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RESEARCH PAPER

Suppression of stretch reflex activity after spinal or systemic treatment with AMPA receptor antagonist NGX424 in rats with developed baclofen tolerance

Masakatsu Oshiro[1], Michael P Hefferan[2], Osamu Kakinohana[2], Nadezda Lukacova[3], Kazuhiro Sugahara[1], Tony L Yaksh[2] and Martin Marsala[2,3]

[1]*Department of Anesthesiology, University of the Ryukyus, Okinawa, Japan,* [2]*Anesthesiology Research Laboratory, University of California, San Diego, La Jolla, CA, USA and* [3]*Institute of Neurobiology, Slovak Academy of Sciences, Kosice, Slovakia*

Correspondence
Martin Marsala, Anesthesiology Research Laboratory-0695, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0695, USA.
E-mail: mmarsala@ucsd.edu

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BACKGROUND AND PURPOSE

Baclofen (a $GABA_B$ receptor agonist) is the most commonly used anti-spasticity agent in clinical practice. While effective when administered spinally or systemically, the development of progressive tolerance represents a serious limitation for its long-term use. The goal of the present study was to characterize the treatment potency after intrathecal or systemic treatment with the selective AMPA receptor antagonist NGX424 on stretch reflex activity (SRA) and background muscle activity (BMA) in rats with developed baclofen tolerance.

EXPERIMENTAL APPROACH

Animals were exposed to 10 min of spinal ischaemia to induce an increase in BMA and SRA. Selected animals were implanted with an intrathecal PE-5 catheter and infused intrathecally with baclofen (1 $\mu g \cdot h^{-1}$) for 14 days. Before and after baclofen infusion, changes in BMA and SRA were measured at 2 day intervals. After development of baclofen tolerance, the animals were injected intrathecally (1 μg) or subcutaneously (3, 6 or 12 $mg \cdot kg^{-1}$) with NGX424, and changes in BMA and SRA were measured.

KEY RESULTS

Intrathecal or systemic delivery of NGX424 significantly suppressed the BMA and SRA in baclofen-tolerant animals. This effect was dose dependent. The magnitude of BMA and SRA suppression seen after 1 μg (intrathecal) or 12 $mg \cdot kg^{-1}$ (s.c.) of NGX424 injection was similar to that seen during the first 5 days of baclofen infusion.

CONCLUSIONS AND IMPLICATIONS

These data demonstrate that the use of NGX424 can represent an effective therapy to modulate chronic spasticity in patients who are refractory or tolerant to baclofen treatment.

LINKED ARTICLE

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Abbreviations

BMA, background muscle activity; EMG, electromyography(ic); EPSC, excitatory post-synaptic potential; $GABA_B$, GABA receptor, B subtype; GluA1, AMPA-type glutamate receptor subunit 1; GluK1, metabotropic glutamate receptor subunit 1; IT, intrathecal; NBQX, 2,3-dihydroxy-6-nitro-7-sulphonylbenzo[*f*]quinoxaline; NGX424, tezampanel; PE,polyethylene; SRA, stretch reflex activity

Introduction

Spinal cord injury can lead to a loss of local spinal segmental inhibition and progressive increase in background muscle tone (BMT) and spasticity (Adams and Hicks, 2005). Spasticity is defined as velocity-dependent increase in muscle tone (i.e. muscle resistance is increasing progressively with increased velocity of muscle stretch) (Lance, 1980; Maurice *et al.*, 2001). In addition to the appearance of clinically defined spasticity, spontaneous or muscle spasm-exacerbated pain is often reported in patients, particularly at chronic stages after spinal injury (Burchiel and Hsu, 2001; Dijkers *et al.*, 2009; Yezierski, 2009). Baclofen (a $GABA_B$ receptor agonist) is one of the most commonly used drug treatments for control of spasticity, and is available for both oral or intrathecal administration (Rawlins, 2004). While effective control of spasticity of different etiology (such as spinal or brain trauma, cerebral palsy, spinal ischaemia, multiple sclerosis, amyotrophic lateral sclerosis) can be achieved with baclofen treatment, a progressive development of tolerance (i.e. required gradual escalation of dose to produce constant anti-spasticity effect), as well as development of baclofen withdrawal syndrome after termination of intrathecal or oral treatment, represents a major limitation of its chronic use (Nielsen *et al.*, 2002; Douglas *et al.*, 2005; D'Aleo *et al.*, 2007; Hansen *et al.*, 2007). Alternative pharmacological treatment for spasticity which would be effective in baclofen-tolerant patients is limited, and a variable degree of success after treatment with morphine (Soni *et al.*, 2003) or tizanidine (Kamen *et al.*, 2008) has been described. There is a clear need for the development of new drug treatments which would be effective in providing anti-spasticity effect as monotherapy or as an alternative therapy in baclofen-tolerant patients.

