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Activation of a G Protein–coupled Inwardly Rectifying K^+ Current and Suppression of I_h Contribute to Dexmedetomidine-induced Inhibition of Rat Hypothalamic Paraventricular Nucleus Neurons

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Background: **α_2-Adrenoceptor agonist has been reported to produce inhibition of arginine vasopressin release, diuresis, and sympatholytic effects. However, its mechanisms of central action remain incompletely understood. Hypothalamic paraventricular nucleus (PVN) neurons, which are in direct contact with noradrenergic synapses and are controlled by the hyperpolarization-activated currents, are called I_h (H current). The effect of dexmedetomidine, a highly selective and potent agonist, at α_2 adrenoceptors on I_h is unknown. The purpose of this study was to examine the effects of dexmedetomidine on the PVN neuron, which is involved in the arginine vasopressin release and autonomic regulation.**

Methods: **The authors investigated the effects of dexmedetomidine on the membrane properties in PVN magnocellular neurons and an I_h in PVN parvocellular neurons with a whole cell patch clamp technique using a rat brain slice preparation.**

Results: **Dexmedetomidine dose-dependently hyperpolarized PVN magnocellular neurons. In the voltage clamp mode, dexmedetomidine induced an outward current, with a reversal potential of −94 mV, and this was shown to depend on the external concentration of K^+. Pretreatment with Ba^{2+} or peptide toxin tertiapin blocked hyperpolarization induced by dexmedetomidine. The effect of dexmedetomidine was blocked by an α_2-adrenoceptor antagonist, yohimbine. I_h was suppressed dose dependently by dexmedetomidine in PVN parvocellular neurons. Pretreatment with Cs^+ occluded the I_h suppression by dexmedetomidine. Yohimbine blocked the I_h suppression by dexmedetomidine. The I_h sensitive to dexmedetomidine was weakly modulated by intracellular cyclic adenosine monophosphate.**

Conclusions: **Dexmedetomidine inhibited PVN magnocellular neurons by activation of the G protein–coupled inwardly rectifying K^+ current and inhibited PVN parvocellular neurons by suppression of I_h.**

THERE has recently been substantial interest in the use of α_2-adrenergic agonists in clinical anesthesia.[1] Several studies have demonstrated that dexmedetomidine, an α_2-adrenergic agonist with an α_2-adrenoceptor/α_1-adrenoceptor binding ratio of 1,620:1,[2] promotes analgesia and anesthesia in humans.[1,3] Although dexmedetomidine has great potential for wide use in human anesthesia, its cellular mechanism of action on the central nervous system (CNS) has not yet been entirely elucidated. Adrenergic mechanisms in the CNS have an important role in the regulation of cardiovascular function and arginine vasopressin (AVP) release. For example, intracerebroventricular administration of clonidine induces hypotension,[4] and the α_{2a}-adrenoceptor subtype has been reported to be involved in central hypotensive effects.[5] Intravenous[6] and spinal[7] administration of dexmedetomidine induce cardiovascular depression and sympatholytic action. Intravenous or intracerebroventricular administration of clonidine or other α_2-adrenergic agonists inhibits AVP release into blood in dogs[4,8] and rats,[9] resulting in diuresis.[9] The AVP is synthesized in the magnocellular neurons of the paraventricular (PVN) and supraoptic nuclei of the hypothalamus.[10] The PVN comprises magnocellular neurons that secrete oxytocin and AVP, neurosecretory parvocellular neurons that secrete hypophysiotropic hormones, and nonneurosecretory preautonomic parvocellular neurons that are involved in the regulation of the autonomic nervous system outflow.[11] The PVN receives a dense noradrenergic innervation originating from the A1 area and other brainstem noradrenergic areas.[12] To our knowledge, the effect of the α_2-adrenergic agonists on the neurons of CNS, which is involved in the control of the release of AVP, has not been studied yet.

It has been demonstrated that slowly activating inward currents are evoked by hyperpolarizing voltage steps in a variety of neurons, including PVN,[13] area postrema,[14] and dorsal root ganglion neurons.[15] Such hyperpolarization-activated currents have been termed I_h (H current).[16] I_h is also called the pacemaker current because it is thought to play a significant role in cell excitability, especially the firing frequency.[16] Activation of I_h at negative potentials can result in a slow depolarization, which is identified as time-dependent rectification during injection of a hyperpolarizing current. Such a depolarizing influence could accelerate neuronal firing discharges. Therefore, the modulation of I_h could affect cell excitability. Recently, we found that PVN parvocellular neuron activity was modulated by I_h channel activity.[13] Despite the fact that clonidine inhibits I_h in central neurons,[15] the effect of dexmedetomidine on the activities of the I_h channels is unknown. We conducted a detailed

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assessment of the actions of dexmedetomidine on neurons of the PVN using whole cell patch clamp techniques.

Materials and Methods

Slice Preparation

The study was approved by the Animal Investigation Committee of Miyazaki University (Miyazaki, Japan) and was conducted according to the animal use guidelines of the American Physiologic Society (Bethesda, Maryland).

Male Wistar rats (postnatal days 14–21) were anesthetized with isoflurane and then killed by decapitation. The brain was rapidly removed and placed in cooled (2°–4°C) standard artificial cerebrospinal fluid (ACSF) containing 140 mM NaCl, 3 mM KCl, 1.3 mM $MgSO_4$, 1.4 mM NaH_2PO_4, 2.4 mM $CaCl_2$, 5 mM HEPES, 3.25 mM NaOH, and 11 mM D-glucose, which had been oxygenated with 100% O_2. The osmolarity was maintained at 290–300 mOsm with a pH of 7.4. This solution was also used as the extracellular solution in most experiments. In some cells, where we tested the effects of dexmedetomidine on I_h, we used a modified ACSF that contained tetraethylammonium (20 mM; blocking the delayed rectifier K^+ current), $BaCl_2$ (1 mM; blocking the inwardly rectifying K^+ current, I_{Kir}), 4-aminopyridine (4-AP; 2 mM; blocking the transient outward K^+ current), tetrodotoxin (1 μM; blocking the Na^+ current), and nominally zero Ca^{2+} (0 mM $CaCl_2$; preventing Ca^{2+} influx). Coronal slices 270 μm in thickness, which included the PVN, were prepared using a vibrating brain slicer (DSK-2000; Dosaka, Kyoto, Japan) and equilibrated for 1–2 h at room temperature in a tissue storage chamber containing ACSF saturated with 100% O_2.

