# Part I

# Basic science

1

# Vascular tone

Alun D. Hughes

Clinical Pharmacology, NHLI, Imperial College, St Mary's Hospital, London

## Introduction

This chapter provides an overview of how vascular smooth muscle cells produce force and how this process is regulated. An overview inevitably involves generalizations and this tends to obscure the considerable diversity that exists in vascular smooth muscle. Such diversity is unsurprising if one recalls the variety of functions performed by blood vessels. Large arteries act as elastic conduits, smaller arteries regulate the distribution of blood flow, the microvasculature largely determines vascular resistance and fluid exchange, while the venous system undertakes a capacitive role and governs venous return to the heart. When these differences are compounded with the differences in behaviour required from blood vessels supplying different tissues, one can see that smooth muscle diversity is a positive asset that allows appropriate responses in a particular circumstance.

Owing to space constraints I have not attempted to provide comprehensive source references in this chapter. Instead, recent reviews have been cited and these should be referred to for more detailed information regarding a particular topic and original sources.

## Types of stimulus for contraction and relaxation

Under physiological circumstances the primary role of differentiated (as opposed to 'synthetic') smooth muscle is to generate force. Normally, the vascular smooth muscle that makes up the bulk of the blood vessel wall is in a state of continual activation. The amount of force generated by smooth muscle is finely regulated by a variety of extracellular and intrinsic factors. The types of stimuli that act on vascular smooth muscle can be grouped into five categories:

1. Agents acting at G protein-coupled receptors
2. Pressure/tension
3. Agents acting directly on ion channels or signalling systems

4. Extracellular matrix components, cell adhesion molecules and integrins
5. Growth factors

**Agents acting at G protein-coupled receptors**

This group includes the majority of classical vasoconstrictors, such as α-adrenoceptor agonists, angiotensin II, serotonin and vasopressin, and vasodilators such as β-adrenoceptor agonists, vasoactive intestinal peptide and calcitonin gene-related peptide. These agents act by binding to receptors that couple to heterotrimeric G proteins ($R_7G$: Morris and Malbon, 1999). $R_7G$ form a protein superfamily; all possess seven transmembrane domains and in consequence are also known as serpentine receptors (Figure 1.1).

Heterotrimeric G proteins act as signal transducers linking the extracellular ligand to a variety of intracellular signals, such as $[Ca^{2+}]_i$, cyclic nucleotides or ion channels. Heterotrimeric G proteins are membrane-associated proteins composed of α, β and γ subunits with the α subunit possessing guanosine triphosphatase (GTPase) activity (Figure 1.1). In the absence of receptor activation they exist in an inactive guanosine diphosphate (GDP)-bound state. The ligand-receptor complex acts as a GDP/GTP exchange factor promoting formation of a dissociated α subunit–GTP complex and a free βγ dimer. The α subunit–GTP complex and the βγ dimer remain associated with the cell membrane and both play signalling roles (Morris and Malbon, 1999). After signalling activation of the intrinsic GTPase of the α subunit catalyses hydrolysis of GTP to GDP which completes the cycle and results in reformation of the inactive αβγ heterotrimer–GDP complex. The system is regulated at two points. Firstly, downstream targets, including receptor kinases (GRKs) and β-arrestins, can negatively feed back on to receptor–G protein interactions (Lefkowitz, 1998). Secondly, regulators of G-protein signalling (RGS) proteins act to enhance the GTPase activity of α subunits (Dohlman and Thorner, 1997). A number of isoforms of both α and βγ subunits exist and preferential coupling of the receptor to a specific αβγ combination probably accounts for the diversity of intracellular events generated by this signalling complex (Hildebrandt, 1997).

**Pressure/tension**

The ability of vascular smooth muscle to respond to increased transmural pressure by increased tone was first recognized by Bayliss in 1902. The current view is that wall tension or stress, rather than pressure *per se* is the stimulus for contraction. The balance between myogenic tone and endothelium-dependent vasodilatation may coordinate the behaviour of arterial networks (Griffith et al., 1987). While the myogenic response is a very important determinant of tone, perhaps particularly in the microvasculature, the biochemical mechanisms underlying its transduction are still poorly understood; stretch-induced production of vasoconstrictors or

