

## Anticonvulsant action of GABA-B receptor agonist SKF97541 differs from that of baclofen

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Short title: GABA-B agonist and seizures in developing rats

## SUMMARY

GABA-B receptor agonist SKF97541 exhibits age-dependent anti- as well as proconvulsant actions in developing rats. It suppressed tonic phase of generalized seizures induced by pentetrazol in 7-, 12- and 18-day-old rats and increased their latency in 7- and 12-day-old animals. Other results in 18-day-old animals are not so clear: SKF97541 blocked the appearance of minimal clonic seizures but tended to decrease latencies of both types of seizures. In addition, it significantly decreases latency of generalized seizures in adult rats. The mixed effects of SKF97541 are in agreement with those of baclofen but there are substantial differences between the actions of these two agonists in individual age groups.

Key words: GABA-B receptor; agonist; convulsions; ontogeny; rat

In spite of the fact that GABA-B receptors represent an important part of GABAergic inhibitory system, there are controversies in the action of a classical GABA-B receptor agonist baclofen. Both anticonvulsant and proconvulsant effects of this agonist were described in vivo as well as in vitro (Ault and Nadler 1983, Ault *et al.* 1986, Watts and Jefferys 1993, Higashima *et al.* 2000, Sokal and Large 2001, Chen *et al.* 2004, Hoskinson *et al.* 2004). It could be due to contradictory effects of pre- and postsynaptic GABA-B receptors but there is also a possibility that it is specific for baclofen. To answer this question we started to work with a more potent GABA-B receptor agonist SKF97541 (Seabrook *et al.* 1990). A simple model – two types of convulsive seizures elicited by an administration of pentetrazol (PTZ, Velíšek *et al.* 1992) – was selected as the first step. An appropriate dose of PTZ elicits minimal, clonic seizures with preserved righting reflexes and, after a longer latency generalized tonic-clonic seizures accompanied by a loss of righting ability. Minimal seizures cannot be induced by PTZ during the first two postnatal weeks in rats whereas generalized tonic-clonic seizures are not age-bound. Our previous study demonstrated only a moderate anticonvulsant action of baclofen against PTZ-induced seizures in immature rats – suppression of the tonic phase of GTCS in 7- and 12-day-old rats and a decreased incidence of GTCS in 25- and 90-day-old animals whereas minimal seizures were not affected (Kubová *et al.* 1996).

Experiments were performed on male Wistar rats 7, 12, 18, 25 and 90 days old. Animals were pretreated with SKF97541 (3-aminopropyl(methyl)phosphinic acid, Tocris, UK) in doses 0.05; 0.1; 0.2; 0.5 and 1.0 mg/kg i.p. (the doses of 0.05 and 0.2 mg/kg were not used in adult rats) and 30 min later pentylenetetrazol (PTZ, Sigma, St.Louis, MO) was injected subcutaneously. Controls received saline instead of SKF97541. Animals were observed in isolation for 30 min after PTZ administration, pattern of seizures, their latency and all behavioral events were registered. Quantification of seizure severity was made by scoring on a 5-point scale (0 – no movements; 1 – isolated myoclonic jerks; 2 – incomplete minimal seizures; 3 – minimal, clonic seizures with preserved righting ability; 4 –

