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Attenuation of Ascending Nociceptive Signals to the Rostroventromedial Medulla Induced by a Novel α_2-Adrenoceptor Agonist, MPV-2426, following Intrathecal Application in Neuropathic Rats

Antti Pertovaara, M.D., Ph.D., Hong Wei, B.M.†*

Background: **In the current study, the potency and spread of the antinociception induced by MPV-2426, a novel α_2-adrenoceptor agonist, was characterized in neuropathic and non-neuropathic animals.**

Methods: **Neuropathy was induced by unilateral ligation of two spinal nerves in the rat. After lumbar intrathecal or systemic administration of MPV-2426, thermally and mechanically evoked responses of nociceptive neurons of the rostroventromedial medulla were recorded during pentobarbitone anesthesia. To obtain a behavioral correlate of neurophysiologic findings, nocifensor reflex responses evoked by thermal and mechanical stimuli were assessed in unanesthetized neuropathic and control animals.**

Results: **After intrathecal administration, MPV-2426 and dexmedetomidine produced a dose-related antinociceptive effect, independent of the submodality of the noxious test stimulus or the pathophysiologic condition. This antinociceptive effect was spatially restricted to the inputs from the lower half of the body, and it was reversed by atipamezole, an α_2-adrenoceptor antagonist. After systemic administration in non-neuropathic animals, MPV-2426 had no antinociceptive effect on responses to rostroventromedial medulla neurons, whereas systemically administered dexmedetomidine produced a dose-related suppression of nociceptive signals to the rostroventromedial medulla, independent of the site of test stimulation. In a behavioral study, intrathecal MPV-2426 produced a dose-dependent suppression of nocifensor responses evoked by noxious mechanical or heat stimuli, whereas systemic administration of MPV-2426 had no effects.**

Conclusions: **Intrathecal MPV-2426 has spatially limited antinociceptive properties in neuropathic and non-neuropathic conditions because of its action on spinal α_2-adrenoceptors. These properties may be advantageous when designing therapy for spatially restricted pain problems. (Key words: α_2-Adrenergic analgesia; antihyperalgesia; experimental neuropathy.)**

α_2-ADRENOCEPTOR agonists have antinociceptive effects.[1–3] This antinociception is in part caused by action on spinal α_2-adrenoceptors. In contrast, the sedative side-effects of α_2-agonists are predominantly a result of supraspinal actions.[4] An α_2-adrenergic compound that diffuses poorly through the blood–brain barrier and within the central nervous system might provide an effective and spatially restricted antinociception with minor side-effects after intrathecal administration. For this purpose, a new α_2-adrenergic compound, MPV-2426, was recently developed from the highly selective and potent α_2-adrenoceptor agonist dexmedetomidine.

The rostroventromedial medulla (RVM) is considered an important relay structure for pain signals. The role of the RVM in descending feedback control of spinal nociception is well-established.[5,6] Nociceptive neurons in the RVM typically have large receptive fields, often covering the whole body,[7] indicating that each RVM neuron receives ascending somatosensory input from a large population of peripheral and spinal neurons. Thus, RVM neurons provide the possibility to study, within single neurons, the spatial distribution of the antinociceptive effects induced by intrathecal application of analgesic compounds in the lumbar level.[8] Additionally, because RVM neurons receive convergent input from various types of primary afferent fibers *via* the spinal dorsal horn, RVM neurons provide the possibility to study the submodality dependence of the antinociceptive effect.

* Professor in Physiology, Department of Physiology, Institute of Biomedicine, University of Turku.

† Research Fellow, Department of Physiology, Institute of Biomedicine, University of Helsinki.

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Address reprint requests to Dr. Pertovaara: Department of Physiology, Institute of Biomedicine, POB 9, University of Helsinki, FIN-00014 Helsinki, Finland. Address electronic mail to: Pertovaa@penger.helsinki.fi

Finally, when the experimental animal has a unilateral neuropathic condition, the study of RVM neurons allows comparison within single neurons of the drug effects on nociceptive input from the control limb with that from the neuropathic limb.

In the current investigation, we attempted to characterize the antinociceptive properties of the novel α_2-adrenoceptor agonist MPV-2426. This was done by comparing the antinociceptive effects of intrathecally administered MPV-2426 *versus* dexmedetomidine on responses of nociceptive RVM neurons. To study submodality dependence and spatial spread of antinociception, the neuronal responses were evoked by noxious mechanical and heat stimuli applied to various body parts. To assess the potency of studied drugs in an experimental neuropathic condition, we tested the drug both in rats with a unilateral neuropathic condition and in sham-operated rats. The central antinociceptive effects on RVM neurons were also determined after systemic administration. Furthermore, to determine the behavioral relevance of neuronal findings, the antinociceptive effects of MVP-2426 were assessed in a corresponding behavioral study.

Materials and Methods

This study was approved by the Institutional Animal Care Committee of the University of Helsinki. Experiments were performed on 250- to 350-g male Hannover-Wistar rats (Finnish National Laboratory Animal Center, Kuopio, Finland). However, three of the animals tested in electrophysiologic experiments with intrathecal MPV-2426, were 250- to 300-g male Sprague-Dawley rats (B & K, Sollentuna, Sweden). Because no difference in results were observed between these two strains of animals, the data were pooled.

Techniques for Providing Neuropathy

The unilateral ligation of two spinal nerves (L5 and L6) was performed during pentobarbital anesthesia (50 mg/kg intraperitoneally; Mebunat, Orion Pharma, Turku, Finland) as described in detail previously.[9] Of the operated rats, only those with unilateral allodynia to mechanical stimulation (hind limb withdrawal thresholds in the operated side < 7 g) were selected for further electrophysiologic and behavioral studies with intrathecal drug administrations (neuropathic group). In one group of rats tested with intrathecal drug administration, a sham operation was performed (*i.e.*, the spinal nerves were exposed but not ligated [sham group]; $n = 4$). The antinociceptive effect of systemically administered MPV-2426 and dexmedetomidine was studied in unoperated, non-neuropathic control rats (unoperated control group).