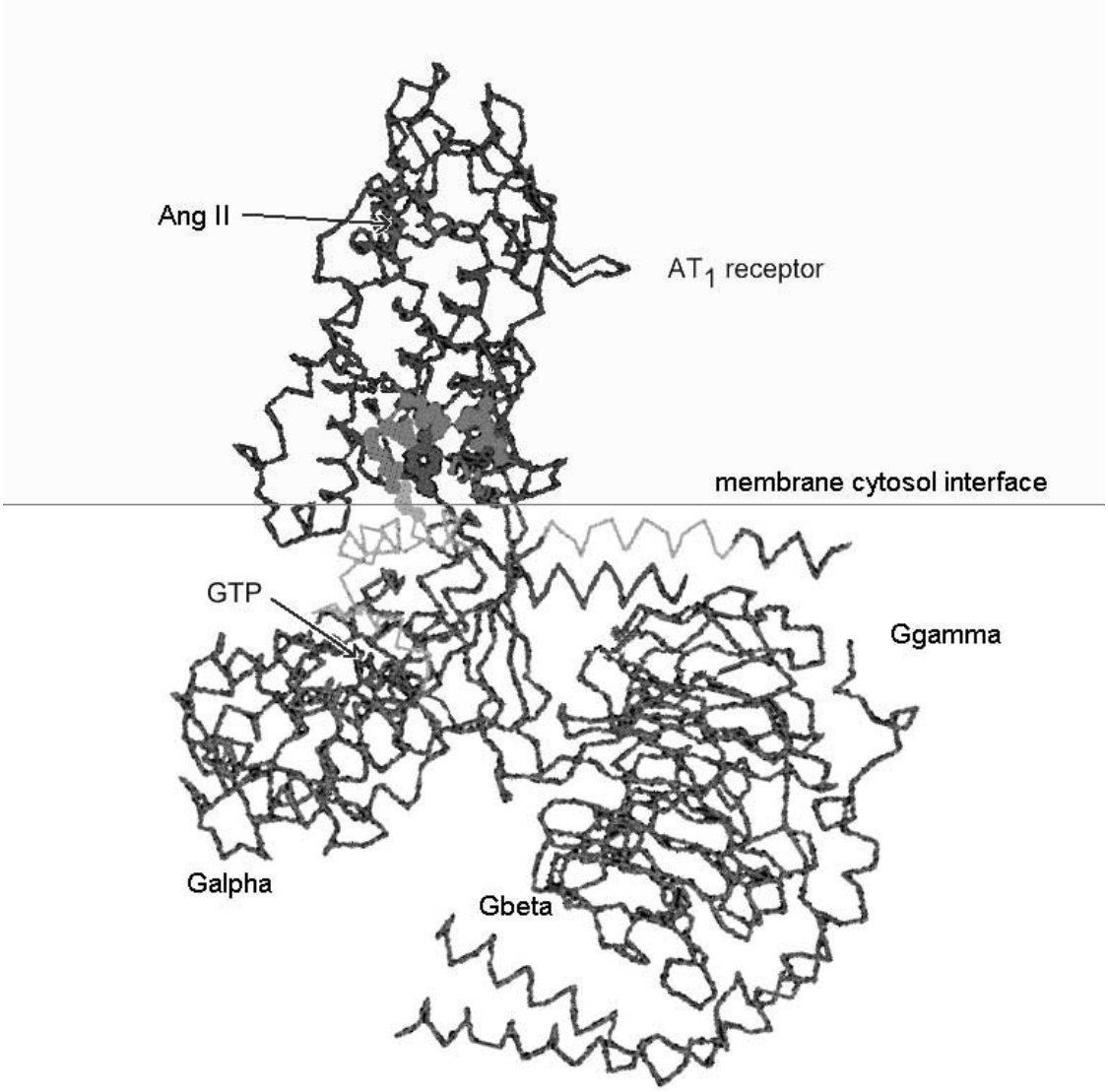


Figure 1.1 Receptor ($R_7G$) and associated heterotrimeric G protein. Example shown is of an angiotensin II type 1 ($AT_1$) receptor and a G protein heterotrimer. The image is based on a model constructed by Paiva, A.C.M. Costa-Neto, C.M. & Oliveira, L. Molecular modeling and mutagenesis studies of angiotensin II/$AT_1$ interaction and signal transduction. On-line Proceedings of the 5th Internet World Congress on Biomedical Sciences '98 at McMaster University, Canada (available from URL:http://www.mcmaster.ca/inabis98/escher/paiva0625/index.html#abstract).

growth factors, stretch sensitivity of ion channels, signalling enzymes and the sensitivity of cell–cell or cell–matrix interactions to tensile stress are all possible candidates for this role.

