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European Journal of Pharmacology 485 (2004) 11–20
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Yohimbine inhibits firing activities of rat dorsal root ganglion neurons by blocking Na^+ channels and vanilloid VR1 receptors

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Received 19 June 2003; received in revised form 10 November 2003; accepted 13 November 2003

Abstract

Yohimbine, an indole alkaloid, is a natural α_2-adrenoceptor antagonist and is frequently used to assess the mechanism of a drug's effect on α-adrenoceptors. Recently, several studies showed that yohimbine exhibited analgesic effects in in vivo animal models. However, the underlying mechanism is not known. We investigated the effects of yohimbine on Na^+ channels and vanilloid VR1 receptors in dorsal root ganglion cells. We found that yohimbine inhibited tetrodotoxin-sensitive Na^+ channels ($Na_V1.2$), the tetrodotoxin-resistant Na^+ channels, including both slow inactivating ($Na_V1.8$) and persistent ($Na_V1.9$) Na^+ channels, and capsaicin-sensitive vanilloid VR1 receptors. Action potential firing activities of dorsal root ganglion neurons evoked by current injection or capsaicin were eliminated by yohimbine. The blocking effects of yohimbine on nociceptive-related ion channels and firing activities of dorsal root ganglion neurons may underlie the ionic mechanism of yohimbine's analgesic effects observed in in vivo studies.

Keywords: Yohimbine; Na^+ channel; Vanilloid VR1 receptor; Dorsal root ganglion; Action potential; Firing activity

1. Introduction

Yohimbine, an indole alkaloid obtained either from the bark of the tree *Pausinystalia yohimbe* or from the root of *Rauwolfia* and initially identified as an aphrodisiac, was introduced in the treatment of erectile dysfunction more than 70 years ago (Hunner, 1926). It is a natural α_2-adrenoceptor antagonist (MacDonald et al., 1997) and is the only α-adrenoceptor antagonist available for the treatment of erectile dysfunction (Filippi et al., 2002). In addition, another application of yohimbine has been proposed recently. In elderly subjects, the lipolytic response to a β-adrenoceptor agonist has been shown to be markedly diminished by the application of yohimbine, whereas the anti-lipolytic response to an α_2-adrenoceptor agonist is fully preserved (Lonnqvist et al., 1990; McCarty, 2002). It is thus suggested that pre-exercise treatment with yohimbine might be of particular value for overweight elderly subjects (McCarty, 2002). Besides clinical applications, yohimbine has been frequently used to assess the involvement of α_2-adrenoceptors in the mechanism of a drug's action.

Yohimbine is relatively selective for the α_2-adrenoceptor (Goldberg and Robertson, 1983). The affinity of yohimbine for the α_{2C}-adrenoceptor is 5-fold greater than that for the α_{2B}-adrenoceptor and 30-fold greater than that for the α_{2A}-adrenoceptor (MacDonald et al., 1997). It has been published that α_2-adrenoceptor agonists produce significant analgesic effects (Furst, 1999) in both animals and humans. For example, clonidine, an α-adrenoceptor agonist, produces analgesia on its own and also potentiates analgesia produced by opiates (Wilcox et al., 1987). The mechanism by which α_2-adrenoceptor agonists exert their antinociceptive effects is thought, at least in part, to be through a presynaptic modulation of primary afferent fibres that convey the nociceptive messages to the spinal cord (Gouarderes et al., 1993; Guyenet et al., 1994). As expected, yohimbine, an α_2-adrenoceptor antagonist, eliminated or attenuated the analgesic effects of an α_2-adrenoceptor agonist (Yaksh et al., 1995). Interestingly, several studies have shown that yohimbine could not completely eliminate clonidine's analgesic effect (Dennis et al., 1980; Tasker and Melzack, 1989).

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doi:10.1016/j.ejphar.2003.11.039

For example, in the formalin test, Tasker and Melzack (1989) showed that yohimbine at a concentration of 2.0 mg/kg only partially antagonized the antinociceptive effects produced by clonidine (Tasker and Melzack, 1989). Furthermore, yohimbine (1.0 and 4.0 mg/kg) administrated alone produced statistically significant antinociceptive effects (Dennis et al., 1980). Those results raise the possibility that yohimbine itself might produce its analgesic effect through mechanisms other than blockade of adrenoceptors. However, the mechanism(s) whereby yohimbine produces its antinociceptive effects in the formalin test in rats is not fully understood. Since yohimbine also has an affinity for 5-HT_{1A} receptors (Winter and Rabin, 1992), Shannon and Lutz (2000) showed that the antinociceptive effect of yohimbine was mediated in part by yohimbine's agonistic action at 5-HT_{1A} receptors. However, other possible mechanisms might exist besides yohimbine's actions on 5-HT_{1A} receptors.

In a study evaluating the effects of alkaloids on voltage-gated Na^+ channels, it was shown that yohimbine had some effects on Na^+ channels in skeletal muscle cells (Korper et al., 1998). This suggests that the direct effect of yohimbine on nociceptive related ion channels may underlie its antinociceptive mechanism. In this study, we have investigated whether yohimbine has any effects on pain sensation and transduction related ion channels such as vanilloid VR1 receptors (Caterina et al., 1997, 2000; Caterina and Julius, 1999, 2001; Davis et al., 2000; Santicioli et al., 1993; Szallasi et al., 1999; Szolcsanyi, 1987) and voltage-gated Na^+ channels (Akopian et al., 1999; Baker and Wood, 2001; Dib-Hajj et al., 2002; Waxman et al., 1999, 2000) in rat dorsal root ganglion neurons. We found that yohimbine not only blocked tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ channels, but also inhibited capsaicin-induced currents recorded in dorsal root ganglion neurons. More importantly, action potential firing activities elicited by current injection or capsaicin treatment were eliminated by yohimbine. These results may account for the antinociceptive effects of yohimbine.

2. Material and methods

2.1. Dissociation and culture of dorsal root ganglion neurons

The animal protocol for this study was approved by the Neurogen Corporation Animal Care and Use Committee. Newborn (1–5 days old) Sprague–Dawley rats (Charles River) were decapitated, and dorsal root ganglions were rapidly removed from spinal cord and put in ice cold media containing Dulbecco's modification of Eagle's medium (DMEM) (Life Technologies, Rockville, MD), 10% fetal bovine serum (Gibco), and antibiotics (10,000 units penicillin G, 25 μg/ml amphotericin B, and 10,000 μg/ml streptomycin). Dorsal root ganglions were then enzymatically digested by shaking at 35 °C in the dissociation solution containing 50,000 units/ml trypsin, 1 mg/ml collagenase, and 0.1 mg/ml DNase for approximately 1.5 h. Single dorsal root ganglion cell suspensions were obtained by gentle trituration and the final dissociated cells were plated onto poly-D-lysine-coated coverslips and were cultured in dorsal root ganglion culture media containing DMEM, 10% fetal bovine serum, 10,000 units penicillin G, 25 μg/ml amphotericin B, 10,000 μg/ml streptomycin, and 50 ng/ml nerve growth factor (NGF). Electrophysiological experiments were performed approximately 24 h after dissociation.

