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$GABA_B$ Receptor Agonist Baclofen Has Non-Specific Antinociceptive Effect in the Model of Peripheral Neuropathy in the Rat

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Summary

Baclofen, which is a specific agonist of the metabotropic $GABA_B$ receptor, is used in clinical practice for the treatment of spasticity of skeletal muscles. It also exerts an analgesic effect, but this effect is still not clear and especially controversial in neuropathic pain. In this work, we studied the antinociceptive effects of baclofen in a model of chronic peripheral neuropathic pain – loose ligation of the sciatic nerve (chronic constriction injury, CCI). As controls we used sham-operated animals. The changes of thermal pain threshold were measured using the plantar test 15-25 days after the operation. The obtained results suggest that baclofen increases pain threshold in both groups. The antinociceptive effect of baclofen was dose-dependent and the maximum response without motor deficits was observed at a dose of 15 mg/kg s.c. In the rats with CCI, significant differences between affected (ipsilateral) and contralateral hind paw were present. This difference was dose-dependent, the highest value (6.2±1.37 s) was found at the dose of 20 mg/kg. Based on our results and previous findings it could be summarized that baclofen has antinociceptive action, which is attenuated in the model of chronic neuropathic pain probably due to the degeneration of GABA interneurons after chronic constriction injury.

Key words

Baclofen • $GABA_B$ receptor • Chronic constriction injury • Antinociception • Rat

Introduction

It is generally accepted that γ-aminobutyric acid (GABA) is involved in spinal antinociception (Sawynok 1987). GABAergic neurons found in laminae I-III are mainly inhibitory interneurons (Gobel 1978). In the superficial layers of the dorsal horn there are also present both ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors (Price *et al.* 1984, Bowery *et al.* 1987). The activation of $GABA_B$ receptors leads to the inhibition of presynaptic mediator release (blockade of voltage-gated calcium channels) (Wojcik *et al.* 1990) and to the inhibition of postsynaptic neuronal activity (activation of G-protein-gated inwardly rectifying potassium channels and inhibition of adenylyl cyclase activity) (Otis *et al.* 1993, Knight and Bowery 1996).

Baclofen (4-amino-3-[cholophenyl] butyric acid; Lioresal) is a specific agonist of heptahelical $GABA_B$ receptor. Baclofen is used for treating the spasticity but besides this action it also induces analgesia in a number

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of animal pain models. The main antinociceptive mechanism of baclofen is thought to be the glutamate release inhibition from Aδ and C primary afferent terminals in substantia gelatinosa (Ataka *et al.* 2000). Moreover, baclofen decreases the NK-1 receptor expression in the spinal dorsal horn (Enna *et al.* 1998).

Our study was carried out in order to determine the effect of baclofen on pain threshold for thermal stimulation in the model of chronic peripheral neuropathic pain – loose ligation of sciatic nerve (chronic constriction injury, CCI) in the rat (Bennett and Xie 1988). Recent studies have shown that baclofen has controversial antinociceptive effects in the models of persistent neuropathic pain (Smith *et al.* 1994, Hwang and Yaksh 1997, Hao *et al.* 1999, Patel *et al.* 2001).

In our experiment, the antinociceptive effect of baclofen was compared with morphine at the dose of 5 mg/kg s.c. This dose is recommended for the sedation in rat (Ben *et al.* 1969) and we used it as a reference drug.

Methods

Animals

Adult male Wistar rats (Velaz, Prague, Czech Republic) weighing 210-240 g were housed with free access to food and water and maintained under a regime with 12 h of light and 12 h of darkness per day. The mean temperature was 22±2 °C and the relative humidity equaled to 55±10 %. The acclimation period was 5 days long. This experiment was approved by the Committee for Animal Care and Use of the Third Faculty of Medicine, Charles University, Prague, and conducted according to the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann 1983).

Surgical procedures

Persistent neuropathic pain in rats was evoked by chronic constriction of sciatic nerve according to the model of Bennett and Xie (1988). Briefly, under pentobarbital anesthesia (40 mg/kg, supplemented when necessary), the right sciatic nerve was exposed and lightly ligated with four ligatures of chromic catgut. In sham-operated animals, the right sciatic nerve was exposed in the same manner, but not ligated. In all animals the left sciatic nerve remained unoperated.

Pain testing

Antinociceptive effects of drugs were determined according to the latency (in seconds) of limb withdrawal to the noxious thermal stimulation (Plantar test, Ugo Basil, Comerio, Italy). Cut-off value of plantar test was set to 22 s to prevent limb injury. The pain thresholds were tested 15-25 days following the surgical procedure.

Drug application

All the drugs were administrated by subcutaneous injection in the following doses: baclofen (Sigma) 1, 5, 10, 15, 20 and 30 mg/kg, morphine (Léčiva Prague, Czech Republic) 5 mg/kg and saline in the same volume as baclofen. Baclofen was administrated in suspension (10 % gum accacia (Sigma) in 0.9 % saline) in volume of 4 ml/kg. Pain thresholds were evaluated three to five times for each hind limb 15, 25, 35, 45, 60 and 90 min after drug administration.

