invited review

A-type potassium currents in smooth muscle

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Amberg, Gregory C., Sang Don Koh, Yuji Imaizumi, Susumu Ohya, and Kenton M. Sanders. A-type potassium currents in smooth muscle. Am J Physiol Cell Physiol 284: C583–C595, 2003; 10.1152/ajpcell.00301.2002.—A-type currents are voltage-gated, calcium-independent potassium (Kv) currents that undergo rapid activation and inactivation. Commonly associated with neuronal and cardiac cell-types, A-type currents have also been identified and characterized in vascular, genitourinary, and gastrointestinal smooth muscle cells. This review examines the molecular identity, biophysical properties, pharmacology, regulation, and physiological function of smooth muscle A-type currents. In general, this review is intended to facilitate the comparison of A-type currents present in different smooth muscles by providing a comprehensive report of the literature to date. This approach should also aid in the identification of areas of research requiring further attention.

smooth muscle; Kv4 channels; potassium channel-interacting proteins

SMOOTH MUSCLE CONTRACTILE ACTIVITY correlates well with the level of free intracellular Ca 2+ available to the contractile apparatus (Ref. 24; see, e.g., Refs. 13 and 52). Ca 2+ may enter the cytosol from the extracellular space or may be released from intracellular stores (i.e., the sarcoplasmic reticulum; e.g., Ref. 99). Ca 2+ influx through voltage-gated Ca 2+ channels is required for physiological patterns of smooth muscle contraction (17, 21, 50, 64). Although release of intracellular Ca 2+ is necessary for effective contraction, it is likely that voltage-dependent entry of Ca 2+ across the plasma membrane is responsible for initiating contractile events (81, 115). Because of this voltage dependence, membrane potential is the primary determinant of smooth muscle tone.

K+ channels are important regulators of membrane potential. Under physiological conditions, membrane potentials of smooth muscles lie positive to the reversal potential for K+ (E K ≈ −80 to −90 mV). Consequently, increasing membrane permeability to K+ results in outward current that opposes depolarization and cellular excitability (i.e., contraction). K+ channels exhibit remarkable molecular and functional diversity, and the electrical behavior of a given tissue is highly influenced by the complement K+ currents present. The broad diversity of electrical activities of smooth muscles largely results from the great diversity of K+ channels expressed in these tissues. Other reviews have examined the general topic of K+ currents in smooth muscles (e.g., Refs. 33, 47, 73, 102, 116). This review is intended to complement and expand on these publications by focusing specifically on rapidly inactivating A-type K+ conductances that are present in various smooth muscles.

GENERAL PROPERTIES OF A-TYPE CURRENTS

Voltage-gated, Ca 2+ -independent K+ (Kv) currents are present in all smooth muscles. By using time dependence as a basis for classification, Kv currents may be divided into two archetypical categories: slow “delayed rectifier” currents and rapid “A-type” currents. Hodgkin and Huxley (44) first used the term delayed rectifier to describe the voltage-dependent K+ current that developed after the Na+ current in response to depolarization of Loligo giant axons. The term delayed rectifier persists and is generally used to describe Kv currents with kinetics similar to the original axonal K+
currents (i.e., delayed onset of activation followed by little or slow inactivation).

A-type currents were first observed in molluscan neurons by Hagiwara and coworkers (41) and later characterized by Connor and Stevens (22), Neher (72), and Thompson (106). The general properties of A-type currents in neurons have been the subject of previous reviews (85, 87). A-type currents are voltage-gated, Ca\(^{2+}\)-independent K\(^+\) currents that are distinguished from typical delayed rectifier currents by rapid rates of inactivation. Kinetically, A-type currents bear closer resemblance to voltage-gated Na\(^+\) currents than to delayed rectifier K\(^+\) currents (72). A-type currents activate at negative membrane potentials, with measurable thresholds typically between \(-45\) and \(-60\) mV. Strong steady-state, voltage-dependent inactivation is a feature typical of A-type currents, and repolarization to potentials negative to \(-50\) mV is typically required for restoration of channel availability (Fig. 1). Inhibition by 4-aminopyridine (4-AP) and insensitivity to extracellular tetraethylammonium (TEA) ions are considered to be pharmacological hallmarks of A-type currents (e.g., Ref. 106), although notable exceptions exist (e.g., Refs. 88 and 91).

### A-TYPE CURRENTS IN NON-SMOOTH MUSCLE TISSUES

A-type currents have been examined extensively in neurons, and these currents are thought to regulate firing frequency (14, 22, 63, 109). In many neurons, A-type channels are unavailable at resting membrane potentials due to pronounced steady-state inactivation. A-type channels become available in these cells during afterhyperpolarizations following action potentials, when the membrane potential becomes sufficiently negative to remove inactivation (22). A-type channels activate during the decay of the afterhyperpolarization and tend to delay depolarization. In this manner, A-type currents prolong the period between action potentials.

A-type currents are also present in atrial and ventricular myocytes and in these cells are referred to as “transient” outward current (\(I_{to}\)). In contrast to neuronal A-type currents, \(I_{to}\) channels are available at resting membrane potentials (Ref. 7; e.g., Refs. 9 and 74). \(I_{to}\) is predominantly responsible for the initial repolarization (phase 1) of the cardiac action potential. Pharmacological blockade of \(I_{to}\) with 4-AP causes an increase in action potential amplitude and duration (34) and increases force generation (94). The distribution of \(I_{to}\) within the myocardium is nonuniform and contributes toward regional variations in action potential waveforms (6, 15, 121).

### A-TYPE CURRENTS IN SMOOTH MUSCLE

Voltage-dependent, transient outward K\(^+\) currents have also been identified in smooth muscle cells (see Table 1). The smooth muscle literature is confusing in that the term transient outward current has been used to designate Ca\(^{2+}\)-activated K\(^+\) currents (elicited by step depolarizations that activate large, initial Ca\(^{2+}\) entry events), slowly inactivating, voltage-dependent K\(^+\) currents, and true A-type currents. In this review we will use the term A-type current to designate rapidly inactivating, voltage-dependent K\(^+\) currents.