2.2. Brain II stable cell line

Rat brain type II Na^+ channel construct ($Na_V1.2$) was kindly provided by Dr. A Goldin (Department of Microbiology and Molecular Genetics, University of California, Irvine, CA). The stable expression of this Na^+ channel construct was established in human embryonic kidney (HEK) 293 cells (ATCC) using 500 μg/ml G418 as the selection agent. Individual clones were then picked from the initial selection pool. The expression of Na^+ channels in the selected clone was confirmed using whole-cell recordings. The expressed Na^+ current is sensitive to low concentration of tetrodotoxin and has biophysical properties consistent with the published data (data not shown).

2.3. Electrophysiology

2.3.1. Voltage-clamp recordings

Voltage-clamp recordings were made in the whole-cell mode with either Axopatch-200B or Axopatch-1D amplifiers (Axon Instruments, Foster City, CA). Recording electrodes were pulled from borosilicate pipettes (World Precision Instruments) on a horizontal puller (Sutter Instrument Model P-87) and had resistances ranging from 1 to 4 MΩ when filled with the internal solution. Voltage protocols were generated by using pClamp 8 (Axon Instruments) software. Data were digitized at 10 or 20 kHz and recorded to a PC. Data was analyzed using Clampfit (Axon Instruments) and Origin softwares (Microcal). Pipette potentials were zeroed before seal formation. Liquid junction potentials were not corrected. The capacitance of dorsal root ganglion neurons in our study was less than 30 pF. Series resistance (R_s) was less than 10 MΩ and compensated up to 90% to reduce series resistance errors. Cells with a series resistance greater than 10 MΩ were discarded. Internal solution for voltage-clamp recording contained (in mM): 130 CsF, 5 EGTA, 10 NaCl, 5 Mg-ATP, 1 Na-GTP, 10 HEPES, and pH = 7.3 adjusted with CsOH. To reduce the Na^+ current amplitude (Trezise et al., 1998), we used low Na^+ external solution for dorsal root ganglion Na^+ current recordings. This solution contained (in mM): 60 NaCl, 80 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH = 7.4 adjusted with NaOH). $CdCl_2$ (200 μM) was used

to block Ca^{2+} currents. To isolate tetrodotoxin-resistant currents, 100 nM tetrodotoxin was included in the bath solution. The external solution used for brain II Na^+ current recordings contained (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH = 7.4 adjusted with NaOH). Ca^{2+}-free external solution was used for vanilloid VR1 receptor current recording, containing (in mM): 140 NaCl, 5 KCl, 2 EGTA, 2 $MgCl_2$, 10 HEPES, and 10 glucose (pH = 7.4 adjusted with NaOH). The osmolarity for all above solutions was approximately 300 mOsm/l. All chemicals are from Sigma, unless otherwise stated.

2.3.2. Current clamp

Cells were also current-clamped in the whole-cell configuration. Recording electrodes (1–4 MΩ) were filled with intracellular solution containing (in mM): 120 K-aspartate, 5 Mg-ATP, 1 Na-GTP, 15 KCl, 1 $MgCl_2$, 5 NaCl, 5 EGTA, 10 HEPES (pH = 7.3 adjusted with KOH). Cells were bathed in the external solution. Recordings were made from cells with a stable resting potential of less than −55 mV. In some cells, spontaneous action potentials were occasionally recorded in the absence of injected current. In the majority of cells, action potentials were induced either by injection of sustained (1 s) depolarizing currents or by application of 100 nM capsaicin. All experiments were conducted at room temperature (22 ± 2 °C).

2.4. Statistical analysis

Pooled data are presented as means ± S.E.M. Statistical comparisons were made using two-way analysis of variance (ANOVA) and two-tailed *t* test with Bonferroni correction; $P < 0.05$ indicated statistical significance.

3. Results

3.1. Effects of yohimbine on tetrodotoxin-resistant Na^+ currents

Dorsal root ganglion neurons contain multiple types of Na^+ channels (Renganathan et al., 2002). We first focused our efforts on testing yohimbine's effects on tetrodotoxin-resistant Na^+ channels, which could be separated from tetrodotoxin-sensitive Na^+ channels by the application of tetrodotoxin. In our experiments, 100 nM tetrodotoxin was used to eliminate tetrodotoxin-sensitive Na^+ currents. A low Na^+ bath solution was applied to reduce the current amplitude in order to minimize voltage-clamp errors. Renganathan et al. (2002) reported that there are two different tetrodotoxin-resistant components in dorsal root ganglion neurons, slow inactivating and persistent. A depolarizing prepulse could be used to inactivate the persistent Na^+ current (Renganathan et al., 2002), and, thus, the slow inactivating Na^+ current could be isolated. In our experiments, both slow inactivating and persistent tetrodotoxin-resistant Na^+ currents were observed. For example, Fig. 1A shows slow inactivating Na^+ currents elicited by depolarization to various test potentials following a −50 mV prepulse. The current–voltage (*I–V*) relationship curve of this slow inactivating tetrodotoxin-resistant Na^+ current is shown in Fig. 1B. In a few cells, however, persistent tetrodotoxin-resistant Na^+ currents were dominant. One such example is shown in Fig. 1C. Persistent tetrodotoxin-resistant Na^+ currents were activated at test potentials as negative as −70 mV and peaked at approximately −20 mV (Fig. 1D). This is consistent with the kinetics of persistent tetrodotoxin-resistant Na^+ currents as previously reported (Renganathan et al., 2002). Application of yohimbine blocked both slow inactivating tetrodotoxin-resistant (Fig. 1B) and persistent tetrodotoxin-resistant (Fig. 1D) Na^+ currents. Due to the complexity in separating these two tetrodotoxin-resistant Na^+ currents, however, no further

