原著

# The effect of $\alpha_2$-adrenergic receptor agonist, dexmedetomidine, on tetrodotoxin resistant $Na^+$ currents in small-sized dorsal root ganglion neurons

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## Abstract

We examined the effects of $\alpha_2$-adrenergic agonist, dexmedetomidine (DEX), on tetrodotoxin-resistant (TTX-r) $Na^+$ currents using small-sized dorsal root ganglion (DRG) cells of the rats. Whole cell patch-clamp configuration was used. After control $Na^+$ current was recorded, DEX was added to the extracellular solution. Yohimbine and prazosin were used to differentiate the contribution of $\alpha_2$-adrenergic receptor from that of $\alpha_1$-adrenergic receptor. The peak TTX-r $Na^+$ currents were significantly and dose dependently inhibited by DEX ($p<0.05$). Yohimbine attenuated the suppressive effects of DEX on TTX-r $Na^+$ currents, while prazosin did not alter the effects of DEX. Thus, DEX inhibited TTX-r $Na^+$ currents by way of $\alpha_2$-adrenergic receptors but not $\alpha_1$-adrenergic receptors. Therefore, it is suggested that DEX has an inhibitory effect on TTX-r $Na^+$ channels through $\alpha_2$-adrenergic receptors.

**Key words;** tetrodotoxin-resistant sodium current, dorsal root ganglion neuron, dexmedetomidine, $\alpha_2$-adrenergic receptor, $\alpha_1$-adrenergic receptor

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## Introduction

$\alpha_2$-Adrenoceptors have an important role in regulating the cardiovascular system[1]. On the other hand, therapeutic application of $\alpha_2$-adrenergic receptor agonists has shown that they have sedative, anxiolytic, analgesic, and sympatholytic actions[2]. The spinal administration of $\alpha_2$ agonists can produce a powerful and selective effect upon nociceptive processing. Based on previous reports, potential sites of the anti-nociceptive action could be brain, spinal cords, dorsal root ganglion (DRG), and sensory neurons[3].

Voltage-gated sodium channels have been found to exist in excitable cells such as neuronal cells and generate active potential. Among these channels, tetrodotoxin-resistant (TTX-r) $Na^+$ channels, which are localized in small DRG neurons, have been shown to exert a pivotal role in mediating nociceptive pathway[4].

Dexmedetomidine (DEX) is a highly selective $\alpha_2$ agonist that has recently been marketed for use in providing short-term sedation in the ICU. DEX created a dose-dependent anti-hyperalgesic effect in animal studies[5~8]. We hypothesized that DEX may have inhibitory effects on TTX-r $Na^+$ channels expressed in DRG neurons. To test this hypothesis, we examined the effects of DEX on TTX-r $Na^+$ currents using small-sized DRG neurons.

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## Materials and methods

### A. Experimental Cell Preparation

Animal use in this study is approved by the institutional animal care and use committee of Kitasato University School of Medicine. Male Sprague-Dawley rats (280 ~ 320g) were killed with diethyl ether (Wako Pure Chemical Industries, Ltd., Osaka, Japan). After lumbar laminectomy, the L4 and L5 DRGs of the rat were removed. The ganglia were immersed immediately and incubated for 90min at 37℃ in Ham's F-12 culture medium (Gibco BRI, Grand Island, NY) containing 2mg/ml collagenase (Worthington Biochemical, Freehold, NJ). Subsequently, the ganglia were incubated for 15min at 37℃ in $Ca^{2+}$- and $Mg^{2+}$-free Hank's balanced salt solution containing 2.5mg/ml trypsin (Sigma Chemical Co., St. Louis, MO). The isolated cells were plated onto polylysine-coated cover glasses (Fisher Scientific, Pittsburgh, PA) and cultured for 1~2hr at 37℃ in Ham's F-12 medium containing 10% fetal bovine serum and penicillin (100 units/ml)-streptomycin (100 μg/ml) under 5%$CO_2$ (pH 7.4). DRG neurons with small diameter (<30μm) were chosen for the study (4~10 cells in each group). Experiments were conducted at 22~24℃.

