

Enhancement of Spontaneous and Heat-Evoked Activity in Spinal Nociceptive Neurons by the Endovanilloid/Endocannabinoid *N*-Arachidonoyldopamine (NADA)

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Huang, Susan M. and J. Michael Walker. Enhancement of spontaneous and heat-evoked activity in spinal nociceptive neurons by the endovanilloid/endocannabinoid *N*-arachidonoyldopamine (NADA). *J Neurophysiol* 95: 1207–1212, 2006. First published November 2, 2005; doi:10.1152/jn.00395.2005. *N*-arachidonoyldopamine (NADA) is an endogenous molecule found in the nervous system that is capable of acting as a vanilloid agonist via the TRPV1 receptor and as a cannabinoid agonist via the CB1 receptor. Using anesthetized rats, we investigated the neural correlates of behavioral thermal hyperalgesia produced by NADA. Extracellular single cell electrophysiology was conducted to assess the effects of peripheral administration of NADA (i.pl.) on nociceptive neurons in the dorsal horn of the spinal cord. Injection of NADA in the hindpaw caused increased spontaneous discharge of spinal nociceptive neurons compared with injection of vehicle. The neurons also displayed magnified responses to application of thermal stimuli ranging from 34 to 52°C. NADA-induced neural hypersensitivity was dose dependent ($EC_{50} = 1.55 \mu\text{g}$) and TRPV1 dependent, as the effect was abolished by co-administration of the TRPV1 antagonist 5'-iodoresiniferatoxin (I-RTX). In contrast, co-administration of the CB1 antagonist SR 141716A did not attenuate this effect. These results suggest that the enhanced responses of spinal nociceptive neurons likely underlie the behavioral thermal hyperalgesia and implicate a possible pain-sensitizing role of endogenous NADA mediated by TRPV1 in the periphery.

INTRODUCTION

N-Arachidonoyldopamine (NADA) was first synthesized for the study of cannabinoid receptors (Bisogno et al. 2000) and subsequently found to activate the vanilloid TRPV1 receptors (De Petrocellis et al. 2000). More recently, NADA was identified as an endogenous molecule in the mammalian nervous system occurring in several brain nuclei and the dorsal root ganglion (Huang et al. 2002). NADA activates TRPV1 with a potency that is 5 to 10 fold higher than that of any other endovanilloid proposed prior to its discovery (De Petrocellis et al. 2000; Huang et al. 2002; Tóth et al. 2003). Additionally, the affinity of NADA for CB1 receptors is in a similar (high-nanomolar) range to that of the endocannabinoid anandamide (Bisogno et al. 2000; Chu et al. 2003). It seems plausible that NADA may act as a potent endovanilloid and a capable endocannabinoid.

NADA may serve naturally to regulate pain sensitivity. It caused thermal hyperalgesia when administered peripherally (Huang et al. 2002), increased substance P and CGRP release in spinal cord slice, (Huang et al. 2002) and trigeminal cultures

(Price et al. 2004), depolarized dorsal root ganglion neurons, and suppressed firing of spinal neurons to mechanical stimuli (Sagar et al. 2004). In addition to influences on nociceptive processing, a range of other physiological effects have been reported. NADA enhanced paired-pulse depression in hippocampal slices (Huang et al. 2002), induced contractile responses in isolated bronchus and urinary bladder (Harrison et al. 2003), caused vasorelaxation (O'Sullivan et al. 2004, 2005), and inhibited activation of NFkB-dependent transcriptional activity in human T cells (Sancho et al. 2004). Most of these effects were capsaicin-like, though some were cannabinoid-like, and still others were suggested to be CB1 and TRPV1 independent. Moreover, it was shown that the potency and the efficacy of NADA could be affected by other factors such as the activity of protein kinase C (Huang et al. 2002; Premkumar et al. 2004). Hence it appears that NADA may participate in a wide array of physiological processes in a rather complex manner. The most salient action of NADA in tests of sensory function is the thermal hyperalgesia observed after peripheral administration, this occurring at sub-microgram doses (Huang et al. 2002). In the present study, we sought to elucidate the neurophysiological basis for this effect. Toward this end, we examined the effects of peripheral administration of NADA on spinal wide dynamic range neurons with the aim of determining the effect of NADA on spontaneous firing and responses to a thermal stimuli ranging from nonnoxious to noxious levels.

