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SHORT COMMUNICATION

Effects of Clonazepam on Picrotoxin-Induced Convulsions

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Summary

The convulsant effects of four doses of picrotoxin (PX) – 2, 3, 4, and 6 mg/kg s.c. – were evaluated in the first part of the study. The 4-mg/kg dose, which elicited minimal seizures in all animals, generalized tonic-clonic (major) seizures in 75 % of rats and fatal outcome in 69 % of rats, was chosen for the second part, i.e. for testing the anticonvulsant action of clonazepam (Rivotril[R] Roche, 0.1 or 1 mg/kg i.p.). Clonazepam exhibited a dose-dependent action against PX-induced seizures, being more efficient against major than against minimal seizures.

Key words

Epileptic seizures – GABAergic system – Clonazepam – Picrotoxin convusions

Benzodiazepines (BZs) exhibit a broad spectrum of pharmacological activities including marked anticonvulsant effects (Haefely *et al.* 1985). Mechanisms of action of these effects are closely connected with the GABAergic system – the most important inhibitory system in the brain (Costa *et al.* 1975). Existence of special receptors necessary for action of BZs was demonstrated in the seventies (Braestrup and Squires 1977, Mohler and Okada 1977). Later, the supramolecular complex of $GABA_A$ receptor/BZ receptor/chloride ionophore was described and the mutual interactions of various binding sites in the complex were studied (for review see Haefely 1987, Olsen and Venter 1986). Picrotoxin binds to a convulsant site on the chloride ionophore (Ticku 1986), i.e. the action of clonazepam against picrotoxin-induced seizures might be used to demonstrate an interaction betwen the benzodiazepine and convulsant binding sites of the supramolecular complex. The results might be compared to the effects of clonazepam on seizures elicited by other drugs interfering with the GABAergic system – bicuculline and 3-mercaptopropionic acid (Kubová *et al.* 1990).

Experiments were performed in albino rats of the Wistar strain, weighing 220–290 g. Picrotoxin (PX, Sigma) was injected subcutaneously as a freshly prepared solution in physiological saline. The relevant dose of PX was determined in the first part of the experiments where the doses of 2, 3, 4, and 6 mg/kg (always as a 1-ml/kg volume) were tested. In agreement with our previous study (Kubová *et al.* 1990), a dose which induced the tonic-clonic convulsions in more than 70 % of the animals (4 mg/kg) was chosen for testing the effects of clonazepam.

Clonazepam (CZP, Rivotril[R] Roche) was administered intraperitoneally in doses of 0.1 and/or 1 mg/kg just before an injection of PX. Control animals were treated only with the solvent (propylene glykol, ethanol and water in the ratio 5:2:3), in an amount corresponding to the higher dose of CZP used. Every experimental group consisted of at least eight animals.

After the injection of PX, the animals were placed into plastic cages and were observed in isolation for 60 min.

The animals' behaviour as well as incidence, character, intensity and latency of epileptic manifestations were recorded. The intensity of the epileptic manifestations was then expressed as an average score for each group. Individual animals were scored according to the same scale that was used for pentylenetetrazol-induced seizures (Pohl and Mareš 1987):

0	no changes
0.5	abnormal behavior (e.g.uneasiness, scratching, tremor)
1	single myoclonic jerks

2 atypical minimal seizures, i.e. only some of their components were present
3 minimal seizures consisting mainly of clonic convulsions involving the head and forelimb muscles and leaving righting reflexes intact
4 major seizures without the tonic phase
5 complete major seizures, i.e. generalized tonic-clonic convulsions with a loss of righting reflexes

Statistical evaluation of the results: The incidence of seizures was evaluated by means of Fisher's exact test (a quadripolar table) and the scores by means of the multiple comparison method according to Holm (1979). Evaluation of latencies was done by ANOVA. Statistical significance was set at the 5 % level.

