Nitric oxide modulates spontaneous cord dorsum potentials in the cat spinal cord

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Abstract

A previous study has shown that lumbar spontaneous cord dorsum potentials (CDPs) are produced by background activity of a neuronal ensemble located in the dorsal horn. Here, the effects produced by intravenous application of the nitric oxide synthase inhibitor L-N\textsuperscript{G}-nitro arginine (L-NOARG, 100 \textmu g/kg) and of the nitric oxide donor 3-morpholinosydnonimine hydrochloride (SIN-1, 500 \textmu g/kg) on spontaneous CDPs were examined. Experiments were performed on pentobarbitaly anesthetized, paralyzed and spinalized cats. The amplitude of spontaneous CDPs increased after L-NOARG, however, decreased after SIN-1. These observations suggest that electrical activity of dorsal horn neurones generating spontaneous CDPs is dependent on nitric oxide production.

Keywords: Dorsal horn neurons; Background activity; Cord dorsum potentials; Free radical gas; Nitric oxide synthase; Spontaneous activity; Non-nociceptive; 3-morpholinosydnonimine hydrochloride

A previous study [8] has shown that the spontaneous cord dorsum potentials (CDPs) are generated by the activation of a population of dorsal horn neurones that share the same functional pathways as those neurones responding to stimulation of low threshold cutaneous afferents. It was not clear, however, the origin of the background activity of this neuronal population.

Some recent findings, obtained by in vivo electrochemical detection, show that nitric oxide (NO) is produced continuously at spinal dorsal horn level [12,13]. Therefore, one can suggest that NO released from dorsal horn neurones could be involved in the modulation of background activity of neurones producing spontaneous CDPs. The purpose of the present study was to provide evidence of the effects produced by a nitric oxide synthase (NOS) inhibitor and a NO donor on the spontaneous CDPs. Disclosure of this modulation could be important since the spontaneous activity of these dorsal horn neurones sets a background level of transmission regulation in the Ia-motoneuron pathway [8].

Guidelines contained in NIH publication 80–23 revised in 1978 on the principles of laboratory animal care were followed throughout. All efforts were made to minimize the number of animals used and their suffering. Experiments were carried out on adult cats (2.5–3.5 kg body weight) initially anesthetized intraperitoneally with pentobarbital sodium (Smith Kline, 35–40 mg/kg). The lumbo-sacral and low thoracic spinal segments were exposed and the left L5 to S1 ventral roots sectioned. The blood pressure (BP) was monitored through the carotid artery. The left radial vein was also cannulated to administer additional doses (10 mg/kg) of pentobarbital to maintain the animals in deep anesthesia. After the surgical procedures, the animals were paralyzed with a single dose of pancuronium bromide (Pavulón, Organon; 0.3 mg/kg, i.v.) and maintained under artificial respiration. In addition, the animals were spinalized at the T12 segment. Adequacy of anesthesia was assessed verifying that the pupils were constricted and that blood pressure was stable (between 90 and 110 mmHg) and not affected by noxious stimulation of the skin. When necessary, a solution of etilefrin (Effortil, Boehringer-Ingelheim) diluted with isotonic saline (1:10) was infused intravenously to maintain...
the blood pressure within this range. The temperature of the animals was kept between 37 and 38°C by means of radiant heat. Spontaneous cord dorsum potentials (spontaneous CDPs) were monopolarly recorded from the surface of the lumbar L6 dorsal horn with silver ball electrodes. The indifferent electrode was placed on the paravertebral muscles. Low noise, high gain differential amplifiers (bandpass filters 0.3 Hz–10 kHz) were used to amplify the potentials. After recording a series of control spontaneous CDPs (1 h), physiological saline (vehicle, 1 ml/kg) was slowly injected i.v. for 1 min and the spontaneous CDPs recorded for 1 h. L-N⁵-nitro arginine (L-NOARG) (100 μg/kg) or 3-morpholinosydnonimine hydrochloride (SIN-1) (500 μg/kg) (Sigma-Aldrich) was dissolved in physiological saline (vehicle, 1 ml/kg) and slowly injected i.v. for 1 min, and the spontaneous CDPs were recorded for 1 h after injection. The present results were derived from those experiments in which blood pressure was reasonably steady, with blood pressure changes of less than 20 mm Hg. Data were stored on a tape (Vetter Digital) and analyzed off-line. Spontaneous CDPs were taken using Axoscope 8.0 (Axon Instruments) and sampled at 1 kHz. Power spectrum (mean square amplitude as a function of frequency) was calculated with the Fast Fourier Transform of the spontaneous CDPs before and after application of vehicle and drugs. The power was expressed as percentage relative to the maximum mean square amplitude. Confidence intervals (95%) were estimated (see Ref. [3] for review) and their magnitude was used as a scale bar against which to assess the significance of the effects of drugs on spontaneous CDPs. Wilcoxon test was used to assess statistical significance. The comparison was considered to be significant if $P < 0.05$.