METHODS

Subjects

A total of 61 male Sprague-Dawley rats (300–470 g; Charles River, Boston, MA) were used to conduct the experiments described herein. All protocols were approved by the Brown University Institutional Animal Care and Use Committee.

Drug preparation and administration

NADA was obtained from Cayman Chemicals (Ann Arbor, MI) and BIOMOL International (Plymouth Meeting, PA). 5'-iodoresiniferatoxin (I-RTX) was obtained from Tocris Cookson (Ellisville, MO) and LC Laboratories (Woburn, MA). SR141716A was a gift from Sanofi Recherche (Montpellier, France). All drugs were dissolved in a vehicle of ethanol: emulphor: saline (1:1:18). In the antagonist studies, either I-RTX or SR 141716A was co-administered

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with NADA in the same solution. Drug(s) or vehicle (50 μ l) was injected (intraplantar) in the ipsilateral hindpaw receptive field of the neuron via a 30 gauge needle. Intraplantar doses of NADA (0.5, 1.5, 3, and 5 μ g) and the dose of I-RTX (1 μ g) employed were based on behavioral assays (Huang et al. 2002). The dose of SR 141716A (10 μ g) was based on its affinity for the CB1 receptor (K_i = 2 nM) (Rinaldi-Carmona et al. 1995) and has been demonstrated in previous studies to be sufficient for peripheral CB1 receptor antagonism (Kelly et al. 2003; Richardson et al. 1998).

Electrophysiological methods

Animals were anesthetized with urethan (1.25g/kg ip) and placed in a stereotaxic frame. Body temperature was maintained throughout the course of the experiment via a rectal probe and a heating pad. Laminectomy was performed over the T₁₂–L₂ vertebrae to expose the lumbar spinal cord. The spinal cord was stabilized with clamps fastened to the vertebral processes immediately rostral and caudal to the exposed spinal segment. The cord was bathed in warm mineral oil. A tungsten recording electrode (5–6 M Ω impedance, FHC, Bowdoinham, ME) was lowered to the spinal cord with a micromanipulator (Narashige, Tokyo, Japan). The electrode was advanced slowly into the cord within the depths of 100–1,000 μ m from the cord surface with a microdrive (Narashige, Tokyo, Japan) while the experimenter gently tapped the ipsilateral hindpaw to search for spinal neurons with receptive fields in the plantar surface of the paw. Action potentials were recorded extracellularly, digitized, and discriminated by the computer. The size and shape of the action potentials were monitored with the aid of a digital oscilloscope (Tektronix, Beaverton, OR) to ensure that only responses from single cells were recorded. Neurons were classified as wide dynamic range nociceptive neurons if they exhibited increasing firing rates to application of increasing intensity of mechanical stimulation (brush, pressure and pinch) to the hindpaw skin. Cells with high ongoing spontaneous discharges were excluded from the study due to problems associated with statistical analysis of heterogeneous populations.

Assessment of spontaneous firing

On identification of a nociceptive neuron with a receptive field on the plantar surface of the hindpaw, spontaneous firing rates were continuously recorded throughout the experiment. After ≥ 10 min of stable recording of predrug neural activity, NADA (5 μ g, 50 μ l, i.pl., n = 3) or vehicle (n = 3) was injected in the center of the receptive field, and postdrug firing was recorded without stimulation.

Assessment of heat-evoked firing

The effects of NADA on thermal stimuli were studied in separate groups of animals from those used for the study of the effects on spontaneous activity. Heat-evoked responses were elicited by applying radiant heat to the receptive field on the plantar surface of the paw. A beam of incandescent light was optically focused and directed at the center of the receptive field (skin blackened with a permanent marker to facilitate heat absorption). The intensity of the stimulation was computer controlled by a digital-to-analog converter connected to a voltage controlled DC power supply. The stimulator was adjusted to obtain increasing paw skin temperatures (from 34 to 52°C over 15 s). Data were acquired for 2 s prior to onset of the stimulus, 15 s during ramp-up, and 15 s after stimulus off-set. After characterization of the nociceptive neurons with mechanical brush, pressure, and pinch, their responsiveness to thermal stimulation was assessed. Cells were classified as heat-responsive nociceptive neurons and included in the study if they exhibited increased firing rates in response to increasing temperatures and if the maximal firing rate occurred at a temperature $>46^\circ\text{C}$. After the establishment of stable baseline responses (3 trials at 10-min intervals), NADA (5 μ g, n = 9; 3 μ g, n = 5; 1.5 μ g, n = 7; 0.5 μ g, n = 3), vehicle (n = 9), TRPV1 antagonist I-RTX 1 μ g +