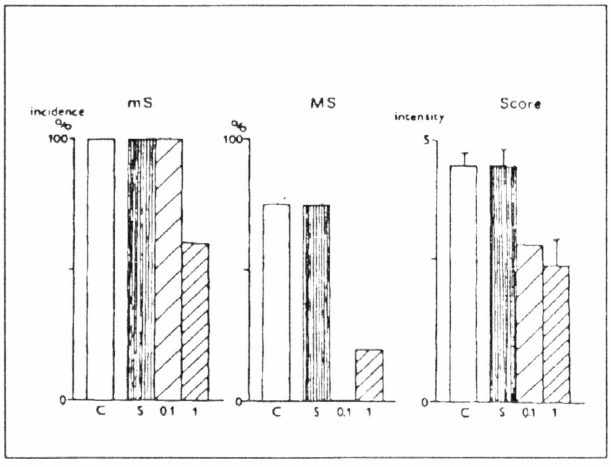

Fig. 1

Effects of CZP on picrotoxin-induced seizures. From left to right: incidence of minimal seizures (mS), incidence of major seizures (MS) and an average score (Score, always M ± S.E.M.). Abscissae: C – control animals given PX only, S – rats pretreated with solvent, 0.1 and 1 – rats pretreated with the two doses of CZP. Ordinates – left and middle: incidence of seizures; right: the five-point scale used for quantification of severity of seizures.

Picrotoxin, in relation to the dose, induced single myoclonic jerks, intensive minimal (mS) and major, generalized tonic-clonic seizures (MS). The tonic phase, which consisted of tonic extension of the forelimbs and flexion of the hindlimbs, was always present.

The two lowest doses of PX induced only mS: the dose of 2 mg/kg in 66.7 % of animals, the dose of 3 mg/kg in all rats. After the dose of 4 mg/kg mS were observed in 100 % and MS in 75 % of animals with a 68.8 % mortality.The highest dose of PX (6 mg/kg) resulted in mS, MS and ultimate death of all animals.

Value of the score was determined mainly by the incidence of MS and it therefore increased with the dose.

Latencies of both types of epileptic seizures decreased in an inverse relation to the dose of PX used.

The 4-mg/kg dose of PX was chosen for testing the effects of CZP. The control group pretreated with the solvent did not differ from rats receiving only PX to any extent. The lower dose of CZP (0.1 mg/kg) abolished MS, but the incidence of mS was not changed. The higher dose of CZP decreased the incidence of mS to 60 % and MS to 20 % (Fig.1).

The mean score was significantly diminished by both doses of CZP tested (Fig.1).

Latencies of both minimal and major seizures were not changed by the solvent. CZP prolonged the latency of minimal seizures in a dose-dependent manner from 879 ± 98 s (M ± S.E.M.) in the controls to 1142 ± 105, and 1252 ± 173 s respectively. Major seizures were observed only in two rats given the higher dose of CZP.

Clonazepam exhibited a potent effect against convulsions induced by interference with the inhibitory GABAergic system, particularly at the receptor level (Kubová *et al.* 1990). In agreement with these results, CZP was effective against picrotoxin-induced convulsions. Picrotoxin, in contrast to bicuculline, which blocks the GABA binding site (Simmonds 1980), binds to the Cl^- channel directly (Twyman *et al.* 1989). Binding studies proved a relationship between convulsant and benzodiazepine binding sites in the $GABA_A$ complex. Picrotoxin inhibits binding of BZs to membranes, this effect being due to a reduction in the number of the binding sites without reduction of their affinity (Chweh *et al.* 1985). This interaction might contribute to the lesser efficacy of CZP against PX-induced seizures in comparison with bicuculline- or metrazol-induced seizures. On the other hand, CZP exhibited a more marked effect against PX-induced convulsions than against seizures elicited by 3-mercaptopropionic acid, an inhibitor of glutamate decarboxylase.

Anticonvulsant effects of CZP were more marked against generalized tonic-clonic seizures than against minimal seizures. This is true also for seizures induced by other convulsants (metrazol, bicuculline – Kubová *et al.* 1990), as well as for other benzodiazepines (e.g midazolam – Kubová and Mareš submitted). Minimal metrazol seizures were taken as a model of human absences (Swinyard *et al.* 1989), but the adequacy of this model was questioned recently (Loscher and Schmidt 1988, Mareš and Zouhar 1988). The generator of minimal seizures, localized hypothetically into the basal forebrain (Browning and Nelson 1986), is probably more resistant to anticonvulsant drugs than the brainstem generator of generalized tonic-clonic seizures.

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Reprint Requests

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