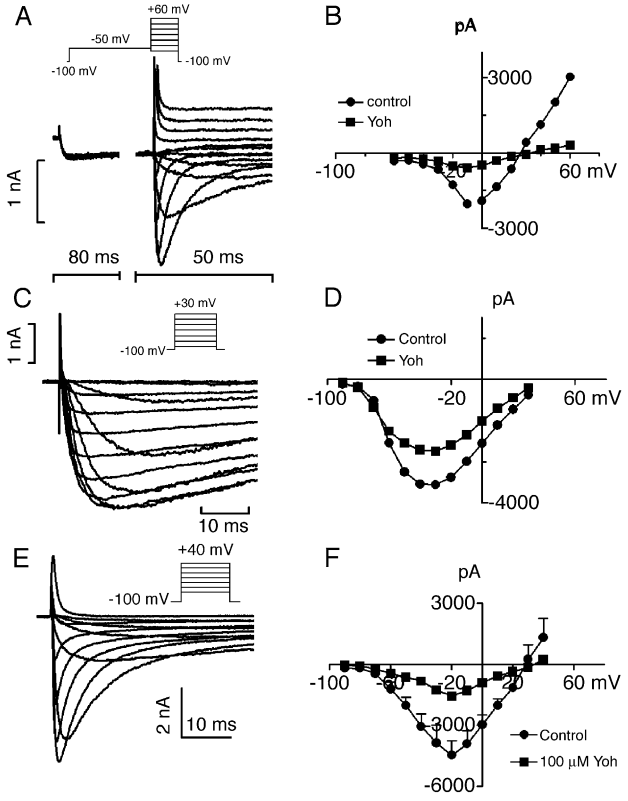

Fig. 1. Tetrodotoxin-resistant Na^+ currents in rat dorsal root ganglion neurons recorded in the presence of 100 nM tetrodotoxin. (A) Slow inactivating tetrodotoxin-resistant Na^+ currents elicited following a-50 mV prepulse. (B) Current–voltage (*I–V*) relationship obtained from the recording shown in A before (filled circle) and after (filled square) 100 μM yohimbine application. (C) Na^+ currents elicited by depolarizing from −90 to +30 mV. Holding potential = −100 mV. Note the predominant component of tetrodotoxin-resistant Na^+ current in this cell is persistent. (D) *I–V* obtained from the recording shown in C before (filled circle) and after (filled square) 30 μM yohimbine application. (E) Typical recording of Na^+ currents in the presence of 100 nM tetrodotoxin. (F) Averaged *I–V* curve of tetrodotoxin-resistant Na^+ currents (mean ± S.E.M, $n = 5$ cells) before (filled circle) and after (filled square) 100 μM yohimbine application.

attempt was made to differentiate them and the effects of yohimbine on the total tetrodotoxin-resistant Na^+ currents were evaluated. Fig. 1E shows a total tetrodotoxin-resistant Na^+ current recorded in the presence of 100 nM tetrodotoxin. The averaged $I–V$ for tetrodotoxin-resistant Na^+ currents from five cells is shown in Fig. 1F. Under our experimental conditions, tetrodotoxin-resistant Na^+ currents peaked at approximately 0 mV and reversed at approximately 30 mV, which is close to the estimated reversal potential of 42 mV (Hille, 2001). Yohimbine, at a 100 μM concentration, blocked peak current amplitude by 64.4% (Fig. 1F).

To investigate if the effect of yohimbine on tetrodotoxin-resistant Na^+ currents was state-dependent, we used two different recording protocols, one to test the tonic block (Fig. 2A–B) and the other to assess the use-dependent block (Fig. 2C–D). Using the tonic protocol, the Na^+ current was activated by stepping from a holding potential of -100 to -10 mV and recorded every 20 s. Fig. 2A shows Na^+ currents elicited under control conditions and in the presence of increasing concentrations of yohimbine. Yohimbine strongly inhibited tetrodotoxin-resistant Na^+ current at 30 μM, and more pronounced effects were observed at higher concentrations (Fig. 2A). The concentration-dependent effect is shown in Fig. 2B. An estimated IC_{50} for the tonic block was 78.0 ± 1.9 μM ($n=8$) when the data were fit with the following logistic equation:

$$\text{Percentage Block} = 100/[1 + (IC_{50}/\text{Dose})^n] \quad (1)$$

where n is the Hill coefficient constant. To test the use-dependent blocking effects, we used a train of 40 depolarizing pulses to activate Na^+ currents with a pulse interval 0.5 s (2 Hz). Fig. 2C shows current amplitude obtained at the 40th pulse in the absence and in the presence of 30, 100, and 300 μM yohimbine. The dose–response curve for the use-dependent activity of yohimbine is shown in Fig. 2D. After the data were fit with the logistic Eq. (1), an IC_{50} of 47.1 ± 1.1 μM ($n=8$) was obtained for the use-dependent block. This result is very similar to the IC_{50} estimated for the tonic block, indicating that there is little or no frequency-dependent effect of yohimbine on tetrodotoxin-resistant Na^+ currents.

To further characterize the use-dependent blockade of yohimbine on tetrodotoxin-resistant Na^+ currents we applied pulse trains of 1, 2, 5, and 10 Hz frequency with each train containing 40 pulses. Fig. 3A and B shows the ratio of current amplitude at each pulse versus the 1st pulse under control conditions and in the presence of 100 μM yohimbine. At both 1 Hz (Fig. 3A) and 10 Hz (Fig. 3B), the current amplitude ratios in the absence and in the presence

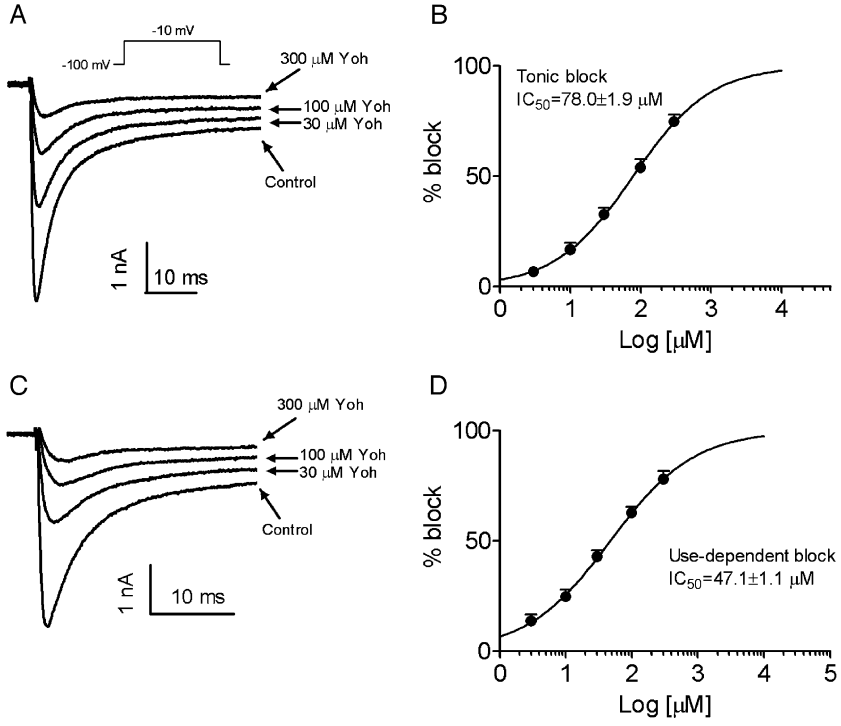

Fig. 2. The tonic and use-dependent blocks of yohimbine on tetrodotoxin-resistant Na^+ currents in dorsal root ganglion neurons. (A) Na^+ currents recorded by depolarizing to -10 mV from a holding potential of -100 mV in the absence and in the presence of yohimbine. (B) Dose–response curve showing that the IC_{50} for the tonic block of yohimbine on tetrodotoxin-resistant Na^+ currents is 78.0 ± 1.9 μM ($n=8$) with a Hill coefficient of 0.79 ± 0.02. (C) The use-dependent block of yohimbine on tetrodotoxin-resistant Na^+ currents. The use-dependent block was assessed at a frequency of 2 Hz. (D) Dose–response curve of the use-dependent block of yohimbine on tetrodotoxin-resistant Na^+ currents ($IC_{50}=47.1 \pm 1.1$ μM and Hill coefficient of 0.69 ± 0.01, $n=8$).