Electrophysiologic Recording

The electrodes were made with a puller (PB-7; Narishige, Tokyo, Japan) from thick-wall borosilicate glass (GD-1.5; Narishige). Their resistance was 3–5 MΩ in the bath, with access resistances in the range of 8–12 MΩ. The electrode solution contained 130 mM K gluconate, 1 mM NaCl, 10 mM EGTA, 1 mM $MgCl_2$, 2 mM Na_2ATP, 1 mM $CaCl_2$, 10 mM HEPES, and 0.5 mM Na_3GTP, pH adjusted to 7.3 with KOH. In some cells, where we tested the effects of cyclic adenosine monophosphate (cAMP), perforated patch recordings were made using amphotericin B. In this case, the patch pipettes were filled with a solution containing 150 mM KCl and 10 mM HEPES, adjusted to pH 7.3 with KOH. An amphotericin B solubilized preparation (Sigma, St. Louis, MO) was added into the pipette solution just before use. The final concentration of amphotericin B in the pipette solution was 300 μg/ml.[17] The series resistance was compensated by 80%. Slices were transferred into the recording chamber and continuously perfused (1.5–2.0 ml/min) with ACSF at room temperature (24°–26°C). Whole cell recordings were made from microscopically identified cells. In each slice, only one neuron was recorded. The transmembrane voltage and current were recorded using a patch clamp amplifier (Axopatch 200B; Axon Instruments, Foster City, CA) and stored on a hard disk *via* an analog–digital converter for later analysis. The signals were also displayed on a thermal rectigraph (model 8M14; San-Ei, Tokyo, Japan). The stored data were analyzed using AxoGraph software (version 8.0; Axon Instruments). The membrane potential and current were low-pass filtered at 2 and 10 kHz, respectively. The liquid-junction potential between the pipette and ACSF was corrected at the beginning of the experiment. After stable recording conditions were obtained, a PVN neuron was identified electrophysiologically as a transient outward rectification-expressing (magnocellular; type 1) neuron or a non-outward rectification-expressing (parvocellular; type 2) neuron according to previously established criteria.[18]

The effects of dexmedetomidine on the membrane potentials were examined at the resting membrane potential. The membrane currents were recorded at a holding potential of −60 mV. Drugs were applied after recording at least 180 s of control events. In evaluating the dose-dependent responses of membrane events, the same neurons were tested from one to three times in another concentration. In these cases, the concentration of the applied drugs was selected at random, and subsequent drug applications were made after the effects of the first drug application had fully reversed.

Drugs and Chemicals

Tetrodotoxin, tetraethylammonium, 4-AP, CsCl, yohimbine, prazosin, 8Br-cAMP, amphotericin B, forskolin, and SQ-22536 were purchased from Sigma Chemical (St. Louis, MO), and ZD7288 was obtained from Tocris Cookson (Ballwin, MO). Dexmedetomidine was provided by Abbott Japan (Osaka, Japan). All other reagents were from Nacalai Tesque (Osaka, Japan). ZD7288 was prepared as a 50 mM stock solution (in H_2O) and stored at −20°C until use. Forskolin was initially solubilized in dimethylsulfoxide and then diluted in ACSF. All other drugs were dissolved in ACSF.

Data and Statistical Analysis

Measurements were acquired using the pCLAMP system (Axon Instruments) and stored on a computer hard disk for off-line analysis. Off-line analysis was performed using Axograph software (Axon Instruments). The amplitude of I_h was obtained by subtraction of the instantaneous currents (I_{INS}) at the end of the capacitative current transient (5–10 ms after stimulus onset) from the steady state currents at the end of the 1-s voltage step (I_{SS}). I_{INS} and I_{SS} were plotted against the membrane potential. To examine the effect of dexmedetomidine on the voltage-dependent activation of I_h, an activation curve was constructed by a tail current analysis, as described by McCor-

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mick and Pape.[19] The tail current amplitudes were normalized (I/I_{max}) and plotted as a function of the membrane potential during the initial hyperpolarizing voltage steps. The resulting data were fitted by the Boltzmann equation: $I/I_{max} = \{1 + \exp[(V_m - V_{1/2})/k]\}^{-1}$, where I_{max} is the maximal tail current, V_m is the membrane potential during the initial voltage steps, $V_{1/2}$ is the voltage at half-maximal conductance, and k is the slope factor. Tail currents were normalized, plotted as a function of the preceding hyperpolarization step voltage, and fitted with Boltzmann curves for derivation of $V_{1/2}$ and k by using a least squares analysis program (Origin; Microcal Software, Northampton, MA).

Data were analyzed statistically using one-way analysis of variance followed by Scheffé F test, Student *t* test, or linear regression. All values described in this study are expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

Results

Effects of Dexmedetomidine on Membrane Potential in PVN Magnocellular Neurons

A representative sample of 34 PVN magnocellular neurons showed a resting membrane potential of −59.2 ± 10.5 mV (range, −56 to −64 mV). The mean input resistance was 932 ± 306 MΩ. Overshooting action potentials were observed in all neurons recorded in current clamp mode.

The effect of dexmedetomidine was reversible on washing the tissue with a drug-free solution for periods up to 1 h. There was no evidence of desensitization with repeated (up to 3 h) or continuous (up to 1 h) applications of dexmedetomidine. A bath application of dexmedetomidine (1 μM) hyperpolarized PVN magnocellular neurons (12.2 ± 1.1 mV, n = 8) was accompanied by decreasing input resistance (24 ± 11.3%). Of all PVN magnocellular neurons tested, only the hyperpolarization of the membrane potentials induced by dexmedetomidine (10 nM–6 μM) was dose related (fig. 1). Figures 1A and B show the effect of two incremental concentrations of dexmedetomidine on a typical neuron in the PVN magnocellular neuron. The most sensitive of these effects, which showed a remarkable change even at low dexmedetomidine concentrations, was the decrease in the firing rate. Dexmedetomidine at 10 and 30 nM decreased the neuronal firing rate by 38% and 72%, respectively (fig. 1A); however, little hyperpolarization and only a small reduction of the input resistance of the

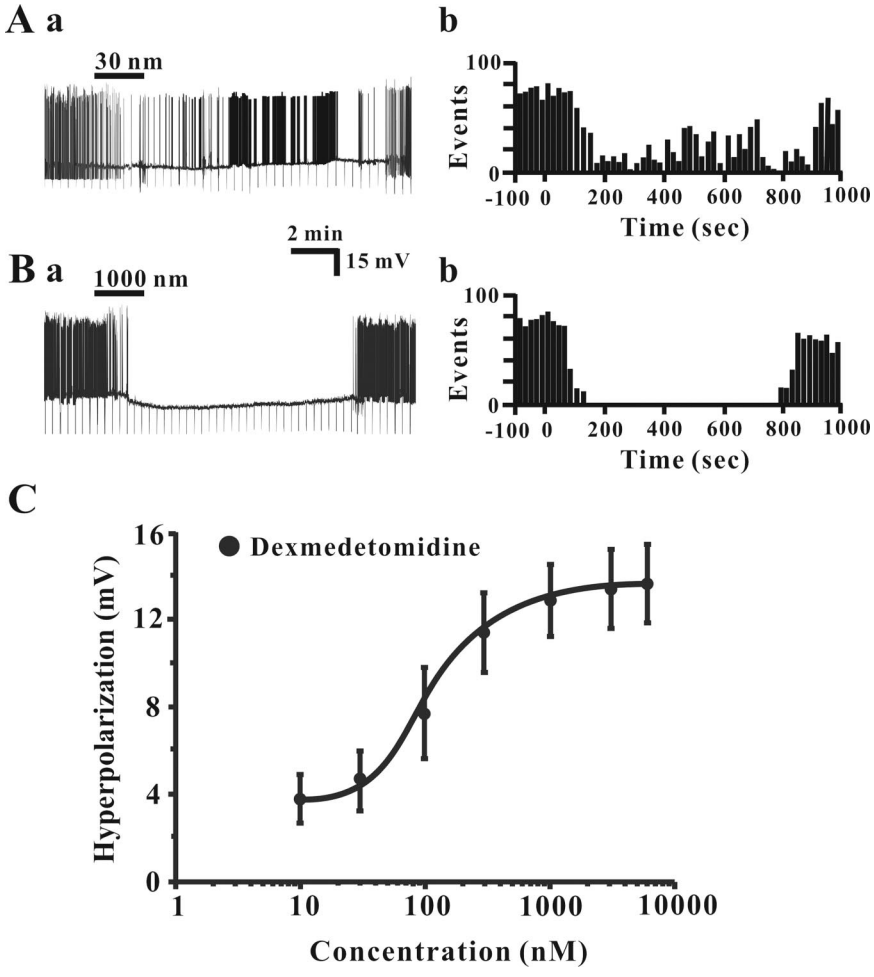

Fig. 1. Effects of dexmedetomidine on hypothalamic paraventricular nucleus magnocellular neurons in current clamp. *A* and *B* are the responses to 30 and 1,000 nM dexmedetomidine, respectively. *Ab* and *Bb* are the instantaneous spike rates of the neurons in *Aa* and *Ba*, respectively. Dexmedetomidine elicited decreases in the firing-action potential accompanied by a decrease in the input resistance (as indicated by responses to hyperpolarizing test pulses of −50 pA, 300 ms duration, administered every 20 s). (*C*) The dose–response curve of dexmedetomidine-induced hyperpolarization. The number of neurons tested for each dose is eight.

