σ Receptor Activation Blocks Potassium Channels and Depresses Neuroexcitability in Rat Intracardiac Neurons

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ABSTRACT
The σ receptors have been implicated in the regulation of the cardiovascular system, and σ-1 receptor transcripts have been found in parasympathetic intracardiac neurons. However, the cellular function of σ-1 receptors in these cells remains to be determined. Effects of σ receptor activation on voltage-activated K⁺ channels and action potential firing were studied in isolated intracardiac neurons using whole-cell patch-clamp recording techniques. Activation of σ receptors reversibly blocked delayed outwardly rectifying potassium channels, large conductance Ca²⁺-sensitive K⁺ channels, and the M-current with maximal inhibition >80%. The inhibition of K⁺ channels by σ ligands was dose-dependent, and the rank order of potency of (+)-pentazocine > ibogaine > 1,3-di-O-tolyguanidin (DTG) suggests that the effect is mediated by σ-1 receptor activation. Preincubation of neurons with the irreversible σ receptor antagonist metaphit blocked DTG-induced inhibition of K⁺ channels, confirming that the effect is mediated by σ receptor activation. Although bath application of σ ligands depolarized intracardiac neurons, the number of action potentials fired by the cells in response to depolarizing current pulses was decreased in the presence of these drugs. Neither dia lysis of the neurons nor application of intracellular 5'-O-(2-thiodiphosphate) triethylamine chloride inhibited the effect of σ receptors on K⁺ channels, which suggests that the signal transduction pathway does not involve a diffusible cytosolic second messenger or a G protein. Together, these data suggest that σ-1 receptors are directly coupled to K⁺ channels in intracardiac neurons. Furthermore, activation of σ-1 receptors depresses the excitability of intracardiac neurons and is thus likely to block parasympathetic input to the heart.

The σ receptors are nonopioid, nonphenylcyclidine receptors that are distributed throughout the central and peripheral nervous system. These receptors have high affinity for numerous drugs in clinical use, including psychotherapeutic agents such as antipsychotics (Su, 1982) and selective serotonin reuptake inhibitors (Narita et al., 1996). At least two subtypes of σ receptors have been classified based on the findings from biochemical and radioligand binding experiments, and they have been designated as σ-1 and σ-2 (Hellewell and Bowen, 1990). However, to date, only the σ-1 receptor has been cloned (Hanner et al., 1996). These receptors have been implicated in physiological processes such as learning and memory and in pathophysiological disorders such as schizophrenia and depression (Snyder and Largent, 1989). More recently, σ receptors have been linked to the development of drug dependence (Ujike et al., 1996).

The σ receptors also seem to play a role in the regulation of the cardiovascular system, as suggested by pharmacological studies using σ receptor ligands. For example, it has been shown that the σ receptor ligand DuP 754 raises the threshold of ventricular fibrillation in rats (Lishmanov et al., 1999). Conversely, the σ receptor agonists (+)-SKF-10,047, 1,3-di-O-tolyguanidin (DTG), and (+)-3-[3-hydroxyphenyl]-1-propyl)piperidine have been shown to produce tachycardia by activating central and peripheral σ receptors (Wu and Martin, 1989; Lishmanov et al., 2000). Some of the effects of σ receptor ligands on the cardiovascular system are likely due to activation of σ receptors on cardiac muscle. The σ receptors have been detected in cardiac myocytes from neonatal and adult rats. 

ABBREVIATIONS: (+)-SKF-10,047, 2S-[2a,6a,11R]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride; DTG, 1,3-di-O-tolyguanidin; Kᵥ, voltage-activated K⁺ channel; Iₘ, M-current; GDP-β-S, guanosine 5'-O-(2-thiodiphosphosphate) triethylamine chloride; PSS, physiological saline solution; TTX, tetrodotoxin; GTP, GTP lithium salt; TEA, tetraethylammonium chloride; BK, large conductance K(Ca) channel; IK, intermediate conductance K(Ca) channel; AKP, afterhyperpolarization; Kₐ, small conductance K(Ca) channel; SK, BK channel; BK, fast inactivating BK current; BKᵢ, noninactivating BK current; SM-21, (-)-tropanyl 2-(4-chlorophenoxyl)butanoate maleate; BD-1047, N-[2-(3,4-dichlorophenyl)ethyl]-N-methyl-2-(dimethylamino)ethylamine dihydrobromide; BD-1063, 1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride.
adult rats, and stimulation of these receptors was shown to elicit direct inotropic and chronotropic effects on cardiac muscle (Ela et al., 1994; Novakova et al., 1995). However, σ receptors have also been found in autonomic neurons that regulate the cardiovascular system (Zhang and Cuevas, 2002), and modulation of the electrical activity of these neurons by endogenous or exogenous σ ligands is likely to have profound effects on cardiovascular function.