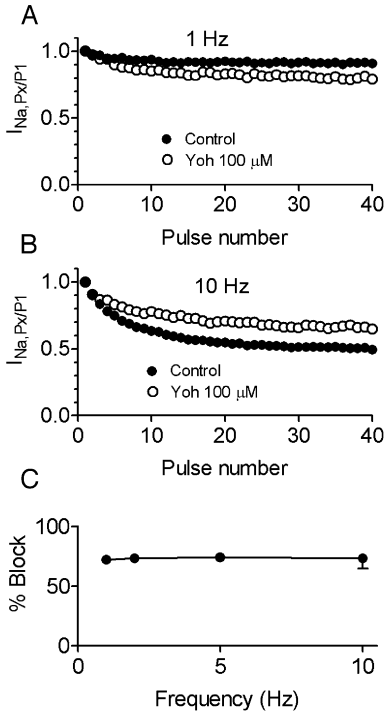

Fig. 3. The use-dependent blockade effect of yohimbine on tetrodotoxin-resistant Na^+ currents. (A–B) The ratios of the current amplitude at the testing pulse versus the 1st are shown at 1 Hz (A) and 10 Hz (B) in the absence (filled circle) and presence (open circle) of 100 μM yohimbine. (C) The percentage block of 100 μM yohimbine on tetrodotoxin-resistant Na^+ currents at different frequencies ($n=5$ for each frequency). No frequency-dependent effects were observed (ANOVA, $P>0.05$).

of 100 μM yohimbine are almost parallel, suggesting that there is little or no frequency-dependent blockade of yohimbine on Na^+ currents. The percentage block of 100 μM yohimbine on tetrodotoxin-resistant Na^+ currents was calculated using the following formula:

$$\text{Percentage Block} = 100 - 100(A/B) \quad (2)$$

where A is the current amplitude measured at the 40th pulse in the presence of 100 μM yohimbine and B is the current amplitude measured at the 40th pulse under control conditions in the same cell. Fig. 3C shows the averaged block ($n=5$) at four different pulse frequencies. ANOVA analysis indicated that there was no frequency-dependent block of yohimbine on tetrodotoxin-resistant Na^+ currents.

Next, we examined whether yohimbine had any effect on voltage-dependent inactivation of tetrodotoxin-resistant Na^+ currents. After a 1.5-s conditioning prepulse ranging from −120 to +40 mV, the steady-state inactivation was assessed by stepping the voltage to −10 mV. Fig. 4A and B shows the steady-state inactivation of tetrodotoxin-resistant Na^+ currents before and after the application of 100 μM yohimbine. The normalized peak currents plotted against prepulse voltages are bimodal (Fig. 4C), suggesting that the tetrodotoxin-resistant Na^+ currents consist of two components (presumably the slow inactivating and persistent components encoded by $Na_V1.8$ and 1.9, respectively) (Akopian et al., 1996; Dib-Hajj et al., 2002; Tate et al., 1998; Wood et al., 2002). The curves were fitted by two Boltzmann functions:

$$I/I_{peak} = 1/(1+\exp((V-V_{1/2})/k)) \quad (3)$$

where I_{peak} is the peak current measured, $V_{1/2}$ is the midpoint of the curve, and k is the slope factor. $V_{1/2}$ values for each component are -88.3 ± 2.1 and -43.8 ± 0.9 mV in control. Yohimbine, at 100 μM, shifted $V_{1/2}$ from −88.3 of control to −90.7 mV and from −43.8 of control to −59.3 mV (Fig. 4C).

3.2. Effects of yohimbine on tetrodotoxin-sensitive Na^+ currents

Yohimbine also exhibited significant inhibitory effects on tetrodotoxin-sensitive Na^+ currents in dorsal root ganglion neurons (data not shown). Since it is pharmacologically difficult to isolate tetrodotoxin-sensitive Na^+ currents in dorsal root ganglion neurons, we used a recombinant rat brain type II Na^+ channel ($Na_V1.2$) expressed in HEK293 cells to examine the yohimbine effect on tetrodotoxin-sensitive Na^+ currents. Fig. 5A shows the dose–response curve of the tonic block of yohimbine on $Na_V1.2$ Na^+ currents. The inset shows original recordings of $Na_V1.2$

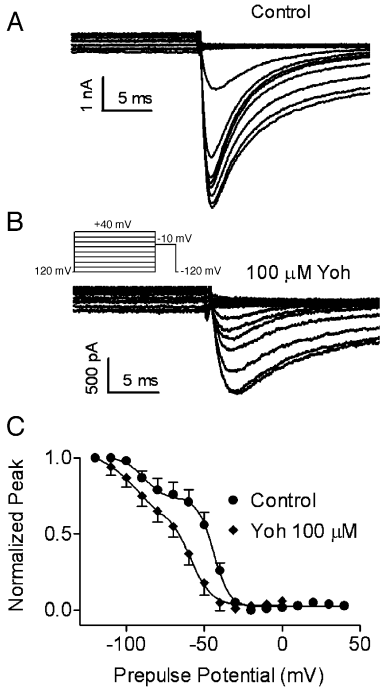

Fig. 4. The voltage-dependent block of yohimbine on tetrodotoxin-resistant Na^+ currents. (A–B) Original recordings of Na^+ currents elicited by depolarizing to −10 mV after a 1.5-s conditioning prepulse ranging from −120 to +40 mV under control conditions (A) and in the presence of 100 μM yohimbine (B). (C) Steady-state inactivation curves in control and in the presence of 100 μM yohimbine ($n=5$). The solid line represents the fitting of two Boltzmann functions.

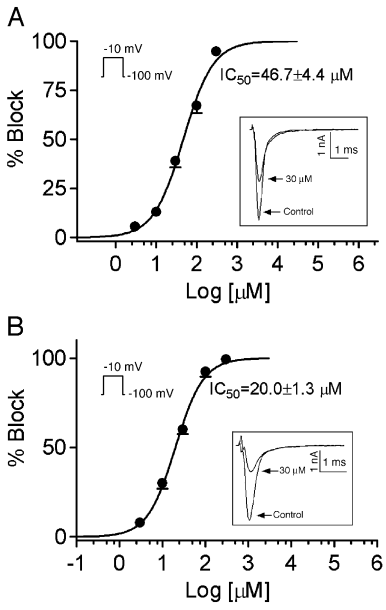

Fig. 5. Effects of yohimbine on tetrodotoxin-sensitive Na^+ currents (rat brain II). (A) Dose–response curve of the tonic block of yohimbine on $Na_V1.2$. $IC_{50} = 46.7 \pm 4.4$ μM and Hill coefficient of 1.18 ± 0.12 ($n = 6$). The inset shows original recordings of $Na_V1.2$ Na^+ currents under control conditions and in the presence of 30 μM yohimbine. (B) Dose–response curve of the use-dependent block of yohimbine on $Na_V1.2$ Na^+ currents. $IC_{50} = 20.0 \pm 1.3$ μM and Hill coefficient of 1.33 ± 0.10 ($n = 6$). The inset shows $Na_V1.2$ Na^+ currents elicited at the frequency of 2 Hz under control conditions and in the presence of 30 μM yohimbine.

elicited by depolarizing from −100 to −10 mV at a frequency of 0.05 Hz in control and in the presence of 30 μM yohimbine. An estimated IC_{50} of 46.7 μM was obtained for yohimbine's tonic block on $Na_V1.2$ Na^+ currents when the data were fitted with the logistic Eq. (1). The use-dependent block was evaluated at a frequency of 2 Hz as shown in Fig. 5B. An IC_{50} of 20 μM was obtained for the use-dependent block (see Fig. 5B). Similar to its effect on tetrodotoxin-resistant Na^+ currents in dorsal root ganglion neurons, yohimbine did not show significant frequency-dependent block on $Na_V1.2$ Na^+ currents either.