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neurons were seen. Higher concentrations of dexmedetomidine (300 nM–6 μM) resulted not only in a greater inhibition of firing but also in the hyperpolarization of the membrane and a decreased input resistance (figs. 1B and C).

Effects of Dexmedetomidine on Membrane Current in PVN Magnocellular Neurons

A bath application of dexmedetomidine (3 μM) produced an outward current (154 ± 66 pA, n = 6) that peaked within 2 min (fig. 2A). To examine the reversal potential for dexmedetomidine-induced hyperpolarization, we calculated from the intersection of the control current–voltage curve and the curve obtained in the presence of dexmedetomidine (1 μM). Figure 2B shows the currents of a PVN neuron in the voltage clamp mode elicited by 1-s ramp pulses between −50 and −130 mV from a holding potential of −60 mV before (control) and after application of dexmedetomidine (1 μM). The current–voltage relation of the PVN magnocellular neurons revealed inward rectification. The reversal potential for dexmedetomidine-induced hyperpolarization in each of eight neurons was deduced from the intersection of the two current–voltage curves. The dexmedetomidine-induced current reversed at −94.1 ± 7.1 mV (fig. 2C), which was close to the K^+ equilibrium potential calculated by the Nernst equation (E_K = −95.2 mV). By the exchange of the external K^+ concentration from 3.0 to 6.0 and 12.0 mM, the reversal potential of the current was shifted to −79.3 ± 11.6 mV and −62.1 ± 16.1 mV, respectively (fig. 2C). The predicted reversal potentials for K^+ conductance are −77.7 and −60.2 mV, respectively, under these conditions. These results suggest that dexmedetomidine elicited an inwardly rectifying K^+ (IRK) current.

The blocking effects of Ba^{2+} (0.3 mM) on dexmedetomidine-induced hyperpolarization were tested on eight PVN magnocellular neurons (fig. 3). After a bath application with $BaCl_2$ (0.3 mM) for 5 min, dexmedetomidine (100 nM) was applied. Ba^{2+} completely blocked dexmedetomidine-induced hyperpolarization in six cells, but a partial inhibition of the baseline firing rate was still observed in two cells (fig. 3B). Ba^{2+} is relatively specific for blocking IRK channels, including IRK and G protein–coupled inwardly rectifying K^+ (GIRK) channels. In neurons, the peptide toxin tertiapin should specifically block GIRK channels, with little effect on IRK channels at concentrations up to 2 μM.[20] To further test whether the IRK channels were the GIRK channel, we applied tertiapin 5 min before the dexmedetomidine application. In the tertiapin (100 nM)–treated neurons, tertiapin eliminated the dexmedetomidine (100 nM)–induced hyperpolarization (n = 8; fig. 3).

Next, we studied whether hyperpolarization induced by dexmedetomidine was mediated by the α_2-adrenergic

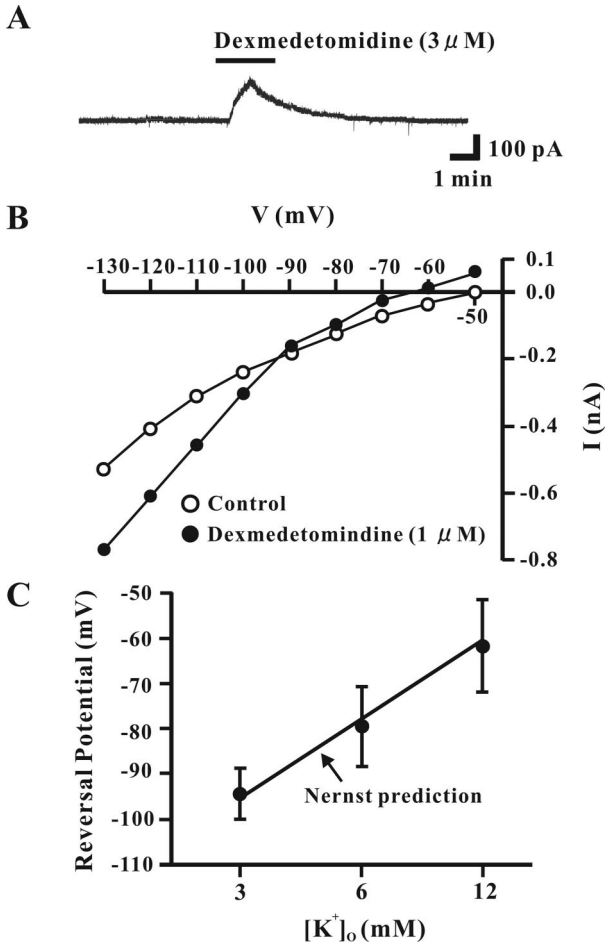

Fig. 2. Dexmedetomidine activates a K^+ conductance in hypothalamic paraventricular nucleus magnocellular neurons. (*A*) Typical responses of paraventricular nucleus neurons to dexmedetomidine (3 μM). Under voltage clamp conditions (at a holding potential of −60 mV), the application of dexmedetomidine (as indicated above; *current trace*) evoked a transient outward current. (*B*) Current (I)–voltage (V) curves obtained in paraventricular nucleus magnocellular neurons in the absence (control) and presence of dexmedetomidine (1 μM). (*C*) Mean reversal potential of dexmedetomidine (1 μM)–induced current at three levels of external K^+ (3, 6, and 12 mM).

receptor. The α_2-adrenergic antagonist yohimbine (100 nm) partially blocked the dexmedetomidine (100 nM)–induced hyperpolarization (42.4 ± 29.6%, n = 6; fig. 4). The application of yohimbine (100 or 1,000 nM) produced a parallel, dose-related shift to the right of the dexmedetomidine dose–response curve (fig. 4B).

