Convulsant action of a benzodiazepine receptor agonist/inverse agonist Ro 19-4603 in developing rats

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Abstract. An inverse benzodiazepine receptor agonist Ro 19-4603, administered intraperitoneally, was found to induce two types of motor seizures, i.e. minimal, predominantly clonic and major, generalized tonic-clonic, in rats at all developmental stages studied (7, 12, 18 and 25 days old). The developmental profile of the two types of seizure was different. Minimal seizures could be induced easily in the two youngest groups, whereas there were no marked differences in the induction of major seizures between the age groups. A lethal outcome was more common in 18- and 25-day-old rats than in younger animals. The convulsant action of the benzodiazepine agonist/inverse agonist Ro 19-4603 shows only quantitative changes during post-natal development in the rat.

Key words: Seizures – Development – Rat – Benzodiazepine – Inverse agonist

Methods

Motor seizures. Experiments were done with specific pathogen free, male, Wistar albino rats (n = 112). Four age groups were used: 7, 12, 18 and 25 days old. Ro 19-4603, a imidazothieno-diazepinone (tert-butyl-5,6-dihydro-5-methyl-6-oxo-4 H-imidazo[1,5-althieno[2,3-f][1,4]diazepine-3-carboxylate; Hoffman La Roche, was freshly dissolved in dimethylsulfoxide (100 mg/ml) and administered intraperitoneally in doses of 25, 50, 75 or 100 mg/kg. Each experimental group consisted of eight animals.

Rats were observed individually for 30 min after the injection. The body temperature was maintained by means of a heating pad. The incidence of the two types of motor seizures, i.e. minimal, predominantly clonic seizures, and major, generalized tonic-clonic seizures, as well as their latencies, were recorded. Other epileptic phenomena (e.g. isolated myoclonic jerks), and behavioral abnormalities, and lethality were also recorded. The severity of epileptic phenomena was scored by means of the following scale (Pohl and Mareš, 1987):

0 - no change
0.5 - abnormal behavior (e.g. scratching, tremor, orienting reaction in a familiar cage)
1 - isolated myoclonic jerks
2 - atypical minimal seizures, i.e. only some elements of minimal seizures
3 - minimal seizures, predominantly clonic, involving head and forelimb muscles, with preservation of righting reflexes
4 - major seizures without a tonic phase
5 - complete major seizures, i.e. generalized tonic-clonic seizures with loss of righting reflexes.

Introduction

Disinhibition is one of the ways to induce epileptic activity in vivo and in vitro (Woodbury 1980; Engel 1989), Heinemann and Jones 1990). Interference with GABA-ergic inhibitory systems results in seizures in adult (review: Meldrum 1975) as well as in immature animals (review: Mareš 1991). We have demonstrated the convulsant action of inhibitors of glutamate decarboxylase, GABA_A receptor antagonists and chloride channel blockers during development in rats (review: Mareš 1991). Because of slight differences between the effects of these drugs it was of interest to study the effect of benzodiazepine receptor agonists/inverse agonists. There is only one abstract in the literature in which a convulsant action of methyl-beta-carboline-3-carboxylate (beta-CCM) in immature rats is described (Cavalheiro et al. 1987). These authors found that this benzodiazepine receptor agonist/inverse agonist was first able to induce clearly discernible seizures (i.e. the adult pattern) in rats at the age of 35 days. Such a late appearance of the convulsant effect is in sharp contrast with the marked anticonvulsant action of benzodiazepine agonists which can already be demonstrated one week postnatally (Kubová and Mareš 1989, 1992). Therefore, we wished to re-examine the action of benzodiazepine receptor agonists/inverse agonists during development. By courtesy of F. Hoffman La Roche AG it was possible to do this with the benzodiazepine receptor agonist/inverse agonist Ro 19-4603 (Pieri 1988).

Abbreviations: DPPC, Dipalmitoylphosphatidylcholine

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Each animal was scored according to the most severe phenomenon observed; then, for each group, an average and a standard error of the mean were calculated.

Incidences of seizures were evaluated by means of Fisher's exact test, latencies were statistically evaluated by analysis of variance (BMDP) with subsequent sequential comparison according to Holm (1979). CD$_{50}$s for major seizures were calculated by means of Finney's probit analysis (BMDP). Seizure severity scores were statistically compared by means of the Kruskal-Wallis nonparametric test. The level of statistical significance was set at five percent.

Electrocorticographic recordings. These experiments were made on 24 male Wistar rats, which had been bred at the same time, when they were 7, 12, 18 and 25 days old. The rats were implanted with electrodes under ether anesthesia according to the method described previously (Schickerová et al. 1984). Flat silver electrodes were placed, epidurally, over the sensorimotor as well as over the occipital cortex of both hemispheres and an indifferent electrode was placed on the nasal bone. All electrodes were fixed with fast-curing, dental acrylic cement. One hour after preparation, the animals were neurologically examined and, if normal, were used for EEG recording. Both reference and bipolar connections were used. Ro 19-4603 was dissolved as described above and injected i.p. at a dose of 100 mg/kg in all age groups. The EEG was recorded for 30 min after the administration of the drug.

At the end of the experiments, the animals were killed with an overdose of ether.

Results

Motor seizures. The first behavioral change observed in all age groups was restlessness accompanied by hyperventilation. This was followed by isolated myoclonic jerks. In rats aged 18 days or more, hyperactivity alternated with periods of behavioral freezing.

Ro 19-4603 induced both basic types of seizures, i.e. minimal, clonic and major, generalized tonic-clonic, in rats of all age groups studied.

Minimal seizures. This type of seizure was elicited, in rats of all age groups, in a dose-dependent manner (Fig. 1); all animals exhibited clonic seizures which involved muscles of the head and the forelimbs. In 18- and 25-day-old rats, the hindlimbs were widely abducted and the tail was erected in a 'Straub-like' fashion. An incomplete pattern of minimal seizures often occurred in younger rats. After lower doses of Ro 19-4603, repetitive minimal seizures were observed in 7- and 12-day-old animals. Atypical minimal seizures, continuing for several minutes (status of minimal seizures), occurred only exceptionally in 7-, 12- and 18-day-old animals. A significantly higher incidence of minimal seizures after the 50-mg/kg dose was observed in 7- and 12-day-old rat pups in comparison with the 25-day-old group and the appearance of these seizures after the 25-mg/kg dose in the two youngest groups demonstrated that minimal seizures can be induced at an early stage of development.

Neither age- or dose-dependence of the latencies of minimal seizures could be found (Table 1).

Major seizures. This type of motor seizure was also elicited in a dose-dependent manner in all age groups (Fig. 1). Generalized tonic-clonic seizures were observed at all developmental stages studied, although the incidence of the complete tonic phase in fore- and hindlimbs was higher in 18- and 25-day-old animals than in younger rats. A dose of 100 mg/kg elicited complete tonic-clonic seizures in 50% and 37.5% of 7- and 12-day-old animals, respectively, whereas complete tonic-clonic seizures occurred in 75% of the 18- and 25-day-old rats. The major seizures started with a short phase of wild running (in the youngest animals this was replaced by swimming-like, poorly coordinated movements) which was followed by a loss of righting reflexes at the beginning of the tonic phase. The tonic phase was usually made up of tonic extension of the forelimbs. Involvement of the hindlimbs was less com-