### Agents acting directly on ion channels or signalling systems

A number of agents, such as $H^+$ ions (intracellular and extracellular pH: Aalkjaer and Peng, 1997), nitric oxide (NO: Ignarro et al., 1999), free radicals and reactive oxygen species (e.g. superoxide anions ($O_2^-$), hydrogen peroxide: Beckham and Koppenol, 1996; Hancock, 1997), have marked effects on ion channels and intracellular signalling systems. These mechanisms play important roles in physiological and pathological responses in the vasculature.

### Extracellular matrix components, cell adhesion molecules and integrins

Cell-to-cell and extracellular matrix-to-cell interactions profoundly affect smooth muscle cell behaviour (Hughes and Schachter, 1994; Braun et al., 1999). Activation of receptors for extracellular matrix proteins (integrins) alters smooth muscle tone. This effect can be mediated by the endothelium (Mogford et al., 1997), or involve direct effects on smooth muscle cells (Mogford et al., 1997; Yip and Marsh, 1997; Wu et al., 1998a).

### Growth factors

Vascular growth factors are potent chemoattractants and mitogens. Factors such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) are thought to play an important part in the blood vessel's response to injury (Ross, 1999). Growth factors can also affect vascular tone (Berk and Alexander, 1989; Hughes and Wijetunge, 1998), although the physiological significance of this action is uncertain.

The majority of growth factors act by binding to and inducing dimerization of transmembranous receptors which are intrinsic tyrosine kinases. Dimerization results in transautophosphorylation of tyrosine residues in the intracellular domain of the receptor and leads to recruitment and activation of a range of signalling molecules (Hughes et al., 1996). Increasing evidence suggests an important role for tyrosine kinases in the regulation of smooth muscle tone, even in response to classical vasoconstrictors (Hughes and Wijetunge, 1998).

## Regulation of $[Ca^{2+}]_i$ in vascular smooth muscle

The pivotal role of $Ca^{2+}$ in muscle contraction has been recognized for many years. $[Ca^{2+}]_i$ can rise as a consequence of an increase in influx of extracellular $Ca^{2+}$, alteration in the amount of intracellularly sequestered $Ca^{2+}$ or a decrease in efflux

of cellular $Ca^{2+}$. In general, most contractile stimulants appear to act by altering influx or release of $Ca^{2+}$. The relative importance of influx or release from stores varies between blood vessels of differing calibre. In the resistance vasculature (i.e. vessels with internal diameters less than 500 $\mu$m) and microvasculature (arterioles and precapillary vessels), $Ca^{2+}$ entry through voltage-operated calcium channels appears to predominate (Hughes, 1995).

## $Ca^{2+}$ influx

### Ion channels, membrane potential and $[Ca^{2+}]_i$

Vascular smooth muscle cells maintain a low $[Ca^{2+}]_i$ ( ~ 100 nmol/L) in the face of an immense electrochemical gradient (extracellular $Ca^{2+}$ ~ 1.6 mmol/L, membrane potential ($E_m$) ~ 60 mV). The smooth muscle cell possesses powerful $Ca^{2+}$-buffering capacity; ~ 99% of the $Ca^{2+}$ entering the cell is estimated to bind to proteins or to be taken up into stores (Kamishima and McCarron, 1996). Despite this, opening of $Ca^{2+}$ channels causes $[Ca^{2+}]_i$ to rise to micromolar levels. This is sufficient to activate the contractile (and other) processes. There is now substantial evidence that $[Ca^{2+}]_i$ is compartmentalized within the cell and that localized increases in $[Ca^{2+}]_i$ are important to cell function, particularly regulation of ion channel opening (Jaggar et al., 1998a).

The major $Ca^{2+}$-permeable channel in vascular smooth muscle is the voltage-operated calcium channel (Hughes, 1995). As its name implies, this channel is primarily regulated by $E_m$ and the likelihood of the channel opening (open probability) increases steeply with depolarization. Consequently, $E_m$ is an important determinant of $Ca^{2+}$ influx in vascular smooth muscle cells.

In the main, $E_m$ in vascular smooth muscle is governed by the membrane permeability to four ions, $K^+$, $Cl^-$, $Na^+$ and $Ca^{2+}$, with $K^+$ being the major determinant of $E_m$ under resting conditions (Figure 1.2).