Electrophysiologic Recordings

Two to three weeks after spinal nerve ligation, the rats ($n = 12$) were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and placed in a standard stereotaxic frame according to the atlas of Paxinos and Watson.[10] Anesthesia was maintained by infusing pentobarbital (15–20 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}) with a Harvard-22 infusion pump. The level of anesthesia was frequently monitored by observing the size of the pupils and by assessing withdrawal responses to noxious pinch. When necessary, the infusion rate of pentobarbital was increased. The rats were spontaneously breathing. A warming blanket was used to maintain body temperature within the physiologic range. Peripheral perfusion was checked by evaluating the color of the ears and extremities. An intrathecal catheter (PE-10) was administered for drug applications into the lumbar level of the spinal cord. In some of the animals the catheter was inserted through the atlantooccipital membrane,[11] and in others the catheter was inserted using a direct lumbar catheterization method.[12] The skull was exposed and a hole drilled for placement of recording electrode in the RVM. The desired recording site in the RVM was 2.0–2.3 mm posterior from the ear bar, 0.10 mm lateral from the midline, and 9.0–11.0 mm ventral from the dura mater. The electrophysiologic single-neuron recording and characterization of neurons was performed as described in detail previously.[8] Briefly, unit activity was recorded extracellularly with lacquer-coated tungsten electrodes (tip impedance 5–10 MOhm at 1 kHz) and then amplified and filtered using standard techniques. The signal was fed through a window discriminator (F. Haer Inc., Bowdoinham, ME) to a rate monitor (bin width, 0.2 s; F. Haer) and to a timed counter (F. Haer). The ratemeter recordings were observed on a digital storage oscilloscope (Tektronix DS420, Portland, OR) and on hard copies of the data from the oscilloscope screen. Only RVM neurons giving an increase in response both to heat and mechanical stimuli within the noxious range were studied further. This cell type of the RVM has properties similar to a neuron type called "ON-neuron," the activation of which presumably contributes to triggering of nocifensor reflexes.[6] Actually, in some of the recordings ($n = 4$) performed during a lowered level of anesthesia,

this was verified by a simultaneous recording of a hind limb withdrawal reflex with a piezo-electric device; the RVM neurons giving accelerated responses to noxious stimuli started their discharge prior to hind limb withdrawal.

Noxious Stimuli

Noxious heat stimuli (peak stimulus temperature, 54°C; baseline temperature, 35°C; rate of stimulus temperature rise, 10°C/s; duration of peak temperature, 5 s) were applied with a feedback-controlled Peltier device[13] to the glabrous skin of each hind paw. A modified clamp producing a pressure of 200 g/0.5 cm^2 was used to deliver noxious mechanical stimulation of 5 s duration to the tail, to one digit in each hind paw, and to one digit in a forepaw. When the pinch was applied to the control hind limb repeatedly at 1-min intervals, the difference in neuronal response evoked by the same stimulus was 6.8 ± 11.7% (n = 8). When applied to the experimenter's skin, the clamp produced a strong painful sensation that was comparable to the intensity of pain produced by the noxious heat stimulus of 54°C in the glabrous skin of the experimenter's hand.

Behavioral Study

Behavioral assessment of antinociceptive effects induced by intrathecal MPV-2426 was performed in neuropathic rats only (N = 8). Prior to actual testing, the rats were habituated to the experimental conditions for 2 days preceding the testing. Tail-flick response latency was determined by applying radiant heat with a commercially available stimulator (Socrel DS-20, Ugo Basile, Varese, Italy) to the dorsal surface of the tail. The latency to the tail-flick was electronically measured. A cutoff latency of 9 s was imposed. Two latency measurements at a 1-min interval were performed at each time point, and the mean of these latencies was used in further calculations.

Heat-induced hind limb withdrawal latency was determined by dipping the hind paw into a hot water bath (48°C) up to the knee level until the rat withdrew its hind paw (hot water-immersion test). A cutoff latency of 30 s was imposed. The left and the right hind paw were consecutively tested twice at each time point, and the average of these latencies for each hind paw was used in further calculations.

Tactile allodynia was determined by applying calibrated monofilaments (Stoelting, Wood Dale, IL) of increasing forces to the foot pad of the neuropathic limb in a rat standing on a metal grid (monofilament test).[14] The lowest force producing hind limb withdrawal was considered the threshold. At each time point, two threshold determinations were performed.

The hind limb withdrawal threshold induced by noxious mechanical stimulation (paw-pressure test) was determined with a Basile Analgesy-meter (Ugo Basile, Varese, Italy). With this device, a mechanical force was consecutively applied to the right and left hind paw. During each measurement, the stimulus force increased at a constant rate (32 g/s), and the force producing a withdrawal was determined in grams. A cutoff force of 500 g was imposed. Two threshold determinations for each hind paw were performed at each time point, and the mean of these thresholds was used in further calculations. In addition, the antinociceptive effect of systemically administered MPV-2426 was determined in non-neuropathic animals (n = 4) using the tail-flick and paw pressure tests.

Drugs

MPV-2426 or 3-(1H-imidazol-4-ylmethyl)-indan-5-ol hydrochloride, a novel α_2-adrenoceptor agonist, and dexmedetomidine, also an α_2-adrenoceptor agonist, were obtained from Orion Pharma. For intrathecal injections with a 50-μl Hamilton syringe, the drugs were dissolved in sterile water to obtain the necessary injection volume of 10 μl (followed by 10–15 μl physiologic saline, depending on the length of the catheter, for flushing the catheter). For systemic injections, the injection volumes of MPV-2426 and dexmedetomidine were 0.2–0.4 ml. Atipamezole, an α_2-adrenoceptor antagonist (Antisedan 5 mg/ml, Orion Pharma), was used at a dose of 1 mg/kg subcutaneously to reverse the MPV-2426- or dexmedetomidine-induced effects. Physiologic saline was used as a control vehicle.