Data analysis

Data are expressed as mean ± S.E.M. of 5-6 animals in each group. To determine the statistical significance, the ANOVA test (Statistica 6.0, StatSoft Inc.) was used. Differences between means were considered statistically significant if $p<0.05$.

Results

Baclofen increases thermal pain threshold in control animals. When administered subcutaneously (s.c.) baclofen showed significant pain threshold elevation in sham operated animals. This effect was observed in the dose of 5 mg/kg. In the dose of 1 mg/kg s.c. the obtained plantar test latency was not significantly different when compared to saline (Fig. 1A). Morphine in the dose of 5 mg/kg s.c. was several times more potent – in this group the cut-off value 22 s was overshot. There were no significant differences between plantar test latency of ipsilateral (sham-operated) and contralateral (unaffected) hind limb in any of the tested compounds.

Baclofen also elevated the plantar test latencies in the model of peripheral neuropathy. The animals with chronic constriction of the sciatic nerve were sensitive to baclofen in a similar manner as the sham-operated ones (Fig. 1B). Of course, the difference compared to sham-operated animals was in the pain thresholds between ipsilateral (ligated) and contralateral (unaffected) hind limbs. The pain threshold was lower in the case of ipsilateral limb. This was observed after the administration of both saline and baclofen. After administration of 1 mg/kg baclofen the plantar test latency was not significantly different compared to saline, but this difference was found in animals with the dose of

5 mg/kg. Morphine in the dose of 5 mg/kg overshot the cut-off value of 22 s.

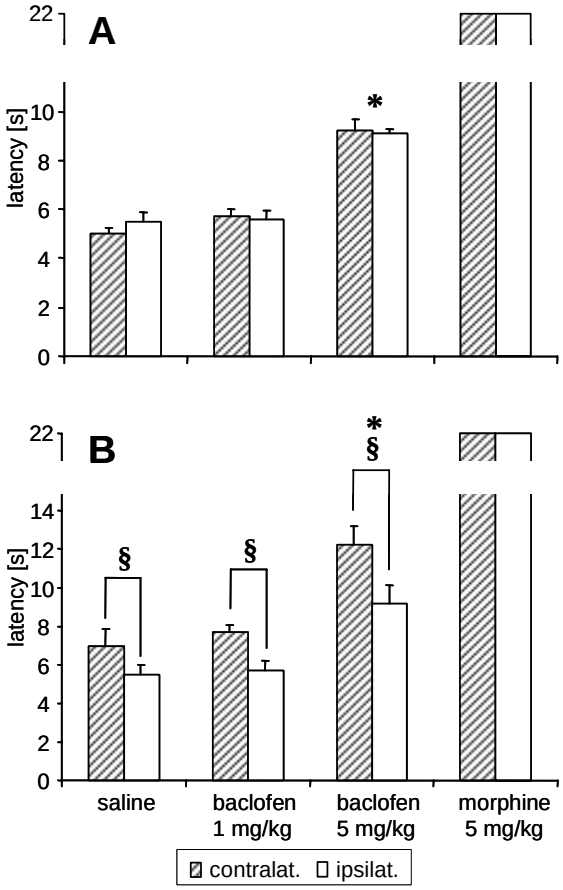

Fig. 1. Plantar test latencies (mean ± S.E.M.; n = 5-6) after drug administration in control (**A**) and ligated (**B**) animals. Baclofen in the dose of 5 mg/kg s.c. increases the thermal pain threshold in both control and neuropathic animals. Hatched bars, left hind limbs latencies (contralateral to the sham-operated (A) or ligated (B) sciatic nerve); open bars, right hind limbs latencies (ipsilateral to the sham-operated (A) or ligated (B) sciatic nerve). After morphine administration cut-off value 22 s was overshot. § p< 0.05; * p < 0.05 compared to saline, ANOVA test.

The elevation of the pain threshold by baclofen is dose-dependent. The changes of plantar test latencies were evaluated after following doses of baclofen: 1, 5, 10, 15, 20 and 30 mg/kg s.c. As illustrated in Figure 2, the increase of the pain threshold was dose-dependent. The significant difference compared to saline was observed for the doses of 5, 10, 15, 20 and 30 mg/kg. After the administration of baclofen in the dose of 30 mg/kg the cut-off value 22 s was overshot. The motor deficit was observed for the doses of 20 and 30 mg/kg. The dose-dependent effect of baclofen was observed in both ipsilateral (ligated) and contralateral (unaffected) hind limbs.

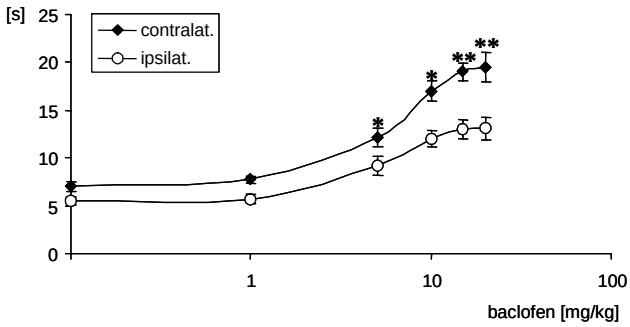

Fig. 2. Antinociceptive activity of baclofen in the model of peripheral neuropathy is dose-dependent. In both ligated and unaffected hind limbs baclofen increases the thermal pain threshold. Solid squares, left hind limbs latencies (contralateral to the ligated sciatic nerve); open circles, right hind limbs latencies (ipsilateral to the ligated sciatic nerve). Data are expressed as mean ± S.E.M. (n = 5-6). * p<0.05 compared to saline; ** p<0.02 compared to saline, ANOVA test.