The σ receptor ligands, including haloperidol, (+)-pentazocine, DTG, and ibogaine, were previously shown to inhibit high-voltage-activated calcium channels in intrinsic cardiac neurons (Zhang and Cuevas, 2002). The pharmacological profile of the σ receptor mediating these effects was consistent with a σ-2 receptor. Although σ-1 receptor transcripts were detected in intracardiac neurons, no cellular effects have been attributed to this receptor. One possible role for σ-1 receptors in these cells is the modulation of voltage-activated K⁺ channels. Recent experiments have shown that currents mediated by Kv1.4 and Kv1.5 are depressed by the σ ligand (+)-SKF-10,047 in oocytes injected with Kv subunits and the σ-1 receptor but not in oocytes injected with the Kv subunits alone (Aydar et al., 2002). Furthermore, σ-1 receptors were shown to alter the kinetic properties of Kv1.4 channels in the absence of ligand, and Kv1.4 and Kv1.5 coimmunoprecipitated with the σ-1 receptor (Aydar et al., 2002), which led those investigators to propose that the σ-1 receptor functions as a ligand-regulated auxiliary K⁺ channel subunit. The inhibition of delayed outwardly rectifying K⁺ channels, such as Kv1.5, by verapamil has been shown to depress action potential firing in intracellular cardiac neurons, and this phenomenon has been implicated in the tachycardia associated with this type IV antiarrhythmic drug (Hogg et al., 1999). However, it remains to be determined whether σ-1 receptors couple to voltage-activated K⁺ channels in intracardiac neurons, and whether σ receptor activation can alter the active membrane properties of these cells. By regulating the function of K⁺ channels in parasympathetic intracardiac neurons, σ-1 receptors, and therefore drugs that act on these receptors, may have significant influence on the heart.

Experiments were undertaken to determine the effects of σ receptors on currents mediated by voltage-activated K⁺ channels (I_K) and the active membrane properties of intracardiac neurons from neonatal rats. The σ receptors were shown to decrease the peak amplitude of currents mediated by delayed outwardly rectifying K⁺ channels (I_KDOR), large conductance Ca²⁺-sensitive K⁺ channels (I_KCa), and the M-current (I_M). The rank-order potency of K⁺ channel inhibition by different σ ligands suggest that the effect is mediated by σ-1 receptors. Neither cell dialysis nor intracellular application of guanosine 5'-O-(2-thiodiphosphate) trilithium salt (GDP-β-S) blocked the effects of σ-1 receptors on I_K, consistent with a direct interaction between σ-1 receptors and the K⁺ channels. Activation of σ-1 receptors also decreased action potential firing and changed the action potential configuration in intracardiac neurons. Therefore, σ-1 receptor activation depresses the excitability of intracardiac neurons and is likely to attenuate parasympathetic input to the heart.

Materials and Methods

Preparation. The isolation and culture of neurons from neonatal rat intracardiac ganglia has been described previously (Cuevas and Adams, 1994). Briefly, neonatal rats (2–7 days old) were euthanized by decapitation, and the hearts were excised and placed in a physiological saline solution containing 140 mM NaCl, 3.0 mM KCl, 2.5 mM CaCl₂, 0.6 mM MgCl₂, 7.7 mM glucose, and 10 mM histidine, pH 7.2 with NaOH. Atria were removed and incubated for 1 h at 37°C in physiological saline solution containing 1 mg/ml collagenase (type 2, Worthington Biochemicals, Freehold, NJ). After enzymatic treatment, clusters of ganglia were dissected from the epicardial ganglion plexus and dispersed by trituration in a high-glucose culture medium (Dulbecco’s modified Eagle’s medium), 10% fetal calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin. The dissociated neurons were then plated onto glass coverslips coated with poly-L-lysine and incubated at 37°C under a 95% air/5% CO₂ atmosphere for 48 to 72 h.

