Pharmacology Biochemistry & Behavior, Vol. 18, pp. 303–306, 1983. Printed in the U.S.A.

Variability in Behavioral Responses to Benzodiazepines in the Rat

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Received 22 October 1982

FILE, S. E. *Variability in behavioral responses to benzodiazepines in the rat.* PHARMACOL BIOCHEM BEHAV **18**(2) 303–306, 1983.—The effects of chlordiazepoxide (10 mg/kg) were assessed in a holeboard by the reductions in head-dipping, rearing and locomotor activity; the correlations among all these measures were significant. Test-retest correlations were significant for all but the time spent head-dipping. On the basis of their behavioral responses to chlordiazepoxide six "strong" and six "weak" responders were identified and used for an in vitro electrophysiological study. There were no differences between the two groups in the extent to which flurazepam potentiated muscimol, but picrotoxin showed a greater antagonism of muscimol in slices from "strong" responders and flurazepam showed a greater reduction of picrotoxin potency. There was a significant correlation between the in vitro picrotoxin shift and the chlordiazepoxide-induced reduction in locomotor activity. The correlations between behavioral responses to chlordiazepoxide and the plasma benzodiazepine concentrations were low and only one (for locomotor activity) reached significance.

Exploratory head-dipping Rears Locomotor activity Test-retest correlations Chlordiazepoxide Flurazepam Picrotoxin Muscimol Plasma concentrations

IT has been known for some time, and somewhat strangely accepted, that some animals do not respond to benzodiazepines. All that has resulted from this realisation is that in some experiments animals are first screened to eliminate any "benzodiazepine non-responders" [2,9]. The theoretical importance of such non-responders seems scarcely to have been explored. If indeed they do exist, is it a stable characteristic of an individual and one that characterises all responses to benzodiazepines? Do such individuals differ in their metabolism of benzodiazepines or in the nature or number of their benzodiazepine receptors? If such individual differences also exist in man the clinical implications are obvious and it would be valuable to know whether evidence of a weak response to a benzodiazepine on one measure (say EEG) could be used to predict the likely success of it as an anxiolytic.

It is the purpose of the present paper to describe preliminary experiments devised to determine: (1) whether a strong or weak response to a benzodiazepine using one behavioral measure is predictive of the strength of response assessed by another measure; (2) whether a strong or weak response to benzodiazepines is a stable individual characteristic; (3) whether "strong" and "weak" responders, defined from behavioral measures, differed in their electrophysiological responses; (4) the role of pharmacokinetic differences in determining behavioral differences in response to benzodiazepines.

The holeboard was chosen as the behavioral test in which to examine these questions, because it provides a reliable and valid measure of directed exploration (head-dipping) that can be assessed independently from locomotor activity and rearing [7,8]. The typical response of rats to an acute dose of benzodiazepines is a reduction in both head-dipping and motor activity [3,4].

METHOD

Animals

Male hooded Lister rats (Olac Ltd), approximately 350 g, were housed in groups of six in an 11 hr light:13 hr dark cycle, with lights on at 0600 hr. Food and water were freely available.

Apparatus

The holeboard was a wooden box with walls 36 cm high and a floor 60×60 cm with 4 equally-spaced holes, 3.8 cm in diameter. The number of head-dips into the holes and the time spent head-dipping were detected by infra-red photocells placed just under the holes; infra-red cells in the walls 4.5 and 11 cm above the floor detected locomotor activity and the number of rears, respectively.

Drugs

Chlordiazepoxide hydrochloride (Roche Products Ltd) was dissolved in distilled water to a concentration of 5 mg/ml and injected intraperitoneally 30 mins before test.

Procedure

Experiment 1

Sixty-six rats were injected with 10 mg/kg chlordiazepoxide and each given a 10 min trial in the holeboard, during which the number of head-dips, the time spent

head-dipping, motor activity and number of rears were automatically recorded. The rats were tested only between 0800 and 1200 h. At the end of each trial any boluses were removed and the floor of the box was thoroughly wiped and dried.