In six experiments, we examined the effects of L-NOARG on the set of dorsal horn neurons producing the spontaneous CDPs. Fig. 1A,B illustrates the results obtained in one of them. Panel A shows recordings of spontaneous CDPs in the absence of any stimulation. The upper trace of Fig. 1A shows the continuous recording of spontaneous CDPs 15 min after i.v. application of L-NOARG. Note that the amplitude of spontaneous negative cord dorsum potentials increased after L-NOARG. To further test this observation, we calculated the power spectra (Fig. 1B) of spontaneous CDPs from: control (15 min), within the first 15 min of vehicle administration, and within the first 15 min of L-NOARG administration. Note that the power increased after L-NOARG (see arrows). Both in this and in five other experiments an increase in the power of spontaneous CDPs was detected after 1 min and the maximal effect developed within 1–2 min. We have not analyzed the maximal duration of the L-NOARG effects but found them practically unchanged within 1 h after injection. Fig. 1C illustrates the mean percentage change in the power spectra of six experiments after L-NOARG (thick lines) and vehicle application with respect to the power spectra obtained in control conditions (taken as 100%). L-NOARG produced a significant increase of 320% ($P < 0.05$, $n = 6$, Wilcoxon test) in the mean percentage change in the power spectra with respect to control, however no statistically significant
significant changes were produced after injection of vehicle. In addition, in all experiments no statistically significant difference was found in mean BP in control conditions and after administration of L-NOARG. These findings indicate that L-NOARG produced a facilitation of the activity of dorsal horn neurons.

To complement this information, we also examined the effects of a NO donor on the activity of these sets of dorsal horn neurons associated with the generation of spontaneous CDPs. Fig. 2 illustrates data obtained in one out of three experiments in which the effect of SIN-1 on spontaneous CDPs was examined. The upper trace of Fig. 2A shows the continuous recording of spontaneous CDPs in control conditions, and the lower trace the recording of spontaneous activity 15 min after i.v. application of SIN-1. Fig. 2B illustrates the power spectra of spontaneous CDPs obtained from: control (15 min), within the first 15 min of vehicle administration, and within the first 15 min of SIN-1 administration. The thin lines correspond to control conditions and vehicle, and the thick line after application of the NO donor. Fig. 2C illustrates the mean percentage change in the power spectra of three experiments after SIN-1 and vehicle application with respect to the power spectra obtained in control conditions (taken as 100%). It can be see that SIN-1 produced a significant inhibitory effect of 78% ($P < 0.05$, $n = 3$, Wilcoxon test) on the spontaneous CDPs in the 4–8 Hz frequency range. This inhibitory change in the power of spontaneous CDPs was detected already after 1 min, and the maximal effect developed within 1–2 min. We have not analyzed the maximal duration of the SIN-1 effects but found them practically unchanged within 1 h after injection.

After application of vehicle, no statistically significant changes in amplitude of spontaneous CDPs were produced.

In three experiments we found that SIN-1 produced a small reduction in BP, however no evidence of possible correlation was found between the decrease in the BP and the maximal decrease in the power of spontaneous CDPs (within the 4–8 Hz frequency range) relative to control. This result is also in agreement with the observation that, although BP decreased after SIN-1, no statistically significant changes in the power of spontaneous CDPs (occurring out of the 4–8 Hz frequency range) were produced (see Fig. 2C). It thus seems that there is a functionally distinct group of dorsal horn neurones, whose activity can be affected by SIN-1.

These results suggest that electrical activity of dorsal horn neurones generating spontaneous CDPs is dependent on NO production, which is consistent with evidence that neuronal NOS has been localized in the dorsal horn [1,2,4,5,7,11,16–18].

To date much of the work examining functions of NO in the spinal cord has focused on nociceptive processing, possibly because the localization within the superficial laminae of dorsal horn makes NO a potential intermediary in the transmission of nociceptive information [9,14,15,19,20]. However, the role played by NO in non-nociceptive transmission has not been elucidated [10,13]. These findings raise the question of the ability of NO to modulate the activity of non-nociceptive dorsal horn neurones (laminae III-VI), which are involved in the generation of the spontaneous cord dorsum potentials (CDPs) [8]. For this reason we have extended our studies in the anesthetized cat, to provide
evidence of the effects of nitric oxide on spontaneous CDPs [8].

The increase in spontaneous activity of dorsal horn neurons observed in the present study following administration of an NOS inhibitor is in full agreement with previous observations made by Hoheisel et al. [6]. These observations suggest that there is a continuous release of NO in the dorsal horn, which tonically depresses the background activity of dorsal horn neurons. We suggest that the NOS inhibitor reduced a possible tonic depression exerted by a set of dorsal horn neurons (containing NOS) on neurones producing the spontaneous CDPs. In this context, some recent findings, obtained by in vivo electrochemical detection, confirm that NO is produced continuously at spinal dorsal horn level [11,12]. Therefore, one can suggest that NO could be released not only under nociceptive circumstances [1,14], but also under non-nociceptive conditions [12].

We conclude that electrical activity of dorsal horn neurones generating spontaneous CDPs is dependent on NO production.

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