generalized clonic seizures with a loss of righting reflexes; 5 – generalized tonic-clonic seizures with a loss of righting reflexes – Pohl and Mareš 1987). Individual age and dose groups were formed by 7-11 rats. Body temperature of immature rats was maintained by means of a heating pad. Statistical evaluation of the incidence of seizures was performed by means of Fisher exact test, latencies and severity of seizures were evaluated by means of ANOVA with subsequent pairwise comparison with Holm-Sidak test (SigmaStat® SPSS). The experiments were approved by Animal Care and Use Committee of the Institute of Physiology to be in agreement with Animal Protection Law of the Czech Republic (compatible with European Community Council directives 86/609/EEC). Control animals in all age groups exhibited generalized tonic-clonic seizures; minimal, predominantly clonic seizures (with preserved righting ability) were elicited in rats 18 and more days old. Pretreatment with SKF97541 resulted in a markedly decreased muscle tone but the animals exhibited phasic activity; they reacted to handling (including subcutaneous injection of PTZ). SKF97541 suppressed tonic phase of generalized tonic-clonic seizures in the three youngest groups; this effect was not observed in 25-day-old rats and it was only moderate in adult animals (Fig.1). The older animals exhibited a tendency to a decreased incidence of generalized seizures (Fig.1). Minimal seizures were suppressed by the two highest doses of SKF97541 (0.5 and 1 mg/kg) in 18-day-old rats; no consistent effect was observed in 25-day-old and adult animals (data not shown). Severity of seizures reflected suppression of the tonic phase of GTCS as well as a decreased incidence of generalized seizures after the highest dose in 25- and 90-day-old rats. The 1-mg/kg dose of SKF97541 led to a significant decrease of score in all five age groups whereas the same effect of the 0.5-mg/kg dose was significant only in the three younger groups. The highest dose of SKF97541 resulted in a significant prolongation of latencies of generalized seizures in 7- and 12-day-old rats; other changes including an outlined decrease of latencies in 18-day-old animals were not significant (Fig.2). In contrast to generalized seizures, minimal seizures started earlier in adult rats pretreated with the 0.1- and 0.5-mg/kg doses of SKF97541 (latencies of

647.5±62 and 705±181 s, respectively, in contrast to a control value of 1008±123 s), changes in 18- and 25-day-old rats did not reach the level of statistical significance.

SKF97541 exhibited mixed anti- and proconvulsant effects in our experiments; it is similar to the actions of baclofen in adult as well as immature animals (Ault and Nadler 1983, Ault *et al.* 1986, Watts and Jefferys 1993, Velíšek *et al.* 1995, Velíšková *et al.* 1996, Higashima *et al.* 2000, Sokal and Large 2001, Chen *et al.* 2004, Hoskinson *et al.* 2004, Mareš and Šlamberová 2006). Effects of SKF97541 on motor performance were not studied as frequently as effects of baclofen but in published papers (mostly in mice) the two drugs exhibit only quantitative differences. Both GABA-B receptor agonists decrease locomotion in open field, induce ataxia and in high doses catalepsy and loss of righting reflexes (Besheer *et al.* 2004, Carter *et al.* 2005). In addition to the basic agreement between the actions of the two GABA-B receptor agonists, SKF97541 exhibits effects which differ from those induced by baclofen: Specific effect against the tonic phase of GTCS even in 18-day-old rats; significant prolongation of GTCS latencies in 7- and 12-day-old rats; suppression of minimal seizures in 18-day-old rats and significant shortening of their latencies in adult rats. It might be due to different target populations of GABA-B receptors for the two agonists but there are no data supporting this possibility and further analysis is needed. There are also quantitative differences between these agonists: SKF9751 is more than 10fold more potent than baclofen according to their action on striatal neurons (Seabrook *et al.* 1990). This difference is even bigger in immature rats in our in vivo experiments, e.g. the 0.2-mg/kg dose of SKF97541 has the same effect as 6-mg/kg dose of baclofen in 7-day-old rats. Our first experiments studying effects of the two agonists on spontaneous EEG activity of immature rats speak also in favor of a marked quantitative difference.

The qualitative changes of action of SKF97541 – effects on the tonic phase of generalized seizures in very immature rats and absence of this effect in older animals are in agreement with data on baclofen action in two different models (Velíšek *et al.* 1995, Velíšková *et al.* 1996). In addition, baclofen potentiates

spike-and-wave episodes induced by low doses of PTZ in adult and 25-day-old rats but it suppresses these episodes in 18-day-old animals (Mareš and Šlamberová 2006). Therefore the effects of the two agonists have to be studied in other models especially in 18-day-old rats.