3.3. Effects of yohimbine on vanilloid VR1 receptors

Effects of yohimbine on Na^+ currents suggest that it may have implications in pain transduction. Vanilloid VR1 receptor, which is expressed in dorsal root ganglion neurons and can be activated by capsaicin, also plays an important role in pain transduction (Caterina and Julius, 1999; Caterina et al., 2000; Davis et al., 2000). Subsequently, we decided to study yohimbine's effect on capsaicin-induced currents in dorsal root ganglion neurons. Fig. 6A shows 1 μM capsaicin-induced currents measured at a holding potential of −60 mV in the absence or in the presence of increasing concentrations of yohimbine. Yohimbine reversibly inhibited capsaicin-induced currents in a dose-dependent manner. The same capsaicin-induced currents were also sensitive to capsazepine, a specific vanilloid VR1 receptor antagonist (data not shown), suggesting that these currents were mediated by vanilloid VR1 receptors. Fig. 6B shows ramp recordings of vanilloid VR1 receptor currents induced by 1 μM capsaicin in the absence or in the presence of 10, 30, 100, and 500 μM yohimbine. The inhibitory effect was similar across the entire ramp voltage, indicating the blocking effect of yohimbine on vanilloid VR1 receptors is voltage independent. Fig. 6C shows the dose–response curve of yohimbine on vanilloid VR1 receptors in dorsal root ganglion neurons. An IC_{50} of 25.6 ± 3.1 μM ($n = 7$) was obtained at a holding potential of −60 mV.

3.4. Potential physiological functions of yohimbine—effects of yohimbine on firing properties of dorsal root ganglion neurons

In neurons, action potential generation and impulse propagation are triggered by a voltage-activated rise in membrane Na^+ conductance. In order to evaluate potential physiological functions of yohimbine, action potential firing

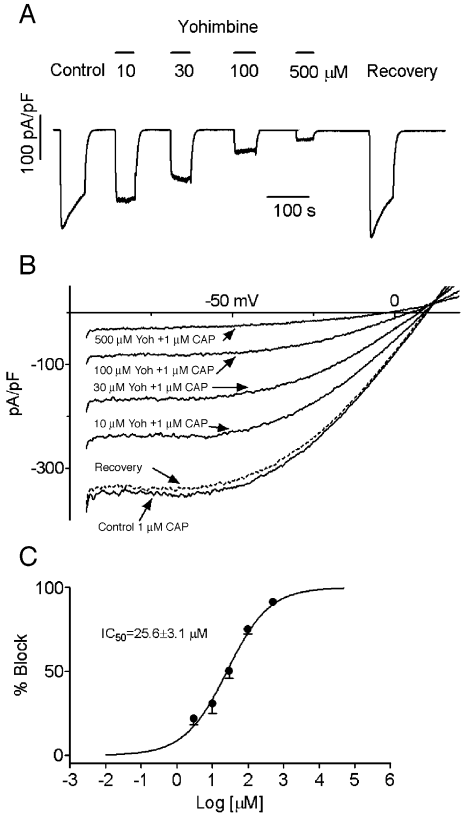

Fig. 6. Effects of yohimhine on vanilloid VR1 receptors. (A) Vanilloid VR1 receptor currents were induced by 1 μM capsaicin at a holding potential of −60 mV with or without application of yohimbine (10, 30, 100, and 500 μM). (B) Representative recordings of vanilloid VR1 receptors elicited by ramp protocol ranging from −100 to +100 mV. Only the inward currents are shown. (C) Percentage block of vanilloid VR1 receptor currents by yohimbine measured at a holding potential of −60 mV. Dose–response curve illustrates the estimated IC_{50} of yohimbine on vanilloid VR1 receptors is 25.6 ± 3.1 μM with a Hill coefficient of 0.73 ± 0.07 ($n = 7$).

activities were studied. Current clamp experiments were carried out in dorsal root ganglion neurons with a resting membrane potential less than −55 mV. Generally, cell membrane potentials were stable and quiescent. A small current ranging from 10 to 80 pA was injected into the cells to induce firing activities. As shown in Fig. 7A, a train of action potentials was elicited by a 30-pA current injection for 1 s. Yohimbine at a concentration of 50 μM significantly and reversibly decreased the firing activity by reducing the number of action potentials (Fig. 7B and C) while having no effect on the resting membrane potential. The firing sparks were reduced from 18 per second (Fig. 7A) to 2 per second (Fig. 7B) and eventually were completely eliminated with prolonged yohimbine incubation (data not shown). Similar results were observed in four other dorsal root ganglion cells. In a separate experiment where dorsal root ganglion neurons exhibited spontaneous firing activities, application of 50 μM yohimbine eliminated these firing activities as well (data not shown). These results indicate that yohimbine could eliminate neuronal firings probably through the inhibition of Na^+ channels.

The potential physiological function of yohimbine on vanilloid VR1 receptors was also tested on capsaicin-induced action potentials under the current clamp configuration. Fig. 8A shows action potentials induced by 100 nM capsaicin. In the presence of 50 μM yohimbine (Fig. 8B), capsaicin failed to induce any action potentials, suggesting that yohimbine was able to eliminate capsaicin-induced firing. Fig. 8C shows continuously recorded action potentials elicited by 100 nM capsaicin. Yohimbine at a concentration of 50 μM completely abolished capsaicin-induced action potential activities (Fig. 8D).

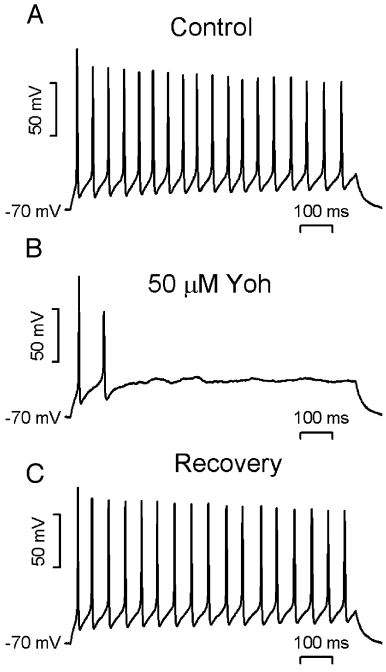

Fig. 7. Effects of yohimbine on action potential firings of dorsal root ganglion neurons. (A) Action potentials were elicited by injection of a 30-pA current for 1 s. A train of spikes with amplitude of approximately 120 mV were observed. (B) Yohimbine (50 μM) drastically decreased the numbers of spikes. (C) Effects of yohimbine on action potentials is reversible upon washout. Both the number and amplitude of action potentials were recovered to the control level upon washout of yohimbine. Action potentials recordings in (A–C) were from the same cell.