A-type currents have been identified in vascular smooth muscle cells of the rabbit (portal vein, pulmonary artery, aorta), rat (pulmonary artery, renal resistance artery), and human (mesenteric artery). See Table 2 for appropriate references. In contrast to the A-type currents of the myocardium (discussed elsewhere; e.g., Refs. 74 and 80), the physiological function of A-type currents in vascular myocytes has not been fully clarified. A-type currents have been identified in genitourinary (GU) smooth muscle cells of the guinea pig (ureter, seminal vesicles, and vas deferens), rabbit (vas deferens), rat (myometrium), and human (myometrium). A-type currents are also present in gastrointestinal (GI) smooth muscle cells of the mouse (fundus, antrum, jejunum, and colon), rat (ileum), guinea pig (colon), and opossum (esophagus), and a “transient delayed rectifier” has also been characterized in the human esophagus. As this review describes, the physiological function of A-type currents in smooth muscles may be related to the maintenance of membrane potential and regulation of excitability.

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**Fig. 1.** General properties of A-type K\(^+\) currents. A: whole cell A-type currents from holding potentials of \(-80\) (a) and \(-40\) mV (b) recorded from mouse antral myocytes. B: steady-state inactivation shown as a plot of normalized peak current \((i/t_{\text{max}})\) as a function of conditioning potential (\(V_c\)) and fit with a Boltzmann function. For voltage dependence of activation, peak K\(^+\) currents at test potentials between \(-80\) and \(+40\) mV were converted into permeabilities using the Goldman-Hodgkin-Katz current equation. Permeabilities were normalized \((P_{\text{K}}/P_{\text{Na}})\), plotted as a function of test potential (\(V_t\)), and fit with a Boltzmann function. Dashed lines mark the voltages of half activation and inactivation. [Reprinted from Amberg et al. (2).]
MOLECULAR IDENTITY OF SMOOTH MUSCLE A-TYPE CONDUCTANCES

K^+ channel α-subunits with A-type properties are found in several K^+ channel families including Shaker (Kv1.4), Shaw (Kv3.4), and Shal (Kv4.1, Kv4.2, and Kv4.3). Kv1.4 and Kv4 channels are responsible for the typical A-type conductances susceptible to 4-AP in a wide range of tissues, whereas Kv3.4 channels are mainly expressed in skeletal muscle but also in the central nervous system. In central and peripheral neurons, Kv1.4 and Kv4 channels distribute primarily to presynaptic and postsynaptic membranes, respectively (93). Differences in the local distribution of specific A-type channel subtypes has been reported to be one of the major determinants of distinct functional characteristics observed in various regions of the heart and central nervous system.

Transcripts of a variety of pore-forming α-subunits with A-type properties (i.e., Kv1.4, Kv3.3, Kv3.4, Kv4.1, Kv4.2, and Kv4.3) have been detected in rat mesenteric and tail arteries (122, 123). Kv β-subunits, which confer A-type properties to some delayed rectifier pore-forming subunits (e.g., Ref. 84), have been found in these two tissues as well. Kv1.4, Kv4.1–4.3, and Kvβ transcripts have been detected in rat pulmonary artery smooth muscle (25, 104, 127), and transcripts encoding the long isoform of Kv4.3 have been detected in rat aortic smooth muscle (77). The distribution of A-type channel transcripts in various smooth muscles is included in Table 3. Evidence regarding the molecular composition of A-type currents in smooth muscles is, however, incomplete, and experiments using antisense techniques or production of A-type channel knockout animals have yet to be performed.

One of the distinguishing features of various A-type currents is the time course of recovery from inactivation. For example, A-type currents mediated by the Kv4 family of channels recover rapidly from inactiva-

