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Benzodiazepine-Receptor Mediated Convulsions in Infant Rats: Effects of Beta-Carbolines

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NUTT, D J AND H LITTLE *Benzodiazepine-receptor mediated convulsions in infant rats Effects of beta-carbolines* PHARMACOL BIOCHEM BEHAV 24(4) 841–844, 1986.—The effects of anticonvulsant and proconvulsant benzodiazepine-receptor ligands were studied in infant rats The agonist flurazepam increased myoclonic twitching of the limbs as has previously been reported In contrast, the convulsant beta-carbolines DMCM and beta-CCM did not produce twitching, but did produce marked increases in locomotor activity and whole body shakes The standard convulsants pentylenetetrazol and bicuculline similarly increased locomotor activity and shaking These findings suggest that the effects of agonist benzodiazepines cannot be interpreted as convulsant-type behaviour In addition, the finding that DMCM and beta-CCM have equivalent effects despite showing preferential affinities for benzodiazepine-receptor subtypes argues against one particular subtype having proconvulsant effects

Benzodiazepine-receptor ligands Flurazepam Beta-carbolines Convulsions

TWO recent publications have reported that benzodiazepines can produce behavioural activation and possibly seizures in infant rats [1,12]. These studies demonstrated increased limb movements in animals treated with chlordiazepoxide, diazepam and flurazepam. Barr and Lithgow [1] interpreted these as being expressions of seizure activity and postulated a paradoxical convulsant effect of benzodiazepines. These have been divided into types 1 and 2, which differ in the ontogenesis. The type 2 receptor is present at birth, whereas the type 1 receptor does not appear in significant number until 7–16 days [8] Barr and Lithgow [1] suggested that their paradoxical "convulsant" effect of flurazepam was due to its interaction with type 2 receptors, which develop before type 1 The data of Pappas and Walsh [12] brought this explanation into question for two reasons. First, they demonstrated that at 35°C vehicle treated animals exhibited as many limb movements as benzodiazepine treated ones Second, the convulsant, pentylenetetrazol (PTZ) produced a very different behavioural picture.

It has recently been demonstrated that there exists a new class of ligand for the benzodiazepine receptor [4] Ligands of this class, such as the beta-carbolines, ethyl and methyl beta-carboline-3-carboxylate (beta-CCE and beta-CCM), FG 7142, and DMCM have opposing actions to the benzodiazepines, being proconvulsant or convulsant and anxiogenic [5, 6, 10]. These drugs gave us the opportunity to produce convulsions directly via the benzodiazepine receptor In addition, an imidazobenzodiazepine (Ro 15-1788) that antagonizes the effects of both conventional benzodiazepines and the beta-carbolines has recently been reported [7,9]

Selectively of binding of beta-carbolines within the benzodiazepine receptor subtype has been described, but it does not correlate with their pharmacological actions. Both beta-CCM and DMCM produce convulsions which are antagonized by Ro 15-1788, but beta-CCM has a preferential affinity for the BDZ 1 subtype [2] while DMCM has been found to have a higher affinity for type 2 [3]. We have investigated the actions of DMCM and beta-CCM in infant rats, and compared them with those of the water soluble benzodiazepine, flurazepam. The effects of Ro 15-1788 on the actions of DMCM were also studied. Finally, we compared the effects of the beta-carbolines with those of PTZ and bicuculline, convulsants which act at the GABA receptor complex but at different sites from the benzodiazepines [11]. The latter convulsants were studied at a single high dose in each case, to compare their full effects with those of the beta-carbolines.

METHOD

Animals

Three-day old infants derived by home-mating of Sprague-Dawley rats (Charles River) were used in all experiments They were kept with mother on an 08.00–22.00 lights on cycle All testing was done on the afternoon of day 3 of age, the day of birth being day 0. The pups weighed 3 and 6 g.