Glass capillaries (World Precision Instruments, Inc. Sarasota, Florida) were processed to pipettes by a micropuller (Sutter Instrument Co. Novato, Ca). The tips were fire-polished using a microforge (MF-900; Narishige, Tokyo, Japan) and had a resistance of 1.5~3.5 MΩ when filled with internal solution.

All investigations were performed as voltage-clamp experiments in the whole-cell configuration of patch-clamp method. Voltage stimuli were generated by personal computer with pClamp8.1software (Axon Instruments, Foster City, CA). The currents were recorded on an EPC8 amplifier (HEKA elektronik, Lambrecht, Germany) in the voltage-clamp mode, and signals were digitized by a 16-bit AD-converter (digidata1322A, Axon Instruments) filtered at 3kHz, sampled at 20kHz using Pclamp8.1softwear (Axon Instrument), and stored on the hard disk of a personal computer, which also served as the stimulus generator.

### B. Solution

Tyrode solution contained the following (mM): Tyrode solution (in mM): NaCl 132, KCl 4.8, $MgCl_2$ 1.2, $CaCl_2$ 1, Hepes 10, Dextrose 5, pH adjusted to 7.3 with NaOH. Extracellular solution (bath and control solution, dissolvent for the drugs) containing (in mM) NaCl 20, choline Cl 100, TEA Cl 20, $CsCl_2$ 5, $CaCl_2$ 0.01, $MgCl_2$ 5, EGTA 11, glucose 5, and HEPES 10, pH adjusted to 7.4 with NaOH. TTX (0.3μM) was added to the extracellular solution when recording TTXr-$Na^+$ currents. Intracellular solution containing (in mM) CsF 130, $MgCl_2$ 5, EGTA 11, NaCl 10, and HEPES 10, pH adjusted to 7.2 by adding CsOH.

After control sodium current was recorded, 10, 25, 50μM of dexmedetomidine (Abbot, North Chicago, IL) was added to the extracellular solution. Yohimbine 10μM and prazosin 1μM were used to differentiate the contribution of $\alpha_2$-adrenergic receptor from that of $\alpha_1$-adrenergic receptor.

NaCl, $MgCl_2$, $CaCl_2$, Hepes, Dextrose, choline Cl, TEA Cl, $CsCl_2$, EGTA, glucose, CsF, CsOH, yohimbine, and prazocine were obtained from Sigma; KCl and NaOH were from Kanto Chemical Co. (Tokyo, Japan); and TTX was from Alomone Labs (Jerusalem, France).

### C. Data analysis

Data were expressed as mean±SD. Analysis of variance (ANOVA) following by Student-Newman-Keuls test was used for statistical comparison. $p<0.05$ was considered to be significant.

To evaluate the voltage dependence of the steady state activation, channel conductance was determined from the whole-cell inward $I_{Na}$ elicited by 50-ms test pulses from −70mV to +40mV from a holding potential of −70mV in 10-mV increments.

## Results

$Na^+$ currents were generated by 10mV depolarizing step (50ms in duration) in a range of test potentials (−70 to +40mV). The peak $Na^+$ current was used to compare the control and the post treatment value.

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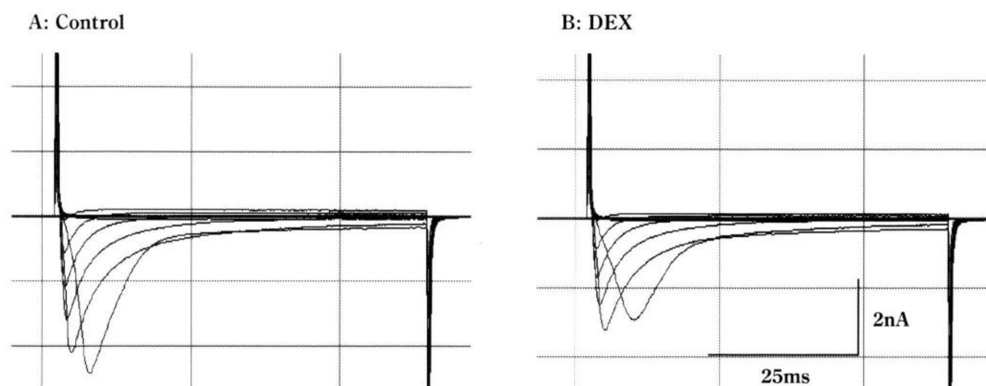


**Figure 1** Current tracings of TTX-r $Na^+$ currents represent before (**A**) and after (**B**) application of 25nM of DEX.