Course of the Electrophysiologic Study

After determination of the receptive field characteristics of the RVM neuron, the predrug responses to noxious mechanical and thermal stimuli were determined twice to ascertain the reproducibility of the responses. When studying the effect of intrathecally administered MPV-2426 or dexmedetomidine in neuropathic rats, noxious pinch of 5 s duration was applied consecutively to the tail, each hind paw, and one forepaw. Noxious heat stimuli of 5 s duration were applied to each hind paw. The order of testing of thermal and mechanical stimulation was varied among the animals. After determination of predrug responses in neuropathic animals, MPV-2426 or dexmedetomidine was applied intrathecally in a cu-

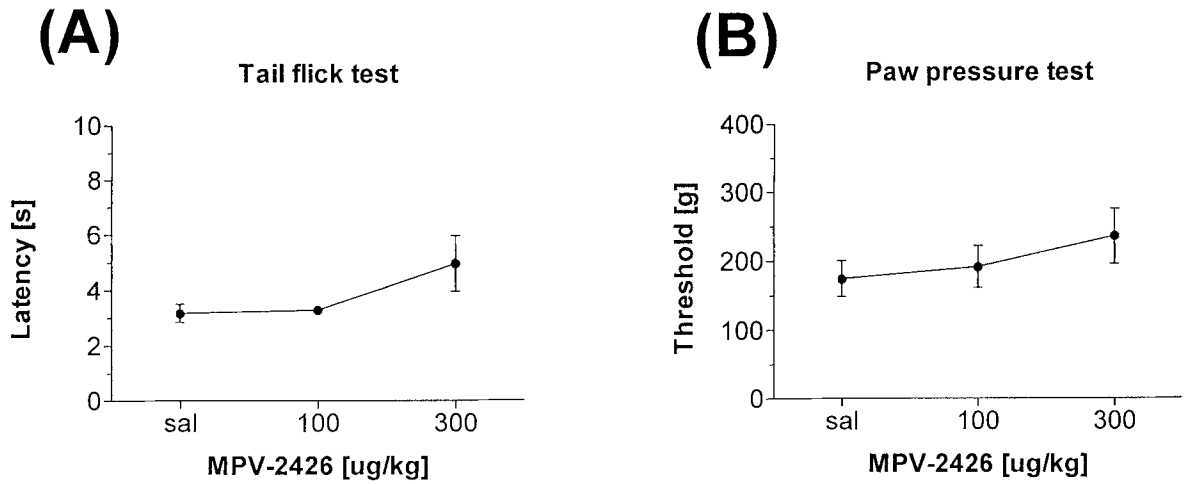

Fig. 1. Effect of systemically administered MPV-2426 or saline on (*A*) the heat-evoked tail-flick response and (*B*) the hind limb withdrawal induced by noxious mechanical stimulation of the paw in unoperated control rats. The drug-induced effects were determined 20–25 min after the subcutaneous injections. The error bars represent ± SEM (n = 4 at all data points).

mulative fashion (1 μg followed by an additional dose of 2 μg), after which 1 mg/kg atipamezole was administered subcutaneously. Determination of postdrug responses started 5 min after the end of each drug administration. The interval between drug injections was 12–15 min. In sham-operated animals only, the time course of the antinociception induced by a single 3-μg dose of MPV-2426 was determined at 10-min intervals for 30 min.

When studying the effects of systemically administered MPV-2426 and dexmedetomidine in unoperated nonneuropathic rats, the study protocol was as described previously with the following exceptions. First, noxious thermal and mechanical stimuli were delivered only to one hind paw. Second, the cumulative drug doses were MPV-2426 100 and 300 μg/kg subcutaneously and dexmedetomidine 30 and 100 μg/kg subcutaneously. Third, after systemic drug injections, the determination of postdrug responses started 10 min after drug administration. At the completion of the study, an electrolytic lesion was made in the recording site to verify that the recording was made within the RVM. The recording sites were within the raphe magnus nucleus and the adjacent nucleus paragigantocellularis pars α at a rostrocaudal level that was between the genu of the seventh nucleus and the inferior olive.

Course of the Behavioral Study

The assessment of pain behavior in unanesthetized neuropathic rats was performed using four tests (order of testing at each time point: monofilament test, hot-water immersion test, tail-flick test, and paw-pressure test) before intrathecal drug injection and 10, 20, 30, and 60 min after the intrathecal injection of drug or saline. The complete testing procedure at each time point took about 5 min. In the results, the actual time point used for each test is reported. The effects of saline (control) and MPV-2426 at doses of 1, 3, and 10 μg were assessed in behavioral tests.

Statistics

One- or two-way analysis of variance (ANOVA) was used to assess the effects of drugs on neuronal responses. However, because the reversal of drug-induced effects by atipamezole was not performed in all occasions (*e.g.*, because of loss of neurons during recordings), ANOVA was performed without the atipamezole condition. In behavioral tests, parametric statistics (one- or two-way ANOVA followed by Tukey test) were used to assess drug-induced effects in the tail-flick test, paw-pressure test, and hot-water immersion test. Nonparametric statistics (Kruskal–Wallis or Friedman's test followed by Mann–Whitney or Wilcoxon's test, respectively) were used to assess drug-induced changes in the monofilament test. $P < 0.05$ was considered to represent a significant difference.

Results

Behavioral Study

Subcutaneous Administration of MPV-2426. The antinociceptive effect of subcutaneously administered