Drugs

Sources were as follows. Flurazepam (Roche, U.K.); Ro 15-1788 (Roche, Switzerland); DMCM (Ferrosan, Denmark);

TABLE 1

THE BEHAVIOURAL EFFECTS OF FLURAZEPAM AND VARIOUS CONVULSANTS IN 3 DAY OLD RATS

Behaviour	Saline	Flurazepam mg/kg 2	10	50	PTZ 100 mg/kg	Vehicle controls	DMCM mg/kg 1	5	20	50	β-CCM mg/kg 2 5	5	Bicuculline mg/kg 5
Shaking	1 (0–1) 9	0 (0–0) 5	0 (0–0) 8	0 (0–0) 8	3* (2–3) 5	0 (0–1) 8	1 (0–2) 6	3* (2–3) 5	2* (1–2) 8	3* (1–3) 7	2 (1–3) 4	2 (0–3) 11	1 (1–2) 5
Twitches	1 (0–1) 9	2 (1–3) 5	1 (1–3) 8	2 (1–3) 5	0 (0–0) 5	0 (0–0) 8	0 (0–0) 9	0 (0–0) 5	0 (0–0) 8	0 (0–1) 7	0 (0–0) 4	0 (0–0) 11	Tonic 5
Locomotor activity	0 (0–1) 9	0 (0–0) 5	0 8	0 (0–1) 8	3* (2–3) 5	0 (0–1) 8	2† (1–3) 6	3† (3–3) 5	2† (2–3) 8	2† (1–2) 7	2‡ (2–3) 4	3† (3–3) 11	2§ (2–3) 5
Loss of righting reflex	0 (0–0) 9	0 (0–1) 5	1 (0–2) 8	1 (0–2) 8	2¶ (1–3) 5	0 (0–0) 8	1 (1–3) 6	0 (0–0) 5	0 (0–0) 8	2 (0–2) 7	0 (0–2) 4	2 (0–2) 11	3† (3–3) 5

Numbers are the median of the rating scores for each animal with interquartile range in parentheses, with n below

Significances tested by the Mann-Whitney 'U' test

p values *<0 05 vs respective controls and each dose of flurazepam †<0 001 vs vehicle controls and each dose of flurazepam ‡<0 002 vs vehicle controls and each dose of flurazepam §<0 02 vs vehicle controls and each dose of flurazepam ¶<0 001 vs vehicle and <0 05 vs flurazepam groups

TABLE 2

THE INTERACTION BETWEEN Ro 15-1788 AND DMCM

Behaviour	Controls vehicle for DMCM	DMCM 2 5 mg/kg⁻¹	Controls vehicle for Ro 15-1788	Ro 15-1788 10 mg/kg⁻¹	DMCM 2 5 mg/kg⁻¹ + Ro 15-1788 10 mg/kg
Shaking	0 (0–1)	1 (1–1)	0 (0–1)	0 (0–1)	0 (0–0)
Twitches	0 (0–1)	1 (0–1)	1 (0–1)	1 (0–2)	1 (1–2)
Locomotor activity	0 (0–1)	3* (2–3)	0 (0–0)	0 (0–1)	0 (0–0)
Loss of righting reflex	0 (0–0)	1 (0–2)	0 (0–0)	0 (0–1)	0 (0–1)
n	5	8	5	6	6

Median ratings (with interquartile range) for animals treated with DMCM, Ro 15-1788, vehicles or DMCM + Ro 15-1788

*Significantly different from DMCM/Ro 15-1788, and from the DMCM vehicle $p<0$ 005

beta-CCM (Glaxo, U K), PTZ and bicuculline (Sigma) Flurazepam and PTZ were dissolved in saline, bicuculline was dissolved in 0 1 N HCl and titrated to pH 3 with NaOH, DMCM and beta-CCM were dissolved in a drop of 0.1 N HCl and then diluted with distilled water Ro 15-1788 was suspended in vehicle (1 drop Tween 80 per 10 ml distilled) All drugs were given IP in a volume of 0 1 ml/10 g Controls (either saline or HCl/distilled water) were run in parallel with the experimental groups

Behavioral Assessments

Infants were removed from their mothers immediately before testing and were then kept at an ambient temperature of 33°C by overhead lights. Drugs were administered IP and the rats were then observed for the next 15 min Ratings of 4 characteristic behaviours were made on a 0–3 scale 0=not observed, 1=mild; 2=moderate, 3=severe. A global rating for the observation period was given for the following behaviours

Shaking A fine tremor of the whole body, usually more pronounced in the head and neck This resembled human shivering

Twitching Distinctive paroxymal myoclonic movements of one or more limbs, discrete and self-limiting Occasionally continuous (status).

Locomotor activity. Paddling movements of all 4 limbs which resulted in forward or rotational locomotion when the animals were upright. These limb movements often persisted when the righting reflex was lost

Loss of righting reflex Failure to maintain upright posture, animals lay on side or back with or without limb movements

All animals were used once daily. They were studied in groups of four, marked individually, in a plastic tray 20×30 cm cm, with a paper towel cover. The drugs and doses to be compared were randomised over the groups of four and the observer who did the rating did not know the treatments given

Statistics

All comparisons were made using the Mann Whitney 'U' test.