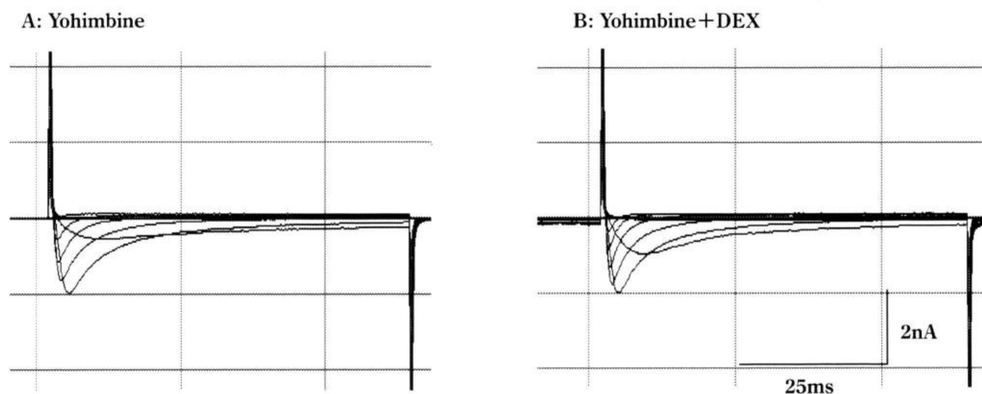


**Figure 2** Current tracings of TTX-r $Na^+$ currents with yohimbine represent before (**A**) and after (**B**) application of 25nM of DEX.

$Na^+$ currents were inhibited by DEX in the external solution with TTX (**Fig. 1**). Current-voltage relations were fitted with a modified Boltzmann's equation to evaluate the slope factor and half-maximal activation potential. There were no significant changes in the current-voltage relationship after administration of DEX (Date not shown).

DEX did not inhibit the $Na^+$ currents when Yohimbine, an $\alpha_2$-adrenergic receptor antagonist, applied to the external solution (**Fig. 2**).

In the extracellular solution without TTX, $Na^+$ currents were significantly inhibited only by 50nM DEX. 10 and 25nM of DEX did not significantly reduce the $Na^+$ currents (**Fig. 3**). TTX-r $Na^+$ currents were dose dependently and significantly inhibited by 55±37%, 64±28%, 73±14% with 10, 25, and 50nM of DEX respectively (**Fig. 3**).

Yohimbine attenuated the suppressive effects of DEX on TTX-r $Na^+$ currents (28±23% inhibition at 25nM), while prazosin, an $\alpha_1$-adrenergic receptor antagonist did not alter the suppressive effect of DEX on TTX-r $Na^+$ currents (**Fig. 4**).

## Discussion

Our results demonstrated that TTX-r $Na^+$ currents were dose-dependently inhibited by DEX. In addition, the suppressive effects of DEX on TTX-r $Na^+$ currents were attenuated by yohimbine, but not by prazosin. $\alpha_2$-Adrenergic agonists produce clinically-relevant effects after binding to $\alpha_2$-adrenergic receptors, which are consisted of three subtypes ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$). These receptor subtypes are distributed ubiquitously, and each may be uniquely responsible of the actions of $\alpha_2$ agonists[2].

On rat DRG, the most common $\alpha_2$AR mRNA were $\alpha_{2C}$ subtype[9]. Although the present study was not