Effects of Dexmedetomidine on I_h Currents in PVN Parvocellular Neurons

The neuron having I_h channels in the PVN exhibited a lack of transient outward rectification in response to a series of depolarizing current pulses delivered at a hyperpolarizing membrane potential (fig. 5A). These neurons were parvocellular neurons in the PVN.[18] More than 80% of parvocellular neurons (91 of 112) displayed a time-dependent inward rectification "sag" during the hyperpolarizing pulses (fig. 5B), which was blocked by

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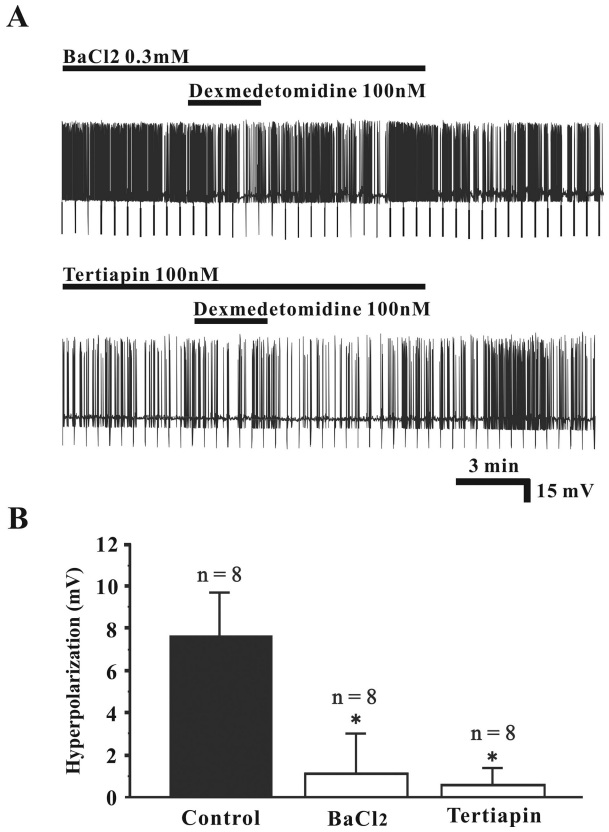

Fig. 3. Effect of preapplication of $BaCl_2$ or tertiapin on dexmedetomidine-induced hyperpolarization. (*A*) $BaCl_2$ (0.3 mM) or tertiapin (100 nM) blocked the dexmedetomidine-mediated hyperpolarization. (*B*) Summary of data showing the effect of $BaCl_2$ or tertiapin on hyperpolarization induced by dexmedetomidine. * $P < 0.05$ *versus* control.

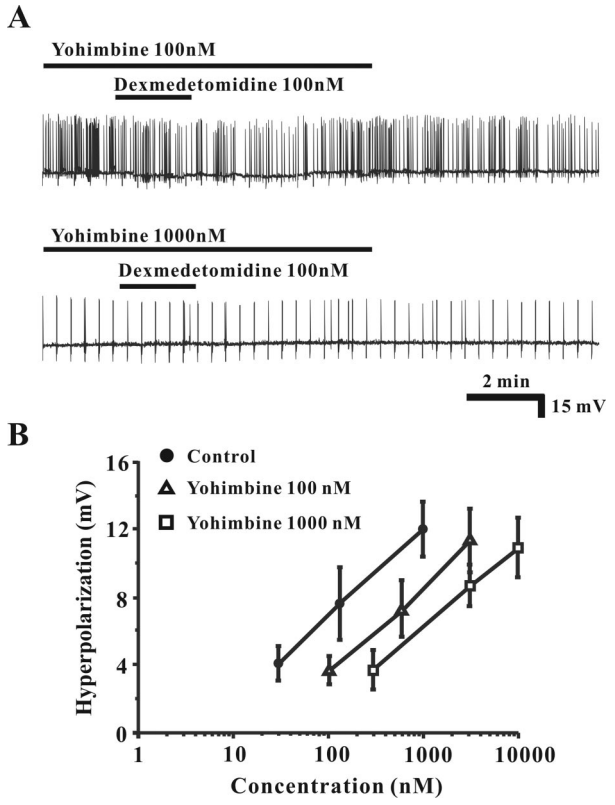

Fig. 4. Antagonism by yohimbine of dexmedetomidine-induced hyperpolarization. (*A*) Effect of dexmedetomidine (100 nM) on the hypothalamic paraventricular nucleus magnocellular neurons in the presence of two different concentrations of yohimbine (100 and 1,000 nM). (*B*) Cumulative dose–response curves for dexmedetomidine in the presence of different concentrations of yohimbine: control (●; n = 8), 100 nM yohimbine (△; n = 8), and 1,000 nM yohimbine (□; n = 8).

50 μM ZD7288. These characteristics are consistent with I_h conductance.[21]

Figure 6 shows typical recordings of I_h in a PVN parvocellular neuron during activation voltage protocols in a modified ACSF that contained tetraethylammonium, $BaCl_2$, 4-AP, and tetrodotoxin and nominally zero Ca^{2+}. Hyperpolarizing step pulses of 1-s duration between −50 and −120 mV from a holding potential of −50 mV in 10-mV increments were used as the activation voltage protocols. A dexmedetomidine (10 μM) application reversibly reduced the I_h amplitude within 5 min. In addition, the tail currents indicated by asterisks in figure 6A, which appeared after stepping back to the holding potential of −50 mV, were reduced in parallel with the reduction of I_h. These effects were stable at approximately 3 min after the application of dexmedetomidine. The effect of dexmedetomidine was not recoverable even after a wash with normal ACSF for half an hour. I_{INS}, as shown in figure 6A (○ = control; ● = dexmedetomidine), and I_{SS}, as shown in figure 6A (□ = control; ■ = dexmedetomidine), were plotted against the membrane potential (fig. 6B). The difference between I_{INS} and I_{SS} in the control (△) and during the application of dexmedetomidine (10 μM; ▲) was plotted against the membrane potential (fig. 6C). The difference between I_{INS} and I_{SS} represents I_h. The mean amplitude of I_h at the −120 mV command voltage was 341 ± 54 pA (n = 9). As shown in figure 6C, the activation of I_h was voltage dependent. The application of dexmedetomidine (0.1–20 μM) suppressed I_h in a dose-dependent manner (fig. 6D).

To examine the effect of dexmedetomidine on the voltage-dependent activation of I_h, an activation curve was constructed by a tail current analysis. A sample activation curve fitted with the Boltzmann equation is shown in figure 6C (inset). I_h was half-activated at −94 mV in the control, and dexmedetomidine caused a small change in the $V_{1/2}$ (to −102 mV). The mean values were as follows: $V_{1/2}$ = −96 ± 4.5 mV, k = 9.2 ± 1.5 in the control and $V_{1/2}$ = −104.6 ± 3.6 mV, k = 8.9 ± 3.3 during the application of dexmedetomidine (10 μM, n = 9). These data revealed that dexmedetomidine produced a significant shift in the $V_{1/2}$ to a more hyperpolarized potential ($P < 0.01$). The slope values were not altered by dexmedetomidine ($P > 0.05$).