### Table 1. Smooth muscle A-type current properties

<table>
<thead>
<tr>
<th>Smooth Muscle</th>
<th>Peak at 0 mV, nA</th>
<th>Threshold, mV</th>
<th>(V_{0.5_{\text{inact}}, \text{mV}})</th>
<th>(\tau_{\text{inact}, \text{ms}})</th>
<th>(\tau_{\text{recov}, \text{ms}})</th>
<th>4-AP, mM</th>
<th>TEA, mM</th>
<th>Single-channel Conductance, pS</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit portal vein</td>
<td>&lt;0.1</td>
<td>-65</td>
<td>-78</td>
<td>-70</td>
<td>800(-70mV)</td>
<td>5</td>
<td>&gt;30</td>
<td>14</td>
<td>(asym K^+)</td>
</tr>
<tr>
<td>Human mesenteric artery</td>
<td>&lt;0.05</td>
<td>+10</td>
<td>-38</td>
<td>-70</td>
<td>254(-100mV)</td>
<td>1</td>
<td>&gt;30</td>
<td>12</td>
<td>95</td>
</tr>
<tr>
<td>Rat pulm artery</td>
<td>-0.2</td>
<td>-30</td>
<td>(\tau_1 \approx 20)</td>
<td>(\tau_2 \approx 120)</td>
<td>10</td>
<td>&gt;10</td>
<td>50</td>
<td>126, 127</td>
<td></td>
</tr>
<tr>
<td>Rabbit pulm artery</td>
<td>-0.2</td>
<td>-50</td>
<td>-49</td>
<td>(\tau_1 \approx 70)</td>
<td>&gt;10 s</td>
<td>2.5</td>
<td>&gt;10</td>
<td>20, 79</td>
<td></td>
</tr>
<tr>
<td>Rabbit portal artery</td>
<td>0.6</td>
<td>-10</td>
<td>-45</td>
<td>(\tau_1 \approx 70)</td>
<td>(\tau_2 \approx 220)</td>
<td>2.5</td>
<td>&gt;10</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Rabbit aorta</td>
<td>-0.1</td>
<td>-20</td>
<td>-50</td>
<td>10</td>
<td>&gt;10</td>
<td>42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonpregnant rat myometrium</td>
<td>&lt;0.1</td>
<td>-60</td>
<td>-77</td>
<td>3</td>
<td>10</td>
<td>&gt;30</td>
<td>119</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnant human myometrium</td>
<td>0*</td>
<td>-40</td>
<td>-69</td>
<td>5</td>
<td>&gt;10</td>
<td>83*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig vas deferens</td>
<td>0.15</td>
<td>-40</td>
<td>-49</td>
<td>(\tau_1 = 220)</td>
<td>(\tau_2 = 1026)</td>
<td>2</td>
<td>&gt;30</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Guinea pig deferens (+30mV)</td>
<td>0.25</td>
<td>-30</td>
<td>-48</td>
<td>(\tau_1 = 30)</td>
<td>(\tau_2 = 120)</td>
<td>1</td>
<td>&gt;10</td>
<td>49, 103*</td>
<td></td>
</tr>
<tr>
<td>Guinea pig seminal vesicle</td>
<td>0.32</td>
<td>-30</td>
<td>-70*</td>
<td>20–30</td>
<td>1</td>
<td>&gt;10</td>
<td>89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newborn rat ileum</td>
<td>2</td>
<td>-50</td>
<td>-71</td>
<td>-30</td>
<td>89(-80mV)</td>
<td>1</td>
<td>&gt;30</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Guinea pig colon</td>
<td>0.3</td>
<td>-60</td>
<td>-58</td>
<td>(\tau_1 \approx 30)</td>
<td>45(-85mV)</td>
<td>&lt;3</td>
<td>&gt;100</td>
<td>12–13 (asym K^+)</td>
<td>113, 114</td>
</tr>
<tr>
<td>Mouse colon</td>
<td>1</td>
<td>-50</td>
<td>-58</td>
<td>(\tau_1 \approx 96)</td>
<td>55(-80mV)</td>
<td>5</td>
<td>&gt;10</td>
<td>19</td>
<td>57, 4</td>
</tr>
<tr>
<td>Opossum esophagus</td>
<td>-0.4</td>
<td>-50</td>
<td>-57</td>
<td>50</td>
<td>125(-75mV)</td>
<td>3</td>
<td>&gt;30</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Human esophagus</td>
<td>-0.15</td>
<td>-40</td>
<td>-43</td>
<td>3</td>
<td>&gt;2</td>
<td>117</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse antrum</td>
<td>1.2</td>
<td>-60</td>
<td>-65</td>
<td>83</td>
<td>252(-80mV)</td>
<td>10</td>
<td>&gt;10</td>
<td>2</td>
<td>126, 127</td>
</tr>
<tr>
<td>Mouse fundus</td>
<td>1.5</td>
<td>-60</td>
<td>-55</td>
<td>71</td>
<td>75(-80mV)</td>
<td>5</td>
<td>&gt;10</td>
<td>Amberg, Koh, and Sanders (unpublished)</td>
<td></td>
</tr>
<tr>
<td>Mouse jejunum</td>
<td>0.37</td>
<td>-50</td>
<td>-56</td>
<td>65</td>
<td>72(-80mV)</td>
<td>5</td>
<td>&gt;10</td>
<td>Amberg, Koh, and Sanders (unpublished)</td>
<td></td>
</tr>
</tbody>
</table>

\(V_{0.5_{\text{inact}}, \text{mV}}\), Voltage of half-inactivation; \(\tau_{\text{inact}, \text{ms}}\), time constant of inactivation; \(\tau_{\text{recov}, \text{ms}}\), time constant of recovery at voltage indicated; \(\tau_1\) and \(\tau_2\), fast and slow time constants of inactivation; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; 3,4-DAP, 3,4-diaminopyridine.
tion (cf. Refs. 92 and 124). The rapid recoveries from inactivation of many GI A-type currents (time constants ~100 ms; Refs. 3, 57, 97, and 114) are comparable to rates reported for cardiac A-type currents (I\textsubscript{to}), which are due to Kv4 channels in most mammals (Refs. 7, 10, and 28; e.g., Ref. 74). For comparison, kinetic profiles of Kv4.2 and Kv4.3 are presented in Table 4. In human mesenteric artery the reported recovery from inactivation is relatively fast (time constant 250 ms; Ref. 95), suggesting that the conductance responsible may be Kv4-derived. The rapid recovery from inactivation of the A-type currents in guinea pig ureter (time constant 100 ms; Ref. 49) is also suggestive of Kv4 channel involvement. In contrast to the currents described above, the slower recovery from inactivation reported in rabbit portal vein (12) and pulmonary artery (79) are considerably slower (time constants 0.8 s and >10 s, respectively). These rates of recovery are more consistent with currents mediated by Kv1 channels. It is important to note that kinetic features of A-type K\textsuperscript{+} currents depend not only on the corresponding α-subunits but also on the presence of and interaction with accessory β-subunits (e.g., Ref. 84). Different heteromeric subunit combinations likely underlie the diversity of A-type currents present in smooth muscles (see Table 1).

KV4 CHANNELS AS A MOLECULAR COMPONENT OF A-TYPE CONDUCTANCES IN SMOOTH MUSCLE

A substantial line of evidence has accumulated suggesting that the Kv4 family of K\textsuperscript{+} channels, particularly Kv4.2 and Kv4.3, may be one of the major components of A-type conductances in many smooth muscles. In GU smooth muscles, Kv4.3L (long isoform) transcripts show greater abundance than those encoding Kv1.4, Kv3.3, Kv3.4, or Kv4.2. A full-length clone of Kv4.3L, obtained by RT-PCR from rat vas deferens