Electrical Recordings. Electrophysiological recording methods used were similar to those described previously (Cuevas et al., 1997). Active membrane properties and voltage-activated K⁺ channel currents in intracardiac neurons were studied under current-clamp and voltage-clamp mode, respectively, using the whole cell patch-clamp technique. Electrical access was achieved through the use of the amphotericin B perforated-patch method to preserve the intracellular integrity and prevent the loss of cytoplasmic components and subsequent alteration of the functional responses of these neurons (Cuevas and Adams, 1994). For perforated patch experiments, a stock solution of 60 mg/ml amphetamine B in dimethyl sulfoxide was prepared and diluted in pipette solution immediately before use to yield a final concentration of 198 μg/ml amphetamine B in 0.33% dimethyl sulfoxide. Final patch pipette resistance was held at ~30 MΩ to activate the channel and repolarized to ~60 mV to deactivate the channels. Membrane voltages and currents were amplified using an Axopatch 200B patch-clamp amplifier (Axon Instruments, Inc., Union City, CA), filtered at 5 kHz (~3 dB; four-pole Bessel filter), and digitized at 20 kHz (Digidata 1200B).

Data Analysis. Analyses of these data were conducted using the SigmaPlot 2000 program (SPSS Inc., Chicago, IL). Data points represent mean ± S.E.M. Statistical difference was determined using paired t test for within-group experiments and unpaired t test for between groups experiments and was considered significant if p < 0.05.

Results

σ Receptor Activation Inhibits Delayed Outward Rectifying K⁺ Currents. Mammalian intracardiac neurons exhibit currents mediated by delayed outwardly rectifying
K⁺ channels (Xi-Moy and Dun, 1995; Hogg et al., 1999) and express various subunits that contribute to these channels, including KCNA5 and KCNA6 (J. Cuevas, unpublished observation). Given that σ-1 receptors have been shown to depress heterologously expressed KCNA6 channels (Aydar et al., 2002) and that inhibition of these channels may have significant effects on the function of intracardiac neurons, it seemed prudent to test the effects of σ receptor activation on currents mediated by voltage-activated K⁺ channels in these cells. Figure 1A shows a family of depolarization-activated K⁺ currents recorded from a single intracardiac neuron in the absence and presence of 100 μM DTG. Bath application of 100 μM DTG depressed peak voltage-activated K⁺ current amplitude at potentials positive to +20 mV. The voltage dependence and kinetics of the currents observed are consistent with the delayed outwardly rectifying K⁺ current (I[K(DR)]) previously reported in intracardiac neurons (Xi-Moy and Dun, 1995). Figure 1B shows a plot of the mean peak I[K(DR)] amplitude in the absence and presence of DTG as a function of voltage. The DTG-induced block of I[K(DR)] was voltage-dependent, with the current being blocked by 26.5 ± 6.2% at +70 mV but only by 1.5 ± 0.1% at +10 mV (n = 7). This difference was statistically significant (p < 0.01).

Concentration-Dependent Inhibition of I[K(DR)] by σ Ligands. Further pharmacological experiments were undertaken to confirm that σ receptors inhibit I[K(DR)] in intracardiac neurons and to identify the σ receptor subtype mediating the observed effects. Figure 2A shows representative currents recorded from three intracardiac neurons in the absence (control) and presence of (+)-pentazocine, ibogaine, and DTG, respectively, at the indicated concentrations. All of the σ ligands tested depressed peak I[K(DR)] in a concentration-dependent manner. A plot of the mean peak I[K(DR)] as a function of drug concentration for several σ ligands is shown in Fig. 2B. Fits of the data using the Hill equation gave half-maximal inhibitory concentration (IC₅₀) values for haloperidol, (+)-pentazocine, ibogaine, (+)-SKF-10,047, and DTG of 9.7 ± 0.5, 76.4 ± 7.7, 218.1 ± 7.0, 295.4 ± 45.4, and 341.3 ± 26.0 μM, respectively, and Hill coefficients of ~0.9 for all drugs. Maximum inhibition of I[K(DR)] by σ ligands was more than 80%. The IC₅₀ values and rank-order potency, in particular the observation that (+)-pentazocine is significantly more potent than ibogaine (p < 0.001), suggests that the effect on I[K(DR)] is mediated by activation of σ-1 receptors.