The reversal potential of the dexmedetomidine-sensitive component of I_h was determined by tail-current analysis, as described previously.[22] As illustrated in figure 7A, after a

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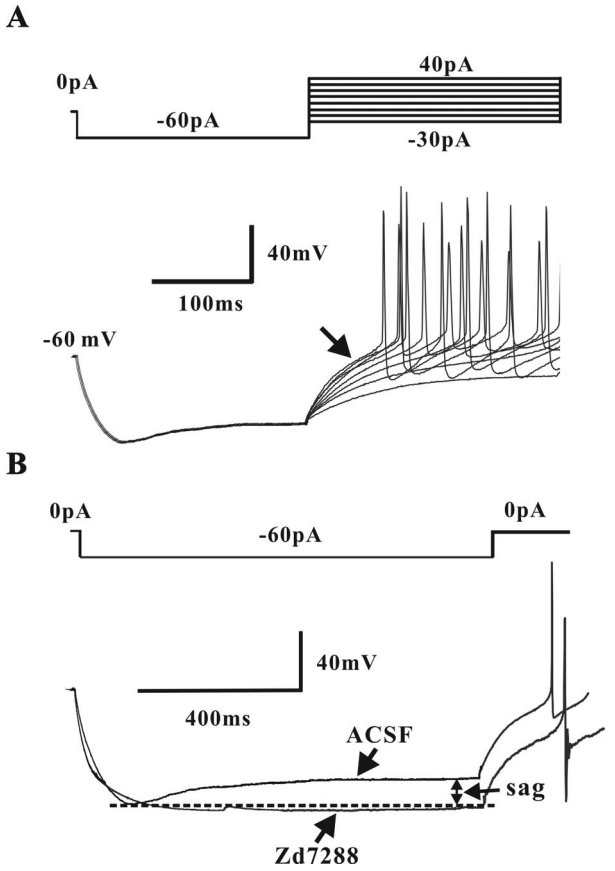

Fig. 5. Electrophysiologic properties of hypothalamic paraventricular nucleus parvocellular neurons that have I_h channels. (*A*) Neuron displaying time-dependent inward rectification and lacked transient outward rectification (*black arrow*) in response to a series of depolarizing current pulses delivered at a hyperpolarized membrane potential. (*B*) Neuron displaying inward rectification (sag), which was blocked by 50 μM ZD7288 in response to a −60-pA hyperpolarizing current pulse. ACSF = artificial cerebrospinal fluid.

step up to −120 mV (250 ms duration) from a holding potential of −60 mV, the membrane voltage was stepped in the range of −70 to −20 mV (50-ms duration, 10-mV increments). The plot of the instantaneous current at each test potential yielded the fully activated current–voltage relation, which was linear (fig. 7B). The reversal potential was −34.8 ± 10.5 mV (n = 6) in standard external solution ($[K^+]_o$ = 3 mM, $[Na^+]_o$ = 144.6 mM). It has been reported that in other central neurons, I_h is carried by Na^+ and K^+.[16] To investigate the ionic basis of I_h, we tested the effects of changing $[K^+]_o$ and $[Na^+]_o$ on I_h. We measured the mean reversal potential of the dexmedetomidine-sensitive current in two other extracellular solutions, one with a low Na^+ concentration ($[K^+]_o$ = 3 mM, $[Na^+]_o$ = 80 mM) and the other with a high K^+ concentration ($[K^+]_o$ = 20 mM, $[Na^+]_o$ = 144.6 mM), using the same method. The reversal potential was shifted toward a more negative potential (−49 ± 2.4 mV; □; n = 6) in the low-Na^+ solution, whereas it was shifted toward a more positive potential (−29 ± 2.2 mV; △; n = 6) in the high-K^+ solution. These results suggest that the channel modulated by dexmedetomidine is permeable to both Na^+ and K^+. I_h is a slowly activating inward current evoked by hyperpolarization, and the current–voltage curve shows rectification in the inward direction; the reversal potential of I_h is close to −35 mV in media of physiologic composition, and I_h is mixed Na^+ and K^+ current; furthermore, the relation between the chord conductance of I_h and voltage can be fitted by a Boltzmann equation. Therefore, we conclude that the dexmedetomidine-sensitive current corresponds to I_h, *i.e.*, dexmedetomidine inhibits I_h in rat PVN parvocellular neurons.

Pharmacologic Profile and Identification of Adrenergic Receptors Involved in the Modulation of I_h

The pharmacologic properties and characterization of the adrenoceptors involved in the inhibition of I_h were explored in PVN parvocellular neurons. We recorded the drug-induced change in the amplitude of I_h evoked by 1-s hyperpolarizing voltage steps from −60 mV to −120 mV. The application of 1 mM Cs^+ decreased the peak amplitude of I_h by 86 ± 26.4% (n = 6), and the effects of dexmedetomidine (20 μM) on I_h were occluded completely by Cs^+-induced blockage of I_h (fig. 8A).

Prazosin (20 μM), an α_1-adrenergic receptor antagonist, had little effect on the I_h amplitude (6.0 ± 10.5%, n = 6) in the same neurons in which dexmedetomidine inhibited I_h (fig. 8B).

Figure 8C shows the antagonism of the dexmedetomidine-induced inhibition of the I_h by yohimbine. When standard ACSF contained 2 mM yohimbine, the application of 20 μM dexmedetomidine resulted in a small reduction of the I_h amplitude (13 ± 7.5%, n = 6), whereas, once the yohimbine had been washed off the neurons, dexmedetomidine produced a significantly larger reduction of the I_h amplitude (67 ± 17.6%, n = 6). These results suggest that the dexmedetomidine-induced inhibition of I_h is mediated by α_2 adrenoceptors.

Modulation of I_h *Sensitive to Dexmedetomidine by Intracellular cAMP*

Finally, we examined the regulation of I_h sensitive to dexmedetomidine by cAMP. To avoid washing out the cytoplasmic biochemicals required for second messenger–mediated responses, perforated patch recordings were performed using amphotericin B. As shown in figure 9, bath application of 8Br-cAMP (1 mM) increased the amplitude of I_h by an average of 7.3 ± 4.5% ($P < 0.05$, n = 8). The value of $V_{1/2}$ shifted by an average of 2.6 ± 1.1 mV ($P < 0.05$, n = 8) in the positive direction (fig. 9B). The application of forskolin (50–100 μM) caused a slight increase in the amplitude of I_h (data not shown; n = 8). This increase in the amplitude of I_h was always less than the increase induced by 1 mM 8Br-cAMP. To test whether physiologic changes in the intracellular cAMP concentration were sufficient to cause maximal activation of I_h

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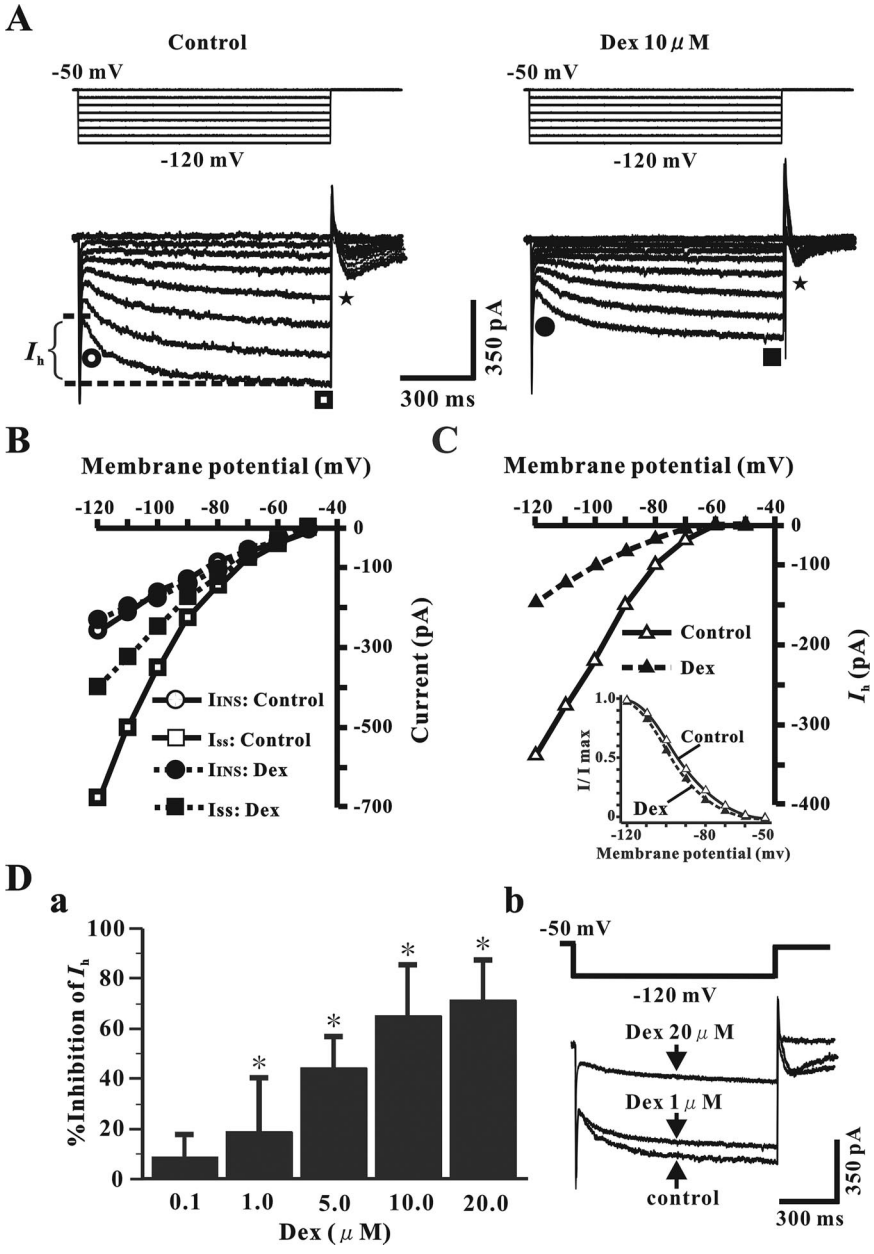

