels without stress and steroid levels of chronically stressed rats after drug treatment. Since a significant elevation is rendered nonsignificant this may be seen as supportive of the hypothesis.

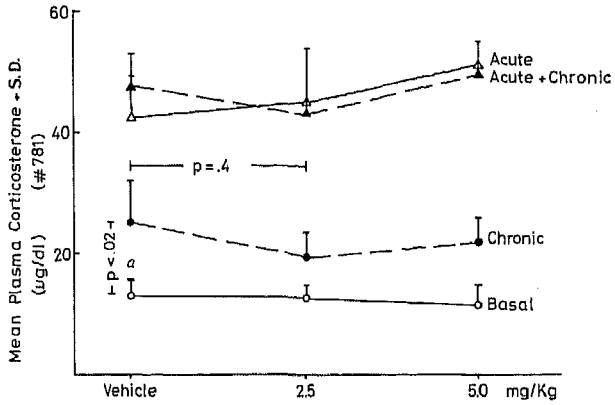

FIG. 4. Effects of acute and chronic stress and zometapine upon circulating corticosterone in the rat. Acutely stressed rats are presented with triangles and chronically stressed rats are presented with broken lines (respective controls are circles and solid lines). Elevations due to acute and chronic stress are presented. A slight but non-significant drug induced reversal of the chronic stress effect may be seen. Symbols as in Fig. 2.

DISCUSSION

Zometapine was behaviorally effective, but only partially effective in reversing a concomitant endocrine abnormality. Thus two measures of three indicated efficacy. Both measures were behavioral, and in both cases anticipated 2 and 3 way interactions indicated the appropriate presence of experimental conditions and drug interactions. Findings were equivocal for one of three measures—the endocrine measure. Since previous studies have indicated that at least certain classes of compounds (e.g., monoamine oxidase inhibitors) may be clinically effective without affecting the endocrine abnormality, this does not rule out potential clinical utility. Indeed, the remaining behavioral results strongly

suggest the existence of such properties. Less consistent behavioral findings might cast some doubt upon this last statement but the present findings follow a pattern of consistency seen previously with other compounds.

Previous tests upon other structurally related anxiolytic drugs which are not effective antidepressants (including oxazepam) did not produce a zometapine-like profile of activity. Rather, such compounds were relatively inert using the particular procedures outlined above. This should not be taken as an indication of an overall lack of effect of anxiolytic drugs upon open field activity, however. Other test procedures point to effectiveness under different conditions [1].

The findings, overall, lend themselves to either of two interpretations. On the one hand, chronic stress may have produced a profound anergia upon the part of the stressed subject. It may have produced, that is to say a "model depression." On the other hand a history of intermittent and mixed modality stress may have produced an adaptive tolerance to additional novel or stressful stimulation. These two rather different interpretations of the results do not challenge the validity of the present findings but do call into question their overall frame. Both for obvious humane considerations and to partially address this issue we are currently developing a procedure with milder and fewer stresses. Preliminary findings suggest that we will be able to maintain the reported behavioral effects and interactions with one week's less stress exposure, although whether drug effects remain as consistent has not fully been determined.

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