In our previous studies, we have developed a rat model of transient spinal cord ischaemia which is characterized by: (i) selective loss of small inhibitory interneurons and resulting development of extensor-type of paraplegia (Taira and Marsala, 1996); (ii) progressive increase in BMT and increase in stretch reflex activity (SRA) as defined by increase in ankle resistance (AR) and corresponding electromyograph (EMG) activity measured during computer-controlled ankle dorsiflexion (Marsala *et al.*, 2005); (iii) effective anti-BMT/SRA effect after bolus intrathecal baclofen or nipecotic acid (GABA uptake inhibitor) injection (Kakinohana *et al.*, 2006); and (iv) development of baclofen tolerance after chronic (14 days) intrathecal infusion of baclofen as defined by the re-appearance of SRA at 7–10 days after initiation of baclofen infusion and loss of anti-SRA effect after single bolus intrathecal baclofen injection (Hefferan *et al.*, 2006). In a more recent study using the same model, we have also demonstrated a potent anti-BMT/SRA effect after intrathecal delivery of the AMPA receptor antagonist tezampanel (NGX424; 0.3–1 μg) and have shown that expression of AMPA receptors (particularly GluA1; receptor nomenclature follows Alexander *et al.*, 2009) on activated astrocytes can play an active role in the local inflammation-evoked exacerbation of α-motoneuronal activity (Hefferan *et al.*, 2007; Codeluppi *et al.*, 2009). Previous studies have demonstrated that NGX424 is a competitive AMPA/KA receptor antagonist, with high affinity for the GluA1–GluA4 AMPA receptor subunits, and moderate affinity for GluK1 (Schoepp *et al.*, 1991; Bleakman *et al.*, 1996; Simmons *et al.*, 1998; Collingridge *et al.*, 2009). In addition to its potent anti-SRA effect, a comparable antihyperalgesic and antinociceptive effect in animal models or in a human intradermal capsaicin-evoked hyperalgesia model has been reported (Sang *et al.*, 1998; Gilron *et al.*, 2000; Lee *et al.*, 2006).

The primary goals of the present study were to: (i) characterize the anti-BMT/SRA effect of NGX424 if administered intrathecally in rats with developed baclofen tolerance; and (ii) characterize the anti-BMT/SRA effect of NGX424 when administered systemically (3, 6 or 12 $mg \cdot kg^{-1}$, s.c.) in animals without any treatment with baclofen or in rats with fully developed baclofen tolerance.

Methods

All animal care and 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 (Indianapolis, IN, USA) and were housed in standard cages with corncob bedding. The animals had access to food and water *ad libitum*, and were housed separately after surgery. A 12 h light/dark cycle (lights on at 7:00 A.M.) was used throughout.

Induction of spinal ischaemia

Spinal ischaemia was induced using the previously described technique (Taira and Marsala, 1996). Briefly, rats were anaesthetized with 4% isoflurane (Hospira, Lake Forest, IL, USA) in air and maintained with 1.5–2% isoflurane. Body temperature was maintained at 37°C using a homeothermic blanket system. Distal arterial blood pressure was monitored by a tail artery catheter (PE-50). The left carotid artery was cannulated with a 20-gauge polytetrafluoroethylene catheter for blood withdrawal. To induce spinal ischaemia, a 2F Fogarty catheter (Am. V. Mueller, CV 1035; Baxter, Irvine, CA, USA) was passed through the left femoral artery to the descending thoracic aorta so that the tip reached the level of the left subclavian artery (10.8–11.4 cm from site of insertion). The intra-aortic balloon catheter was inflated with 0.05 mL of saline for 10 min, and occlusion confirmed by an immediate and sustained drop in distal arterial blood pressure measured in the tail artery. Systemic hypotension (40 mmHg) was induced during occlusion by withdrawing blood from the carotid artery to the glass collecting circuit and kept at 37.5°C. After ischaemia, the balloon was deflated, and the blood was re-infused during a 60 s period. After blood re-infusion, 4 mg of protamine sulphate was administered subcutaneously. Stabilization of arterial blood pressure was then monitored for an additional 10 min, after which the arterial lines were removed and wounds were closed. The rats were then allowed to recover.

Identification of changes in BMT and SRA after ischaemia

Seven to ten days after ischaemia, the animals were tested for changes in BMT and SRA. Increase in BMT was identified by the appearance of ongoing spontaneous background EMG activity measured in the gastrocnemius muscle, as well as continuous dorsal plantar flexion of paw digits in the absence of any stimulus. Increase in SRA was identified as an increase in AR during computer-controlled ankle dorsiflexion (i.e. active AR), which correlated with increased EMG activity (active EMG) measured in the gastrocnemius muscle during the same time-frame.

Direct measurement of AR during computer-controlled ankle dorsiflexion was performed as described previously (Marsala *et al.*, 2005). Briefly, rats after spinal ischaemia were individually placed in a plastic restrainer, and one hind paw was securely fastened to the paw attachment metal plate, which is interconnected loosely to the 'bridging' force transducer (LCL454G, 0–454 g range; or LCL816G, 0–816 g range; Omega, Stamford, CT, USA). After a 20 min acclimation period, rotational force was applied to the paw attachment unit using a computer-controlled stepping motor (MDrive 34 with onboard electronics; microstep resolution to 256 microsteps/full step; Intelligent Motion Systems, Marlborough, CT, USA), causing the ankle to dorsiflex (Figure 1A). The resistance of the ankle was measured during 40° of dorsiflexion during 3 s ($13.3°{\cdot}s^{-1}$), and data were collected directly to a computer using custom software (Spasticity, version 2.01; Ellipse, Kosice, Slovak Republic).