Electrogenic pumps such as the Na-K-ATPase or the Na/Ca exchanger also have an influence on $E_m$ and the Na-K-ATPase may contribute up to 10 mV under certain circumstances (Hermsmeyer, 1982).

Most studies of isolated blood vessels carried out under isometric conditions in vitro give values for resting $E_m$ of $\sim -60$ mV, although more depolarized potentials have been recorded in pressurized arteries (Harder, 1984). In vivo smaller arteries would be expected to be relatively depolarized as a result of 'myogenic' depolarization and prevailing tonic contractile influences such as the sympathetic nervous system and circulating factors. Measurements of $E_m$ in vivo are consistent with this, with $E_m$ being in the range $\sim -40$ mV (Bryant et al., 1985). This has important consequences for our understanding of the action of some drugs, e.g. dihydropyridine, which act preferentially on depolarized cells.

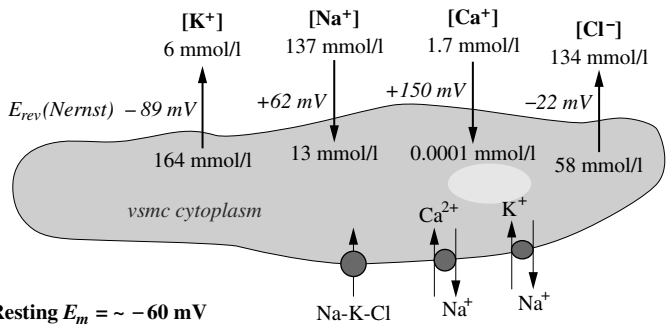


Figure 1.2 Determinants of resting membrane potential ($E_m$) in vascular smooth muscle. Diagram shows the concentration gradients and equilibrium (reversal) potentials ($E_{rev}$) of the major ions. The major ion pumps are also indicated.

Unlike most excitable cells, vascular smooth muscle cells rarely display action potentials (for an exception, see Yamaguchi and Jensen, 1993), but show graded depolarization to stimuli. In most cases the vascular muscle cells in the blood vessel wall act as an electrically coupled multiunit. This electrical coupling is due to the existence of intercellular connections (gap junctions) between smooth muscle cells. Gap junctions are formed from the apposition of two hemichannels (connexons), each composed of six transmembrane proteins (connexins). Numerous connexins have been described, but connexin 43 is the most common type in arterial smooth muscle (Brink, 1998; Gustafsson and Holstein-Rathlou, 1999). As a result of the electrical coupling a blood vessel behaves like a three-dimensional electrical cable through which potential changes can propagate (Holman et al., 1990; Tomita, 1990; Gustafsson and Holstein-Rathlou, 1999). Estimates of the cable properties of smooth muscles vary, but values for the length constant of electrical conduction $\lambda$ are generally in the range 1–2 mm. It has been suggested that smooth muscle cells and endothelial cells may also be electrically coupled via gap junctions in some blood vessels (Gustafsson and Holstein-Rathlou, 1999).

## Major ion channel species in vascular smooth muscle

### K channels

K channels make up a large family of channels encoded by multiple gene families (Standen and Quayle, 1998). K channels consist of four $\alpha$ subunits that are associated with $\beta$ subunits to make a hetero-octomer (Figure 1.3). The $\alpha$ subunits form the channel pore while the $\beta$ subunits modify channel gating properties. Four major types of K channel are present in vascular smooth muscle: voltage-dependent ($K_V$) channels, $Ca^{2+}$-activated ($K_{Ca}$) channels, inward rectifier ($K_{IR}$) channels and ATP-sensitive ($K_{ATP}$) channels. The presence of relatively large