5 μ g NADA (n = 5), CB1 antagonist SR 141716A 10 μ g + 5 μ g NADA (n = 5), 1 μ g I-RTX (n = 4), or 10 μ g SR 141716A (n = 4) was injected into the receptive field of the ipsilateral hindpaw. To account for possible changes in the contour of the paw surface from injection, periodic adjustments were made to maintain optimal focus of the light beam at low beam intensity. Postdrug heat-evoked responses were assessed at 10-min intervals for 90 min. Data from the three trials at 20, 30, and 40 min were analyzed as postinjection heat-evoked responses, and the three trials conducted prior to injection as baseline responses. Heat-evoked firing was taken as the response over the 30-s period post stimulus-onset. Injection of 5 μ g NADA (n = 4) into the paw contralateral to the recording site was also conducted to assess the possibility of a systemic action of the drug.

Recording site

The location of recording site for each neuron was reconstructed using the atlas of the rat nervous system (Paxinos and Watson 1986) based on the depth of electrode penetration into the cord and the distance of the electrode from the midline.

Statistical analysis

Data were analyzed by repeated-measures ANOVA with BMDP Statistical Software (SPSS, Chicago, IL). The Hyunh-Feldt (1976) correction was applied to interaction terms of repeated measures factors where applicable. The Tukey comparison was employed for post hoc analysis. The Mann-Whitney test was used to analyze data on spontaneous firing (n = 3/group). P < 0.05 was considered statistically significant. For the construction of the log dose-response curve, data points were fitted with a variable slope sigmoidal dose-response curve-fitting algorithm using Hill equation sometimes known as the four-parameter logistic equation (GraphPad Prism4, San Diego, CA)

$$Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{\left[1 + \left(\frac{10^{\text{LogEC}_{50}}}{10^x} \right)^{\text{HillSlope}} \right]}$$

Bottom was fixed as the firing rate under control conditions; top reflects the apparent maximum effect. Top, slope (*Hillslope*), and EC₅₀ values were derived from the algorithm. Analysis of the stimulus-response functions were conducted by fitting the linear portion of the stimulus-response function with linear regression, and nonoverlapping 95% confidence intervals were used to assess statistical significance (GraphPad Prism4, San Diego, CA).

RESULTS

A total of 61 neurons were recorded, one from each animal. The depth of the recording sites ranged 100–1,000 μ m from the dorsal surface of the spinal cord with a mean of 550 ± 33 (SE) μ m. Reconstruction of the recording sites indicated that all neurons were located in laminae I–V of the dorsal horn of the spinal cord, 41/61 were in laminae IV and V. All, except one, were in the inner portion of the gray matter. All cells included in the study exhibited characteristics of wide dynamic range nociceptive neurons, in that increased firing rates were observed with increased intensity of stimulus, either to mechanical (brush, pressure, pinch) or thermal (heat ramp) stimulation. The cells exhibited no or low spontaneous firing prior to drug treatment.

NADA evokes spontaneous firing in spinal nociceptive neurons

At 10 min after intraplantar injection of 5 μ g of NADA (n = 9), increased spontaneous activity of spinal nociceptive neu-

rons was observed compared with injection of vehicle when data from the prestimulus period were analyzed [mean = 10.7 ± 3.5 , 3.4 ± 0.8 Hz, respectively; $n = 9/\text{group}$; $F(3,48) = 4.00$, $P < 0.05$; Fig. 1A]. The effect dissipated by 20-min postinjection, after which time no significant difference in spontaneous firing was observed. Results from animals in which neural activity was continuously monitored without heat-ramp stimulation revealed that whereas vehicle injections caused a transient increase (<2 min) in firing, injection of NADA caused a sustained increase in spontaneous firing (>10 min). The effect was most prominent in the first 10 min immediately after injection into the hindpaw receptive field with mean firing rates of 17.8 ± 3.0 Hz compared with 5.0 ± 1.0 Hz for vehicle-injected group for most of this period ($P < 0.05$; $n = 3/\text{group}$; Fig. 1B)