To identify the mechanical component of measured AR, all animals were anaesthetized with 2.5–3% isoflurane at the end of the experiment, and the relative contribution of mechanical versus neurogenic component (isoflurane sensitive) was calculated. Data generated before and after NGX424 treatment were expressed as % of neurogenic component contributing to measured resistance (see Figure 1 for details). Each recorded value was the average of three repetitions. To record EMG activity, a pair of tungsten electrodes were inserted percutaneously into the gastrocnemius muscle 1 cm apart. EMG signals were bandpass filtered (100 Hz to 10 kHz) and recorded before, during and after ankle dorsiflexion. EMG responses were recorded with an alternating current-coupled differential amplifier (model DB4; World Precision Instruments, Sarasota, FL, USA) and stored on a computer for subsequent analysis. EMG was recorded concurrently with AR measurement during dorsiflexion, and expressed as the average of three measurements. Digitized EMG signal was full-wave rectified, and values within given time interval (bin) were averaged and used for statistical analysis. Similarly, as for AR, integrated EMG data recorded before and after induction of isoflurane anaesthesia were used to determine the maximum possible effect.

Intrathecal catheterization

At 10–15 days after spinal cord ischaemia, intrathecal catheters were implanted in animals with identified increase in BMT and SRA as described previously (Yaksh and Rudy., 1976). Under isoflurane anaesthesia, an 8.5 cm PE-5 catheter (Spectranetics, Colorado Springs, CO, USA) connected to 1 cm of polyethylene-60 tube was inserted through an incision in the atlanto-occipital membrane of the cisterna magna. The PE-60 catheter end was then connected to the mini-osmotic pump using the manufacturer's instructions (Alzet model #2002, Cupertino, CA, USA) and placed subcutaneously behind the head. Animals receiving intrathecal bolus of NGX424 ((TorryPines Therapeutics, Inc., San Diego, CA, USA) injections were implanted with

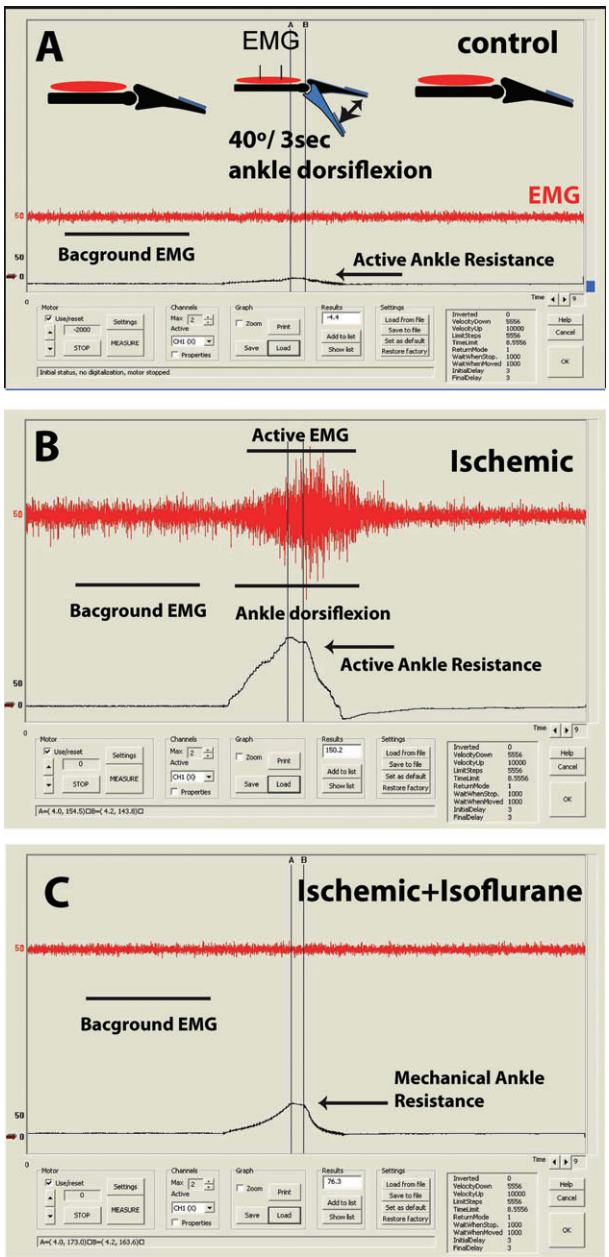

Figure 1

Quantification of changes in background EMG and SRA in rats with spinal ischaemic injury. (A,B) At 4–10 days after spinal cord ischaemia, an increase in background EMG measured in the gastrocnemius muscle in the absence of any peripheral stimulus is seen (compare A: control to B: ischaemic; background EMG). (B) In ischaemic animals, changes in SRA are identified by the appearance of burst EMG activity (active EMG) which correlates with increased AR (active AR) measured during computer-controlled ankle dorsiflexion (40°·3 s^{-1}). (C) To identify the mechanical component in measured AR (mechanical AR) during ankle dorsiflexion, animals were anaesthetized with isoflurane at the end of the experiment and the magnitude of active AR suppression measured. A decrease in active AR, active EMG during ankle dorsiflexion and background EMG measured under isoflurane anaesthesia is then used as 100% possible effect and is defined in each animal. All drug treatment data generated after NGX424 treatment are then expressed as % of maximum effect seen under isoflurane anaesthesia.

