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Development of baclofen tolerance in a rat model of chronic spasticity and rigidity

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Abstract

Systemic or spinal treatment with baclofen has been associated with the development of tolerance in patients with chronic spasticity. In the present study, we used a rat model of spinal ischemia-induced spasticity to characterize the development of baclofen tolerance after chronic intrathecal (i.t.) baclofen infusion. Following the induction of spinal ischemia and the development of behavioral spasticity, animals were implanted with i.t. catheters connected to osmotic pumps to continuously infuse baclofen (1.0 μg/0.5 μl/h). Hindleg peripheral muscle resistance (PMR) was measured periodically after initiation of chronic infusion and after bolus i.t. baclofen injection (1.0 μg). Peripheral muscle resistance was significantly decreased at the onset of baclofen infusion, however, after 5–7 days of infusion a progressive return of spasticity was noted, where baseline PMR values returned to preinfusion levels. At the same time, the efficacy of bolus i.t. baclofen treatment also decreased, where after 5 days of baclofen infusion 1.0 μg (i.t.) baclofen only reduced PMR by 10% (compared to 40–50% preinfusion). Baclofen efficacy progressively returned once continuous infusion was stopped. These data demonstrate that transient spinal ischemia leads to the development of spasticity which is sensitive to spinal baclofen. Chronic i.t. infusion leads to a progressive development of tolerance. This model offers potential to study tolerance mechanisms after spinal injury, and aid in drug discovery for use in baclofen-tolerant patients.

Keywords: Spasticity; Rat; Spinal cord ischemia; Baclofen; Tolerance; $GABA_B$ receptor

Spasticity and rigidity are frequent neurological deficits associated with traumatic or ischemic injury to the brain or spinal cord, as well as neurological diseases such as multiple sclerosis, cerebral palsy, and Parkinson's disease [3,6,8]. Spasticity is characterized by muscle hypertonia and displays increased resistance to externally imposed movement with increasing speed of stretch [18,36], while rigidity is defined as continuous involuntary sustained muscle contraction with a constant degree of resistance when the muscle is stretched at different velocities [26]. Despite diverse etiologies, a common denominator is increased peripheral muscle tone secondary to exaggerated α-motoneuron activity. Current clinical strategies to control spasticity and rigidity include surgical approaches like myelotomies and dorsal or ventral rhizotomies [20], and/or systemic or spinal treatment with various pharmacological agents like tizanidine, diazepam, dantrolene, and baclofen.

Baclofen is a potent agonist at the $GABA_B$ receptor and is presently a frequently used clinical drug in the treatment of spasticity and rigidity [28]. Like other GPCRs (e.g. opioid receptors), experimental and clinical data show that tolerance develops with continued $GABA_B$ receptor activation [10,12]. In clinical studies, for example, it has been shown that patients that use chronic baclofen therapy to control spasticity often require a 2–4-fold dose increase to achieve a satisfactory control of spasticity during the first 12–36 months after treatment initiation [7,29]. Studies of chronic baclofen treatment in *naive* animals have produced a number of similar reports of tolerance (e.g. refs. [22,39]). However, to the best of our knowledge there are no well-defined baclofen tolerance studies which employ a rodent spinal injury model of spasticity.

Previous experimental studies have demonstrated the development of prominent rigidity and spasticity after transient spinal cord ischemia using the rat or cat spinal ischemia models [14,37].

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A comparable development of baclofen-sensitive spasticity in patients after transient aortic cross clamp (to replace aortic aneurysm) was reported [19].

In the current study we used a well-characterized rat model of spinal ischemia-induced spasticity/rigidity coupled with direct muscle resistance measurement [25,37] to investigate: (i) the efficacy of chronic intrathecal (i.t.) baclofen to modulate spasticity and rigidity; and (ii) the development of tolerance during chronic i.t. baclofen treatment.

All experimental procedures were approved by the Animal Care Committee at the University of California, San Diego. Male Sprague–Dawley rats (300–350 g) were obtained from Harlan Industries (Indianapolis, IN), housed in standard cages with corn-cob bedding with free access to food/water and a 12-light:12-h dark cycle.

Spinal ischemia was induced using a previously described technique [37]. Briefly, under isoflurane anesthesia, spinal ischemia was induced by aortic balloon occlusion (Am. V. Mueller, CV 1035) for 10 min combined with systemic hypotension (40 mmHg). After ischemia, rats were allowed to recover and the presence of spasticity and rigidity was identified 7–10 days later by a significant increase in muscle resistance during ankle flexion.