Experiment 2

On the basis of their responses on all 4 measures in the holeboard the 12 rats (from the 66 tested in experiment (1) showing the strongest response to chlordiazepoxide (i.e. the lowest scores) and the 12 showing the weakest were selected for a second chlordiazepoxide injection and holeboard test, 10 days after the first. On the basis of their responses on all 4 measures the six rats showing the strongest response to chlordiazepoxide and the six showing the weakest were selected for an in vitro study.

The in vitro experiment was performed blind with regard to the behavioral results. Two cuneate slices were obtained from each rat and maintained in a Krebs bicarbonate buffer [10]. Superfusion of the GABA analog muscimol caused depolarisation of the dorsal funiculus fibres in a dose-related manner [11]. On one slice from each rat the potency of picrotoxin as an antagonist of muscimol was determined. On the other slice, the effects of flurazepam were determined on both the potency of muscimol and on the potency of picrotoxin as an antagonist of muscimol.

Experiment 3

Sixteen rats were injected with 10 mg/kg chlordiazepoxide, given a 10 min trial in the holeboard and immediately at the end of this test blood samples were taken for estimations of plasma benzodiazepine concentrations. These were estimated by HPLC with UV detection [1].

RESULTS

Table 1 shows the correlation coefficients among the four measures taken in the holeboard. It can be seen that the correlation between the two measures of exploration (number of head-dips and time spent head-dipping) was very high (0.80). Similarly the correlation between locomotor activity and rearing was very high (0.94). However, although the correlations between head-dipping and locomotor activity or rearing all reached significance they were considerably lower (see Table 1).

Thus, whilst a significant correlation exists for the group as a whole the ability to predict for an individual rat the strength of its response to the locomotor depressant effects of chlordiazepoxide on the basis of the strength of its decrease in exploration is relatively low.

Table 2 shows the test:retest correlations for the 4 measures in the holeboard, in response to a 10 mg/kg dose of chlordiazepoxide. These were significant for all but the time spent head-dipping and ranged from 0.28 to 0.61. Thus again, for the group as a whole, a significant correlation exists and in general a strong response to chlordiazepoxide on one occasion is predictive of a strong response on a subsequent occasion.

The responses of "strong" and "weak" responders, identified from their behavioral responses in the holeboard, were then compared in the in vitro phase of the experiment. Flurazepam 10 μM consistently potentiated musicmol (see Simmonds, this symposium), as measured by a parallel displacement of the muscimol dose-response line to the left.

TABLE 1

CORRELATIONS AMONG 4 MEASURES IN HOLEBOARD

	Head-dipping			
	Time	No	Rears	Motor
Time		0.80	0.41	0.38
No	0.80		0.57	0.55
Rears	0.41	0.57		0.94

All significant $p<0.001$.
n=66.

TABLE 2

TEST-RETEST CORRELATIONS (r) BETWEEN TWO TRIALS IN THE HOLEBOARD (n=24), TOGETHER WITH 't' VALUES AND PROBABILITY LEVELS (p)

	Head-dipping			
	Time	No	Rears	Motor
r	0.28	0.61	0.55	0.42
t	1.4	4.6	3.7	2.4
p	n.s.	0.001	0.001	0.025

There was no difference between the two groups of rats in the extent of this potentiation (see Table 3). However, picrotoxin showed a greater antagonism of muscimol in the slices from the "strong" responders (two-tailed t-test, $0.1>p>0.05$). Likewise, flurazepam tended to cause a greater reduction in picrotoxin potency in slices from the "strong" responders, but again, this did not quite reach significance ($0.1>p>0.05$). The end result was that in the presence of flurazepam the difference between "strong" and "weak" responders in the potency of picrotoxin was removed.

The extent of the picrotoxin shift was then correlated with each of the 4 behavioral measures from the second test in the holeboard. From Table 4 it can be seen that the correlation with motor activity was significant, but that the correlations with the other behaviors were not. A similar pattern was seen with the scores from the first holeboard test, where only the correlation with motor activity reached significance ($r=0.60$, $p<0.05$).

In one rat the plasma chlordiazepoxide concentration was only 0.58 μg/ml, but the rest of the values fell in the range of 1.10 to 2.7 μg/ml. Table 5 shows the correlation coefficients calculated for each of the 4 behavioral measures from the holeboard with the plasma concentrations of chlordiazepoxide and with the combined concentrations of chlordiazepoxide and demoxepam (its major metabolite). It can be seen that the correlations were close to each other and ranged from -0.31 to -0.52. In other words, the higher the plasma concentration the lower the behavioral score, i.e. the stronger the response to chlordiazepoxide. However, only for motor activity were the correlations significant and for this measure pharmacokinetic differences accounted for only 25% of the variance in the behavioral scores.