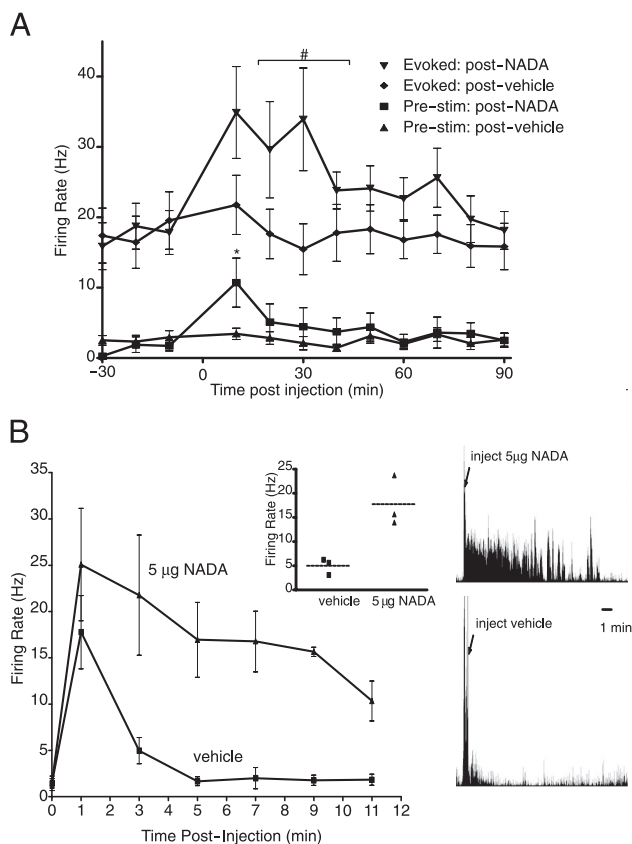


FIG. 1. A: peripheral administration of *N*-arachidonoyldopamine (NADA) increased spontaneous and heat-evoked activity of spinal nociceptive neurons. Spontaneous and heat-evoked firing was assessed at 10-min intervals prior to and post injection. Data presented are the mean firing rates over the 2-s period prior to stimulus onset for prestimulus (spontaneous) activity, and over the 30 s after stimulus onset for evoked-firing. NADA ($5 \mu\text{g}$) significantly enhanced the prestimulus activity of the neurons at 10 min after injection but not at later time points ($P < 0.05$). In contrast to prestimulus activity, a significant increase in heat-evoked firing could be observed at 20–40 min after injection of $5 \mu\text{g}$ NADA ($P < 0.05$). *, significant difference from postvehicle prestimulus response. #, significant difference from postvehicle heat-evoked response. B: continuous recording of spinal wide dynamic range neurons showing sustained increase in the spontaneous firing rate after intraplantar injection of $5 \mu\text{g}$ NADA but not after injection of vehicle ($P < 0.05$; $n = 3$ per group). Left inset: scatter plot showing the sample distribution and mean firing rates over the 1st 10 min after NADA or vehicle injection. Right inset: example histograms showing the activity of single neurons prior to and after intraplantar injection of $5 \mu\text{g}$ NADA (top) or $50 \mu\text{l}$ vehicle (bottom) into the receptive field in the hindpaw.

NADA enhances heat-evoked firing of spinal nociceptive neurons

Peripheral administration of NADA (i.pl., $50 \mu\text{l}$) dose-dependently increased the heat-evoked firing in spinal nociceptive neurons with an EC_{50} of $1.55 \mu\text{g}$ [$R = 0.999$; $F(4,28) = 2.95$, $P < 0.05$; Fig. 2] at 20–40 min after injection. Six of nine neurons exhibited a $>30\%$ increase in response at the $5 \mu\text{g}$ dose, 4/5 at $3 \mu\text{g}$, 3/7 at $1.5 \mu\text{g}$, 0/3 at $0.5 \mu\text{g}$, 1/9 at $0 \mu\text{g}$. An example neuron illustrating the enhancement of heat-evoked activity by NADA is shown in Fig. 3. This increased firing was not due to a systemic effect of the drug as when NADA was injected in the contralateral paw, the effect was similar to that observed after injection of vehicle in the ipsilateral paw. The mean differences in the overall number of action potentials during a stimulus trial compared with predrug baseline were -26 ± 44 and 25 ± 22 for ipsilateral injection of vehicle and contralateral injection of $5 \mu\text{g}$ NADA, respectively, versus 355 ± 136 for ipsilateral injection of $5 \mu\text{g}$ NADA.

