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Anticonvulsant action of $GABA_B$ receptor positive modulator CGP7930 in immature rats

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Summary $GABA_B$ receptors mediate inhibition at early stages of development but mixed anti-and proconvulsant action of their agonists affecting all receptors was found in immature rats. Positive allosteric modulators of $GABA_B$ receptors potentiate only already active $GABA_B$ receptors and therefore more specific action is expected. Possible anticonvulsant action of CGP7930 was studied in a model of pentetrazol-induced seizures previously used for studies with agonists baclofen and SKF97541.

Pentetrazol (100 mg/kg) was administered subcutaneously in male rats 7, 12, 18, 25 and 90 days old pretreated with CGP7930 in doses 1—40 mg/kg i.p.

High doses of CGP7930 suppressed generalized tonic—clonic seizures in all five age groups. Animals 18 and less days old exhibited a specific suppression of the tonic phase after lower doses of CGP7930. Twelve-day-old rats were the most sensitive to anticonvulsant effect of CGP7930 (even the 2-mg/kg dose suppressed the tonic phase whereas 20-mg/kg dose was active in other age groups). Minimal clonic seizures (mS) were moderately potentiated by low doses of CGP7930 in 18-day-old but suppressed by the highest dose in 25-day-old rats. The 60-mg/kg dose of PTZ induced only mS in 4 out of 9 25-day-old rats; the 40-mg/kg dose of CGP7930 combined with this lower dose of PTZ resulted in the only proconvulsant effect — generalized tonic—clonic seizures appeared in two rats.

Results from 12-day-old rats suggest a possibility to find an age-specific anticonvulsant among positive allosteric modulators of $GABA_B$ receptors.

Introduction

Baclofen, a $GABA_B$ receptor agonist, is used for a long time in the treatment of spasticity. Recently, therapeutic spectrum of baclofen was extended so that Froestl in his review presents 17 possible indications for this drug (Froestl, 2010). Possible anticonvulsant action has to be also taken into account. In contrast to this presumption data concerning

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epileptic seizures in adult rodents are controversive — anticonvulsant (in vivo — De Sarro et al., 2000; Chen et al., 2004; Gasior et al., 2004), proconvulsant (in vitro hippocampus — Ault and Nadler, 1983; dentate gyrus in vitro — Mott et al., 1989; in vivo — Sokal and Large, 2001) and even convulsant (direct intracortical injection — van Rijn et al., 1987) actions of baclofen were described. Our studies in immature rats yielded similar results — baclofen and another agonist SKF97541 exhibit a mixture of anti- and proconvulsant effects against pentetrazol (PTZ)-induced seizures (Mareš, 2008). The action of these two agonists is not identical: SKF97541 possesses more anticonvulsant and less proconvulsant activities than baclofen. The anticonvulsant effect of SKF97541 against PTZ-induced seizures is best expressed in 12-day-old rats (Mareš, 2008) what is in agreement with our data in another model — cortical epileptic afterdischarges (Mareš and Tabashidze, 2008).

$GABA_B$ receptors are localized both post- and presynaptically (as homo- and heteroreceptors). This localization forms a background for a mixed anti- and proconvulsant actions of $GABA_B$ receptor agonists. An uneven development of pre- and postsynaptic $GABA_B$ receptors (Fukuda et al., 1993) suggests a possible use of activation of $GABA_B$ receptors against seizures in immature brain. $GABA_B$ receptor agonists activate all $GABA_B$ receptors and in addition to positive effects they exhibit also unwanted side effects. Attention was shifted to allosteric positive modulators; they potentiate only active $GABA_B$ receptors and therefore their actions should be more specific and differ from those of agonists. Expected milder side effects (Marshall, 2005; Pin and Prézeau, 2007) were demonstrated in mice (Jacobson and Cryan, 2008). Developmental data are missing, therefore we started to study effects of a positive allosteric modulator of $GABA_B$ receptors CGP7930 (Urwyler et al., 2001, 2005) in the same model as in our studies with receptor agonists baclofen and SKF97541, i.e. convulsions induced by systemic administration of pentetrazol (Mareš, 2008). High dose of pentetrazol elicits two types of seizures: minimal clonic seizures restricted to muscles of head and forelimbs with only a moderate tonic component (axial muscles) and preserved righting reflexes and generalized tonic—clonic seizures with a loss of righting ability. Minimal clonic seizures (mS) are present in animals 18 and more days old whereas tonic—clonic seizures (GTCS) can be elicited at all ages (Velíšek et al., 1992).