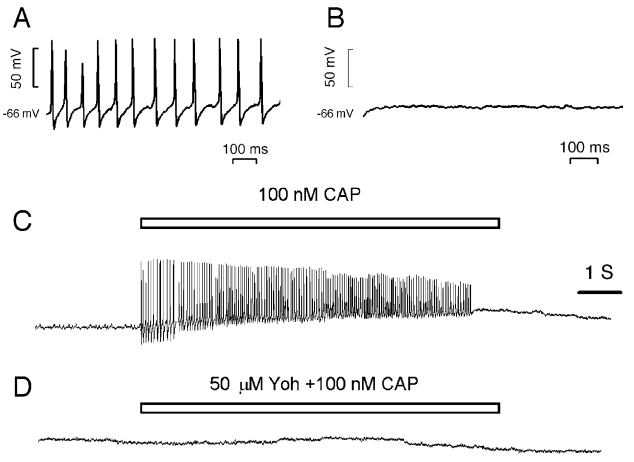

Fig. 8. Effects of yohimbine on action potential firings induced by capsaicin. (A) Representative recording of action potentials elicited by 100 nM capsaicin in 1 s. (B) Application of 100 nM capsaicin in the presence of 50 μM yohimbine failed to induce action potentials. Recordings shown in (A) and (B) are from the same cell. (C) Continuously recorded action potentials elicited 100 nM capsaicin. (D) Yohimbine (50 μM) abolished the capsaicin-induced firing activities. Effects of yohimbine were reversible (data not shown). Recordings shown in (C) and (D) are from the same cell.

4. Discussion

The present study shows that dorsal root ganglion neurons in neonatal rats express both tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents. The tetrodotoxin-resistant Na^+ currents consist of two components; slow inactivating and persistent. The α_2-adrenoceptor antagonist yohimbine blocked tetrodotoxin-resistant and tetrodotoxin-sensitive Na^+ currents in dorsal root ganglion neurons and recombinant tetrodotoxin-sensitive Na^+ currents ($Na_V1.2$) expressed in HEK293 cells. Yohimbine shifted the steady-state inactivation curves of slow inactivating and persistent tetrodotoxin-resistant Na^+ currents to the hyperpolarized direction. No frequency-dependent effects of yohimbine were observed. Consistent with the observed effects of yohimbine on Na^+ channels, current-induced or spontaneous firing activities in dorsal root ganglion neurons were eliminated by yohimbine. Yohimbine also blocked the firing activities elicited by capsaicin. Since dorsal root ganglion neurons play an important role in nociceptive transmission, our results indicate that inhibition of Na^+ channel and vanilloid VR1 receptor by yohimbine may account for its antinociceptive effects observed in in vivo studies (Dennis et al., 1980; Tasker and Melzack, 1989). These results also suggest that yohimbine, and probably other related alka-

loids, is able to block Na^+ currents and vanilloid VR1 receptors, and therefore, potentially interferes with pain transduction pathways.

It has been established that yohimbine is a natural α_2-adrenoceptor antagonist. Application of yohimbine as an aphrodisiac compound started before the turn of the last century (Hunner, 1926). The mechanism for the treatment of erectile dysfunction is believed to be through interfering with nitric oxide (NO) release from the endothelium, which counteracts endothelium-1-induced contractions (Filippi et al., 2002). It has been proposed recently that pre-exercise administration of yohimbine may enhance the efficacy of exercise training as a fat loss strategy by boosting lipolysis in overweight elderly subjects (McCarty, 2002). Besides these clinical implications, antinociceptive effects of yohimbine have also been reported. However, the underlying mechanisms are not fully understood (Dennis et al., 1980; Tasker and Melzack, 1989).

While the effects of yohimbine on adrenoceptors have been established, less is known about whether yohimbine has any direct effects on ion channels. Previous reports showed that yohimbine at a 100 μM concentration did not affect L-type Ca^{2+} currents (Liang et al., 1998). In an attempt to test whether clonidine's effect on nonmylinated nerve fiber's activities is through α_2-adrenoceptors, Erne-Brand et al. (1999) found that yohimbine at 10 μM decreased action potential amplitude, suggesting that yohimbine may directly affect ion channel activities. The detailed mechanisms, however, were not investigated. Korper et al. (1998) compared the effects of several alkaloids on Na^+ currents in frog skeletal muscle cells. They found that at 10 μM concentration yohimbine had an inhibitory effect on Na^+ channels that was greater than strychnine but smaller than ajmaline. The detailed investigation regarding the effects of those alkaloids on Na^+ channels was not provided.

The present study is the first detailed investigation on the effects of yohimbine on pain related ion channels. We showed that yohimbine blocked both tetrodotoxin-sensitive and tetrodotoxin-resistant Na^+ currents and vanilloid VR1 receptors at μM concentrations. It is known that two components of tetrodotoxin-resistant Na^+ currents are present in dorsal root ganglion neurons (Baker and Wood, 2001; Benn et al., 2001; Renganathan et al., 2002; Tate et al., 1998) and are attributed to $Na_V1.9$ and $Na_V1.8$. Since there is no appropriate pharmacological tool to separate the two components, we did not attempt to isolate each component of tetrodotoxin-resistant Na^+ currents. However, at least two lines of evidence in the present study suggest that both components of tetrodotoxin-resistant Na^+ currents are present in the dorsal root ganglion neurons used in our experiments, and yohimbine blocks both of them. First, the currents shown in Fig. 1A and C have different inactivating kinetics, indicating different components of tetrodotoxin-resistant Na^+ currents were present. Second, bi-model steady-state inactivation curves in Fig. 4 indicate that there are two tetrodotoxin-resistant components. The $V_{1/2}$ for each component was shifted by yohimbine, indicating that yohimbine blocked both tetrodotoxin-resistant components.

Both slow inactivating and persistent tetrodotoxin-resistant Na^+ currents have been suggested to be involved in pain transduction process (Akopian et al., 1999; Baker and Wood, 2001; Dib-Hajj et al., 2002; Waxman et al., 1999, 2000). The $Na_V1.8$ knockout mouse showed reduced hyperalgesia in inflammatory but not neuropathic pain models, and a complete absence of sensitivity to noxious pressure (Akopian et al., 1999). The persistent $Na_V1.9$ is restricted in small dorsal root ganglion neurons. Computer simulation suggests that $Na_V1.9$ plays an important role in resting membrane potential and the excitability of dorsal root ganglion neurons (Dib-Hajj et al., 2002; Fang et al., 2002; Herzog et al., 2001). These simulations predict a strong influence on the resting membrane potential, shifting it from -70 mV to approximately -50 mV. Even a small fraction of persistent tetrodotoxin-resistant Na^+ channels could exert a significant depolarizing influence (Herzog et al., 2001).