a double lumen PE-5 catheter (Spectranetics); one arm was externalized on the neck for bolus drug delivery, and the second arm was connected to a mini-osmotic pump as described. The animals were infused with saline (0.5 µL·h^{-1}) or baclofen (Sigma, St Louis, MO, USA; 1.0 µg in 0.5 µL·h^{-1}) for 14 days (Hefferan *et al.*, 2006).

Experimental timeline/drug treatment design

Three experimental treatment studies were performed.

In the first study (study A; n = 8), animals with identified increase in BMT/SRA were intrathecally infused with baclofen for 14 days. After development of baclofen tolerance, as defined by re-appearance of BMT/SRA, the animals received a single intrathecal bolus of NGX424 (1 µg) delivered in 10 µL of saline and followed by an additional 10 µL of saline injection to flush the catheter. The presence and degree of BMT/SRA were measured before and after baclofen infusion, and for 2 h in 10 min intervals after intrathecal bolus injection of NGX424. Control animals (n = 6) were infused intrathecally with saline for 14 days, and then received a single intrathecal bolus of NGX424 (1 µg).

In the second study (study B; n = 18), animals were injected subcutaneously with NGX424 (3, 6 or 12 mg·kg^{-1} in 500 µL of saline). Before and after injection, BMT/SRA was measured for 2 h in 10 min intervals. Control animals (n = 6) were injected with saline 500 µL subcutaneously.

In the third study (study C; n = 18), animals were intrathecally infused with baclofen for 14 days. After development of baclofen tolerance, the animals received a single subcutaneous injection of NGX424 (3, 6 or 12 mg·kg^{-1}) delivered in 500 µL of saline. The presence and degree of BMT/SRA were measured for 2 h in 10 min intervals after NGX424 injection. Control animals (n = 6) were infused intrathecally with saline for 14 days, and then received a single subcutaneous injection of NGX424 (3, 6 or 12 mg·kg^{-1} in 500 µL of saline).

In a separate group of naive non-paralysed animals, whisker, corneal reflexes and motor activity were tested after s.c. injection of 12 mg·kg^{-1} NGX424. A cotton-tipped applicator was used to gently displace the whiskers or touch the outer edge of the eye while monitoring reactions. Responses were graded as follows: 4, normal; 3, mildly impaired; 2, consistently impaired (rarely present); 1, absent. Effects on ambulatory motor function were assessed in an open-field paradigm and graded as follows: 4, normal; 3, moderate muscle weakness; 2, severe muscle weakness; 1, flaccidity.

Statistical analysis

Multiple comparisons were performed using one-way ANOVA followed by Student–Newman–Keuls test. All results are shown as mean ± SEM. $P < 0.05$ was considered statistically significant.

Results

Transient spinal ischaemia leads to progressive increase in BMT and SRA

Animals exposed to 10 min of spinal ischaemia showed progressive increase in BMT/SRA at 4–10 days after aortic occlusion. The increase in BMT was identified by an increase in background EMG activity measured in the gastrocnemius muscle in the absence of any stimulus (compare Figure 1A: control to B: ischaemic; background EMG). The increase in SRA was identified by: (i) burst EMG activity; and (ii) concomitant increase in AR measured during computer-controlled ankle dorsiflexion from 0–40° (compare Figure 1A: control to B: ischaemic; active EMG and active AR). Induction of isoflurane anaesthesia effectively suppressed both BMT and SRA measured during ankle dorsiflexion (Figure 1C). Residual AR measured during ankle dorsiflexion after induction of anaesthesia is the result of mechanical resistance (Figure 1C; mechanical AR). In control non-ischaemic animals, only low-level EMG activity (0.1–0.5 mV) was measured and was similar to isoflurane-anaesthetized ischaemic rats (compare Figure 1A and C; background EMG). In control naive animals, AR measured during ankle dorsiflexion did not exceed 6–10 g (Figure 1A; active AR). These data are similar to our earlier observations (Kakinohana *et al.*, 2006).