After injection of NADA in the hindpaw, spinal nociceptive neurons responded more vigorously to thermal stimuli (Fig. 3). For example, at 20- to 40-min postinjection, the mean firing rates of these neurons to 48 and 50°C were 39.1 ± 7.6 and 55.2 ± 9.8 Hz for $5 \mu\text{g}$ NADA-injected animals versus 18.9 ± 3.5 and 34.2 ± 7.0 Hz for vehicle-injected animals. Moreover, spinal nociceptive neurons appeared to encode an increase in temperature with a disproportionately greater increase in firing. This is evident when stimulus-response functions over the noxious temperature range (46 – 52°C) were plotted. There was a significant difference in the slopes of the functions before and after drug injection (mean slope 682 ± 22.8 vs. 1138 ± 111 ; $n = 9$; $P < 0.05$ based on nonoverlapping 95% confidence intervals; Fig. 4A). This phenomenon was more prominent at the lower end of the noxious temperature range, possibly due to neurons achieving their maximal firing rates at the highest temperatures. The increased slope of the stimulus-response function produced by NADA was not observed with injection of vehicle (mean slope 694.7 ± 71.01 vs. 796.2 ± 102.9 ; $n = 9$; Fig. 4B).

The pharmacological bases for the effects described above were examined with TRPV1 and CB1 antagonists. Co-administration of $1 \mu\text{g}$ of the TRPV1 antagonist I-RTX with $5 \mu\text{g}$ NADA ($n = 5$) blocked the increase in overall firing induced by injection of NADA alone [$F(1,12) = 5.96$, $P < 0.05$; Fig. 5]. By contrast, the effect of administration of I-RTX alone ($n = 4$) was no different from injection of vehicle. Conversely, co-administration of $10 \mu\text{g}$ of the CB1 antagonist SR141716A with $5 \mu\text{g}$ NADA ($n = 5$) did not block the NADA-induced enhancement in firing (Fig. 5). Firing rates after SR141716A alone ($n = 4$) did not differ significantly from those observed after administration of vehicle.

DISCUSSION

Peripheral administration of NADA enhanced the firing rates of spinal nociceptive neurons, both in the absence of any external stimuli and in response to thermal stimuli in the periphery. The enhanced heat-evoked firing was dose dependent with an EC_{50} of $1.55 \mu\text{g}$. NADA-induced neural hypersensitivity was TRPV1 dependent as co-administration of the TRPV1 antagonist I-RTX abolished the effect. In contrast,

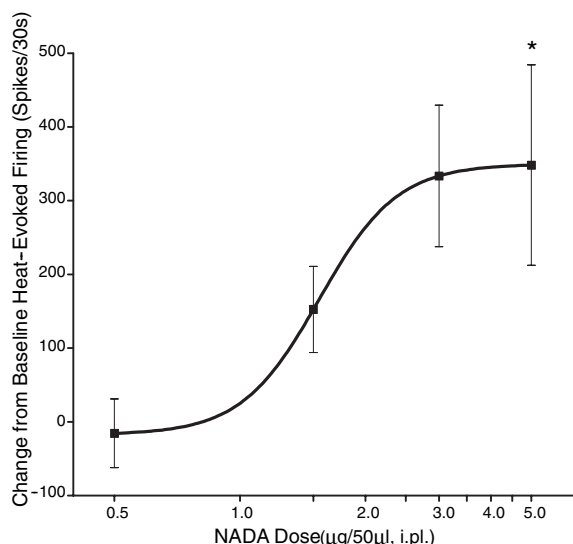


FIG. 2. Peripheral administration of NADA dose-dependently enhanced heat-evoked firing in spinal nociceptive neurons. NADA was injected into the receptive field of heat-responsive dorsal horn nociceptive neurons in the ipsilateral hindpaw of anesthetized rats. Firing over the 30 s from the onset of the heat ramp as a change from predrug (baseline) firing was analyzed. Increasing concentrations of NADA caused neurons to respond with increasing vigor with an estimated EC_{50} of $1.55 \mu\text{g}$ ($R = 0.999$). Significant difference from vehicle indicated by $*$ ($P < 0.05$; $5 \mu\text{g}$ $n = 9$, $3 \mu\text{g}$ $n = 5$, $1.5 \mu\text{g}$ $n = 7$, $0.5 \mu\text{g}$ $n = 3$, $0 \mu\text{g}$ $n = 9$).