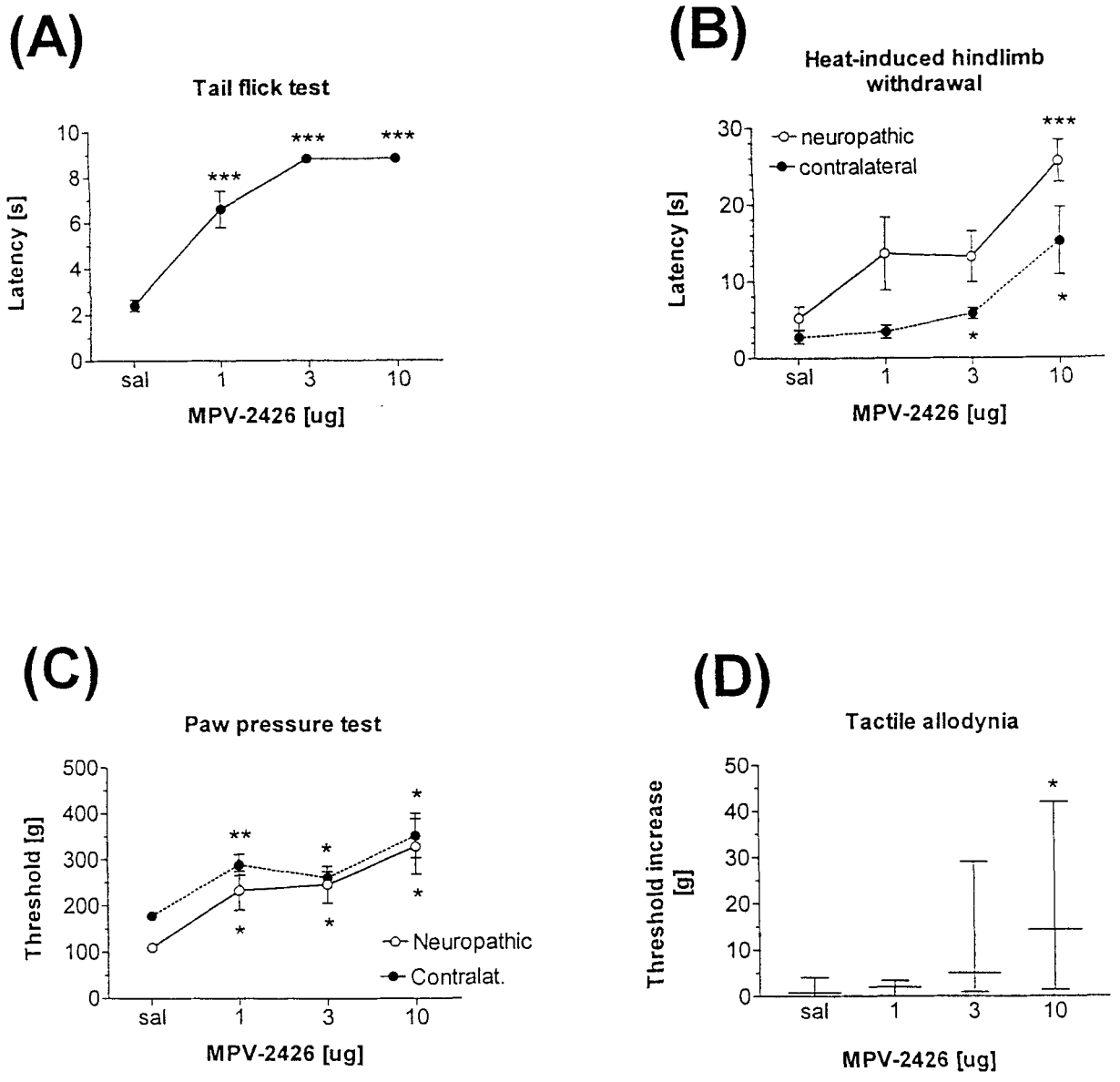

Fig. 2. Effect of intrathecally administered MPV-2426 or saline (control) in unanesthetized, neuropathic rats. (*A*) The mean latencies of the heat-evoked tail-flick. (*B*) The mean latencies of the heat-evoked hind limb withdrawal. (*C*) The mean hind limb withdrawal thresholds induced by noxious mechanical stimulation of the paw. (*D*) The median hind limb withdrawal thresholds of the neuropathic limb induced by innocuous mechanical stimulation of the paw. The drug-induced effects were determined 20–25 min after injections. The error bars in *A–C* represent ± SEM, and in *D* they represent the range. At all data points with MPV-2426 n = 5 and with saline n = 4. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ (in *A–C*, Tukey test; reference value, saline; in *D*, Mann–Whitney U test).

MPV-2426 was behaviorally assessed in non-neuropathic animals. MPV-2426 (100–300 μg/kg) had no significant effect on the radiant heat-induced tail-flick latency ($F_{2,11} = 2.7$; one-way ANOVA; fig. 1A) or on hind limb withdrawal induced by noxious mechanical stimulation of the paw ($F_{2,11} = 0.93$; one-way ANOVA; fig. 1B).

Intrathecal Administration of MPV-2426. The antinociceptive effect of intrathecal MPV-2426 was behaviorally assessed in unanesthetized neuropathic rats. Before drug treatment, the rats had a marked tactile allodynia and a significant mechanical hyperalgesia in the neuropathic limb, whereas hot-water immersion test