Effects of yohimbine on Na^+ channels are not frequency-dependent. This, however, should not influence the yohimbine's analgesic effects. C-fibers have relatively low action potential firing frequencies, therefore, frequency dependence of a Na^+ channel blocker can be anticipated to be of limited importance for analgesia (Raymond et al., 1990; Schmelz et al., 1995; Weiser and Wilson, 2002).

Effects on tetrodotoxin-sensitive Na^+ channels may also contribute to analgesic effects. In neuropathic pain states, inhibition of tetrodotoxin-sensitive Na^+ channels may become more important because the tetrodotoxin-resistant Na^+ current amplitudes and mRNA levels in C-type dorsal root ganglion neurons were significantly reduced after axotomy of the sciatic nerve in rats (Cummins and Waxman, 1997; Dib-Hajj et al., 1996; Rizzo et al., 1995; Waxman et al., 1994). However, in the same experiment, tetrodotoxin-sensitive (brain type III) Na^+ channel mRNA levels and current amplitudes were markedly elevated (Cummins and Waxman, 1997; Dib-Hajj et al., 1996; Waxman et al., 1994). Thus, blockade of tetrodotoxin-sensitive Na^+ channels by yohimbine suggests that yohimbine may have effects on neuropathic pain.

Effects of yohimbine on vanilloid VR1 receptors provide more evidence that yohimbine may exert analgesic effects directly at the site of noxious sensation. Yohimbine abolished the firing activities induced by capsaicin or current injection, further indicating that yohimbine is able to interfere with pain sensation or transduction.

Binding studies suggest that yohimbine has much higher affinity for α_2-adrenoceptors, ranging from approximately 1 nM for α_{2c}- to 23 nM for α_{2a}-adrenoceptors, than for Na^+ channels or vanilloid VR1 receptors reported here (MacDonald et al., 1997). Therefore, at low concentration of yohimbine, its biological action is largely through antagonizing α-adrenoceptors. Only when a high concentration of yohimbine is administered, its other activities, such as

inhibition of Na^+ channels and vanilloid VR1 receptors discussed here, should be considered for fully understanding its pharmacological properties.

In conclusion, yohimbine exhibited strong inhibition on tetrodotoxin-sensitive ($Na_V1.2$) Na^+ channels, tetrodotoxin-resistant Na^+ channels that contain both slow inactivating ($Na_V1.8$) and persistent ($Na_V1.8$) components, and capsaicin-induced vanilloid VR1 receptor currents. Action potential firing activities evoked by current injection or capsaicin were eliminated by yohimbine. This study is the first demonstrating that yohimbine blocked nociceptive related ion channels. The results provide a potential ionic mechanism for antinociceptive effects of yohimbine observed in in vivo studies.

Acknowledgements

The authors would like to thank Rita Patel, Lauren Danner, Michele Matchett, Marci Crandall, and Chu Ngo for their technical support.

References

Akopian, A.N., Sivilotti, L., Wood, J.N., 1996. A tetrodotoxin-resistant voltage-gated sodium channel expressed by sensory neurons. Nature 379, 257–262.

Akopian, A.N., Souslova, V., England, S., Okuse, K., Ogata, N., Ure, J., Smith, A., Kerr, B.J., McMahon, S.B., Boyce, S., Hill, R., Stanfa, L.C., Dickenson, A.H., Wood, J.N., 1999. The tetrodotoxin-resistant sodium channel SNS has a specialized function in pain pathways. Nat. Neurosci. 2, 541–548.

Baker, M.D., Wood, J.N., 2001. Involvement of Na^+ channels in pain pathways. Trends Pharmacol. Sci. 22, 27–31.

Benn, S.C., Costigan, M., Tate, S., Fitzgerald, M., Woolf, C.J., 2001. Developmental expression of the TTX-resistant voltage-gated sodium channels Nav1.8 (SNS) and Nav1.9 (SNS2) in primary sensory neurons. J. Neurosci. 21, 6077–6085.

Caterina, M.J., Julius, D., 1999. Sense and specificity: a molecular identity for nociceptors. Curr. Opin. Neurobiol. 9, 525–530.

Caterina, M.J., Julius, D., 2001. The vanilloid receptor: a molecular gateway to the pain pathway. Annu. Rev. Neurosci. 24, 487–517.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Caterina, M.J., Leffler, A., Malmberg, A.B., Martin, W.J., Trafton, J., Petersen-Zeitz, K.R., Koltzenburg, M., Basbaum, A.I., Julius, D., 2000. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288, 306–313.

Cummins, T.R., Waxman, S.G., 1997. Downregulation of tetrodotoxin-resistant sodium currents and upregulation of a rapidly repriming tetrodotoxin-sensitive sodium current in small spinal sensory neurons after nerve injury. J. Neurosci. 17, 3503–3514.

Davis, J.B., Gray, J., Gunthorpe, M.J., Hatcher, J.P., Davey, P.T., Overend, P., Harries, M.H., Latcham, J., Clapham, C., Atkinson, K., Hughes, S.A., Rance, K., Grau, E., Harper, A.J., Pugh, P.L., Rogers, D.C., Bingham, S., Randall, A., Sheardown, S.A., 2000. Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405, 183–187.

Dennis, S.G., Melzack, R., Gutman, S., Boucher, F., 1980. Pain modulation by adrenergic agents and morphine as measured by three pain tests. Life Sci. 26, 1247–1259.

Dib-Hajj, S., Black, J.A., Felts, P., Waxman, S.G., 1996. Down-regulation of transcripts for Na channel alpha-SNS in spinal sensory neurons following axotomy. Proc. Natl. Acad. Sci. U. S. A. 93, 14950–14954.

Dib-Hajj, S., Black, J.A., Cummins, T.R., Waxman, S.G., 2002. NaN/Nav1.9: a sodium channel with unique properties. Trends Neurosci. 25, 253–259.

Erne-Brand, F., Jirounek, P., Drewe, J., Hampl, K., Schneider, M.C., 1999. Mechanism of antinociceptive action of clonidine in nonmyelinated nerve fibres. Eur. J. Pharmacol. 383, 1–8.

Fang, X., Djouhri, L., Black, J.A., Dib-Hajj, S.D., Waxman, S.G., Lawson, S.N., 2002. The presence and role of the tetrodotoxin-resistant sodium channel Na(v)1.9 (NaN) in nociceptive primary afferent neurons. J. Neurosci. 22, 7425–7433.

Filippi, S., Luconi, M., Granchi, S., Natali, A., Tozzi, P., Forti, G., Ledda, F., Maggi, M., 2002. Endothelium-dependency of yohimbine-induced corpus cavernosum relaxation. Int. J. Impot. Res. 14, 295–307.

Furst, S., 1999. Transmitters involved in antinociception in the spinal cord. Brain Res. Bull. 48, 129–141.

