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Short Communication

Heterotopic inputs facilitate poststimulus afterdischarges of spinal WDR neurons in rats with chronic nerve constriction

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ARTICLE INFO

Article history:
Accepted 27 April 2006
Available online 23 June 2006

Keywords:
Afterdischarge
Noxious evoked response
WDR neuron
Heterotopic afference
Neuropathic rat

ABSTRACT

Heterotopic inputs activate spinal wide dynamic range (WDR) neurons in rats with chronic constriction of one sciatic nerve (CCI rats). A possible contribution from these inputs, to long-lasting afterdischarges (ADs) of noxious evoked responses, was investigated during reversible input blockade from adjacent saphenous nerve and contralateral peripheral nerve territories. The results show significant AD reduction or suppression, indicating that heterotopic afferences contribute to mechanisms underlying prolonged ADs.

The sensitization of central neurons after peripheral nerve damage reflects altered local network dynamic properties, and the most significant signs are spontaneous unit hyperactivity and hyperexcitability. Wherever spontaneous hyperactivity shows diverse degrees of enhanced ongoing activity (Laird and Bennett, 1993; Omana-Zapata et al., 1997; Sotgiu et al., 1992), hyperexcitability results in an enhanced strength of response to noxious stimuli as compound occurrence of initial discharge (ID) amplitude and afterdischarge (AD) duration increase (Laird and Bennett, 1993; Sotgiu et al., 1992, 1995). Furthermore, the unmasking of heterotopic afferences from territories outside neuronal peripheral receptive fields (PRFs) is another marker of sensitization (Biella and Sotgiu, 1995; Sotgiu and Biella, 1995, 1998). Indeed, the spinal wide dynamic range (WDR) neurons receive excitatory inputs from a wider source range than that provided solely by their PRFs (Fitzgerald, 1982; Markus and Pomeranz, 1987). Indeed, normally silent inputs become effective after nerve injury in rats with chronic constriction of one sciatic nerve (CCI). In these rats, noxious stimulation of the adjacent saphenous nerve (Sotgiu and Biella, 1995) or of contralateral saphenous or sciatic nerve territories (Sotgiu and Biella, 1998) could activate WDR neurons ipsilateral to injury. This behavior is not observed in intact or sham rats (Sotgiu and Biella, 1995). The heterotopic input efficacy has been attributed to the sensitization of target neurons, allowing subthreshold inputs to become effective (Sotgiu and Biella, 1998). The heterotopic afference contribution to the maintenance of target neuron sensitization has also been reported (Sotgiu et al., 2004).

These data indicate a potential heterotopic input involvement in the neuronal dynamic adaptive processes in CCI rats. Thus, it could be interesting to investigate the influence of these inputs on the different expressions of altered neuronal activity associated with painful sensory disorders, such as spontaneous pain, hyperalgesia, and prolonged pain (Laird and Bennett, 1993; Pitcher and Henry, 2000; Sotgiu et al., 1992).

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doi:10.1016/j.brainres.2006.04.130

In this study, the influence on AD duration was explored; AD is considered a determinant issue for pain perception (Woolf and King, 1987). In fact, the persistence of neural response after the termination of the stimulus prolongs the transfer of nociceptive transmission to the brain (Laird and Bennett, 1993; Sotgiu et al., 1992), giving a physiological basis for abnormally prolonged pain (Laird and Bennett, 1993; Sotgiu et al., 1992, 1995).

The 28 adult male Sprague–Dawley rats used underwent, 2 weeks before the electrophysiological experiments, one sciatic nerve constriction (CCI), as described by Bennett and Xie (1988). At 7th, 10th, and 13th postoperative days, abnormal pain-related behavior was evaluated on the injured paw by the hot-plate (HP) test, measuring the paw licking latency (PLL in s), and by von Frey calibrated filaments, measuring the withdrawal threshold (WT in g).

The experimental procedures for surgical preparation and neuronal recording have been described in Sotgiu and Biella (1995, 1998).

We selected WDR neurons recorded ipsilaterally to the constricted nerve at spinal level L5–L6, responding to noxious mechanical stimuli (calibrated pinching of 400 g/2 mm^2 through Somedic Algometer) applied for 10 s to ipsilateral sciatic area and to heterotopic areas supplied by ipsilateral saphenous nerves and by contralateral sciatic and saphenous nerves (72% of the sample tested).

Noxious evoked activity was calculated by subtracting the spontaneous activity mean values.

Two protocols were followed.

In 16 rats, after neuron responses to contralateral sciatic and saphenous nerves territories stimulation had been recorded, lidocaine 2% (0.05 + 0.05 ml) was injected subcutaneously (s.c.) in those areas.