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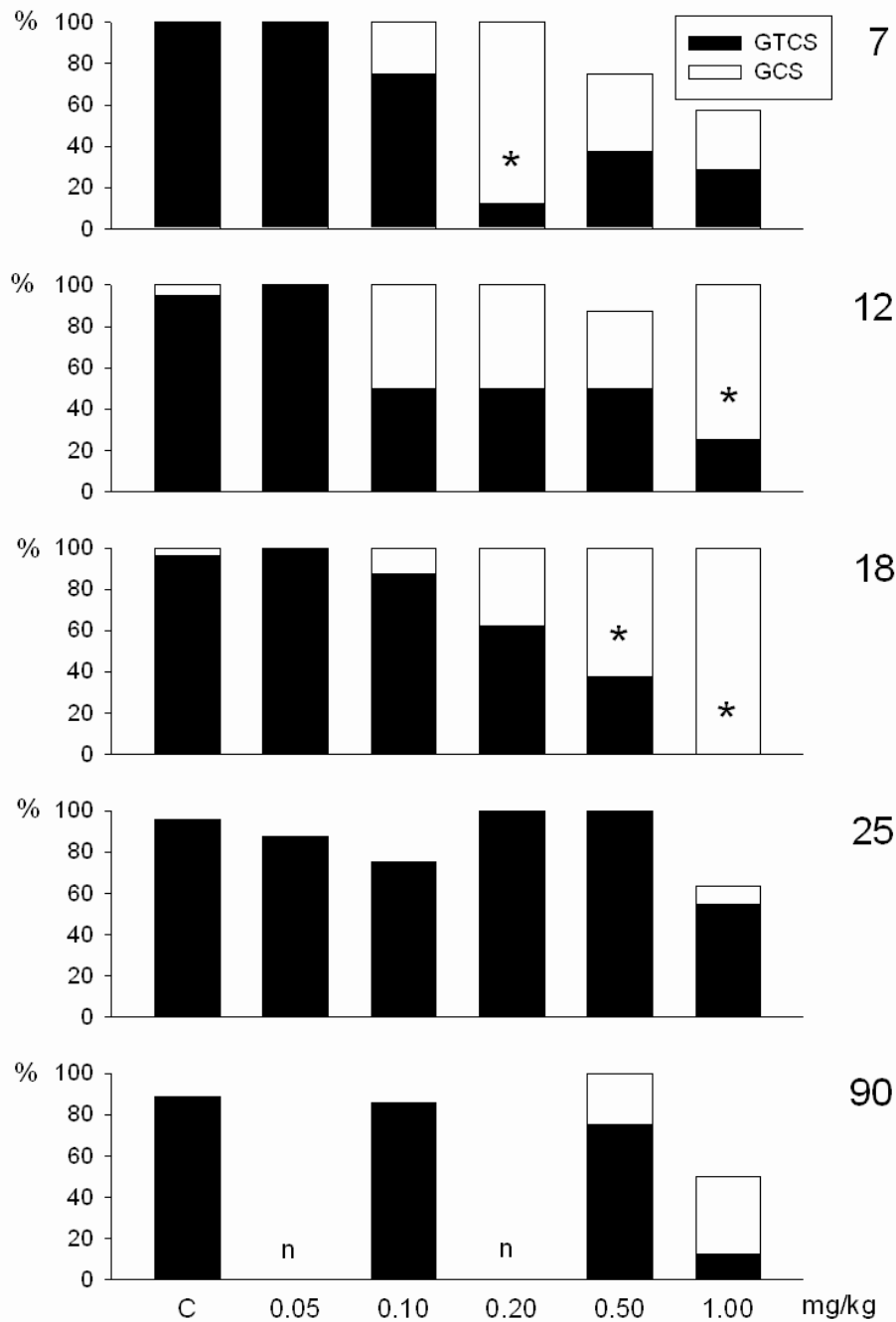
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Text to Figures:

1. Incidence of generalized seizures in rats 7, 12, 18, 25 and 90 days old (from top to bottom). Black parts of columns represent complete generalized tonic-clonic seizures, white parts of columns - generalized seizures without the tonic phase). Abscissae: doses of SKF97541, C means control animals; ordinates: percentage of animals exhibiting seizures. Asterisks denote a significant difference in the incidence of complete generalized tonic-clonic seizures in comparison with the age-matched control group.
2. Latencies of generalized tonic-clonic seizures (mean + S.E.M.) in rats 7, 12, 18, 25 and 90 days old (from top to bottom). Details as in Fig.1, only ordinates – latencies in seconds.

## Incidence of generalized seizures - SKF97541



## Latencies of GTCS - SKF97541