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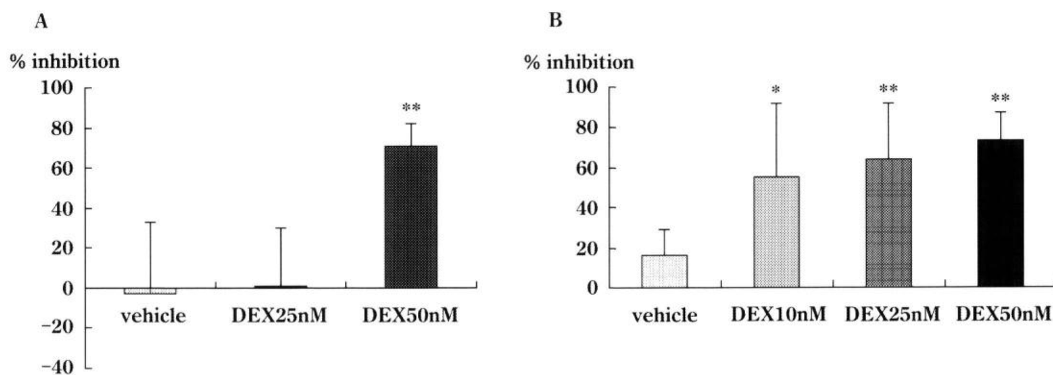


**Figure 3** Changes in $Na^+$ currents by DEX in the external solution without TTX (A: n=5; vehicle, n=8; DEX25nM, n=4 DEX50nM), with TTX (B: n=5; vehicle, n=10; DEX10nM, n=8; DEX25nM, n=10; DEX50nM). Columns are mean±SD. *$p<0.05$ versus vehicle. **$p<0.01$ versus vehicle.

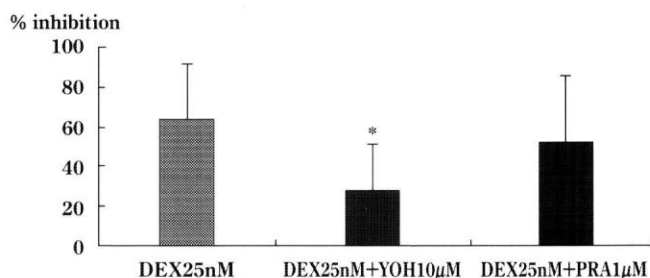


**Figure 4** Changes in $Na^+$ currents by DEX in the external solution with TTX and α-AR antagonist (n=8; DEX25nM, n=10; DEX25nM+Yohimbine (YOH) 10μM, n=5; DEX25nM+Prazosin (PRA) 1μM). Columns are mean±SD. *$p<0.05$ versus DEX25nM.

designed to specify the receptor subtypes, DEX would likely to have effects on the $\alpha_{2C}$ adrenergic receptor subtype of DRG cells based on these previous reports.

The neurons in the DRG are composed of two major categories on the basis of their microscopic observations, the large-light cells and the small-dark cells[10]. It has been shown that peripheral C-fibers originate from neurons in the size range of small-dark cell population and that peripheral Aα and Aβ fibers originate from neurons in the size range of the large-light cell population[11,12]. Small DRG neurons co-express two types of $Na^+$ current: (i) a rapidly-inactivating, TTX-sensitive fast current and (ii) a slowly-activating and -inactivating, TTX-resistant slow current[13,14]. Large cells express TTX-sensitive $Na^+$ current. The biophysical and pharmacological properties of these two types of $Na^+$ channels are of particular importance of the CNS and in the mechanism of actions of drugs on the CNS neurons[13,15].

Sensitization of primary afferent neurons underlies much of the pain and tenderness associated with tissue injury and inflammation[16]. Recent studies suggest that an increase in a voltage-gated $Na^+$ current might underlie the increased excitability of primary afferent neurons following injury[17,18]. Hyperalgesic agents such as $PGE_2$, 5-HT, and adenosine sensitize primary afferent nociceptors by increasing the magnitude of TTX-r $Na^+$ current, and increase its rate of activation and inactivation[19]. From these points of view, the preferential inhibition of TTX-r $Na^+$ current in rat DRG cells by DEX demonstrated in our study, may suggest a mechanism of DEX in exerting analgesic effects.

## In conclusion

DEX inhibited TTX-r $Na^+$ currents, and this effect may be mediated by $\alpha_2$-adrenergic receptors but not

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$\alpha_1$-adrenergic receptors. Therefore, it is suggested that the antinociceptive property of DEX attributes to the inhibitory effects of DEX on TTX-r $Na^+$ channels through $\alpha_2$-adrenergic receptors.

**Acknowledgment**: The authors would like to thank Prof. T. Kawakami and Associate Prof. H. Hiruma for their advice.

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