At 14–21 days following spinal cord ischemia, i.t. catheters were implanted as previously described [13,40] except with catheter modifications. For use with miniosmotic pumps, catheters were constructed from double-lumen PE-5 (8.5 cm for midlumbar termination), with one channel connected to ~4 cm of PE-10 for externalization (allowing bolus drug/vehicle delivery) and the other channel connected to ~2 cm of PE-50 (to connect the osmotic pump). Under isoflurane anesthesia, the PE-5 was inserted through the atlanto-occipital membrane and the PE-10 externalized behind the head and sealed. The PE-50 was heat-sealed and placed subcutaneously for later connection to the osmotic pump. Rats were housed separately and monitored for normal forelimb gait, feeding and grooming behavior. Beginning 4–5 days later, peripheral muscle resistance (PMR) was measured in response to acute i.t. baclofen (1.0 μg bolus). Animals were then reanesthetized with isoflurane and the miniosmotic pump connected using the manufacturer's instructions (Alzet model #2002, Cupertino, CA, USA) and placed in a subcutaneous pouch behind the head. Animals were infused with saline (0.5 μl/h) or baclofen (Sigma, USA; 1.0 μg/0.5 μl/h) for up to 14 days [17].

Direct measurement of hindlimb muscle resistance was performed as described previously [25]. For each experiment, one rat was placed in a plastic restrainer and the right hindpaw securely fastened to the paw attachment unit which was loosely interconnected to a force transducer (LCL 454G: 0–454 g range; or LCL816 G: 0–816 g range; Omega, USA). After a 20-min acclimation period, a computer-controlled stepping motor (MDrive 34^{TM} Intelligent Motion Systems, Inc., Marlborough, CT, USA) was used to flex the ankle 40° during 3 s (13.3 °/s). Custom PC computer software (Spasticity v2.01, SK) simultaneously recorded data from the force transducer. Each recorded value was the average of three repetitions. Because of the presence of continuous muscle tone (i.e. rigidity) in animals after ischemia, this technique does not distinguish between spasticity and rigidity and concurrently assesses both components.

Bolus drug injections were performed using a hand-operated microinjector connected to the external PE-10. Baclofen (Sigma, USA) was dissolved in 0.9% sterile saline and delivered in a volume of 10 μl followed by 10 μl of sterile saline flush. All equipment was sterilized with 70% alcohol before injection and thoroughly rinsed with 0.9% sterile saline. After injection, the i.t. catheter was immediately resealed with the stainless steel plug. Measurements were taken before and every 20 min after drug administration for up to 1 h.

Peripheral muscle resistance was measured before, and routinely (every 2–5 days) after initiation of chronic baclofen or vehicle infusion. This provided data to show the effect of continuous i.t. infusion on baseline PMR. The efficacy of bolus baclofen delivery during the development of tolerance was also studied: on each day that baseline PMR was measured, the same rat was given a bolus injection of baclofen (1.0 μg; i.t.) and PMR was measured up to 1 h after injection.

Muscle resistance results are presented as raw data or percent of baseline resistance. Statistical testing was performed using Sigmastat® 2.03 for Windows® (SPSS, Chicago, IL, USA). Multiple comparisons were performed using one-way analysis of variance followed by Student–Newman–Keuls test. All results are shown as mean ± S.E.M. $P < 0.05$ was considered statistically significant.

Animals exposed to a 10-min spinal ischemic insult displayed a progressive development of extensor-type paraplegia by 3–7 days after ischemia. Measurement of PMR during ankle rotation (40 °/3 s) demonstrated a clear increase in ischemic-injured animals as compared to control (control: 0–25 g versus ischemic: 100–400 g). Apart from spastic/rigid paraplegia, all animals appeared healthy with normal grooming and feeding behavior, regular weight gain, and normal bladder/bowel function.

Before chronic infusion, bolus delivery of i.t. baclofen (1.0 μg) reduced PMR by 40–45% in spastic rats. Fig. 1A and B show sample recordings demonstrating the effect of bolus baclofen injection before chronic infusion was started. Identical treatment of the same animal after 8 days of baclofen infusion resulted in little change in PMR (Fig. 1C and D). Fig. 1E shows the baseline PMR time-course of the same animal and the effects of bolus baclofen injection during baclofen infusion.

Group data are presented in Fig. 2. After 3 days of i.t. baclofen infusion, bolus baclofen injection reduced PMR by about 30% and by only 10% after 5 days of baclofen infusion. In contrast, spastic animals receiving saline infusion showed no change in baclofen efficacy over 9 days of saline treatment. By 8 days after the osmotic pump was removed, the acute i.t. injection of 1.0 μg baclofen attenuated PMR by 35–40%, a response not significantly different than obtained on day 0.

Apart from the above-mentioned effects of bolus i.t. baclofen, continuous baclofen treatment initially reduced the animals' baseline PMR measurements (Fig. 3). This reduction reached a maximum on day 3 of infusion, when baseline PMR was approximately 60% of pre-infusion values; the reduction was still statistically significant on day 5. However, after 8 days of