An advantage of positive allosteric modulators is their moderate anxiolytic action and effects in treatment of drug abuse, i.e. positive psychotropic effects. Such effects may be very useful because of high psychiatric comorbidity in epileptic patients (Karouni et al., 2010).

Methods

Animals

Male rats of Wistar strain 7, 12, 18, 25 days old and adults from the breeding of the Institute of Physiology ASCR were used in the experiments. Rat pups 7 days old correspond with prenatal stage of development of human brain, 12 days old ones with early postnatal period and 18 and 25 days old with preschool and early school age of children (Dobbing, 1970). The project was approved by Animal Care and Use Committee of The Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic which is fully compatible with European Community Council directives 86/609/EEC.

Drugs

CGP 7930 (3,5-bis(1,1-dimethylethyl)-4-hydroxy-b,b-dimethylbenzenepropanol, Tocris Bioscience, UK) was dissolved in dimethylsulfoxide (DMSO) in concentration of 5 mg/1 ml. Pentetrazol (PTZ, Sigma, St. Louis, MO) was dissolved in distilled water in concentration of 50 mg/1 ml. Solutions were freshly prepared immediately before the administration.

Experimental procedures

Because there are no data on effects of CGP7930 in immature brain animals were pretreated with CGP 7930 in a wide range of doses (1, 2, 5, 10, 20 or 40 mg/kg i.p.), control rats received DMSO in a volume of 8 ml/kg corresponding to the highest dose of CGP 7930. PTZ (100 mg/kg s.c.) was injected 30 min later. A lower dose of PTZ (60 mg/kg i.p.) inducing in a minority of 25-day-old rats (in 18-day-old and adult rats it is impossible to find a dose eliciting only minimal clonic seizures) minimal clonic seizures (but never generalized tonic—clonic seizures) was administered to study an effect on this type of seizures as well as possible proconvulsant effects of CGP7930. To know duration of the action of CGP7930 (there are no pharmacokinetic data in immature rats on disposal) against seizures induced by the 100-mg/kg dose of PTZ the highest, 40-mg/kg dose was studied in 12- and 25-day-old rats with intervals of 60, 120, 240, and 360 min between pretreatment with CGP7930 or DMSO and PTZ. Rat pups up to the postnatal day 18 were returned to their mothers for a time between the two injections.

Animals were directly observed in isolation for 30 min after PTZ administration by an experienced observer (P.M.), body temperature of rats 18 days old and younger was maintained by means of a pad heated electrically to 34 °C, i.e. the temperature in the nest. Isolated myoclonic jerks and incidence and latency of two types of seizures (minimal clonic and generalized tonic—clonic) were recorded. Severity of seizures was classified using a five-point scale (Pohl and Mareš, 1987). Each age and dose group was formed by 8—10 animals.

Statistics

One way ANOVA with subsequent pairwise comparison by means of Holm—Sidak test was used for latencies and severity of PTZ-induced seizures, incidence of seizures was evaluated with Fisher Exact Test. Level of significance was set at 5%.

Results

Control rats

Animals were pretreated with dimethylsulfoxide. The 100-mg/kg dose of PTZ induced minimal clonic seizures (mS) as the first seizure type in animals 18, 25 and 90 days old. The incidence of mS in 18-day-old rats was only 40% whereas majority of 25-day-old rats and all adult animals exhibited this type of seizures (Fig. 1). Generalized tonic—clonic seizures (GTCS) were observed in all rats in all age groups of control rats with the exception of adult animals where the incidence of GTCS was only 80% (Fig. 2).