### Table 2. Tissues with reported A-type currents

<table>
<thead>
<tr>
<th>Smooth Muscle</th>
<th>Resting Membrane Potential, mV (Ref.)</th>
<th>Phasic</th>
<th>A-Type Current Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit portal vein</td>
<td>−50 (59)</td>
<td>Yes</td>
<td>12</td>
</tr>
<tr>
<td>Human mesenteric artery</td>
<td>−45 (cells; 95)</td>
<td>No</td>
<td>95</td>
</tr>
<tr>
<td>Rat pulmonary artery</td>
<td>−44 (cells; 96)</td>
<td>No</td>
<td>125–127</td>
</tr>
<tr>
<td>Rabbit pulmonary artery</td>
<td>−55 cells (20)</td>
<td>No</td>
<td>29, 31, 79</td>
</tr>
<tr>
<td>Rat renal resistance</td>
<td>−50 (65)</td>
<td>No</td>
<td>37</td>
</tr>
<tr>
<td>Rabbit aorta</td>
<td>−50 (19)</td>
<td>No</td>
<td>42</td>
</tr>
<tr>
<td>Rat myometrium</td>
<td>−68 (early pregnancy; 60)</td>
<td>Yes</td>
<td>30, 67, 83, 100, 119</td>
</tr>
<tr>
<td></td>
<td>−55 (late pregnancy; 60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human myometrium</td>
<td>−51 (71)</td>
<td>Yes</td>
<td>29, 53, 54</td>
</tr>
<tr>
<td>Guinea pig ureter</td>
<td>−66 (−60 mV before spike; 32)</td>
<td>Yes</td>
<td>32, 48, 49, 62, 68, 103</td>
</tr>
<tr>
<td>Guinea pig vas deferens</td>
<td>−54 (75)</td>
<td>Yes</td>
<td>68</td>
</tr>
<tr>
<td>Rabbit vas deferens</td>
<td>−50 (68)</td>
<td>Yes</td>
<td>68</td>
</tr>
<tr>
<td>Guinea pig seminal vesicles</td>
<td>−50 (51)</td>
<td>Yes</td>
<td>89</td>
</tr>
<tr>
<td>Newborn rat ileum</td>
<td>−60 (36)</td>
<td>Yes</td>
<td>97</td>
</tr>
<tr>
<td>Guinea pig colon</td>
<td>−58 (57)</td>
<td>Yes</td>
<td>68, 113, 114</td>
</tr>
<tr>
<td>Mouse colon</td>
<td>−58 (57)</td>
<td>Yes</td>
<td>4, 56, 57</td>
</tr>
<tr>
<td>Opossum esophagus</td>
<td>−49 (23)</td>
<td>Yes</td>
<td>1</td>
</tr>
<tr>
<td>Human esophagus</td>
<td>−59 (2)</td>
<td>Yes</td>
<td>117</td>
</tr>
<tr>
<td>Mouse antrum</td>
<td>−59 (2)</td>
<td>Yes</td>
<td>2</td>
</tr>
<tr>
<td>Mouse fundus</td>
<td>−45 (Amberg, Koh, and Sanders)</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−58 (120)</td>
<td>Yes</td>
<td>3</td>
</tr>
</tbody>
</table>

Kv, voltage-gated Ca\textsuperscript{2+}-independent K\textsuperscript{+} current; NCS, neuronal Ca\textsuperscript{2+} sensor; KChIP, K\textsuperscript{+} channel-interacting protein.

### Table 3. A-type channel and accessory subunit expression in smooth muscle

<table>
<thead>
<tr>
<th>Smooth Muscle</th>
<th>Transcript</th>
<th>Protein</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat mesenteric artery</td>
<td>Kv1.4, Kv3.3, Kv3.4, Kv4.2, Kv4.3, Kvβ1–β3</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>Rat tail artery</td>
<td>Kv1.4, Kv3.3, Kv3.4, Kv4.2, Kv4.3, Kvβ1–β3</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Rat pulmonary artery</td>
<td>Kv1.4, Kv4.1–4.3</td>
<td>25, 104, 127</td>
<td></td>
</tr>
<tr>
<td>Rat aorta</td>
<td>Kv4.3L</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Rat vas deferens</td>
<td>Kv4.3L &gt; Kv4.2</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Rat urinary bladder</td>
<td>Kv4.3L</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Rat myometrium</td>
<td>Kv4.3L &gt; Kv4.2 ~ Kv4.1</td>
<td>Kv4.3 ~ 100</td>
<td></td>
</tr>
<tr>
<td>Rat stomach</td>
<td>Kv4.3L</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Rat colon</td>
<td>Kv4.3L</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td>Mouse fundus</td>
<td>Kv4.1, Kv4.2, Kv4.3L, NCS-1, KChIP1, 3, 4</td>
<td>Kv4.2, Kv4.3 76; Amberg, Koh, and Sanders, unpublished</td>
<td></td>
</tr>
<tr>
<td>Mouse antrum</td>
<td>Kv4.3L &gt; Kv4.2 &gt; Kv4.1, NCS-1, KChIP1, 3, 4</td>
<td>Kv4.2, Kv4.3 2, 76</td>
<td></td>
</tr>
<tr>
<td>Mouse jejenum</td>
<td>Kv4.3L &gt; Kv4.2 &gt; Kv4.1, NCS-1, KChIP1 &gt; KChIP2–4</td>
<td>Kv4.2, Kv4.3 3, 76</td>
<td></td>
</tr>
<tr>
<td>Mouse colon</td>
<td>Kv4.3L &gt; Kv4.2 &gt; Kv4.1, NCS-1, KChIP1 &gt; KChIP2–4</td>
<td>Kv4.3 &gt; Kv4.2 3, 76</td>
<td></td>
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</table>
smooth muscle, produced a 4-AP-sensitive A-type current when heterologously expressed in HEK-293 cells (77). This study also identified Kv4.3L transcripts in rat urinary bladder smooth muscle. The most complete study addressing the molecular underpinnings of a G protein-coupled receptor in smooth muscle A-type current focused on rat myometrium (100). Kv4 transcripts were detected in uterine smooth muscle tissue. Real-time PCR showed that Kv4.3 transcripts were more than twice as abundant as those encoding Kv4.1 and Kv4.2; Western blot analysis confirmed Kv4.3 expression. Short Kv4.3 isoforms were not detected in myometrial smooth muscle.