The σ Receptor Antagonist Metaphit Depresses the Effect of DTG on K⁺ Channels. To confirm the fact that the effect of σ ligands on I[K(DR)] was mediated by activation of σ receptors and not the result of direct channel block, experiments were done using the irreversible σ receptor antagonist metaphit. Metaphit rapidly and specifically acetylates σ receptors and inhibits ligand binding to the receptor (Bluth et al., 1989). In intracardiac neurons, metaphit has been shown to block σ-2 receptor-mediated attenuation of voltage-gated Ca²⁺ channel currents (Zhang and Cuevas, 2002). Isolated intracardiac neurons were preincubated in 50 μM metaphit (in PSS) for 10 min at room temperature. After washout of drug, I[K(DR)] was activated by depolarizing cells to +50 mV from a holding potential of −90 mV in the absence and presence of 100 μM DTG. Figure 3A shows representative currents recorded from two different intracardiac neurons without (top) or with (bottom) metaphit preincubation. Upon application of DTG, I[K(DR)] peak amplitude was depressed under both conditions, but in cells preincubated in metaphit the response to DTG was obtunded. Figure 3B shows a bar graph of degree of block of I[K(DR)] by 100 μM DTG in control neurons (DTG; n = 4) or neurons preincubated in metaphit (metaphit + DTG; n = 5). DTG decreased mean I[K(DR)] by 31 ± 3% in control cells, whereas in cells exposed to metaphit the decrease was 18 ± 3%. The difference in DTG attenuation of I[K(DR)] for the two conditions was statistically significant (p < 0.05). Several other σ receptor antagonists, including SM-21, BD-1047, and BD-1063 were tested to de-
termine whether these agents could block the effects of DTG on $I_{K\text{DR}}$. However, all of these drugs themselves depressed peak amplitude of $I_{K\text{DR}}$, suggesting that they are either acting as partial agonists of the $\sigma$-1 receptors, as reported previously for BD-1047 (Zambon et al., 1997), or having direct effects on the $K^+$ channels.

**Effect of Intracellular Dialysis with GTP and GDP-$\beta$-S on $\sigma$ Receptor Inhibition of $I_{K\text{DR}}$.** Controversy exists in the literature as to whether $\sigma$ receptors couple to G proteins and whether these second messengers are involved in the regulation of ion channels by $\sigma$ receptors. To determine whether $\sigma$ receptor-mediated inhibition of $K^+$ channels in intrinsic cardiac neurons is dependent on G protein activation, neurons were dialyzed with pipette solution containing either 100 $\mu$M GTP or the G protein inhibitor GDP-$\beta$-S (100 $\mu$M). Cell dialysis with 100 $\mu$M GDP-$\beta$-S has previously been shown to successfully block G protein-mediated signal transduction in intrinsic cardiac neurons (Cuevas and Adams, 1994, 1997; Zhang and Cuevas, 2002). Figure 4A shows representative currents in response to step depolarizations from $-90$ to $+50$ mV recorded from two neurons dialyzed with either GTP (top traces) or GDP-$\beta$-S (bottom traces) in the absence and presence of 100 $\mu$M DTG. The inhibition of $I_{K\text{DR}}$ evoked by this concentration of DTG under dialyzing conditions, with GTP in the pipette, was similar to that observed when the intracellular milieu was preserved ($\sim$30%). The DTG-induced attenuation of $I_{K\text{DR}}$ was present when the cells were dialyzed with GDP-$\beta$-S (Fig. 4A, bottom traces). A summary of the peak $I_{K\text{DR}}$ amplitudes elicited upon depolarization to $+50$ mV under the different experimental conditions and normalized to their respective control values is presented in Fig. 4B. The difference observed between the two groups was not statistical significant. The current-voltage relationships for six neurons dialyzed with either GTP or GDP-$\beta$-S are shown in Fig. 4C and D, respectively. Whereas dialyzing cells with GDP-$\beta$-S decreased the peak $I_{K\text{DR}}$ amplitude, compared with the cells dialyzed with GTP, DTG attenuated peak amplitudes under both condi-