Fig. 6. Dexmedetomidine suppresses the activation of I_h. (*A*) Current traces elicited by the hyperpolarizing voltage steps indicated before and during the application of dexmedetomidine (Dex; 10 μM) in the modified artificial cerebrospinal fluid. Note the reduced size and time course of activation in the steady state currents and the reduction of the tail currents (*stars*) during the application of dexmedetomidine. (*B*) Plots of instantaneous current (I_{INS}, ○ and ●, are shown in *A*) and steady state currents (I_{SS}, □ and ■) against the membrane potential. (*C*) Plots of the difference between I_{INS} and I_{SS} in the control (△) and at 5 min after starting the application of dexmedetomidine (▲) against the membrane potential. Note that the difference between I_{INS} and I_{SS} corresponds to I_h. The *inset* shows the activation curve for I_h before and during the application of dexmedetomidine. (*Da*) Dose-dependent inhibition of I_h by dexmedetomidine (n = 6, each concentration) (0.1 μM *vs.* 1, 5, 10, 20 μM; * $P < 0.05$). (*Db*) Representative examples of raw current traces in response to 1 s step to −120 mV from −50 mV.

channels, we bath-applied the adenylate cyclase inhibitor SQ22536 (50 μM). SQ22536 caused no significant changes in the amplitude or activation curves of I_h (n = 8). The amplitude of I_h was changed to 104.7 ± 12.2% of the control level ($P > 0.05$, n = 8). The value of $V_{1/2}$ was changed from −95.8 ± 2.3 to −95.2 ± 2.5 mV ($P > 0.05$, n = 8). From these results, we concluded that I_h sensitive to dexmedetomidine in hypothalamic PVN parvocellular neurons is only weakly modulated by the intracellular cAMP system.

Discussion

Summary

Under whole cell voltage clamp conditions in a neonatal rat hypothalamic slice preparation, we demonstrated that dexmedetomidine, a highly selective α_2-adrenoceptor agonist, activated an inwardly rectifying K^+ current leading to membrane hyperpolarization in PVN magnocellular neurons. A second component of the dexmedetomidine current was due to inhibition of I_h carried by a mixture of Na^+ and K^+ in PVN parvocellular neurons. This effect was blocked by external Cs^+ and ZD7288, but not by Ba^{2+}, and was primarily due to a decrease in the maximal I_h, although it was also accompanied by a slight hyperpolarizing shift in the half-activation potential. This I_h was weakly activated by intracellular cAMP.

Dexmedetomidine Activates a G Protein–coupled Inwardly Rectifying K^+ Current

We showed that dexmedetomidine activated a GIRK in PVN magnocellular neurons. This facilitates significant

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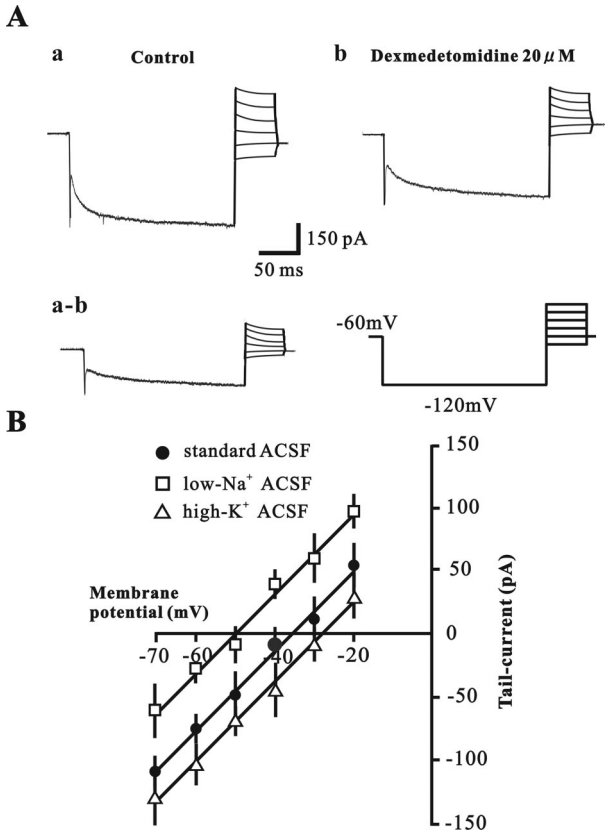

Fig. 7. Reversal potential of the dexmedetomidine-sensitive current. (*A*) Representative recordings for measuring the reversal potential. In the voltage clamp mode, whole cell currents of a hypothalamic paraventricular nucleus parvocellular neuron were recorded in response to a twin-pulse protocol comprising a 200-ms hyperpolarizing prepulse to −120 mV from a holding potential of −60 mV, which made the chord conductance underlying the inward current constant, followed by a test pulse to different voltage steps from −70 to −20 mV in 10-mV increments before and during application of 20 μM dexmedetomidine. (*a–b*) Tail current of the dexmedetomidine-sensitive current was obtained by subtracting the current recorded in the presence of dexmedetomidine (*b*) from the control current shown in *a*. (*B*) Examples of the relation between the tail current amplitude and test voltage steps in standard artificial cerebrospinal fluid (ACSF; ●) and low-Na^+ ($[Na^+]_o$ = 80 mM; □) and high-K^+ ($[K^+]_o$ = 20 mM; △) extracellular solutions.