Smooth muscles from the rodent GI tract express the three known Kv4 isoforms. Transcripts encoding Kv4 channels have been detected in isolated myocytes from the murine antrum, jejunum, and colon (2, 3, 57). Kv1.4 transcripts were not detected in colonic myocytes (57). At the tissue level, real-time PCR demonstrated a relative predominance of Kv4.3 transcripts over Kv4.1 and Kv4.2 in the mouse colon (3). Although Kv4.3 transcripts may be alternatively spliced (e.g., Ref. 78), only the long form was detected in mouse GI smooth muscles. Kv4.3 transcripts were also detected in rat stomach and colon (77). Myocyte-specific expression of Kv4 isoforms in the mouse GI tract has been confirmed with assays of protein expression, using commercially available Kv4.2- and Kv4.3-specific antibodies (2, 3). The intensity of Kv4-like immunoreactivity in myocytes in different regions of the mouse GI tract mirrored that of respective A-type current densities (i.e., greater Kv4-like immunoreactivity in antrum and colon than in jejunum). Immunohistochemical findings also corresponded well with the results from quantitative PCR in that Kv4.3-like immunoreactivity was more intense than Kv4.2-like immunoreactivity in mouse colonic myocytes.

The discovery of KChIPs (K⁺ channel-interacting proteins) as β-subunits specific for Kv4 channels (5) was a pivotal step in understanding the mechanisms responsible for the heterogeneity of Kv4-mediated A-type currents. KChIPs, which interact with the NH₂ terminus of Kv4 proteins (8), enhance surface expression, thereby increasing A-type current densities when coexpressed in heterologous systems and in native tissues (5, 26, 43, 63). It has been shown that a defect in the KChIP2 gene results in complete loss of Isca in cardiac myocytes (58). In contrast to KChAP (K⁺ channel-associated protein), a chaperone for Kv1.3, Kv2.1, and Kv4.3 channels (61), KChIPs are specific for the Kv4 family of K⁺ channels. KChIPs have high sequence homology to neuronal Ca²⁺ sensor-1 (NCS-1; frequenin) and calsenilin/DREAM, which belong to the superfamily of EF-hand-containing proteins (16, 101). It has been demonstrated that frequenin (NCS-1) is responsible for Ca²⁺-dependent enhancement of Kv4 expression and modulation of kinetic behavior (70). Quantitative analyses of transcriptional expression of neuronal Ca²⁺-binding proteins (NCBPs) indicated that KChIP1 and KChIP3, as well as other NCBPs, are extensively expressed in mouse GI smooth muscles (76).

In addition to enhancing surface expression, KChIPs also modulate Kv4 channel kinetic behavior (5, 8, 11). A variety of modulation patterns have been observed depending on the specific combination of Kv4 and KChIP proteins. For Kv4.2, coexpression with KChIP1 slowed the rate of inactivation, accelerated the time course of recovery from inactivation, and shifted the voltage of half-activation to more negative potentials (5, 8, 69). The effects of KChIP1 on Kv4.3L are similar to that of Kv4.2, although there are quantitative differences between the heteromeric subunit combinations (43). On the other hand, coexpression of KChIP1 with Kv4.1 accelerated inactivation and shifted the voltage of half-activation in the positive direction (69). More strikingly, coexpression of a novel KChIP4 isoform nearly abolished Kv4 channel inactivation (46). Thus the assembly of different KChIP and Kv4 combinations likely contributes to the diversity and complexity of native A-type currents in neuronal, cardiac, and smooth muscle cells.

Observed levels of Kv4 transcripts were found to be the same in murine colon and jejunum; however, the density of A-type current in colonic myocytes was approximately twice that of jejunum, and Kv4-like immunoreactivity was substantially greater in the colon (3). Studies were performed to determine whether differences in A-type current density could be due to differential expression of KChIPs (3). KChIP2 expression has recently been shown to mirror the transmural gradient of Isca in canine and human ventricles (86). In analogous fashion, KChIPs appear to mediate the density of A-type current in mouse colonic and jejunal myocytes (3). KChIP transcripts, which were detected in isolated colonic and jejunal myocytes (3, 76), were

Table 4. Kinetic profile of Kv4.2 and Kv4.3 α-subunits heterologously expressed in Xenopus oocytes, CHO cells, and HEK cells

<table>
<thead>
<tr>
<th>α-Subunit (Expression System)</th>
<th>V₀.5act, mV</th>
<th>V₀.05inact, mV</th>
<th>τ₀act, ms</th>
<th>τ₀inact, ms</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kv4.2 (oocyte)</td>
<td>7</td>
<td>29</td>
<td>224(−100 mV)</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Kv4.2 (CHO)</td>
<td>28*</td>
<td>36</td>
<td>245(−90 mV)</td>
<td>5, 82*</td>
<td></td>
</tr>
<tr>
<td>Kv4.2 (HEK)</td>
<td>−39</td>
<td>45</td>
<td>278(−70 mV)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Kv4.3 (oocyte)</td>
<td>1.1</td>
<td>257(−90 mV)</td>
<td>118</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kv4.3 (CHO)</td>
<td>12</td>
<td>33</td>
<td>175(−70 mV)</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>Kv4.3 (HEK)</td>
<td>−33</td>
<td>102</td>
<td>187(−70 mV)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Kv4.2/4.3 (HEK)</td>
<td>−29</td>
<td>67</td>
<td>132(−70 mV)</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

V₀.5act, voltage at half-activation; CHO, Chinese hamster ovary; HEK, human embryonic kidney.
approximately twice as abundant in colon as in jejunum. Thus, in mouse colon and jejunum, A-type current density parallels KChIP expression.