Intrathecal bolus injection of NGX424 suppresses BMT and SRA in baclofen-tolerant animals

At day 5 after initiation of baclofen infusion, a significant decrease in active AR (on average 32 ± 7%) ($P < 0.05$; compared to pre-baclofen baseline) and in ankle rotation-evoked EMG activity (active EMG) (on average 69 ± 5%) ($P < 0.05$; compared to pre-baclofen baseline) was measured (Figure 2A,B). At 10 days after baclofen infusion, the ankle dorsiflexion-evoked resistance and EMG activity returned near completely to pre-baclofen treatment levels (Figure 2A,B). Only moderate, but not significant, changes in BMT activity were seen during the course of baclofen infusion (Figure 2B: background EMG). In animals with developed baclofen

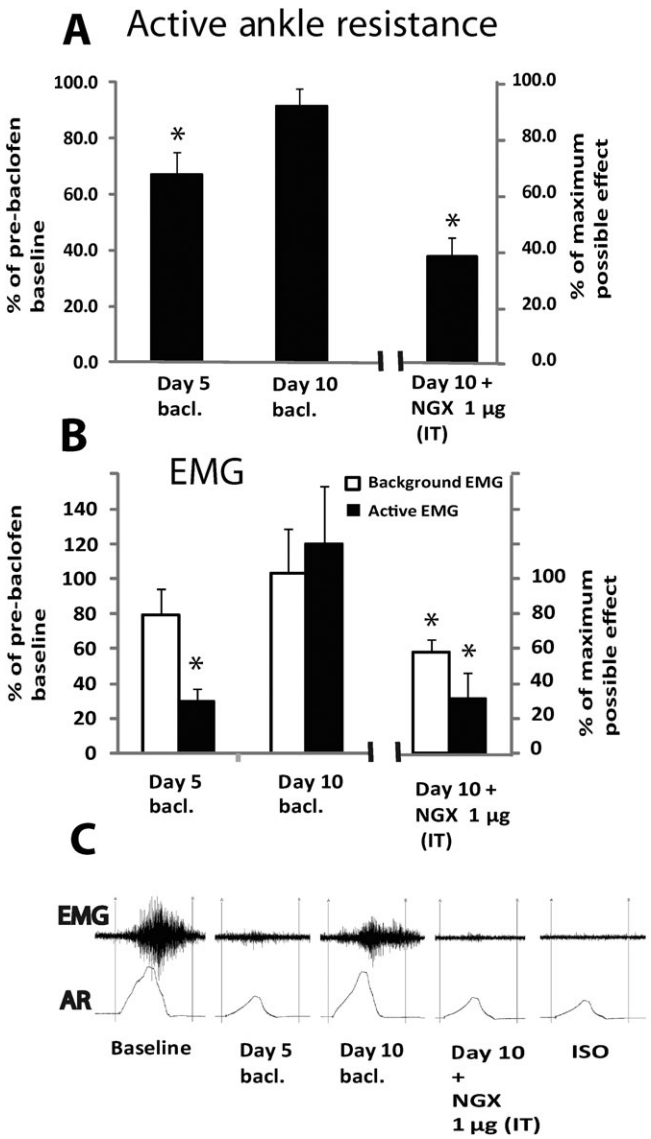

Figure 2

Development of baclofen tolerance and suppression of background EMG activity and SRA after intrathecal NGX424 treatment. (A,B) At day 5 after initiation of baclofen infusion, a significant decrease in active AR (AAR) and EMG activity (active EMG) can be seen. At 10 days after baclofen infusion, the AAR and active EMG activity returned almost completely to pre-baclofen treatment levels. Intrathecal injection of NGX424 (1 µg) led to a potent and significant suppression of AAR, active EMG and background EMG activity (A,B). For statistical analysis, effect of NGX424 treatment on AAR, active EMG and background EMG activity was compared to baseline data recorded at day 10 just before NGX424 administration, and then expressed as % of maximum possible effect measured after induction of isoflurane anaesthesia (*$P < 0.05$; Student's *t*-test). (C) A typical EMG and AR recording pattern in an animal before and after development of baclofen tolerance, and the effect of intrathecal NGX424 treatment followed by induction of isoflurane anaesthesia (ISO) at day 10 after initiation of baclofen infusion (A–C: NGX424 = NGX).

tolerance, intrathecal injection of NGX424 (1 μg) led to a potent and significant suppression of BMT and SRA (Figure 2A,B). The peak maximum possible effect was seen between 5 and 10 min after NGX424 injection, and was still seen at 45 min after treatment. The time-course of treatment effect seen after a single bolus intrathecal injection of NGX424 (1 μg) was similar to what we have reported in baclofen-non-tolerant animals (Hefferan *et al.*, 2007). In control animals infused intrathecally for 14 days with saline, no significant change in baseline BMT/SRA was seen, and the anti-BMT/SRA effect after intrathecal injection of NGX424 (1 μg) was similar to that reported for spastic animals without any treatment with baclofen (Hefferan *et al.*, 2007).

Figure 2C shows a typical EMG and AR recording pattern in an animal before and after development of baclofen tolerance, and the effect of intrathecal NGX424 (1 μg) treatment at day 10 after initiation of baclofen infusion.

Systemic delivery of NGX424 leads to dose-dependent suppression in BMT and SRA in baclofen non-treated animals

Subcutaneous injection of NGX424 (3, 6 or 12 mg·kg^{-1}) provided a dose-dependent suppression of BMT/SRA with the most potent maximum possible effect seen at the 12 mg·kg^{-1} dose (Figure 3A,B). Figure 3C shows sample EMG and AR recording patterns in an animal before and after 3, 6 or 12 mg·kg^{-1} NGX424 injection.