TABLE 3

ELECTROPHYSIOLOGICAL RESPONSES IN CUNEATE NUCLEUS SLICES FROM "STRONG" AND "WEAK" RESPONDERS TO CHLORDIAZEPOXIDE, ASSESSED FROM THEIR BEHAVIOURAL RESPONSES IN THE HOLEBOARD

	Strong responder	Weak responder	Δ
1. Potentiation of muscimol by flurazepam 1 μM (left shift of dose-response line, log units)	0.243 ± 0.036 (5)	0.199 ± 0.039 (6)	NS
2. Antagonism of muscimol by picrotoxin 30 μM (right shift of dose-response line, log units)	1.225 ± 0.035 (5)	1.096 ± 0.052 (6)	$0.1>p>0.05$
3. Change in 2. in the presence of flurazepam 1 μM.	*−0.140 ± 0.060 (5) †−0.383 ± 0.076 (5)	0.034 ± 0.056 (6) −0.166 ± 0.064 (6)	$0.1>p>0.05$ $0.1>p>0.05$

*Reference line for picrotoxin effect was that obtained in presence of flurazepam.
†Reference line was the control dose-response line (see Simmonds, this symposium).

TABLE 4

CORRELATIONS (r) BETWEEN IN VITRO PICROTOXIN SHIFT AND BEHAVIORAL SCORES IN THE HOLEBOARD (n=11), AND SIGNIFICANCE LEVELS (p)

	Head-dipping			
	Time	No.	Rears	Motor Activity
r	0.17	−0.19	0.15	−0.58
p	n.s.	n.s.	n.s.	<0.05

TABLE 5

CORRELATIONS (r) AND PROBABILITY LEVELS (p) BETWEEN PLASMA BENZODIAZEPINE CONCENTRATIONS AND 4 MEASURES IN THE HOLEBOARD

		Head-dipping			
		Time	No.	Motor Activity	Rears
Chlordiazepoxide	r	−0.32	−0.37	−0.52	−0.43
	p	n.s.	n.s.	0.025	0.05
CDP + demoxepam	r	−0.31	−0.040	−0.51	−0.41
	p	n.s.	n.s.	0.025	n.s.

DISCUSSION

The response of rats in a holeboard to an acute dose of benzodiazepines is a reduction in head-dipping and motor activity and rearing. There were significant correlations among all the measures, indicating that in general "strong" and "weak" responders to benzodiazepines could be identified. However, although the correlation between the two measures of exploration (number of head-dips and time spent head-dipping) was very high, as was the correlation between motor activity and rearing, the correlations between head-dipping and the other two measures were lower. This suggests that different factors may control the benzodiazepine-induced reduction in exploratory head-dipping from those controlling the decrease in motor activity. Results from other studies are helping to separate out these two classes of behavior: picrotoxin antagonises the chlordiazepoxide-induced reduction in motor activity, but is without effect on the reduction in head-dipping [5]; and RO 15-1788, the specific benzodiazepine antagonist, antagonises the chlordiazepoxide reduction in motor activity, whereas at doses of 4 mg/kg and above in combination with chlordiazepoxide it enhances exploratory head-dipping [6]. The results of Experiment 1 suggest that whilst in general "strong" and "weak" responders to benzodiazepines can be identified on the basis of one category of response (e.g. reduction in exploration) it would not be easy to make a prediction for a given individual as to the extent of a chlordiazepoxide-induced reduction in a different behavioral category (e.g. reduction in motor activity).

The results from Experiment 2 suggest that whilst there is significant stability in an individual's strength of response to benzodiazepines for any given measure, this does not account for more than 36% of the variance in behavioral scores. The test:retest correlations based on responses to chlordiazepoxide can be compared with the test:retest correlations found in undrugged animals, where head-dipping correlated 0.47 and motor activity 0.83 [7]. The results from the in vitro study suggest that "strong" and "weak" responders, identified by their behavioral responses to benzodiazepines, may differ in their electrophysiological responses and that these differences are due to different sensitivities to picrotoxin. The electrophysiological results were only of borderline significance, but since only six rats in each group were tested and the in vitro experiments spanned three weeks these differences indicate considerable stability in in-

dividual differences and some that are certainly worthy of further investigation.