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**Fig. 2.** Dose-dependent inhibition of $I_{K\text{DR}}$ by $\sigma$ receptor ligands in rat intracardiac neurons. A, whole cell $K^+$ currents evoked from three intracardiac neuron by step depolarizations to $+50$ mV from a holding potential of $-90$ mV in the absence (control) and presence of (+)-pentazocine, ibogaine, and DTG at the indicated concentrations. B, peak whole cell $I_{K\text{DR}}$ current amplitude, evoked by depolarizing to $+50$ mV from $-90$ mV, normalized to control, and plotted as a function of $\sigma$ ligands concentration. Data points represent mean $\pm$ S.E.M. for four or more observations. The curves represent best fit to the data using the Hill equation. Half-maximal inhibition was $9.7 \pm 0.5$ $\mu$M for haloperidol, $76.4 \pm 7.7$ $\mu$M for (+)-pentazocine, $218.1 \pm 7.0$ $\mu$M for ibogaine, $295.4 \pm 45.4$ $\mu$M for (+)-SKF-10,047, and $341.3 \pm 26.0$ $\mu$M for DTG. The corresponding Hill coefficients were $1.01 \pm 0.05, 0.69 \pm 0.05, 1.14 \pm 0.04, 0.84 \pm 0.11, \text{and } 0.97 \pm 0.07$, respectively.
Fig. 3. Attenuation of DTG-mediated inhibition of $I_{K_{DR}}$ by the $\sigma$ receptor antagonist metaphyt. A, depolarization-activated (−90 to +50 mV) $I_{K_{DR}}$ recorded from two intracardiac neurons in the absence (control) and presence of 100 µM DTG (DTG). Bottom traces are from a neuron preincubated in metaphyt (50 µM in PSS, 10 min). B, bar graph of the percent inhibition of mean peak $I_{K_{DR}}$ (±S.E.) produced by 100 µM DTG in control cells that were not exposed to metaphyt (DTG) or cells preincubated in metaphyt (50 µM; 10 min; metaphyt + DTG). $I_{K_{DR}}$ was evoked by step depolarizations (−90 to +50 mV). Data were collected from seven neurons for each condition, and the asterisk denotes significant difference between the groups ($p < 0.05$).

Fig. 4. The $\sigma$ receptor inhibition of $I_{K_{DR}}$ is not blocked by intracellular GDP-β-S. A, depolarization activate (−90 to +50 mV) $K^+$ currents recorded from neurons dialyzed with pipette solutions containing either 100 µM GTP or GDP-β-S in the absence (control) or presence of 100 µM DTG (DTG). B, mean (±S.E.M.) peak $I_{K_{DR}}$, recorded from neurons dialyzed with either GTP ($n = 6$) or GDP-β-S ($n = 6$) in the presence of 100 µM DTG. Currents are normalized to their respective controls (absence of drug). Mean (±S.E.) peak $I_{K_{DR}}$, current density as a function of depolarization voltage (−90-mV holding potential) recorded from neurons dialyzed with pipette solution containing either 100 µM GTP (C, $n = 6$) or 100 µM GDP-β-S (D, $n = 6$) in the absence (C) and presence (D) of 100 µM DTG.
tions to a similar extent. These data suggest that neither cell
dialysis nor inhibition of G protein activation blocks \( \sigma \)-1
receptor-mediated inhibition of \( I_{K(Ca)} \). Thus, neither a diffus-
ible cytosolic second messenger nor a G protein couples \( \sigma \)-1
receptors to \( I_{K(Ca)} \).

**Effects of \( \sigma \) Receptor Ligands on Action Potential Firing.** Studies have shown that inhibition of \( I_{K(DR)} \) by ve-
rapamil in intracardiac neurons can depress action potential firing (Hogg et al., 1999). Experiments were thus conduced
to determine whether \( \sigma \) receptor modulation \( I_{K(DR)} \) has a
similar effect on neuroexcitability in these cells. The effects of
\( \sigma \) ligands on the active membrane properties of isolated in-
tracardiac neurons were studied using the amphotericin B
perforated patch method under current-clamp mode. Figure
5A shows a family of action potentials elicited from a single
intracardiac neuron in response to depolarizing current pulses (100 pA; 400 ms) in the absence (control) and presence
of 100 \( \mu \)M DTG (DTG), and after washout of drug (wash).
DTG depolarized the neuron and decreased the number of
action potentials evoked by the current injection in a rapidly
reversible manner. In similar experiments, 100 \( \mu \)M DTG
depolarized neurons from a control value of \(-50.8 \pm 2.1\) to
\(-49.2 \pm 2.4\) mV (n = 9). DTG also decreased the number of
action potentials evoked by depolarizing membrane pulses by
85\% (Fig. 5B). Both of these changes were statistically sig-
nificant (\( p < 0.01 \) and \( p < 0.001 \), respectively). DTG also
altered the action potential configuration by decreasing both
the action potential overshoot and afterhyperpolarization
(AHP) and by slowing both the rate of depolarization and rate
of repolarization (Fig. 5A, inset; Table 1). The effects of DTG
were mimicked by 10 \( \mu \)M haloperidol, 200 \( \mu \)M ibogaine, and
50 \( \mu \)M (+)-pentazocine, which depressed action potential
firing by 65, 85, and 65\%, respectively and changed the
configurations of the waveform in a reversible manner. The
effects of the \( \sigma \) ligands on the action potential firing and configures are summarized in Table 1.