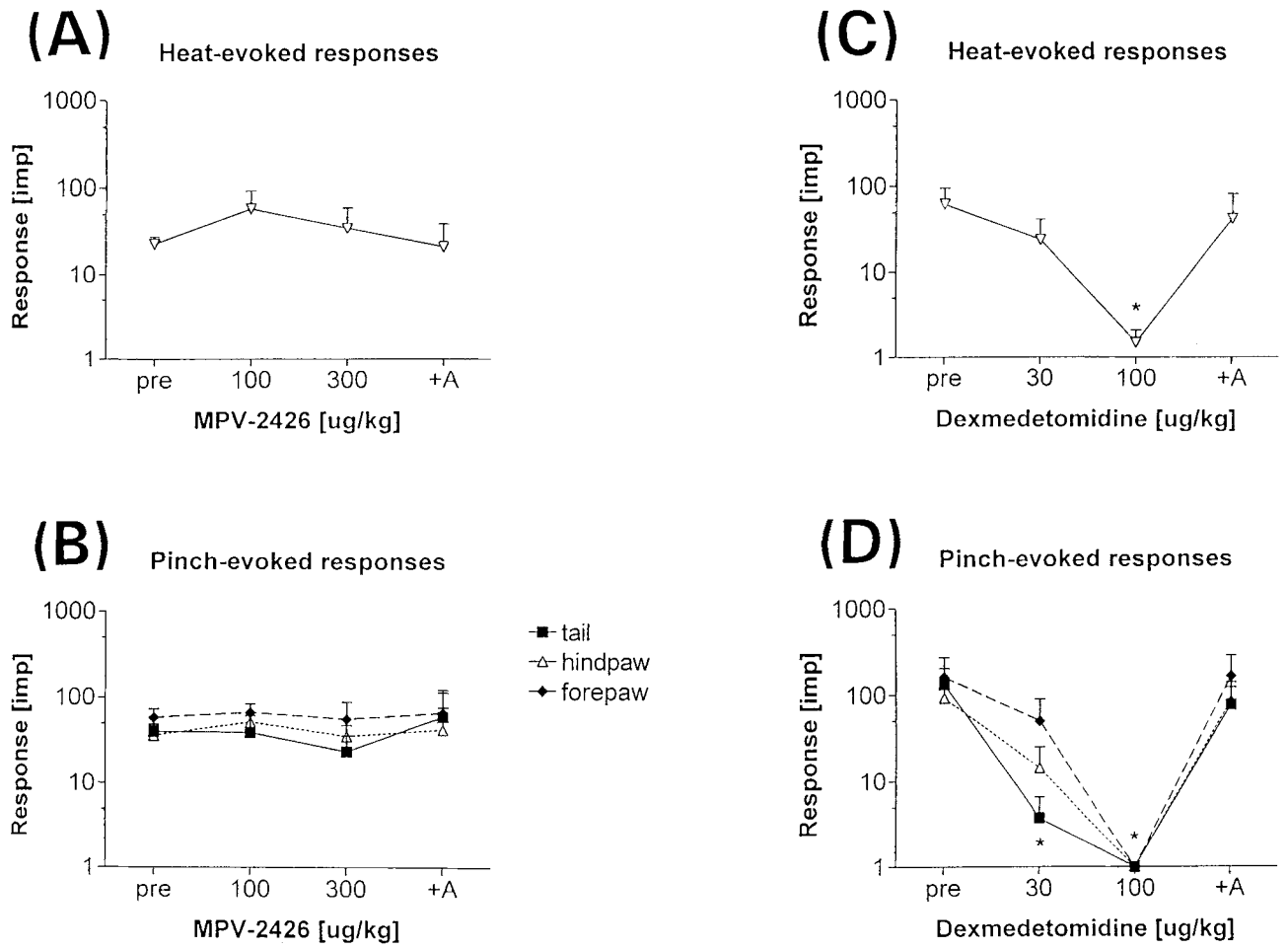

Fig. 3. Average responses of rostroventromedial medulla (RVM) neurons after systemic administrations of (*A, B*) MPV-2426 or (*C, D*) dexmedetomidine in unoperated, non-neuropathic animals. (*A, C*) Noxious heat-evoked responses to stimulation of the hind limb. (*B, D*) Noxious pinch-evoked responses to stimulation of various body parts. Error bars represent SEM. In each graph and time point, n = 4. +A = +atipamezole (1 mg/kg subcutaneously). *$P < 0.05$ (Mann–Whitney U test; ref, the corresponding prevalue).

revealed no significant heat hyperalgesia in the neuropathic hind limb.

In the tail-flick test, intrathecal MPV-2426 produced a dose-dependent antinociception ($F_{3,18} = 40.74$; $P < 0.0001$; one-way ANOVA; fig. 2A). Heat-induced hind limb withdrawal was also dose-dependently attenuated by intrathecal MPV-2426 ($F_{3,30} = 10.82$; $P < 0.0001$; two-way ANOVA; fig. 2B), and this antinociceptive effect was significantly stronger on the neuropathic side ($F_{1,30} = 12.99$; $P < 0.002$; two-way ANOVA), independent of the drug dose ($F_{3,30} = 0.76$).

Hind limb withdrawal response induced by noxious mechanical stimulation was dose-dependently attenuated by intrathecal MPV-2426 ($F_{3,30} = 10.36$; $P < 0.0001$; two-way ANOVA), and this attenuation was of the same magnitude in the neuropathic and control limbs ($F_{1,30} = 2.68$; not significant; two-way ANOVA; fig. 2C). In the test of tactile allodynia, Kruskal–Wallis ANOVA indicated that the main effect of drug dose on the monofilament-induced withdrawal threshold was significant (Kruskal–Wallis ANOVA = 8.717; $P < 0.04$; fig. 2D). Friedman ANOVA indicated that the main effect of time in the test of tactile allodynia was significant at 3- and 10-μg doses of MPV-2426 (*e.g.*, 3 μg, Friedman ANOVA = 13.642; $P < 0.01$; data not shown).

Electrophysiologic Study

Systemic Drug Administrations in Nonneuropathic Animals. Cumulative administration of MPV-2426 at doses of 100–300 μg/mg subcutaneously

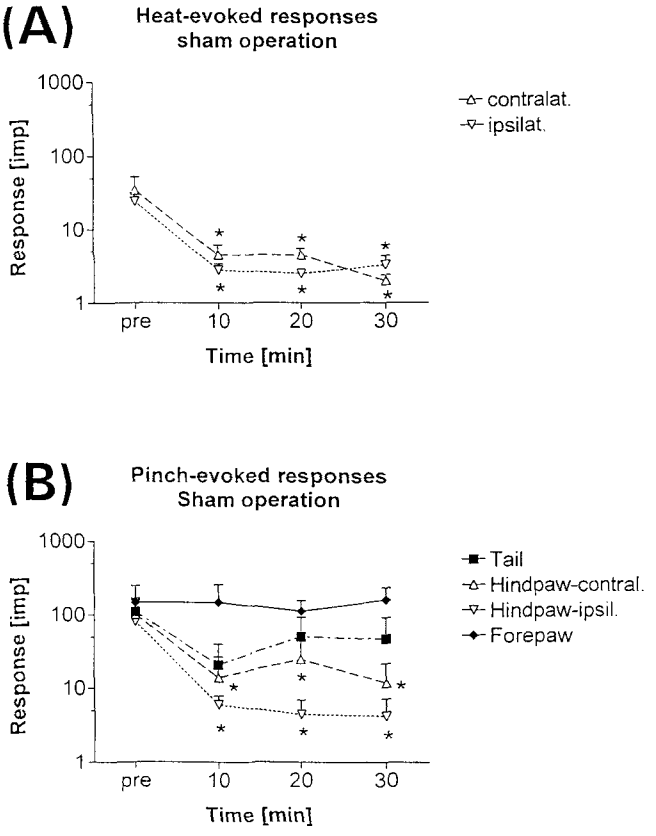

Fig. 4. Average responses of rostroventromedial medulla (RVM) neurons after intrathecal administration of MPV-2426 in sham-operated, non-neuropathic rats. In each graph and time point, n = 4. For other explanations, see the legend for figure 3.

did not produce any significant change in the pinch- or heat-evoked responses to nociceptive RVM neurons (figs. 3A and 3B). Independent of the test stimulus site, cumulative administration of dexmedetomidine at doses of 30–100 μg/kg subcutaneously produced a significant dose-related attenuation of pinch- and heat-evoked responses to nociceptive RVM neurons (figs. 3C and 3D). The antinociception induced by systemically administered dexmedetomidine was completely reversed by atipamezole (1 mg/kg subcutaneously).