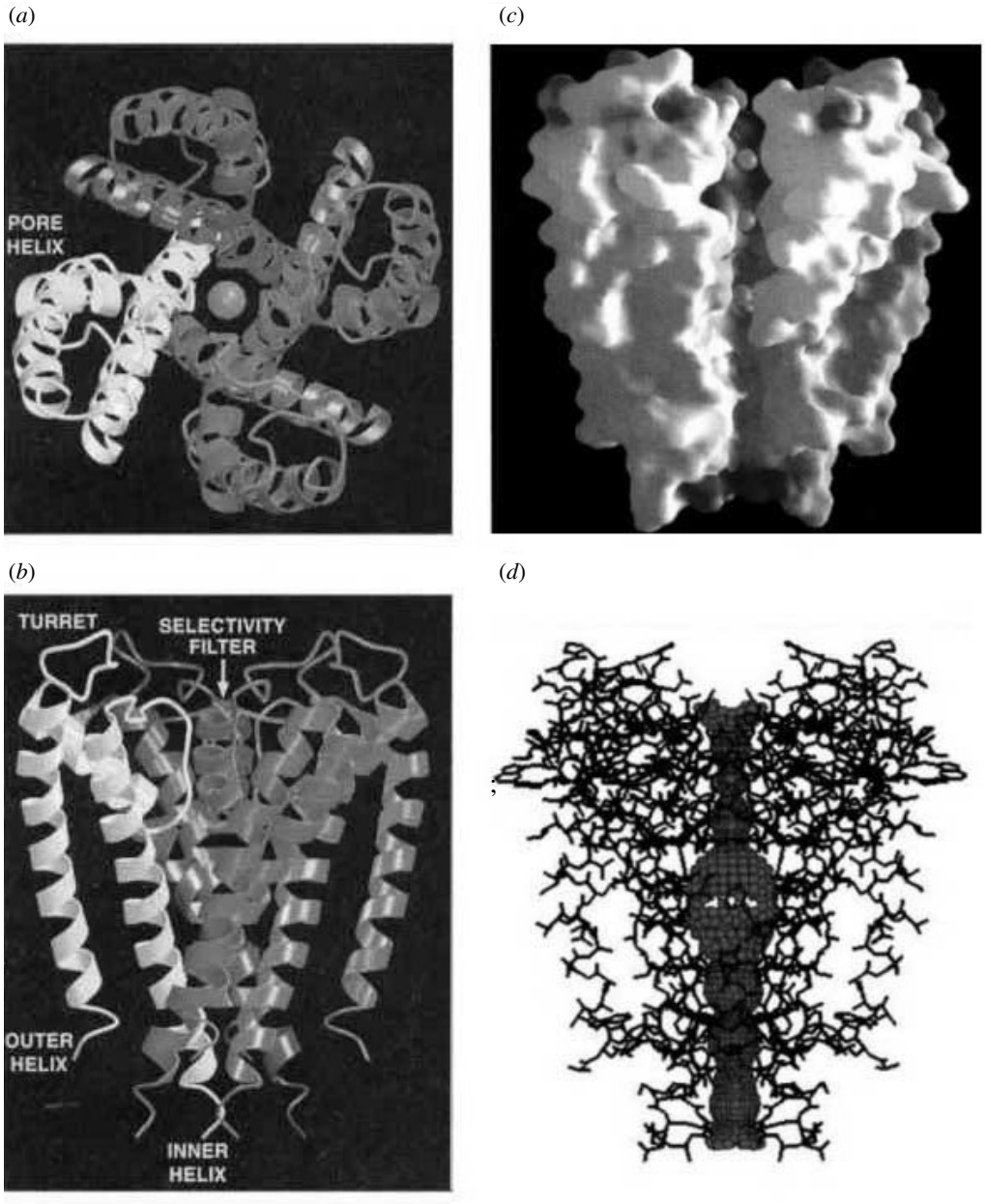


Figure 1.3 Views of the KcsA channel tetramer, molecular surface of KcsA and contour of the pore. (*a*) Stereoview of a ribbon representation illustrating the three-dimensional fold of the KcsA tetramer viewed from the extracellular side (above). The four subunits are distinguished by colour. (*b*) Stereoview from another (side) perspective, perpendicular to that in (*a*). Original diagrams were prepared with MOLSCRIPT and RASTER-3D. (*c*) A cutaway stereoview displaying the solvent-accessible surface of the K channel. (*d*) Stereoview of the entire internal pore. This display was created with the program HOLE. Modified from Doyle et al. (1998) with permission.

numbers of K channels (plus the low density of L-type calcium channels and the absence of voltage-operated Na channels) probably accounts for the rarity of action potentials recorded from vascular smooth muscle cells. There is considerable evidence that K channels dominate $E_m$ in vascular smooth muscle under resting conditions. Recent evidence has highlighted the role of $K_{Ca}$ channels in myogenic tone (Jaggar et al., 1998a) and hypoxic vasoconstriction in the lung (Dumas et al., 1999; McCulloch et al., 1999). Many vasoactive agents affect K channel opening. This may account for the ability of these agents to alter $E_m$ (Standen and Quayle, 1998). In some cases (e.g. NO) this may involve direct effects on the channels; in other cases protein kinases, such as protein kinase C, tyrosine kinases or cyclic nucleotide-dependent kinases, appear to mediate this effect. In addition, a num-ber of therapeutic vasodilators act on K channels. Examples include minoxidil and nicorandil which open $K_{ATP}$ channels (Standen and Quayle, 1998), and thiazide diuretics which open $K_{Ca}$ channels (Table 1.1: Calder et al., 1993).