hyperpolarization of the membrane potential and a decrease in input resistance. The functional significance of the modulation of K^+ currents in the PVN magnocellular neurons has not been established; however, intravenous administration of α_2-adrenoceptor agonists produces a decrease in plasma[9] and cerebrospinal fluid[23] concentration of AVP. Intravenous administration of clonidine induces water diuresis and decreases plasma AVP levels, probably because of central inhibition of AVP release.[23] Many of the magnocellular neurons in the PVN project to the posterior pituitary and release AVP into the peripheral circulation.[10] It has been suggested that the PVN contains a large number of α_2 adrenoceptors[12] and may be involved in the regulation of the autonomic nervous and endocrine systems.[11] Therefore, it is possible that the diuretic responses induced by α_2-adrenoceptor agonists are, at least in part, mediated by the inhibitory actions on PVN magnocellular neurons. Our results are consistent with another report regarding the activating effect on IRK channels of dexmedetomidine in the locus coeruleus.[24] The effects of dexmedetomidine on these IRK channels were inhibited by Ba^{2+}. The peptide toxin tertiapin also blocked the effect of dexmedetomidine. These results suggest that dexmedetomidine activated GIRK channels, which is supported by the fact that the hypnotic–anesthetic action of dexmedetomidine can be attenuated by pretreatment with either an intracerebroventricular injection of pertussis toxin, a specific inactivator of inhibitory G proteins, or an intraperitoneal injection of 4-AP, a K^+ channel blocker.[25] The effect of dexmedetomidine seems to be mediated *via* α_2 adrenoceptors, because yohimbine, antagonists at α_2 adrenoceptors, produced a parallel, dose-related shift to the right of the dexmedetomidine dose–response curve. These results suggest that dexmedetomidine activated GIRK channels mediated by α_2 adrenoceptors and contributed to both the reduction in spike frequency and the hyperpolarization of the membrane potential of PVN magnocellular neurons. The best known function of GIRK channels is to provide a mechanism for the slow inhibitory modulation of the overall excitability of the cell. It has been suggested that the noradrenergic input to AVP neurons may regulate cardiovascular function, either by AVP effects on CNS autonomic centers[26,27] or by direct effects of circulating AVP on blood pressure.[28] The cardiovascular depression induced by dexmedetomidine may be, at least in part, mediated by inhibition of PVN magnocellular neurons. Diuretic and natriuretic action of clonidine is due to both central inhibition of AVP release in combination with a second action of the drug that leads to inhibition of tubular reabsorption of sodium and water either in the thin loop of Henle or in the proximal tubule.[9] Intravenous administration of dexmedetomidine during the perioperative period may prevent oliguria mediated by inhibition of PVN magnocellular neurons.

Dexmedetomidine Inhibits a Hyperpolarization-activated Inward Current (I_h)

This is the first study showing the effects of dexmedetomidine on I_h. The autonomic beating of the heart and a considerable number of rhythmic activities in the brain are controlled by the I_h.[16,20,29] Originally described in cardiac atrial cells, I_h is a noninactivating, inwardly rectifying current carried by both Na^+ and K^+ ions.[16] Four different isoforms (HCN1–4) of the I_h channels have been cloned,[30,31] and we previously reported that the messenger RNA of three isoforms (HCN1–3) is expressed in rat PVN parvocellular neurons.[13] Dexmedetomidine dose-dependently elicited a decrease in I_h that was due primarily to inhibition of the maximal current amplitude in the PVN parvocellular neurons tested.

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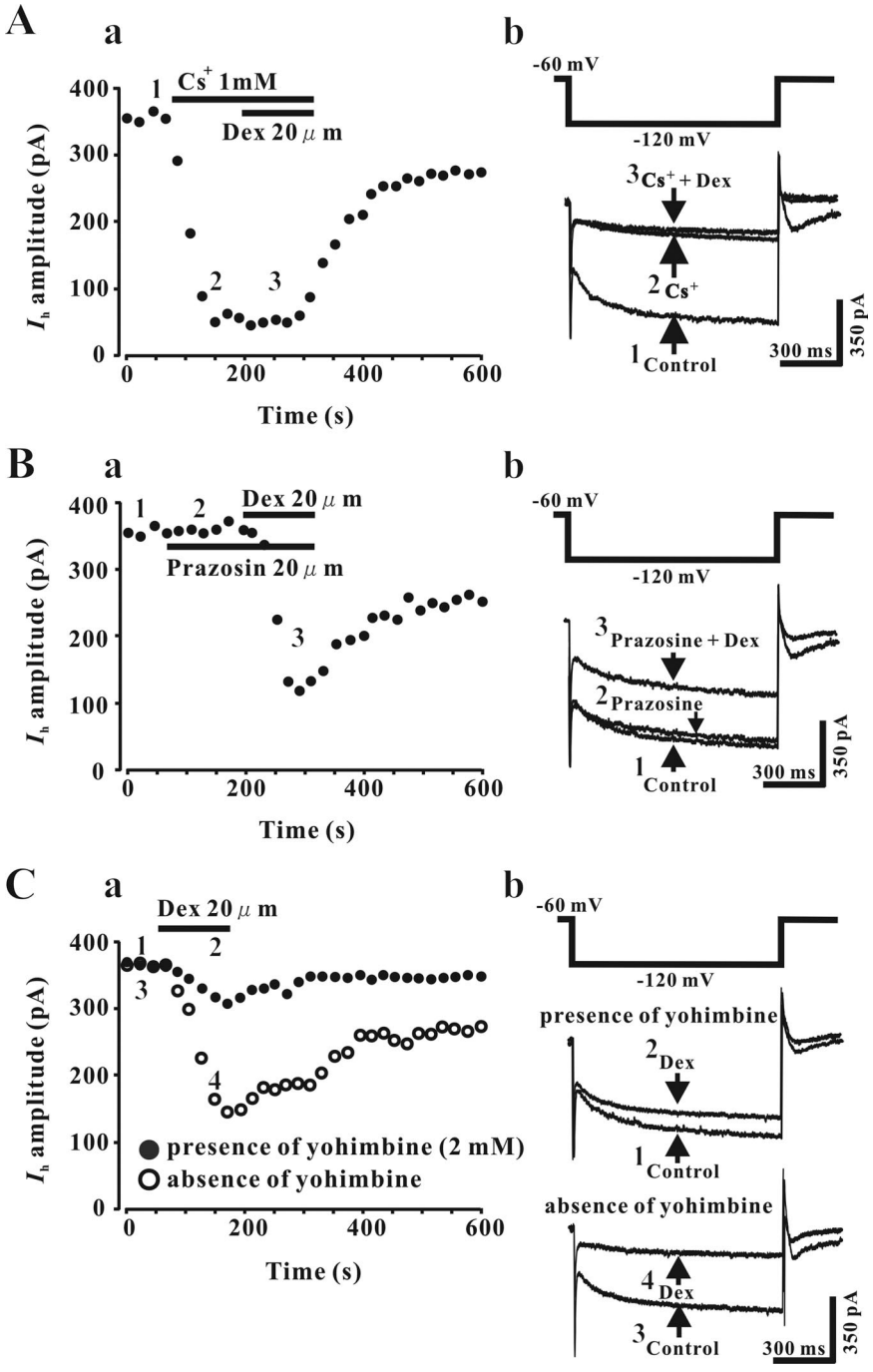

Fig. 8. Pharmacologic profile involved in the inhibition of I_h. (*A–C*) *a* shows plots of I_h amplitude in response to 1-s steps to −120 mV from −60 mV *versus* time. Amplitude of I_h determined by subtracting that of instantaneous current (I_{INS}) from that of the total current at the end of the pulse (steady state current [I_{SS}]). Each drug was applied by superfusion during the period indicated by a *horizontal bar*. Each *b* shows representative examples of raw current traces in response to 1-s steps to −120 mV from −60 mV. *Numbers* by each trace (*b*) in *A–C* designate where the trace was taken from in each *a*. (*A*) Occlusion of dexmedetomidine (Dex)-induced inhibition of I_h by Cs^+. (*B*) Effect of prazosin, a selective α_1-adrenergic receptor antagonist. Prazosin (20 μM) had little effect on the inward current, whereas 20 μM dexmedetomidine induced a decrease in I_h. (*C*) Antagonism of dexmedetomidine-induced inhibition of I_h by yohimbine. Application of 20 μM dexmedetomidine resulted in a small decline of I_h in the presence of 2 mM yohimbine (●), whereas after the yohimbine had been washed off the neuron for 10 min, 20 μM dexmedetomidine produced a larger reduction of I_h (○).