Intrathecal Administration of MPV-2426 in Sham-operated Rats. In sham-operated rats, the time course of antinociception induced by 3 μg of MPV-2426 after intrathecal administration was assessed. Within 10 min, MPV-2426 induced a significant bilateral antinociceptive effect on neuronal responses evoked by thermal stimulation of the hind paws (fig. 4). The attenuation of pinch-evoked responses by MPV-2426 was dependent on the site of stimulation ($F_{3,48} = 4.23$; $P < 0.01$); that is, only response from the hind limbs were significantly attenuated during the 30-min observation period. The antinociceptive effect lasted at least 30 min (the maximum observation period).

General Observations in Neuropathic Animals. All neurons selected for the study gave accelerating (on-) responses to noxious pinch and noxious heat but not to stimuli that were in the innocuous range. The responses evoked from the neuropathic hind limb were not significantly different from responses evoked from the contralateral limb, independent of the submodality (Mann-Whitney U test). Moreover, the magnitudes of responses evoked by mechanical and thermal stimulation of the hind limb were not significantly different (Mann-Whitney U test). The mean spontaneous discharge of RVM neurons of 3.1 ± 6.1 Hz (n = 13) in neuropathic animals was not significantly higher than that in non-neuropathic animals (2.3 ± 4.8 Hz; n = 12; *t* test).

Intrathecal Administration of MPV-2426 in Neuropathic Animals. MPV-2426 produced a significant dose-related antinociceptive effect for both thermally and mechanically induced responses to nociceptive RVM neurons (figs. 5 and 6). The MPV-2426-induced suppression of heat-evoked responses was significant ($F_{2,38} = 4.89$; $P < 0.02$; two-way ANOVA) and was not dependent on the side of test stimulation (neuropathic *vs.* control hind limb; $F_{1,38} = 1.34$; not significant; fig. 6A). The effect of intrathecal MPV-2426 on pinch-evoked responses was also significant ($F_{2,76} = 2.9$; $P < 0.05$) and dependent on the site of test stimulation ($F_{3,76} = 3.55$; $P < 0.02$). The pinch-evoked responses from both hind limbs (fig. 6B) were significantly suppressed after MPV-2426, whereas the pinch-evoked responses from the tail or the forepaw were not significantly changed by MPV-2426 in the dose range used (1–3 μg). Independent of the submodality of the test stimulus or the test side, the antinociception induced by MPV-2426 was completely reversed by atipamezole (1 mg/kg subcutaneously).

Intrathecal Administration of Dexmedetomidine in Neuropathic Animals. Dexmedetomidine produced a dose-related (1–3 μg intrathecally) antinociceptive effect on heat- and pinch-evoked responses (figs. 6C and 6D). The significant suppression of heat-evoked responses from the hind limbs ($F_{2,24} = 3.47$; $P < 0.05$) was not dependent on the side of stimulation ($F_{1,24} = 0.02$; not significant). According to two-way ANOVA, the effect of intrathecal dexmedetomidine on pinch-evoked responses was highly significant ($F_{2,48} = 7.75$; $P < 0.002$). This dexmedetomidine-induced antinociceptive effect was not dependent on the site of stimulation