In naive control animals injected with 12 mg·kg^{-1} of NGX424, analysis of whisker and corneal reflex showed no significant alteration for 120 min after injection. Analysis of open-field motor performance showed severe to moderate motor weakness lasting for 100 min after NGX424 injection (Figure 4).

Systemic delivery of NGX424 leads to dose-dependent suppression in BMT and SRA in baclofen-tolerant animals

Subcutaneous injection of NGX424 (3, 6 or 12 mg·kg^{-1}), when injected in animals with fully developed baclofen tolerance, provided a similar dose-dependent suppression of BMT/SRA as seen in animals without treatment with baclofen. After 3, 6 or 12 mg·kg^{-1} of NGX424, the suppression of BMT and active AR expressed as a maximum possible effect was: 3 mg, 39 ± 15% ($P < 0.05$) and 25 ± 15%; 6 mg, 46 ± 12% ($P < 0.05$) and 39 ± 8% ($P < 0.05$); 12 mg, 62 ± 15% ($P < 0.01$) and 51 ± 15% ($P < 0.05$) respectively.

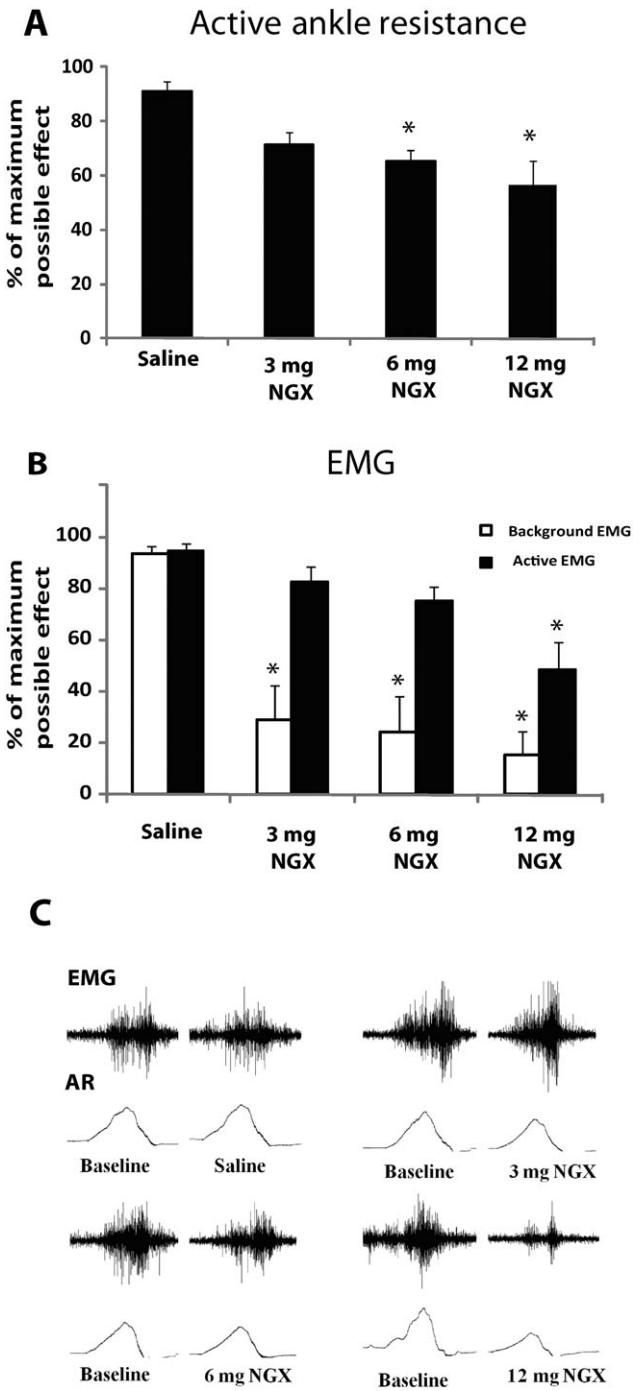

Figure 3

Potent suppression of background EMG activity and SRA after systemic NGX424 treatment. (A,B) Subcutaneous injection of NGX424 (3, 6 or 12 mg·kg^{-1}) provided a dose-dependent suppression in active AR and active EMG measured during computer-controlled ankle dorsiflexion, as well as in background EMG activity. The most potent effect was seen at 12 mg·kg^{-1} dose. For statistical analysis, the effect of NGX424 treatment was compared to baseline data recorded just before NGX424 (or saline) administration, and then expressed as % of maximum possible effect measured after induction of isoflurane anaesthesia (*$P < 0.05$; paired Student's *t*-test). (C) A sample EMG and AR recording pattern in an animal before and after subcutaneous 3, 6 or 12 mg·kg^{-1} NGX424 injection (A–C: NGX424 = NGX).