co-administration of the cannabinoid CB1 receptor antagonist SR141716A did not block this response.

The findings in this study paralleled our previous observation of behavioral thermal hyperalgesia from peripheral injection of NADA (Huang et al. 2002). The potency of NADA-induced enhancement of neural firing was similar to that for behavioral thermal hyperalgesia (EC_{50} of 1.55 vs. $1.53 \mu\text{g}$, respectively). Moreover, the reversal of NADA-induced enhanced firing of nociceptive neurons by I-RTX agrees with the reversal of NADA-induced thermal hyperalgesia by capsaizipine and I-RTX in the behavioral study (Huang et al. 2002). The matching dose dependency and pharmacology suggest that the changes observed in the firing properties of spinal nociceptive neurons underlie the enhanced behavioral nocifensive responses to thermal stimuli.

The data in this study are in agreement with previous studies which reported NADA-evoked increase in intracellular calcium levels in DRG neurons (Huang et al. 2002) and NADA-evoked release of substance P and CGRP in spinal cord slices and trigeminal neuronal culture in a TRPV1-dependent (Huang et al. 2002; Price et al. 2004) and CB1-independent (Price et al. 2004) manner. When taken as a whole, the evidence suggests that NADA, when present in the vicinity of peripheral or spinal sensory neurons, facilitates pain sensitivity predominantly via a TRPV1 mechanism. TRPV1 has been reported to be present on central and peripheral terminals of primary afferent neurons and skin keratinocytes (Denda et al. 2001; Guo et al. 1999; Szallasi et al. 1995; Tominaga et al. 1998). Even though CB1 receptors are also present on peripheral nerves (Hohmann and Herkenham 1999; Sanudo et al. 1999), we did not find NADA-induced hypersensitivity to be mediated by CB1 receptors, evident by the lack of antagonism with SR141716A.

Sagar et al. (2004) found that NADA depolarized and increased intracellular calcium levels in cultured DRG neu-

rons. However, they found that the effects were blocked by both TRPV1 and CB1 antagonists. Sagar et al. (2004) also found that peripheral administration of NADA suppressed firing of spinal neurons to mechanical stimuli and that this suppression was blocked by CB1 antagonist but not TRPV1 antagonist when the stimulus was in the innocuous range and vice versa when the stimulus was in the noxious range of mechanical pressure. It seems that the consequence of NADA on nociceptive neurons may differ depending on the mode of stimulation perhaps leading to thermal hyperalgesia accompanied with mechanical insensitivity, although no behavioral data on the effects of NADA on mechanical stimuli have been reported to date. Alternatively, the population of neurons that were recorded in this study may be different from that reported by Sagar et al. (2004). It is also possible that NADA exerts a complex pattern of effects on nociceptive processing as Bisogno et al. (2000) reported analgesia in mice after systemic administration, and Sancho et al. (2004) recently reported NADA-mediated inhibition of IL-2 and NF κ B transcription in T cells, suggesting a role of NADA in immunosuppression and anti-inflammation possibly via a TRPV1- and CB1-independent mechanism.