RESULTS

Significant differences in the actions of the various drugs were found on the different behaviours (Table 1) Shaking was significantly greater with DMCM at doses of more than 5 mg–1 kg^{-1} and with PTZ group when compared both with their respective controls and with all flurazepam groups. A lesser and non-significant increase was observed for bicuculline and both beta-CCM groups Controls were not significantly different from any flurazepam group.

Twitching was seen predominantly in the control and flurazepam groups Although no single flurazepam dose had significantly different effects from those of the controls or the single beta-carboline doses, when the twitching scores of the flurazepam groups were pooled they were significantly different from the pooled twitch scores of the DMCM groups ($p<0$ 01) and from the saline groups ($p<0.01$). The bicuculline group rapidly developed tonic seizures, after a period of 1–2 min of shaking and locomotor activity They were therefore qualitatively different from the other groups

Locomotor activity was seen predominantly in those animals treated with convulsants. In each of these groups a significantly elevated level of locomotion was observed as compared with both their respective controls and all flurazepam groups. No significant dose-related effects were seen with either DMCM or beta-CCM In many cases locomotor movements continued after loss of righting reflex.

Righting reflex loss showed some differences between convulsants, but none between the benzodiazepine receptor ligands, flurazepam, DMCM or beta-CCM PTZ and bicuculline both significantly increased the time spent on side or back In the base of bicuculline most animals rolled onto their back at the onset of the tonic seizure

The effects of Ro 15-1788 against DMCM were investigated in a separate series of experiments (Table 2) Ro 15-1788, 10 mg/kg^{-1}, IP, was given immediately prior to the DMCM injections Ro 15-1788 alone did not cause any significant changes in any of the ratings DMCM alone caused a significant increase in locomotor activity, which was not seen when Ro 15-1788 was given in addition

The considerable quantitative variability seen in these results has also been found by other authors in this type of study, for example in Fig. 3 of the study by Pappas and Walsh [12]. However, the qualitative differences which we found in the effects of the drugs were fully consistent.

DISCUSSION

These results throw some doubt on the suggestions of Barr and Lithgow [1] that benzodiazepines produce behavioral convulsions in infant rats. Under our experimental conditions with flurazepam the limb twitching that they interpreted as behavioural convulsions were rated as elevated compared with saline controls, but this did not reach significance for any dose of flurazepam However, when the results from all of the doses of flurazepam were pooled there was a significant increase in twitch rating compared with values for controls or for DMCM treatments Our findings therefore agree with those of Barr and Lithgow [1] in that we found that flurazepam increased limb movements (although weakly), but we also found that this effect was qualitatively different from those of the classical convulsants, PTZ and bicuculline and from those of the beta-carbolines. Twitching was strikingly absent in those groups receiving either of the latter types of compound

Barr and Lithgow [1] postulated that the 'behavioural convulsions' produced by benzodiazepines might suggest that the type 1 receptor mediates proconvulsant effects, which are later masked by the type 2, anticonvulsant type Our results showed that although DMCM and beta-CCM show differential receptor selectivity, both produced the same type of seizures activity in infant rats This argues against different benzodiazepine receptors having different effects. In addition, the fact that Ro 15-1788 blocked the effects of DMCM (Table 2) showed that these were mediated via a benzodiazepine receptor

The behavioural effects of the beta-carbolines beta-CCM and DMCM were similar to those seen with PTZ and with bicuculline In particular, rapid paddling limb movements with a latency of 1–3 min were seen The rats would frequently rush around the test arena before losing their righting reflex Even when on their side or back the limb movements would continue. With bicuculline these movements were initially obvious, but later suppressed by the tonic seizures These results suggest that in infant rats clonic seizures may be expressed as increased locomotor activity rather than the convulsions seen in adults.

There is little evidence that in infant rats flurazepam produces what would generally be recognized as seizures The behavioural effects of this drug was clearly distinguishable from those of classical convulsants and convulsant beta-carboline which share a common profile of action It is clear that flurazepam and vehicle produced limb twitching, whereas the beta-carbolines and other convulsants did not The mechanisms underlying this phenomenon are unclear, but do not necessarily reflect central seizure activity. They may relate to temperature changes or peripheral actions

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