Dexmedetomidine also produced a statistically significant hyperpolarizing shift in the voltage dependence of I_h activation that was similar to that observed in rat hypoglossal motoneurons induced by clonidine.[32] The reversal potential (approximately −35 mV) of I_h in PVN parvocellular neurons was consistent with the mixed Na^+ and K^+ permeability and close to the reversal potential values reported for I_h in other central neurons.[15,33] The preapplication of Cs^+ blocked the inhibition of I_h induced by dexmedetomidine. This is consistent with the report that the extracellular application of Cs^+ in the millimolar concentration range blocks I_h in various central neurons in the brain.[16] The attenuation of the dexmedetomidine inhibition by yohimbine supports the existence of an α_2 mechanism. This is consistent with other central neurons[15,32,33] relevant to clonidine.

The molecular identification of genes encoding I_h channels[34] has enabled studies of the electrophysiologic properties of specific channel types that mediate I_h. The regulation of these HCN channels may occur *via* cAMP, cyclic guanosine monophosphate, and/or Ca^{2+}.[16,29,35,36] Among the HCN channel family (HCN1–4), HCN1 and HCN2 are most prominently expressed in the CNS, with

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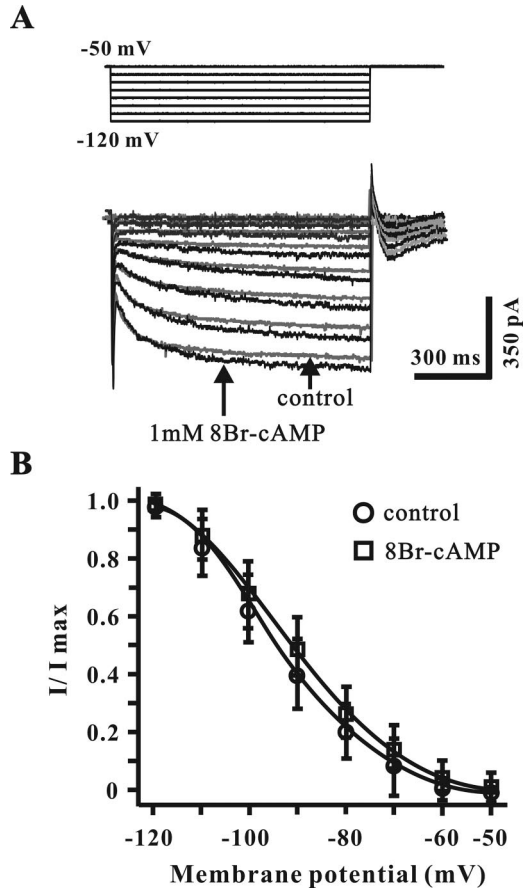

Fig. 9. Weak modulation of I_h by intracellular cyclic adenosine monophosphate (cAMP). (*A*) Currents elicited by hyperpolarizing voltage steps (1-s duration, from −50 to −120 mV with 10-mV decrements) before (*gray*) and during (*black*) the bath application of 8Br-cAMP (1 mM). Note a slight increase in the amplitude of I_h induced by 8Br-cAMP at millimolar concentrations. (*B*) Activation curve of the conductance underlying I_h constructed with plots of the mean normalized tail currents as a function of the membrane potential before (○) and during (□) the application of 8Br-cAMP (n = 8 cells in each). Each of the resulting values was fitted to the Boltzmann equation and yielded an activation with $V_{1/2}$ = −96.1 ± 1.7 and −93.5 ± 2.0 mV, k = 9.4 ± 2.0 and 10.1 ± 1.4 before and during the application of 8Br-cAMP, respectively.

the HCN1 subunit more selectively localized than HCN2.[31] The activation of HCN2 is highly accelerated by increases of cytoplasmic cAMP. By contrast, HCN1 is only weakly modulated by cAMP.[31,34] The half activation concentration of cAMP for HCN2 channels is 0.5 μM.[30] In the current study, we showed that a relatively high concentration of 8Br-cAMP (1 mM) caused a slight increase in the amplitude of I_h with a positive shift of the voltage dependence. Forskolin caused smaller effects on I_h than those induced by a high concentration of 8Br-cAMP. These results suggested that the sensitivity of the I_h channel subtype in PVN parvocellular neurons to intracellular cAMP could be much less than that of HCN2 channels. We also found that there were no significant changes in either the amplitude or the activation kinetics of I_h during the application of adenylate cyclase inhibitor SQ22536,[37] suggesting that the likelihood of the tonic up-regulation of I_h channels by the intracellular cAMP system is even smaller. The intravenous anesthetic propofol selectively inhibits HCN channels containing HCN1 subunits in cortical pyramidal neurons.[38] The inhalational anesthetic halothane modulates neuronal I_h, and the corresponding HCN channel subunits are HCN1 and HCN2.[39] The precise contribution of HCN channel inhibition to dexmedetomidine action in PVN parvocellular neurons will require modeling in networks or experiments in animals with targeted disruption of HCN subunits.

Among the various autonomic responses, increases or decreases in blood pressure, heart rate, and sympathetic nerve activity are elicited by various stimulations in the rat PVN,[40] indicating a clear relation between outputs from the PVN and autonomic activity. Dexmedetomidine[5] and clonidine[41] induce cardiovascular depression and sympatholytic action mediated by a central mechanism. Dexmedetomidine may affect the autonomic nervous system activity mediated by the inhibition of I_h in PVN parvocellular neurons. Because I_h in PVN neurons can be modulated weakly by the cAMP system, any chemical substances or neurotransmitters that alter cAMP levels will have a small effect on the resting potential, spontaneous activity, and rebound potential latency. This feature of I_h may stabilize spontaneous discharge rates in PVN parvocellular neurons. In contrast to these compounds, dexmedetomidine and other anesthetics such as halothane,[39] enflurane,[42] and propofol[38] can directly reduce the I_h conductance. These drugs should induce a hyperpolarization of the membrane potential, a decrease in the slope steepness of slow depolarization, and a longer latency of rebound action potentials. As a result, the spontaneous discharge rate of neurons should decrease when neurons are exposed to these drugs. Perineural administration of the I_h channel blocker ZD7288 significantly reduced mechanical allodynia induced by partial sciatic nerve injury and hind paw incision.[43] It has been suggested that the medial parvocellular subdivision of the PVN is also involved in the arousal mechanisms.[44] These results suggest that antinociceptive, hypnotic, and sedative effects induced by anesthetics may be, at least in part, mediated by activation of GIRK and/or blockage of the I_h channel.

In conclusion, the current study demonstrated that dexmedetomidine binds to α_2 adrenoceptors on the cell membrane of PVN neurons, which leads to activation of the GIRK channels and inhibition of I_h channels, resulting in hyperpolarization of the membrane.

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