The stimulus-response functions indicated that the neural responses to thermal stimuli were magnified after peripheral administration of NADA. The increase in spontaneous discharges, which occurred immediately after injection of NADA, may in whole or part establish the delayed sensitized response to thermal stimuli. Enhanced spontaneous and/or evoked ac-

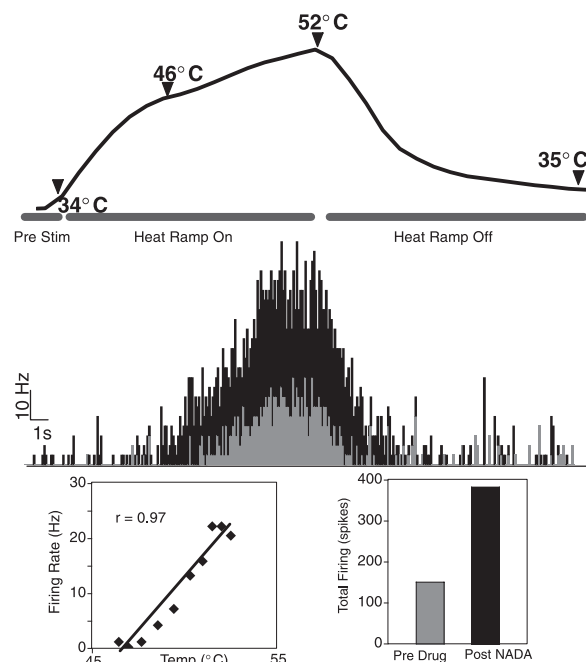


FIG. 3. Example of the response of a single dorsal horn nociceptive neuron to thermal stimulation. *Top*: plot of the temperatures delivered to the hindpaw over the 32 s of data collection (2 s prestimulus, 30 s poststimulus onset). The temperature of the skin was increased from 34°C to 52°C over 15 s. *Middle*: histograms depicting the mean firing rates of the neuron over the 3 trials prior to (\square) and at 20–40 min after (\blacksquare) injection of $5 \mu\text{g}$ NADA in the receptive field in the ipsilateral hindpaw. *Bottom left*: plot of firing rate by log stimulus intensity (46 to 52°C) demonstrating the systematic encoding of increasing temperatures ($R = 0.97$) which formed the basis for classifying the neuron as a heat responsive wide-dynamic range neuron. *Bottom right*: overall firing over the 32 s period for the 3 predrug trials vs. the 3 postdrug trials at 20–40 min.

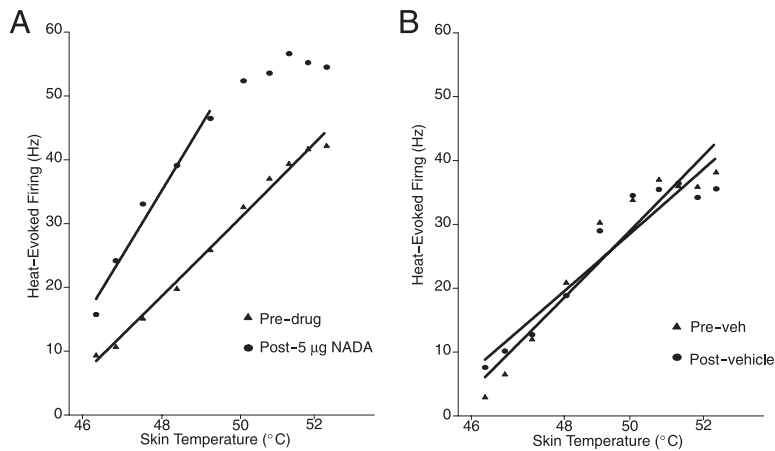


FIG. 4. Stimulus-response functions of spinal nociceptive neurons over the noxious temperature range (46–52°C). *A*: injection of vehicle did not change the firing rates or affect the stimulus response function of these neurons ($n = 9$). *B*: neurons showed a linear relationship in firing rate to stimulus intensity (log) prior to injection. After injection of 5 µg NADA in the hindpaw receptive field, enhanced firing to thermal stimuli was observed ($n = 9$). Not only was there elevated firing at a given temperature, but there was also an increase in the slope of the stimulus-response function (nonoverlapping 95% confidence intervals). At the higher temperatures the neurons appeared to have reached their maximal firing rates after injection of NADA.