PLASTICITY OF A-TYPE CURRENT EXPRESSION

Electrical remodeling of myometrial smooth muscle results from pregnancy-associated changing hormone levels (e.g., Refs. 107 and 111). In most species, including humans, plasma levels of 17β-estradiol and progesterone increase during pregnancy (110). These changes are associated with a decline in A-type current densities in myometrial smooth muscle cells during the transition from a nonpregnant to a late-pregnant state (119). The changes in myometrial A-type currents have been mimicked by administration of 17β-estradiol to immature female rats. However, progesterone was without effect (30). In cultured human myometrial cells, chronic exposure to estrogen or progesterone induced a hyperpolarizing shift in the A-type current steady-state inactivation curve, whereas simultaneous exposure of estrogen and progesterone was without effect (54). Acute effects of estrogen and progesterone were not evident. As noted above, Kv4.3 channels are thought to underlie the A-type current in rat myometrium. In this species, at the end of pregnancy, Kv4.3 mRNA and Kv4.3 protein levels were reduced (100), with the Kv4.3 protein being predominantly confined to the perinuclear region. Administering 17β-estradiol, but not progesterone, to ovariectomized rats reduced Kv4.3 transcript and protein levels (100). As with pregnant rats (e.g., Ref. 119), ovariectomized rats treated with 17β-estradiol had drastically diminished A-type currents. Taken together, these data suggest that estrogen is a negative regulator of A-type channel expression in the rat myometrium.

BIOPHYSICAL PROPERTIES OF SMOOTH MUSCLE A-TYPE CURRENTS

A-type currents recorded from vascular smooth muscle cells tend to be relatively small in amplitude with rather variable biophysical properties (see Table 1). Activation thresholds for vascular A-type currents have ranged between −65 (rabbit portal vein) and +10 mV (human mesenteric artery). With the exception of rabbit portal vein, vascular A-type current activation thresholds and voltages of half-inactivation were positive to resting membrane potentials (Tables 1 and 2). Cells of the rabbit portal vein are an exception to this generalization, however, because the A-type current in these cells appears to be completely inactivated at the resting membrane potential (12). In contrast to other vascular smooth muscles in which A-type currents have been recorded, portal vein smooth muscle displays spontaneous phasic electrical activity (see Table 2); however, the role of A-type current in the spontaneous activity of this tissue has not been clarified.

Vascular smooth muscle A-type currents activate and inactivate (e.g., 20 to 220 ms) rapidly. Inactivation of A-type currents in rat pulmonary and renal arteries was best described by the sum of two exponentials, and inactivation of similar currents in rabbit portal vein was fit with a single component. Recovery from inactivation was slow (0.8 to 10 s) in rabbit portal vein and pulmonary artery. Recovery from inactivation was considerably faster in human mesenteric artery (time constant 250 ms). The single-channel conductance(s) responsible for A-type currents in vascular smooth muscle cells have not been identified.

A-type currents have also been recorded in GU smooth muscle cells. Like vascular muscle cells, these currents are relatively small in amplitude (see Table 1). Activation thresholds were between −60 and −30 mV and occurred at potentials just positive to the resting membrane potential (Table 2), near the firing threshold of GU smooth muscles. The A-type current in nonpregnant rat myometrium appears to be an exception, and significant activation occurred negative to the resting membrane potential. Although the reported voltages of half-inactivation for these currents are often negative to the resting membrane potential, GU A-type currents are not fully inactivated at physiologically relevant membrane potentials.

Inactivation of rat myometrial and guinea pig seminal vesicle A-type currents were best fit with a single exponential, whereas those of the guinea pig ureter required two exponentials. Recovery from inactivation was relatively fast in guinea pig ureter with a time constant near 100 ms. In guinea pig vas deferens, recovery was slower and best described by the sum of two exponentials. In myocytes of the guinea pig ureter, a 14-pS (asymmetrical K+ channel with A-type properties has been recorded in cell-attached patches (49).

A-type currents dominate macroscopic voltage-dependent outward currents of GI smooth muscle cells isolated from rat ileum (97) and murine colon (57), antrum (2), and fundus (Amberg GC, Koh SD, and Sanders KM, unpublished observation) (see Table 1). Smaller A-type currents were reported in guinea pig colon (114), opossum esophagus (1), human esophagus (117), and mouse jejunum (3). GI A-type currents were resolved at potentials between −60 and −50 mV, which corresponds with the resting potentials of many GI smooth muscles (Table 2). These potentials are also near the voltages of half-inactivation for the A-type currents. As a result, there is sustained availability of A-type channels at physiologically relevant membrane potentials throughout the GI tract.

Once activated, GI A-type currents inactivated rapidly with time constants <100 ms. A-type currents of mouse (57) and guinea pig (114) colonic myocytes were best fit by the sum of two exponentials. At negative potentials, GI A-type currents typically recovered from inactivation quickly, with time constants <100 ms. However, opossum esophagus and mouse antral A-currents had time constants for recovery from inactivation in excess of 125 ms. A 19-pS K+ (symmetrical K+) channel with A-type properties has been identified in cell-attached patches of murine colonic myocytes (see Fig. 2; Ref. 4). A 12-pS K+ (asymmetrical K+) channel is thought to be responsible for the A-type current in the guinea pig colon (113).
PHARMACOLOGY OF SMOOTH MUSCLE
A-TYPE CURRENTS

All smooth muscle A-type currents showed sensitivity to 4-AP (10 mM) and insensitivity to external TEA (10 mM). 3,4-Diaminopyridine blocked seminal vesicle myocyte A-type currents (89). As with other tissues [e.g., ventricle (18)], the effects of 4-AP on some smooth muscle A-type currents [e.g., guinea pig ureter (49), opossum esophagus (1), and guinea pig (114) and mouse colon (57)] were complex (see Fig. 3). 4-AP slowed activation and decreased the peak amplitudes of these A-type currents, but channel block by 4-AP was relieved during prolonged depolarization, resulting in partial recovery from block. These observations are consistent with closed channel block by 4-AP that is relieved when the channels open (see Ref. 57 for further discussion).

The effects of inorganic cations (e.g., Cd$^{2+}$ and La$^{3+}$) have been tested on A-type currents of smooth muscle cells (1, 3, 12, 49, 95). From studies in neurons, inorganic cations are known to shift A-type current voltage dependencies of activation and inactivation to more depolarized potentials (e.g., Ref. 66). In considering investigations of A-type currents, it is important to note the presence of inorganic cations, such as Cd$^{2+}$ or Mn$^{2+}$, which are often included to minimize contamination by Ca$^{2+}$-activated K$^+$ currents, since these ions will affect the voltage-dependence of activation and inactivation.