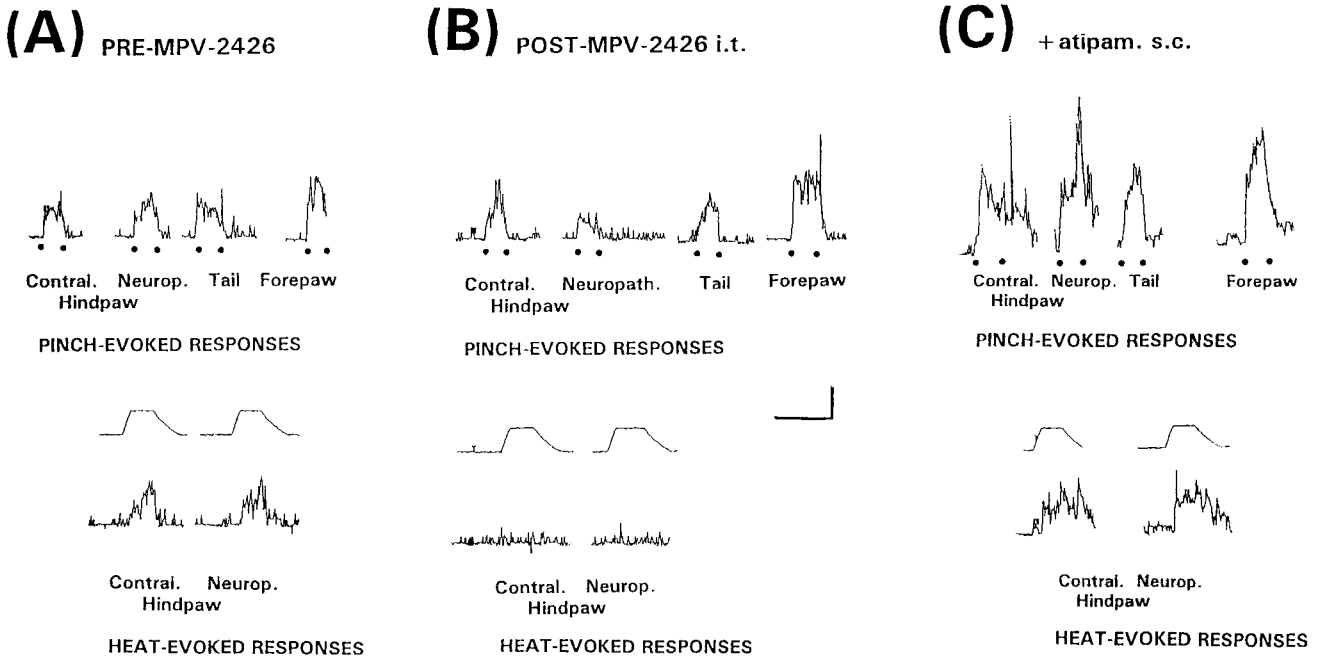

Fig. 5. Ratemeter recordings of a single rostroventromedial medulla (RVM) neuron in various experimental conditions in a neuropathic animal. (*A*) Before application of the drug. (*B*) After administration of MPV-2426 (1 μg intrathecally). (*C*) After administration of atipamezole (1 mg/kg subcutaneously). (*Upper*) Represents responses evoked by noxious pinch. (*Lower*) Represents responses evoked by noxious heat. In each graph, ratemeter recording of the response is shown. (*Upper*) The dots indicate the onset and offset of the pinch. (*Lower*) The temperature change in the thermode (from 35 to 54°C and back) is shown above the ratemeter recordings. The horizontal calibration bar represents 10 s, and the vertical one represents 25 Hz.

($F_{3,48}$ = 1.43; not significant; two-way ANOVA), although the forepaw-induced responses appeared to be only slightly attenuated when compared with the dexmedetomidine-induced suppression of pinch-evoked responses from the hind limbs or tail. The dexmedetomidine-induced antinociception was completely reversed by atipamezole (1 mg/kg subcutaneously).

Discussion

Suppression of Neuronal Responses by MPV-2426

The current neurophysiologic results indicate that administration of MPV-2426 and dexmedetomidine to the lumbar spinal cord has a significant suppressive effect on ascending nociceptive responses to the RVM neurons evoked by both thermal and mechanical stimuli. The antinociception induced by intrathecally administered MPV-2426 and dexmedetomidine was significant for signals both from the neuropathic and control side. Additionally, the suppression of responses to nociceptive RVM neurons induced by intrathecal MPV-2426 was equally effective in neuropathic and in sham-operated animals. No marked differences in the antinociceptive potencies between intrathecally administered MPV-2426 and dexmedetomidine were observed. However, the antinociception induced by intrathecally administered MPV-2426 was spatially more restricted than that induced by intrathecally administered dexmedetomidine. Moreover, systemic administrations revealed another significant difference in the antinociceptive effects between these compounds. After systemic administration, MPV-2426 had no effect on nociceptive responses to RVM neurons, whereas subcutaneous dexmedetomidine produced a strong, dose-related antinociceptive effect that was independent of the submodality or the site of stimulation. The antinociceptive effects induced by MPV-2426 as well by dexmedetomidine were determined to be the result of action on α_2-adrenoceptors because atipamezole, an α_2-adrenoceptor antagonist, reversed their antinociceptive effects. Furthermore, the antinociception induced by MPV-2426 was caused by a spinal action because systemic administration outside of the blood–brain barrier was without effect and because the antinociception induced by MPV-2426 administered into the spinal cord was spatially restricted to the lower