**Role of M-Currents in the Effects of \( \sigma \) Receptor Ac-
tivation.** One possible mechanism by which \( \sigma \)-1 receptor
activation depolarizes intracardiac neurons is via a block of
the M-current. Previous studies have shown that inhibition
of \( I_{M} \) by muscarinic receptor activation depolarizes in-
tracardiac neurons (Cuevas et al., 1997). The M-channels mediat-
ing \( I_{M} \) are noninactivating and close slowly in response to
membrane repolarization from depolarizing holding poten-
tials. The closing of these channels in intracardiac neurons
upon repolarization results in a characteristic inward cur-
rent relaxation (Cuevas et al., 1997). Figure 6A shows whole
cell currents recorded from a single cell in response to rep-
olarizing steps to \(-60\) mV from a holding potential of \(-30\) mV
in the absence (control) and presence of 1 mM DTG. The
inward relaxation observed under control conditions is abol-
ished by application of DTG. Similarly, 20 \( \mu \)M linopirdine, a
specific blocker of \( I_{M} \) (Wallace et al., 2002), eliminates the
inward relaxation (Fig. 6B). Figure 6, C and D, shows bar
graphs of mean peak \( I_{M} \) recorded under control conditions
(absence of drug) and when either DTG (\( n = 4 \)) or linopirdine
(\( n = 6 \)) was added, respectively. \( I_{M} \) was completely blocked by
application of either drug, and this effect was reversible after
washout in both cases (data not shown).

**Effects of \( \sigma \) Receptor Activation on BK Currents.** In
central and peripheral neurons, action potential AHPs are
mediated by the opening of \( \text{Ca}^{2+} \)-activated \( K^{+} \) channels
\( [I_{K(Ca)}] \). Thus far, three types of \( \text{Ca}^{2+} \)-activated \( K^{+} \) channels
have been identified on the basis of their pharmacology and
single channel conductances. These channels have been
named according to their unitary conductance: large (big)
conductance K(Ca) channels (BK), small conductance K(Ca) channels (SK), and intermediate conductance (IK). Although both BK and SK channels have been found in mammalian intracardiac neurons, neonatal rat intracardiac neurons only express BK channels (Franciolini et al., 2001; Jelson et al., 2003). The fact that /H9268 receptor activation decreases the AHP suggests that /H9268 receptors may modulate these channels.

TEA, at micromolar concentrations (200–500 M), has been shown to preferentially block BK channels in intracardiac neurons and to block the AHPs more effectively than either charybdotoxin or iberiotoxin (Franciolini et al., 2001). Thus, 500 M TEA was used to distinguish between outward K channels mediated by BK and other K channel types [i.e., IK(DR) and IM]. Outward currents were evoked by stepping to 50 mV from a 90-mV holding potential in the absence and presence of 500 M TEA. The net TEA-sensitive current, and thus the current mediated by BK channels, was determined by subtracting the current remaining after TEA application from that observed under control conditions (absence of TEA). Two distinct TEA-sensitive currents, which differed in their time-dependent inactivation kinetics, were seen in our experiments. These currents were expressed in different pop-

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Asterisks denote significant difference (*p < 0.05 and **p < 0.01).

![Fig. 6](image-url)

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The /H9268 receptor ligand DTG inhibits the M-current in rat intracardiac neurons. A, slow inward current relaxation (M-current) induced by repolarizing step (−30 to −60 mV; 250 ms) recorded from a single intracardiac neuron before (control) and after application of 1 mM DTG (DTG). B, M-current recorded in the absence (control) and in the presence of 20 M linopirdine (linopirdine). Bar graph of mean current amplitudes in the absence (control) or presence of 1 mM DTG (C; n = 4) and 20 M linopirdine (D; n = 6). The amplitude of the M-current was defined as the difference between the peak current observed at the start of the relaxation (A, left trace, black arrow) and the holding current at the end of the 250-ms repolarizing step (A, left trace, gray arrow). Asterisks denote significant difference from control (p < 0.001).
ulations of neurons, with the fast inactivating current (BK) being expressed in six of 10 cells and the slowly inactivating or noninactivating currents (BK) being expressed in the remaining four cells. Currents representative of each BK channel subtype are shown in Fig. 7, A and B. Both the rapidly decaying and the slowly decaying TEA-sensitive currents were blocked by 1 mM DTG. Figure 7, C and D, shows bar graphs of the mean peak and sustained current amplitude obtained from the BK, (n = 6) and BK, (n = 4) species, respectively. Both phases of the current were reduced in the two current types when 1 mM of DTG was applied.