A-type potassium currents in mouse GI smooth muscle were inhibited by flecainide (Refs. 2 and 3; see Fig. 3). Flecainide, an antiarrhythmic agent used in clinical practice, blocks A-type potassium currents formed by the Kv4 family of K$^+$ channels near therapeutically relevant concentrations (i.e., low micromolar; Refs. 39 and 124). In the mouse, the IC$_{50}$ for inhibition of peak current was 11 μM in colon (3) and 35 μM in antrum (2). Data with flecainide are highly suggestive of a role for Kv4 isoforms in GI A-type currents; however, the most convincing pharmacological evidence for the involvement of Kv4 channels is block by heteropodatoxin (90) or phrixotoxin (27), two spider venom-derived inhibitors that are highly specific for Kv4 channels. However, limited availability of these toxins has precluded their use in the pharmacological profiling of smooth muscle A-type currents.

The A-type currents of rabbit vas deferens and guinea pig ureter, vas deferens, and colon were inhibi-
CaMKII activity in colonic cells appears to in
and increasing the phosphorylated state (4). Basal
current properties by blocking phosphatase activity
A and FK506) and protein phosphatase 1 (okadaic acid)
inactivation (56). Inhibitors of calcineurin (cyclosporin
CaMKII inhibitor KN-93 greatly increased the rate of
rate of inactivation of colonic A-type currents (Fig. 4),
of colonic myocytes with exogenous CaMKII slowed the
Ca2+
A-type currents in most of the smooth muscles in which
these channels are expressed. In mouse colonic myo-
cytes, the A-type current is regulated by Ca2+/calmo-
dulin-dependent kinase II (CaMKII; Ref. 56) and by the
Ca2+-dependent phosphatase calcineurin (4). Dialysis
of colonic myocytes with exogenous CaMKII slowed the
rate of inactivation of colonic A-type currents (Fig. 4),
whereas bath application of the membrane-permeant
CaMKII inhibitor KN-93 greatly increased the rate of
inactivation (56). Inhibitors of calcineurin (cyclosporin
A and FK506) and protein phosphatase 1 (okadaic acid)
slowed the inactivation of 19-pS channels with A-type
current properties by blocking phosphatase activity
and increasing the phosphorylated state (4). Basal
CaMKII activity in colonic cells appears to influence
A-type K+ channels, because inhibition of CaMKII
increased the rate of inactivation of whole cell A-type
currents in amphotericin-perforated patch experi-
ments (56). It is possible that a portion of the basal
activity results from Ca2+-independent activity of au-
tophosphorylated CaMKII, a well-characterized fea-
ture of this kinase (e.g., Ref. 98).

In the presence of external TEA, A-type currents of
myocytes dialyzed with autophosphorylated CaMKII
did not inactivate completely during 500-ms test poten-
tials (Fig. 4). However, after inhibition of CaMKII
with KN-93, inactivation was complete within a few
hundred milliseconds in the presence of TEA. Slowing
of inactivation due to endogenous CaMKII activity
resulted in incomplete inactivation of A-type current.
Thus kinetic modulation by CaMKII functionally
transforms this current from a rapidly inactivating
A-type current into a conductance that performs more
like a delayed rectifier with a significant sustained
component. This transformation converts the A-type
current into a conductance that would tend to have
sustained influence on excitability at negative mem-
brane potentials. KChIPs, which regulate A-type cur-
rent densities in mouse colon and jejunum, have been
shown to produce kinetic effects qualitatively similar
to the effects of CaMKII (5, 46).

PHYSIOLOGICAL FUNCTION OF SMOOTH MUSCLE
A-TYPE CURRENTS

The availability of A-type channels to contribute to
physiological events in smooth muscle cells is a func-
tion of the intrinsic voltage-dependent properties of the
channels and the voltage range of the smooth muscle
tissue. The physiological significance of A-type cur-
rents in vascular smooth muscles, therefore, is contro-
versial. In rabbit portal vein, where the resting mem-
brane potential is reported to be −50 mV (e.g., Ref. 59),
A-type currents should be completely inactivated (12).
In human mesenteric artery, application of 4-AP in-
duced firing of action potentials and eliminated tran-
sient afterhyperpolarizations following the action po-
tentials (95). Those authors suggested that a high-
threshold A-type current may suppress membrane
excitability in these cells. A similar argument may be
made for the A-type current in rat renal resistance
arteries (37). In rabbit (79) and rat (125) pulmonary
arterial smooth muscle tissue, 4-AP caused depolariza-
tion. However, this effect may not result from inhibi-
tion of an A-type current. In rabbit pulmonary artery,
quinine had little effect on the resting membrane po-

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**Fig. 4.** Dialysis with autophosphorylated Ca2+/cal-
modulin-dependent kinase II (CaMKII) slows the inacti-
vation of mouse colonic A-type currents. **A:** A-type cur-
rents recorded from a myocyte dialyzed with autophosphorylated CaMKII (10 nM) immediately
upon establishing whole cell recording (early) and after 200 s of dialysis (late). **B:** A-type currents recorded from a
myocyte dialyzed with autophosphorylated CaMKII (10 nM) alone, during exposure to TEA (10 mM), and
during exposure to TEA plus the CaMKII inhibitor KN-93 (5 μM). [Reprinted from Koh et al. (56).]
Fig. 5. Effects of A-type current inhibition on the electrical activity of intact murine antral smooth muscle. Flecainide (30 μM) was applied after control in standard oxygenated bathing solution supplemented with N^0^-nitro-L-arginine, atropine, nicardipine, glibenclamide, and TEA. A representative slow wave before and after flecainide is shown at right with an expanded time scale; for clarity, the flecainide slow wave is shifted to the right by ~2 s. Dashed line denotes the membrane potential between slow waves under control. [Reprinted from Amberg et al. (2).]