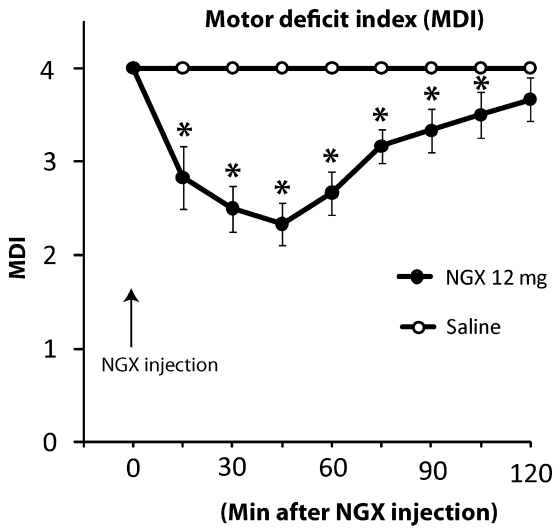

Figure 4

Systemic administration of NGX424 in naive non-injured animals led to a transient motor weakness. Subcutaneous injection of NGX424 (12 mg·kg^{-1}) led to significant motor weakness lasting for 100 min after NGX424 administration (*P < 0.05).

Discussion

Utilization of a rat spinal ischaemia model as a model of chronic spasticity

Transient spinal cord ischaemia or hypoxia may lead to a progressive and selective loss of small and medium-sized inhibitory interneurons in previously ischaemia/hypoxia-exposed spinal cord segments. In contrast, α-motoneurons show long-term survival and relative resistance (compared to interneurons) to the same level of ischaemic insult (Taira and Marsala, 1996). The resulting loss of local segmental inhibition can functionally be presented as prominent increase in BMT and SRA. A comparable functional deficit in several spinal ischaemia models from other laboratories has been reported (Gelfan and Tarlov, 1963; Murayama and Smith, 1965; Matsushita and Smith, 1970; Zivin *et al.*, 1982). In more recent studies, we have developed and characterized a computer-controlled AR meter that permits the objective measurement of AR and concomitant changes in EMG activity during ankle dorsiflexion in fully awake rats (Marsala *et al.*, 2005). Using this system, we have demonstrated, in addition to increased BMT, the presence of neurologically defined spasticity during periacute to chronic (2–3 weeks to 13 months) stages after ischaemic injury as evidenced by the presence of velocity-dependent increase in AR and EMG activity measured during ankle dorsiflexion.

As shown in numerous clinical studies, we have, using this ischaemic model, also demonstrated a similar anti-BMT/SRA effect after intrathecal treatment with baclofen, nipecotic acid (GABA uptake inhibitor), tizanidine (α_2-adrenoceptor agonist) or after L2–L6 dorsal rhizotomy (Marsala *et al.*, 2005; Kakinohana *et al.*, 2006; Fuchigami *et al.*, 2007). Jointly, these data demonstrate that this spinal ischaemic model represents an appropriate preparation to study objectively the efficacy of new treatments (pharmacological, gene therapy based, surgical) targeted to modulate acute, but also chronic, increases in background muscle activity and SRA of spinal origin.

Effective suppression of BMT and SRA with NGX424 in baclofen-tolerant animals

In a previous study, we have demonstrated the development of baclofen tolerance after chronic (14 days) intrathecal infusion of baclofen as shown by the re-appearance of BMT/SRA at 7–10 days after initiation of intrathecal baclofen infusion. In addition, the presence of baclofen tolerance was validated by the loss of an anti-SRA effect after single bolus intrathecal injection of baclofen of an otherwise effective anti-SRA dose (1 μg) (Hefferan *et al.*, 2006). Similar development of baclofen tolerance in naive rats infused intrathecally for 7 days with 1 μg·h^{-1} of baclofen was reported (Kroin *et al.*, 1993).

Data from the present study show that intrathecal or systemic NGX424 treatment is effective in suppressing BMT/SRA in baclofen non-treated, but also in baclofen-tolerant animals. Comparison of the efficacy after systemic treatment with 3, 6 or 12 mg·kg^{-1} between baclofen non-treated and baclofen-tolerant animals shows similar potency, and on average a 45–60% suppression of BMT/SRA was measured after 12 mg·kg^{-1} dose in both groups. Similarly, intrathecal treatment with 1 μg of NGX424 showed a potent anti-BMT/SRA effect in baclofen-tolerant animals. In a previous study, we have shown that the LD_{50} for anti-SRA effect after IT NGX424 is 0.44 μg in baclofen non-treated animals. Thus, as seen after systemic delivery, a comparable efficacy is achieved after intrathecal NGX424 treatment in both baclofen-non-tolerant and baclofen-tolerant animals. Analysis of whisker and corneal reflex in control naive animals after 12 mg·kg^{-1} NGX424 showed no detectable change for 120 min after administration. A previous systematic study from another laboratory showed transient impairment of motor performance as measured by rotorod test after 17–34 μmol·kg^{-1} i.p. (5.1–10.2 mg·kg^{-1}) NGX424 administration (Lee *et al.*, 2006). These observations are consistent with the potent anti-spasticity effect and transient motor weakness in control naive animals seen in our current study.