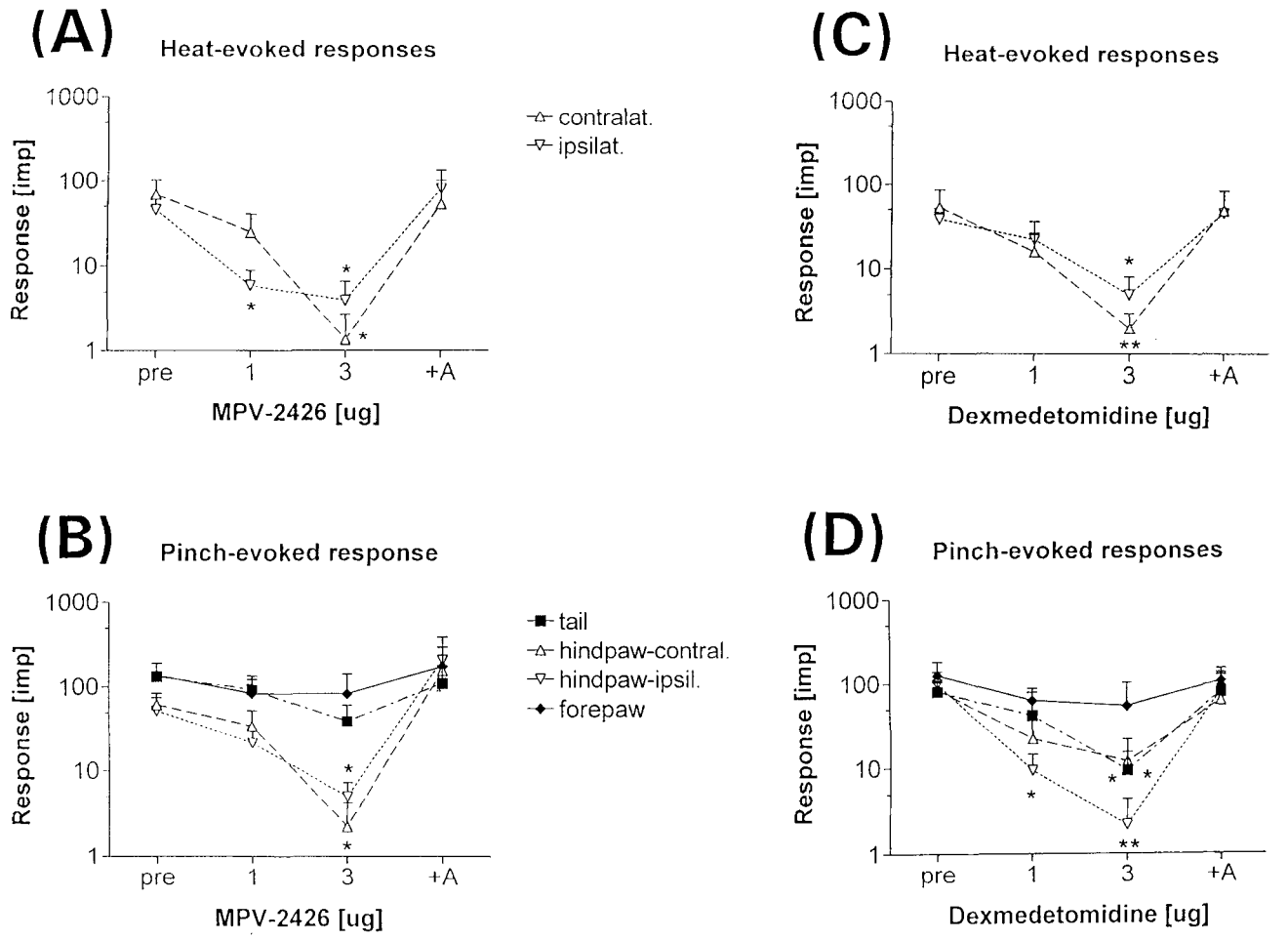

Fig. 6. Average responses of rostroventromedial medulla (RVM) neurons after intrathecal administration of (*A, B*) MPV-2426 or (*C, D*) dexmedetomidine in neuropathic animals. (*A, C*) Noxious heat-evoked responses to stimulation of hind limbs. (*B, D*) Noxious pinch-evoked responses to stimulation of various body parts. +A = +atipamezole (1 mg/kg subcutaneously); contralat. = contralateral to neuropathy; ipsilat. = neuropathic side; pre = response before drug administration. The error bars represent SEM (in *A* and *B*: pre and 1 μg, n = 7; 3 μg, n = 5; +A, n = 4; in *C* and *D*: n = 5 at all drug doses). *$P < 0.05$, **$P < 0.01$ (Mann–Whitney U test; ref, the corresponding prevalue).

half of the body. This finding corresponds with the extensive literature describing attenuation of nociceptive responses to spinal dorsal horn neurons after direct spinal administration of various α_2-adrenoceptor agonists.[1-3]

Comparison of Neurophysiologic and Behavioral Findings

The most prominent behavioral symptom produced by ligation of two spinal nerves was allodynia and hyperalgesia to mechanical stimulation. In contrast to some previous studies with this model,[9] but in agreement with two other recent studies,[15,16] no significant heat hyperalgesia was observed, indicating that the development of heat hyperalgesia is variable and is dependent on various experimental parameters that differ among laboratories.

The current behavioral findings on the effect of intrathecally and subcutaneously administered MPV-2426 correspond to a large extent with the electrophysiologic observations. In general, mechanically and thermally evoked behavioral and neuronal responses from the hind limbs were attenuated by intrathecal but not subcutaneous MPV-2426. The most prominent difference between behavioral and neuronal findings was seen in the tail-flick responses. The heat-induced tail-flick was strongly attenuated by intrathecal MPV-2426, whereas the effect of MPV-2426 on nociceptive responses to RVM neurons evoked by noxious mechanical stimulation of the tail

was not significant. This discrepancy suggests that the spatial spread of antinociception induced by intrathecal MPV-2426 may be larger for heat-evoked than for mechanically evoked responses. Furthermore, it should be noted that systemic drug administrations were performed only in non-neuropathic animals. Therefore, we cannot exclude the possibility that systemically administered MPV-2426 might have an action in neuropathic animals that is different from that in non-neuropathic ones.

Role of RVM Neurons in Neuropathy

Although all animals receiving intrathecally administered drugs had a behaviorally verified mechanical allodynia (hind limb withdrawal threshold < 7 g), the responses from the neuropathic side were not significantly different from those evoked from the contralateral (non-neuropathic) side independent of the submodality. In agreement with this finding, constriction injury-induced mononeuropathy did not induce a significant change in the electrically evoked responses to nociceptive neurons of the bulboreticular neurons (including the RVM),[17] whereas transection of the sciatic nerve produced an enhancement of responses to nociceptive neurons in or adjacent to the RVM evoked from the innervation area of the neighboring intact nerve.[18] These findings suggest that the stimulus-evoked responses to the RVM do not significantly contribute to the hyperalgesia induced by spinal nerve ligation or chronic constriction of the sciatic nerve, whereas RVM neurons may have a more important role in adjacent hyperalgesia induced by deafferentation. Conversely, because tonic inactivation of the RVM has produced a selective attenuation of tactile allodynia in the currently used model of experimental neuropathy,[19] it is possible that a tonic descending influence from the RVM disinhibits or facilitates spinal circuitry, mediating enhanced reflex responses to tactile stimulation in neuropathic animals.

It should be noted that the nociceptive input to the RVM neurons ascends from a subpopulation of spinal dorsal horn neurons.[20] Thus, the results of the current study may not be applicable to nociceptive signals ascending into some other supraspinal structures such as the thalamus, hypothalamus, or parabrachial nucleus. Additionally, it should be noted that the electrophysiologic recordings were performed during anesthesia, and general anesthesia may significantly influence the response properties of RVM neurons.[21]

The results indicate that intrathecal administration of MPV-2426 and dexmedetomidine attenuates ascending nociceptive signals from neuropathic and from non-neuropathic limbs to the RVM because of action on spinal α_2 receptors. Because of its pharmacokinetic properties, MPV-2426 has a limited spatial distribution within the central nervous system and through the blood-brain barrier. This characteristic may be of benefit in targeting the therapeutic (antinociceptive) action of MPV-2426 to the desired site. There were no differences in the responses to nociceptive RVM neurons evoked from the neuropathic *versus* the non-neuropathic limb. This finding suggests that the phasic response properties of RVM neurons do not explain the hyperalgesia and allodynia induced by spinal nerve ligation, although the possibility remains that descending influence from or adjacent to the RVM may tonically contribute to development or maintenance of neuropathic symptoms.

Addendum: The new name for MPV-2426 is radolmidine.

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