There are differences in baclofen effects on ligated and unaffected hind limbs. As mentioned in the previous paragraph, baclofen increases thermal pain threshold in a dose-dependent manner in both ligated and unaffected hind limbs. Moreover, the intensity of the increase (i.e. differences in the latencies between the two hind limbs for different doses, antinociceptive potential) differs between the two limbs. Figure 3 (gaps between ligated and unaffected limbs) shows that baclofen increases pain threshold significantly more in contralateral (unaffected) hind limb. These gaps are dose-dependent with the maximum obtained value 6.2±1.37 s for the dose of 20 mg/kg.

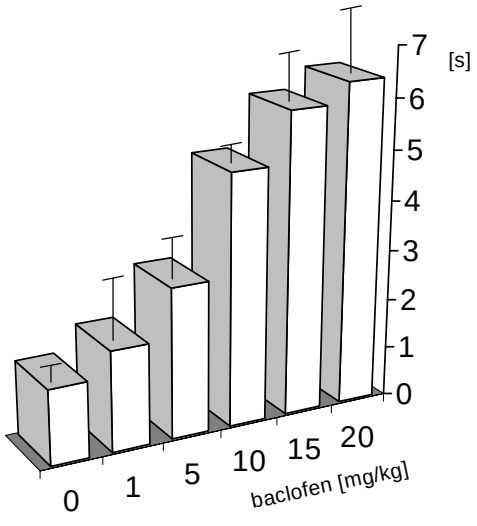

Fig. 3. Plantar test latencies gaps (mean ± S.E.M.; n = 5-6) between contralateral (unaffected) and ipsilateral (ligated) hind limbs. Values represent the difference between unaffected and ligated hind limbs for different doses of baclofen. These gaps are dose-dependent.

Discussion

The chronic ligation of sciatic nerve is a widely used model of peripheral neuropathic pain. It is generally accepted that this model may mimic many important characteristics of neurogenic pain in patients after peripheral nerve injury (Cui *et al.* 1997, Zarrindast *et al.* 2000).

The plantar test enables measurement of the effects of various conditions on the defensive paw withdrawal reflex, which is under spinal and supraspinal control (Franěk *et al.* 2002). The fact that baclofen modulates the pain threshold in non-affected animals, has already been described (Malcangio *et al.* 1991). Our results confirm the antinociceptive effects of baclofen in the intact animals, and this effect is mediated at least partially by spinal cord neurons (Gobel 1978). It has been suggested that one of possible mechanisms of baclofen-induced antinociception is the inhibition of excitatory neurotransmitters released from primary afferent fibers (Aran and Hammond 1991).

The present study shows that a subcutaneous injection of baclofen, a typical $GABA_B$ receptor agonist (Bowery 1993), increases the pain threshold for the thermal stimulation in CCI rats. Similar results have been previously described for the mechanical stimulation (Smith *et al.* 1994, Patel *et al.* 2001). Our results confirm some antinociceptive action of baclofen in the neuropathic rats.

However, the main finding of the present study is that the baclofen-induced increase in the latency was significantly higher in the intact hind limbs compared to the ligated ones in CCI rats. It means that antinociceptive effect of baclofen is attenuated under neuropathic conditions. Although Smith *et al.* (1994) found neither changes in the number of $GABA_B$ binding sites nor changes in the affinity of [^{3}H] GABA to $GABA_B$ receptors in the spinal cord after chronic constriction of the sciatic nerve, it has not been corroborated by later studies. Our results are likely to be explained by the findings of Castro-Lopes. They described the decrease in the number of $GABA_B$ binding sites (affinity of [^{3}H] GABA to $GABA_B$ receptor) in lamina II of the spinal cord 2-4 weeks after the sciatic nerve injury (Castro-Lopes *et al.* 1995). It is also known that the damage of primary afferent fibers (CCI) might lead to a degeneration of spinal neurons (Sugimoto *et al.* 1990), and that the number of GABA-immunoreactive cells in laminae I-III of the spinal cord significantly falls after sciatic nerve transsection (Castro-Lopes *et al.* 1993).

On the other hand, the antinociceptive effect of baclofen was found in our study to be dose-dependent even in both hind limbs after CCI. The curves of the dose-dependence had similar shape in both the intact and injured hind limb with a shift of the injured limb curve to lower values. The dose-dependent effect in the injured hind limb suggests an absence of a saturation of GABAergic neurones. It is concluded that besides the decrease in the number of GABAergic neurons and/or binding sites, other factors might play an important role in the attenuation of the antinociceptive effect of baclofen by chronic neuropathy.

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