In ureteral myocytes, studied under current clamp, increasing the availability of A-type current by changing the resting potential from −50 to −70 mV caused a slight delay in action potential onset (103). From a resting potential of −70 mV, but not −50 mV, 4-AP exposure increased the rate of action potential depolarization by a few milliseconds, whereas other action potential components were unaffected (49, 103). However, in intact ureter, 4-AP decreased the maximum rate of rise of action potentials and reduced the initial spike amplitude (32). In contrast to current-clamped myocytes, where late components of the action potential were unaffected by A-type current inhibition, the plateau phase of the action potential in the intact ureter was greatly extended by 4-AP (32). This effect was thought to result from either inhibition of a sustained steady-state component of the A-type current or an increase in initial Ca^{2+} influx during the action potential (32). Further investigation is required to adequately explain the observed differences between intact tissue and isolated myocyte current-clamp experiments in the guinea pig ureter.

Very little is known about the physiological significance of the A-type current in myometrial smooth muscle. However, it is clear that the A-type current is predominantly a feature of the nonpregnant myometrium (see plasticity of A-type current expression). Although A-type currents have been reported in myocytes isolated from pregnant rat and human uteri, they are largely overshadowed by nonactivating K^+ currents (e.g., Ref. 119). It is likely that decreases in A-type currents, in concert with changes in other conductances, contribute toward the increased excitability of the myometrium at parturition (100).

In GI smooth muscles, reported A-type current activation thresholds and voltages of half-inactivation are near the resting membrane potential (Tables 1 and 2). Because activation occurs at potentials where steady-state inactivation is incomplete, a small fraction of these A-type currents is available (i.e., there is “window current”) at physiological relevant membrane potentials (Fig. 1). These small steady-state currents can have significant influences on membrane potential as a result of the high input resistances typical of GI smooth muscle cells (i.e., gigaohm).
A-type currents contribute toward the resting membrane potential in guinea pig colon (114), opossum esophagus (1), and in mouse antrum (2) and colon (57). These conclusions are supported by experimental evidence in intact tissue preparations (mouse antrum and colon) and in current-clamped isolated smooth muscle cells (opossum esophagus, guinea pig colon, and mouse antrum). In this respect, the A-type currents assume a role more typically associated with noninactivating delayed rectifier K\(^+\) currents. At first consideration, this function would seem unlikely for a rapidly inactivating K\(^+\) current; however, these findings are in agreement with steady-state activation and inactivation analyses that have revealed sustained currents (i.e., window currents) within the physiological range of potentials. Small tonic currents would be expected to arise through these channels within the window current region of the current-voltage relationship.

With the exception of mouse fundus, the electrical activities of these GI tissues are spontaneously phasic (Table 2). In mouse colon tissue, 4-AP exposure induced a slight depolarization and abolished the quiescent periods between slow wave depolarizations (57). These observations suggest that the contribution of the A-type current to resting membrane potential is critical for maintaining the phasic nature of this tissue. In contrast, after blockade of enteric neurotransmission, flecaainide inhibition of the A-type current in mouse antrum caused a decrease in resting membrane potential without loss of phasic activity (Fig. 5; Ref. 2). Similar results were obtained in the mouse antrum when 4-AP was used to block the A-type current (Amberg GC, Koh SD, and Sanders KM, unpublished observation). Thus A-type currents are not a requirement for phasic activity in the GI tract. Because flecaainide blocks Na\(^+\) channels, as well as other K\(^+\) channels, results obtained using this compound may result from inhibition of non-A-type channels, and other pharmacological agents are needed to manipulate A-type currents. The use of 4-AP brings similar uncertainties with regard to nonspecificity, thus highlighting the need for greater availability of specific blockers and use of transgenic technologies in assessing physiological functions of A-type currents.

In current-clamped myocytes isolated from guinea pig colon (114) and opossum esophagus (1), evoked action potentials peaked \(~30\) ms faster after 4-AP exposure, which may have led to an increase in spiking frequency (116). In mouse antral myocytes, inhibiting the A-type current did not accelerate the upstroke of evoked action potentials (2). These observations may reflect fundamental differences between GI smooth muscles or may result from differences in experimental parameters. The changes in electrical activity observed after inhibition of GI A-type currents likely results from general membrane depolarization as opposed to specific modulation of spike waveforms by A-type currents. In contrast to the succinct depolarizations observed in isolated myocyte current-clamp experiments, in tissue preparations at 37°C, action potential spikes during slow wave complexes generally occur after relatively slow depolarizations (see Fig. 6). Under these conditions, most of the A-type current, which is small to begin with, will have accumulated in an inactive state by the time action potential thresholds are reached. Furthermore, at elevated temperatures (e.g., 37°C), current availability at negative potentials is increased (35, 108) and inactivation kinetics are more rapid (1). Consequently, at physiological temperatures, A-type current contributions toward resting membrane potentials will be increased, whereas faster rates of inactivation will preclude availability at depolarized potentials. Thus, in vivo, it is likely that A-type currents modulate action potentials indirectly by regulating resting membrane potential. This role for A-type currents is highly dependent on the window current that results from overlapping of voltage dependence of activation and inactivation.

**CONCLUSION**

Although not ubiquitous in smooth muscles, A-type currents clearly are important regulators of membrane excitability in a variety of smooth muscles. The physiological role of these channels in smooth muscles deviates from the functions traditionally associated with A-type currents. Considerably more information is needed about the molecular species responsible for A-type currents in a variety of smooth muscles, and studies to determine second messenger regulation of these conductances still must be performed on most of the cells in which these conductances have been identified. To better understand the physiological significance of A-type current, improved blockers for A-type currents are needed. Known specific antagonists are in short supply and difficult to obtain in quantities necessary for physiological experiments. Alternatively, employment of transgenic technologies and other molecular techniques may allow a more rigorous understanding of the physiological function of these currents in intact smooth muscle organs. Finally, little is known about the presence and function of A-type currents in human smooth muscles.


45. Holmqvist MH, Cao J, Knoppers MH, Jurman ME, Diste- rano PS, Rhodes KJ, Xie Y, and An WF. Kinetic modulation of Kv4-mediated A-current by arachidonic acid is dependent on

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