### Cl channels

$Cl^-$ ions are actively concentrated inside the vascular smooth muscle cell, probably as a result of the activity of the Na-K-2Cl cotransporter and $HCO_3^-/Cl^-$ exchange. Consequently the equilibrium potential for $Cl^-$ ion ($E_{Cl}$) is around $-25$ mV. Opening Cl channels will therefore depolarize smooth muscle cells. Two classes of Cl channels have been identified in vascular smooth muscle – a $Ca^{2+}$-activated Cl channel ($Cl_{Ca}$: Large and Wang, 1996) and volume-sensitive Cl channels (Yamakazi et al., 1998; Lamb et al., 1999). $Cl_{Ca}$ has not been identified at the molecular level, but it is a small conductance channel (Klockner, 1993), that opens in response to a rise in $[Ca^{2+}]_i$. Opening of this channel has been implicated in agonist-induced depolarization (Large and Wang, 1996). A number of relatively nonselective blockers of this channel have been described, but in general much remains to be learned about the biophysics, physiological role and regulation of this channel.

Volume-regulated chloride channels form a family currently containing nine members (Jentsch et al., 1999). One of these, CLCN3, has been demonstrated in vascular smooth muscle cells (Yamakazi et al., 1998; Lamb et al., 1999). It has been suggested that this channel contributes to pressure-induced depolarization and plays a role in myogenic responses (Yamakazi et al., 1998).

### Voltage-operated sodium channels

There is little evidence that tetrodotoxin-sensitive voltage-operated $Na^+$ channels like those found in neurons or cardiac myocytes contribute to $E_m$ in vascular smooth muscle cells. However, relatively nonselective channels permeable to

**Table 1.1** Ion channels in vascular smooth muscle

| Channel | Physiological role | Opener | Inhibitor/blocker |
|---|---|---|---|
| *Potassium channels* | | | |
| $K_V$ | Regulation of membrane potential<br>Hypoxic pulmonary vasoconstriction | Depolarization | 4-Aminopyridine, quinidine, phenylcyclidine, tedisamil, tetraethylammonium |
| $K_{IR}$ | Resting membrane potential $K^+$-induced dilatation | Depolarization | $Ba^{2+}$ |
| $K_{Ca}$ | Myogenic tone<br>'Brake' on agonist-induced depolarization | $[Ca^{2+}]_i$<br>Depolarization<br>Thiazides<br>NS004 | Charybdotoxin, iberiotoxin |
| $K_{ATP}$ | Metabolic regulation of tone<br>Reactive hyperaemia<br>Autoregulation<br>Endotoxic shock | $[ATP]_I$<br>Cromakalim, diazoxide, minoxidil, nicorandil, pinacidil, RP-49356 | Sulphonylureas, U-37883A, $Ba^{2+}$ |
| *Chloride channels* | | | |
| $Cl_{Ca}$ | Agonist-induced depolarization in some smooth muscle | $[Ca^{2+}]_i$ | Niflumic acids, stilbenes (e.g. DIDS, SITS), furosemide (frusemide) |
| $Cl_v$ | Pressure-induced depolarization | Increased in cell volume | Stilbenes, 5-nitro-2-(3-phenylpropylamino)-benzoate |
| *Cation channels* | | | |
| Receptor-operated channels<br>$Ca^{2+}$-activated | Agonist-induced depolarization | G protein-linked receptors<br>$[Ca^{2+}]_i$ | Inorganic cations (e.g. $Ni^{2+}$, $Gd^{3+}$) |
| *Calcium channels* | | | |
| L-type | Myogenic tone<br>Agonist-induced calcium entry | Depolarization, dihydropyridine agonists (e.g. Bay K8644a) | Dihydropyridine antagonists, phenylalkylamines, benzothiazepines |
| T-type | 'Pacemaker' activity? | Depolarization | Mibefradil |