**Discussion**

The results presented here show that in neonatal rat intracardiac neurons activation of σ-1 receptors reversibly inhibited I_{K(DR)}, I_{K(Ca)}, and I_{M}, and multiple K^+ channel types are frequently affected in individual cells (Soriani et al., 1999a,b; Wilke et al., 1999). All of these K^+ channel subtypes, except A-type K^+ channels, which are not expressed in intracardiac neurons, were shown to be affected by σ receptor activation in our study.

Although σ receptors have been shown to block K^+ channels in native cells, the σ receptor subtype mediating these effects has not been definitively identified. The rank-order potency for IK inhibition by ligands reported here, haloperidol > (+)-pentazocine > ibogaine > DTG, suggests that the effect is mediated by σ-1 receptor activation. In contrast, the rank-order potency for σ ligand inhibition of Ca^{2+} channels in intracardiac neurons is haloperidol > ibogaine > (+)-pentazocine > DTG, which is consistent with a σ-2 receptor-mediated effect. The IC_{50} values for the various σ ligands tested here are in agreement with those reported in the literature for modulations of voltage-gated K^+ channels. For example, in frog pituitary melanotrophs, (+)-pentazocine inhibited delayed outwardly rectifying K^+ channels with an IC_{50} of 37 μM, compared with the IC_{50} of 42 μM reported here. Similarly, 100 μM (+)-SKF-10,047 blocked approximately 50% of the voltage-activated K^+ currents recorded in

**Fig. 7.** The σ receptor activation blocks BK in rat intracardiac neurons. Traces of BK-mediated currents recorded from two neurons expressing fast inactivating (A) and slowly inactivating BK channels (B), in the absence (control) and presence of 1 mM DTG (DTG). BK-mediated currents were evoked by step depolarizations to +50 mV from a holding potential of −90 mV and defined as the net TEA-sensitive current. The net TEA-sensitive current was determined by subtracting the inward current recorded in the presence of 500 μM TEA (with or without DTG) from the outward current obtained in the absence of TEA (with or without DTG). Bar graph of the peak and sustained BK current amplitudes recorded in the absence (control) and presence of DTG (DTG; 1 mM) in cells expressing fast inactivating (C; n = 6) or slowly inactivating (D; n = 4) BK channels. Asterisks denote significant difference from control (p < 0.05).
neurohypophysial nerve terminals (Wilke et al., 1999). Thus, σ-1 receptors are likely to be responsible for inhibition of K+ channels in intracardiac neurons and in other native cells studied. The conclusion that σ receptors mediate the inhibition of IK by σ ligands in intracardiac neurons is strengthened by the observation that metaphtin, an irreversible, antagonist of σ receptors, blocks the effects of DTG on IK in these cells.

Considerable controversy exists as to the mechanisms by which σ receptors modulate K+ channels. In frog pituitary melanotrophs, cell dialysis with GTP-β-S and preincubation in cholina toxin were shown to inhibit σ receptor effects on IA and IK(DR) (Soriani et al., 1999a, b), suggesting that σ receptors couple to the K+ channels via a G protein. G proteins have also been implicated in both σ receptor-mediated activation of phospholipase C and regulation of Ca2+ release from intracellular stores (Morin-Surun et al., 1999; Hayashi et al., 2000). However, neither cell dialysis nor application of intracellular GTP-β-S blocked the inhibition of IK by σ-1 receptors in intracardiac neurons. Thus, σ receptors couple to K+ channels in these cells via a membrane-delimited signal transduction cascade that does not involve a G protein. Our observation is in agreement with the direct protein-protein interaction between σ-1 receptors and K+ channels shown by Jackson and colleagues (Lupardus et al., 2000; Aydar et al., 2002).