Goldberg, M.R., Robertson, D., 1983. Yohimbine: a pharmacological probe for study of the alpha 2-adrenoreceptor. Pharmacol. Rev. 35, 143–180.

Gouarderes, C., Jhamandas, K., Cridland, R., Cros, J., Quirion, R., Zajac, J.M., 1993. Opioid and substance P receptor adaptations in the rat spinal cord following sub-chronic intrathecal treatment with morphine and naloxone. Neuroscience 54, 799–807.

Guyenet, P.G., Stornetta, R.L., Riley, T., Norton, F.R., Rosin, D.L., Lynch, K.R., 1994. Alpha 2A-adrenergic receptors are present in lower brainstem catecholaminergic and serotonergic neurons innervating spinal cord. Brain Res. 638, 285–294.

Herzog, R.I., Cummins, T.R., Waxman, S.G., 2001. Persistent TTX-resistant Na^+ current affects resting potential and response to depolarization in simulated spinal sensory neurons. J. Neurophysiol. 86, 1351–1364.

Hille, B., 2001. Ion Channels of Excitable Membranes. Sinauer Associates Inc., (Sunderland, MA).

Hunner, M.A., 1926. A Practical Treatise on Disorders of the Sexual Function of the Male and Female. FA Davis, Philadelphia.

Korper, S., Wink, M., Fink, R.H., 1998. Differential effects of alkaloids on sodium currents of isolated single skeletal muscle fibers. FEBS Lett. 436, 251–255.

Liang, Y., Wu, B.W., Dun, W., 1998. Blocking effects of phentolamine on L-type calcium current and ATP-sensitive potassium current in guinea pig ventricular myocytes. Zhongguo Yaoli Xuebao 19, 154–157.

Lonnqvist, F., Nyberg, B., Wahrenberg, H., Arner, P., 1990. Catecholamine-induced lipolysis in adipose tissue of the elderly. J. Clin. Invest. 85, 1614–1621.

MacDonald, E., Kobilka, B.K., Scheinin, M., 1997. Gene targeting-homing in on alpha 2-adrenoceptor-subtype function. Trends Pharmacol. Sci. 18, 211–219.

McCarty, M.F., 2002. Pre-exercise administration of yohimbine may enhance the efficacy of exercise training as a fat loss strategy by boosting lipolysis. Med. Hypotheses 58, 491–495.

Raymond, S.A., Thalhammer, J.G., Popitz-Bergez, F., Strichartz, G.R., 1990. Changes in axonal impulse conduction correlate with sensory modality in primary afferent fibers in the rat. Brain Res. 526, 318–321.

Renganathan, M., Cummins, T.R., Waxman, S.G., 2002. Nitric oxide blocks fast, slow, and persistent Na^+ channels in C-type DRG neurons by *S*-nitrosylation. J. Neurophysiol. 87, 761–775.

Rizzo, M.A., Kocsis, J.D., Waxman, S.G., 1995. Selective loss of slow and enhancement of fast Na^+ currents in cutaneous afferent dorsal root ganglion neurones following axotomy. Neurobiol. Dis. 2, 87–96.

Santicioli, P., Del Bianco, E., Figini, M., Bevan, S., Maggi, C.A., 1993. Effect of capsazepine on the release of calcitonin gene-related peptide-like immunoreactivity (CGRP-LI) induced by low pH, capsaicin and potassium in rat soleus muscle. Br. J. Pharmacol. 110, 609–612.

Schmelz, M., Forster, C., Schmidt, R., Ringkamp, M., Handwerker, H.O., Torebjork, H.E., 1995. Delayed responses to electrical stimuli reflect C-fiber responsiveness in human microneurography. Exp. Brain Res. 104, 331–336.

Shannon, H.E., Lutz, E.A., 2000. Yohimbine produces antinociception in the formalin test in rats: involvement of serotonin(1A) receptors. Psychopharmacology (Berl.) 149, 93–97.

Szallasi, A., Blumberg, P.M., Annicelli, L.L., Krause, J.E., Cortright, D.N., 1999. The cloned rat vanilloid receptor VR1 mediates both R-type binding and C-type calcium response in dorsal root ganglion neurons. Mol. Pharmacol. 56, 581–587.

Szolcsanyi, J., 1987. Selective responsiveness of polymodal nociceptors of the rabbit ear to capsaicin, bradykinin and ultra-violet irradiation. J. Physiol. 388, 9–23.

Tasker, R.A., Melzack, R., 1989. Different alpha-receptor subtypes are involved in clonidine-produced analgesia in different pain tests. Life Sci. 44, 9–17.

Tate, S., Benn, S., Hick, C., Trezise, D., John, V., Mannion, R.J., Costigan, M., Plumpton, D., Grose, D., Gladwell, Z., Kendall, G., Dale, K., Bountra, C., Woolf, C.J., 1998. Two sodium channels contribute to the TTX-R sodium current in primary sensory neurons. Nat. Neurosci. 1, 653–655.

Trezise, D.J., John, V.H., Xie, X.M., 1998. Voltage- and use-dependent inhibition of Na^+ channels in rat sensory neurones by 4030W92, a new antihyperalgesic agent. Br. J. Pharmacol. 124, 953–963.

Waxman, S.G., Kocsis, J.D., Black, J.A., 1994. Type III sodium channel mRNA is expressed in embryonic but not adult spinal sensory neurons, and is reexpressed following axotomy. J. Neurophysiol. 72, 466–470.

Waxman, S.G., Dib-Hajj, S., Cummins, T.D., Black, J.A., 1999. Sodium channels and pain. Proc. Natl. Acad. Sci. U. S. A. 96, 7635–7639.

Waxman, S.G., Cummins, T.R., Dib-Hajj, S.D., Black, J.A., 2000. Voltage-gated sodium channels and the molecular pathogenesis of pain: a review. J. Rehabil. Res. Dev. 37, 517–528.

Weiser, T., Wilson, N., 2002. Inhibition of tetrodotoxin (TTX)-resistant and TTX-sensitive neuronal Na(+) channels by the secretolytic ambroxol. Mol. Pharmacol. 62, 433–438.

Wilcox, G.L., Carlsson, K.H., Jochim, A., Jurna, I., 1987. Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. Brain Res. 405, 84–93.

Winter, J.C., Rabin, R.A., 1992. Yohimbine as a serotonergic agent: evidence from receptor binding and drug discrimination. J. Pharmacol. Exp. Ther. 263, 682–689.

Wood, J.N., Akopian, A.N., Baker, M., Ding, Y., Geoghegan, F., Nassar, M., Malik-Hall, M., Okuse, K., Poon, L., Ravenall, S., Sukumaran, M., Souslova, V., 2002. Sodium channels in primary sensory neurons: relationship to pain states. Novartis Found. Symp. 241, 159–168.

Yaksh, T.L., Pogrel, J.W., Lee, Y.W., Chaplan, S.R., 1995. Reversal of nerve ligation-induced allodynia by spinal alpha-2 adrenoceptor agonists. J. Pharmacol. Exp. Ther. 272, 207–214.