tivity in spinal neurons is often observed after peripheral application of pro-inflammatory or algescic agents such as Freund's Adjuvant, formalin, carrageenan, and histamine (e.g., Carstens 1997; Dickenson and Sullivan 1987; Menetrey and Basson 1982; Torsney and Fitzgerald 2002). Administration of the TRPV1 antagonist capsaizepine attenuated pain behavior, spinal c-fos expression, and spinal nociceptive responses in carrageenan- and formalin-induced inflammatory pain models (Kelly and Chapman 2002; Kwak et al. 1998; Santos and Calixto 1997). TRPV1-knockout mice exhibit impaired sensitivity to noxious thermal pain and fail to develop thermal hyperalgesia in mustard-oil-induced, Freund's adjuvant-induced, and carrageenan-induced inflammation (Caterina et al. 2000; Davis et al. 2000). Furthermore, PKC isoforms are upregulated and found to be involved in chronic pain (Aley et al. 2000; Martin et al. 1999) and sensitize TRPV1 receptors (Cesare and McNaughton 1996; Cesare et al. 1999; Numazaki et al. 2002; Olah et al. 2002; Premkumar and Ahern 2000; Tominaga et al. 2001; Vellani et al. 2001). Several cellular effects of NADA have been shown to be potentiated by activators of PKC (Huang et al. 2002; Premkumar et al. 2004),

and rapid repeated exposure to NADA potentiated cellular responses (Premkumar et al. 2004). Hence our results are consistent with a predominantly pro-inflammatory and -nociceptive role of NADA at peripheral sites and the notion of possible involvement of endogenous NADA acting via TRPV1 in nociceptive neuronal sensitization.

In summary, the present study examined the neurophysiological basis for the thermal hyperalgesic effects of peripherally administered NADA. The increased spontaneous firing and the hypersensitivity of spinal nociceptive neurons and the TRPV1 dependency are consistent with the behavioral thermal hyperalgesia observed in our previous study (Huang et al. 2002). The results suggest a possible pain-sensitizing role of endogenous NADA mediated by TRPV1 in the periphery.

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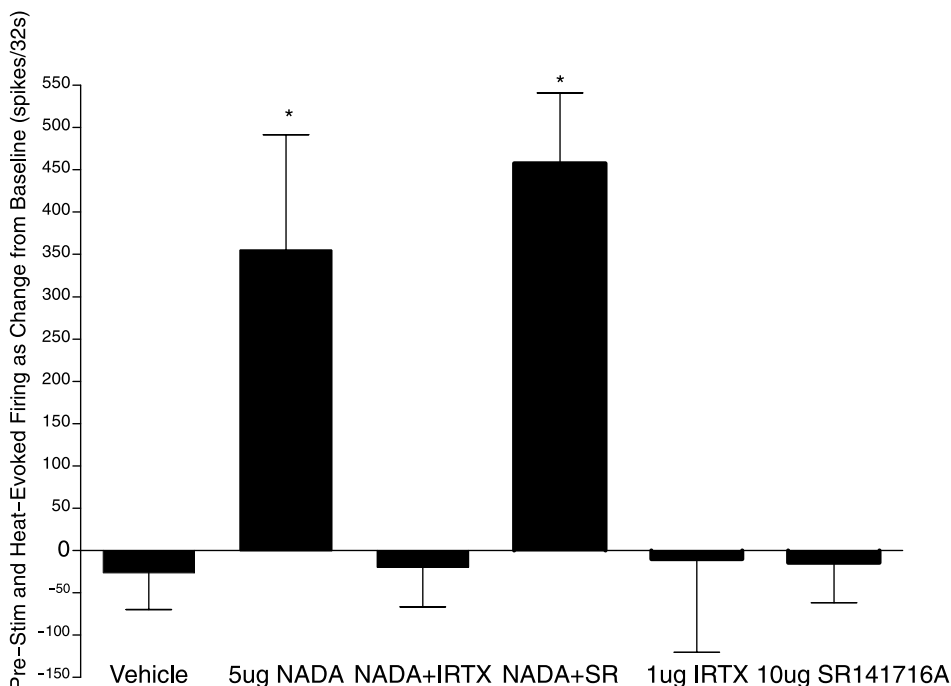


FIG. 5. NADA-induced enhanced firing of spinal nociceptive neurons was TRPV1 dependent and CB1 independent. Injection of 5 µg NADA in the hindpaw (50 µl, i.pl.) increased the overall (prestimulus and heat-evoked) firing of spinal nociceptive neurons. Co-administration of the TRPV1 antagonist I-RTX (1 µg) in the paw completely blocked the enhanced firing ($n = 5$). Co-administration of the CB1 antagonist SR 141716A (10 µg) in the paw did not attenuate the response ($n = 5$). *, significant difference from vehicle ($P < 0.05$).

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