The significant correlation between the picrotoxin shift measured in vitro and the effects of chlordiazepoxide on locomotor activity in the holeboard, and the absence of correlation with head-dipping, further emphasises the difference between these two behavioral measures and the link between picrotoxin and the sedative effects of benzodiazepines, as measured by a decrease in locomotor activity.

Finally, the results from Experiment 3 showed that whilst there was a considerable range of plasma benzodiazepine concentrations following the fixed dose of chlordiazepoxide these differences could account for only around 25% of the variance in behavioral scores. This correlation was found in rats following an acute dose of chlordiazepoxide; even this limited degree of correlation is lost when drug-experienced rats are considered. Thus the same reduction in locomotor activity (approximately 60%) was found in rats with blood chlordiazepoxide concentrations of 1.8,3.7 and 9.0 μg/ml, corresponding to an acute dose of 5 mg/kg, 5 days of 10 mg/kg and 20 days of 50 mg/kg chlordiazepoxide, respectively [4].

In conclusion, whilst in general "strong" and "weak" responders to benzodiazepines can be identified the correlations between different behavioral responses to benzodiazepines, whilst significant, are not high enough to be reliable predictors in individual cases. Pharmacokinetic differences could not account for the differences seen in the behavioral response, but differences in electrophysiological responses to picrotoxin might correlate with the differences in behavior, especially in the effects on locomotor activity.

ACKNOWLEDGMENTS

SEF is a Wellcome Trust Senior Lecturer. These experiments were partly supported by a project grant from the Medical Research Council of Great Britain. I am grateful to David Holt and Diana Harvey for expert technical assistance, to Dr. D. Greenblatt for conducting the chlordiazepoxide determinations, and to Roche products Ltd. for the gift of chlordiazepoxide.

REFERENCES

1. Divoll, M., D. J. Greenblatt and P. J. Orsulak. Methods for measuring benzodiazepines in plasma. *Ther. Drug Monitoring*, in press.
2. Duka, T., R. Cumin, W. Haefely and A. Herz. Naloxone blocks the effect of diazepam and meprobamate on conflict behaviour in rats. *Pharmacol Biochem Behav* **15:** 115–117, 1981.
3. File, S. E. Rapid development of tolerance to the sedative effects of lorazepam and triazolam in rats. *Psychopharmacology* **73:** 240–245, 1981.
4. File, S. E. Behavioural pharmacology of the benzodiazepines. Paper read to CINP meeting, Hong-Kong, Oct. 1981. *Prog Neuropsychopharmacol Biol Psychiatry*, in press.
5. File, S. E. Chlordiazepoxide-induced ataxia, muscle relaxation and sedation: effects of muscimol, picrotoxin and naloxone. *Pharmacol Biochem Behav* **17:** 1165–1170, 1982.
6. File, S. E., R. G. Lister and D. J. Nutt. Intrinsic actions of benzodiazepine antagonists. *Neurosci Lett* **32:** 165–168, 1982.
7. File, S. E. and A. G. Wardill. The reliability of the holeboard apparatus. *Psychopharmacologia* **44:** 47–51, 1975.
8. File, S. E. and A. G. Wardill. Validity of head-dipping as a measure of exploration in a modified holeboard. *Psychopharmacologia* **44:** 53–59.
9. Polc, P., E. P. Bonetti. L. Pieri, R. Cumin, R. M. Angioi, H. Mohler and W. E. Haefely. Caffeine antagonises several central effects of diazepam. *Life Sci* **28:** 2265–2275, 1981.
10. Simmonds, M. A. Presynaptic actions of aminobutyric acid and some antagonists in a slice preparation of cuneate nucleus. *Br J Pharmac* **63:** 495–502, 1978.
11. Simmonds, M. A. Evidence that bicuculline and picrotoxin act at separate sites to antagonise γ-aminobutyric acid in rat cuneate nucleus. *Neuropharmacology* **19:** 39–45, 1980.