In 14 rats, before the recording session, the ipsilateral saphenous nerve was carefully isolated (leaving the perinevrium intact) and sheltered with saline-soaked cotton. A catheter connected to a lidocaine syringe and secured to muscle membrane was juxtaposed with the neighboring nerve. After neuron response to ipsilateral saphenous nerve territory stimulation had been recorded, lidocaine 2% (0.3–0.4 ml) was ejected onto the nerve through the catheter. In this protocol, the lidocaine is ejected directly onto the nerve rather than onto the innervated periphery; this is done to avoid the results being altered due to the drug spreading onto nearby sciatic territory.

The frequency discharge of the IDs and the afterdischarge durations, defined as the time interval from the end of stimulation to the return to the prestimulus discharge frequency value, were compared before and at 10, 30, 60, and 90 min from the lidocaine treatments.

In each experiment, one or two lidocaine treatments (at 120 min intervals) were performed.

The data are presented as the mean ± standard deviation (SD). Statistical evaluation was made by ANOVA for variance analysis with post hoc Duncan test; the criterion for significance was $P < 0.05$.

This study adhered to the Ethical Guidelines of the IASP (Zimmermann, 1983).

All the CCI rats showed thermal and mechanical hyperalgesia on the injured side, as verified by a significant decrease in paw licking latency (PLL), compared to the value prior to surgery (8–11 s vs. 19–22 s), and by a significant decrease in withdrawal threshold to mechanical stimuli, compared to before surgery values (1.5–7 g vs. 14–29 g).

A total of 60 neurons recorded at a depth of 650–850 μm were studied.

Contralateral blockade. The 32 sciatic neurons analyzed responded to the noxious stimulation of ipsilateral sciatic area with ID mean frequency of 44.3 ± 5.2 spikes/s and ADs lasting 60.5 ± 5.1 s and to the noxious stimulation of contralateral territory with ID mean frequency of 40.9 ± 5.1 spikes/s and ADs lasting 58.8 ± 4.3 s. After 10 min from the lidocaine application, during the blockade of the contralateral afferences (confirmed by failure to evoke responses), the

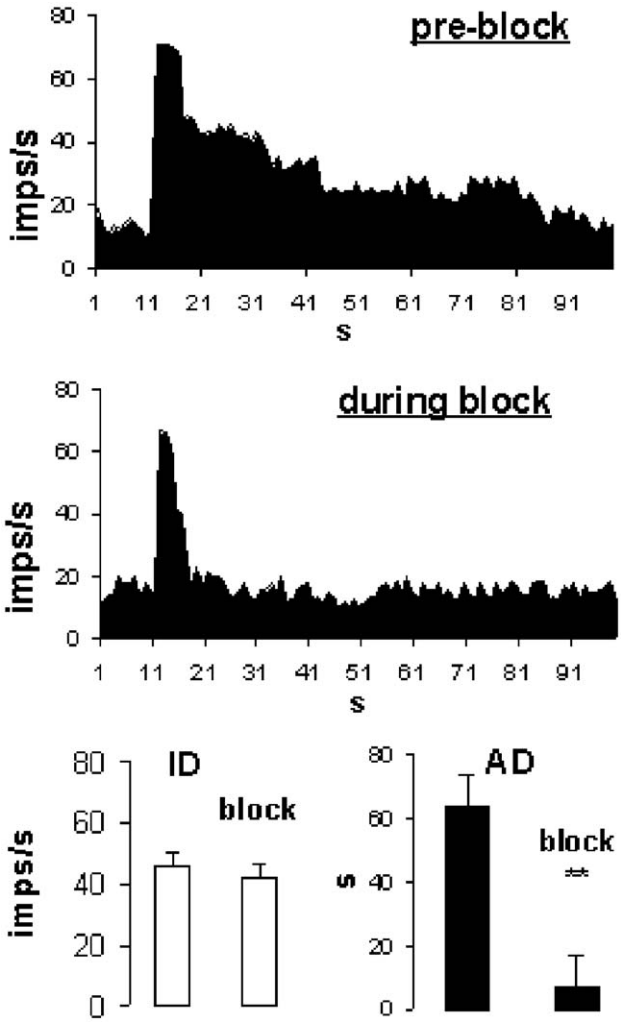

Fig. 1 – Contralateral saphenous and sciatic territory blockade. Upper row: pulse rate histograms of one WDR neuron, illustrating the responses to noxious stimulation of the sciatic nerve territory, before and during the blockade. Bottom row: noxious evoked responses of WDR neurons. Data pooled from 32 neurons presented as mean ± SD. ID (initial discharges) in Imp/s, AD (afterdischarges) in seconds before and during the blockade (block). $^{**}P < 0.0001$. In this and in the following figure, recording and stimulation on the side ipsilateral to the constricted nerve after 10 min from the lidocaine.