The net effect of σ receptor modulation of ion channels in intracardiac neurons at rest is a depolarization of the membrane potential. We have previously shown that the IM contributes to the resting membrane potential of intracardiac neurons and that inhibition of this channel depolarizes the cells (Cuevas et al., 1997). Given that application of DTG blocks IM, σ receptor modulation of these channels likely accounts for the depolarizations reported here. Further support for this conclusion comes from the fact that neither inhibition IK(DR) nor IK(Ca1) has been linked to changes in the resting membrane potential of these neurons (Xu and Adams, 1992; Hogg et al., 1999; Jelson et al., 2003). Inhibition of voltage-gated Ca2+ channels by σ-2 receptor activation is also unlikely to account for this depolarization since removal of extracellular Ca2+ does not alter the resting membrane potential of these cells (DeHaven and Cuevas, 2004).

The block of IK by σ receptors in intracardiac neuron is associated with decreased action potential firing, whereas previous studies have shown that σ receptor block of IK enhances neuroexcitability (Soriani et al., 1998). Our laboratory has shown that inhibition of IM is associated with increased excitability of intracardiac neurons (Cuevas et al., 1997), and thus σ receptor modulation of non-IM K+ channels must be responsible for the decreased excitability observed. The depressed neuroexcitability reported here likely results from modulation of IK(DR) by σ receptors. Verapamil has been shown to convert tonic and adapting intracardiac neurons into phasic neurons via a direct block of IK(DR) (Hogg et al., 1999). However, high concentrations of verapamil failed to abolish action potential firing in intracardiac neurons, whereas high concentrations of σ ligands completely blocked the genesis of action potentials in these cells. The concentrations of (+)-pentazocine (50 μM) and ibogaine (200 μM) required to appreciably decrease action potential firing and alter the action potential configuration in our study suggest that the effects of σ ligands on the active membrane properties of these cells are primarily mediated by σ-1 receptors. However, inhibition of voltage-activated K+ channels alone cannot explain the complete block of action potential firing at high concentrations of σ ligands, and thus σ receptors are likely to affect other channel types that regulate action potential firing (e.g., voltage-gated sodium channels).

The inhibition of IK(Ca1) by σ receptors is also likely to contribute to changes in the active membrane properties of intracardiac neurons. Two types of σ receptor-regulated BK currents were found in rat intracardiac neurons, one which exhibited rapid time-dependent inactivation, BK1, and a second that showed little or no inactivation, BK2. The presence of distinct subpopulations of BK channels with dissimilar inactivation kinetics has been reported in rat adrenal chromaffin cells (Solaro et al., 1995). The inhibition of BK currents by σ receptors would account, at least in part, for the increase in action potential duration and decrease in AHPs reported here, since inhibition of BK by 200 μM TEA produces similar effects on the action potentials of these cells (Franciolini et al., 2001). Although inhibition of voltage-gated Ca2+ channels may contribute to the decrease in IK(Ca1) amplitude observed in our studies, it is unlikely to exclusively account for the block of BK channels observed here. Evidence for this conclusion comes from the fact that concentrations of Cd2+ (100 μM) that inhibit all Ca2+ channels in these neurons (Cuevas and Adams, 1997) fail to eliminate the AHPs in these cells (Franciolini et al., 2001). Furthermore, in our study, 100 μM Cd2+ blocked <5% of IK, whereas 500 μM TEA or Ca2+-free extracellular solution blocked ~25% of IK (data not shown). Together, these data suggest that σ-1 receptors are likely having a direct effect on BK channels in intracardiac neurons. However, σ receptor modulation of other mech-ianisms of Ca2+ entry or homeostasis must also be examined to confirm a direct effect on IK(Ca1).

There has been considerable speculation about the role of σ receptors in the cardiovascular system. Although the endogenous ligand responsible for activating σ receptors under physiologic and pathophysiological conditions remains to be determined, there are data suggesting that some putative σ receptor ligands may affect the heart and coronary vasculature. For example, pregrenolone, a putative σ ligand (Maurice et al., 2001), increases heart rate and cardiac output in anesthetized dogs (Hogskilde et al., 1991). Such effects may in part be due to activation of σ receptors on intracardiac neurons. Similarly, various drugs that act on σ receptors, such as haloperidol, have significant effects on the heart (Monassier and Bousquet, 2002). Our observations raise the possibility that the cardiovascular effects of these compounds may be mediated by their modulation of the electrical activity of intrinsic cardiac neurons. In conclusion, stimulation of σ-1 receptors inhibits multiple voltage-gated K+ channel subtypes and depresses excitability in intracardiac neurons. Thus, activation of σ-1 receptors in these cells is likely to attenuate parasympathetic input to the heart, and consequently, to affect cardiovascular function.

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