Mechanism of NGX424 anti-BMT and anti-SRA effect may include blockade of AMPA receptors on neurons and glia

Previous immunohistological studies have identified AMPA receptor subunits on spinal α-motoneurons, interneurons and presynaptic Ia afferents (Tachibana *et al.*, 1994; Nagy *et al.*, 2004; Polgar *et al.*, 2008). Consistent with the role of AMPA receptors in spinal excitatory transmission, *in vitro* experiments demonstrated that: (i) stimulation of cultured motoneurons leads to significant acetylcholine release (Fontana *et al.*, 2001); and (ii) 2,3-dihydroxy-6-nitro-7-sulphonylbenzo[*f*]quinoxaline (NBQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (AMPA and kainate receptor antagonists) suppressed the monosynaptic reflex in the isolated spinal cord tail preparation (Ault and Hildebrand., 1993; Pinco and Lev-Tov, 1993). In *in vivo* experiments, it has been shown that: (i) short-term (2 h) intrathecal infusion of AMPA produces reversible spastic paraplegia in the rat (Nakamura *et al.*, 1994); (ii) systemic or intrathecal NBQX suppressed spasticity in a genetic mutant rat model of spasticity (Turski and Stephens, 1993); and (iii) intrathecal 6,7-dinitroquinoxaline-2,3-dione or NBQX blocked the monosynaptic reflex in naive, spinally hemisected or completely transected rats (Schwarz *et al.*, 1995; Kocsis *et al.*, 2003).

In addition to neuronal expression of AMPA receptors, there is increasing evidence on an important functional role of AMPA receptors in spinal astrocytes. Histological studies have identified GluA1 immunoreactive hypertrophic astrocytes in the CA1 hippocampal region after transient forebrain ischaemia, or in cerebellar Bergman glia (Gottlieb and Matute, 1997; Matsui *et al.*, 2005). Using immunofluorescence and electron microscopy, we have demonstrated comparable GluA1 expression in reactive astrocytes in the lumbar spinal cord in animals with validated increase in BMT and SRA (Hefferan *et al.*, 2007). Activation of cultured astrocytes with NMDA or AMPA led to a significant increase in intracellular Ca^{2+} in functional *in vitro* assays, and this effect was blocked by respective antagonists (Hu *et al.*, 2004; Matsui *et al.*, 2005). More importantly, an increase in $[Ca^{2+}]$ induces glutamate release from astrocytes, and such an increase was associated with increased frequency of AMPA receptor-mediated miniature EPSPs in neighbouring neurons in the hippocampus. In our previous study, we demonstrated a comparable AMPA-evoked glutamate release in cultured astrocytes which was effectively blocked by NGX424 (Hefferan *et al.*, 2007). Jointly, these data demonstrate the existence of a positive feedback system which is likely to be involved not only in the initiation, but also in the maintenance of increased glutamate release from local segmental astrocytes and the resulting ongoing increase in α-motoneuronal excitatory drive. Based on these data, we speculate that the potent anti-BMT and anti-SRA effect seen after intrathecal or systemic NGX424 treatment may include: (i) its direct blockade of AMPA receptors on α-motoneurons and/or primary afferents; and/or (ii) blockade of AMPA receptors on astrocytes and consequent inhibition of secondary astrocyte-derived glutamate release.

Potential clinical utilization of AMPA receptor antagonism in treatment of spasticity

As demonstrated in our present and previous studies, systemic or intrathecal treatment with NGX424 has a potent anti-BMT and anti-SRA effect in animals with or without developed baclofen tolerance. Because the mechanism of NGX424 functional effect is distinct from that of baclofen (i.e. blockade of the Ca ionophore on GluA2 subunits of AMPA receptors vs. G protein-coupled effects), NGX424 or other AMPA receptor blockers could be used in several clinical drug treatment paradigms: (i) systemic or intrathecal treatment used as monotherapy; (ii) permanent or transient replacement therapy in baclofen-tolerant patients and in patients with baclofen withdrawal; or (iii) combined treatments using an AMPA antagonist with other clinically used anti-spasticity agents (baclofen, tizanidine, dantrolene, gabapentine, tiagabine, morphine). While the long-term anti-SRA effect of NGX424 after repetitive treatment was not assessed in our recent study, using the same experimental design as used in our current study, we have found that after 28 days of daily NGX424 injection (12 $mg \cdot kg^{-1}$; s.c.), there was no change in potency of the anti-SRA effect at 28 days (unpublished data).

Previous clinical studies with NGX424 demonstrated a significant treatment effect after 1.2 $mg \cdot kg^{-1}$ i.v. or 40 mg s.c. in a patient with acute migraine as measured by the headache response rate. The most common side effects were dizziness and sedation/drowsiness (Sang *et al.*, 2004). A comparable antinociceptive effect in pre-clinical animal models and a human model using intradermal capsaicin has been reported (Sang *et al.*, 1998; Lee *et al.*, 2006).

In conclusion, our present data showed that systemic or intrathecal treatment with NGX424 (tezampanel) was effective in suppressing BMT and SRA in a rat spinal ischaemia model. Comparable efficacy was seen in animals without any treatment with baclofen and in baclofen-tolerant animals. These data indicated that the use of NGX424 could represent an alternative treatment in patients with spasticity induced by spinal injury and in patients who are refractory or tolerant to baclofen treatment.

Future studies are needed to define the long-term safety of spinally delivered NGX424.

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Conflict of interest

None to declare.

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