T-type Ca^{2+} channels in non-vascular smooth muscles

CH Fry, G Sui & C Wu

Institute of Urology, University College London, London W1W 7EY, UK

Key words: T-type Ca^{2+} channel; smooth muscle, bladder, urinary tract, gastrointestinal tract, myometrium, airways smooth muscle

Address for correspondence:

CH Fry

Institute of Urology,

University College London,

London W1W 7EY

UK

Tel: +44 20 7679 9376

Fax: +44 20 7679 9584

e-mail: .fry@ucl.ac.uk

Abstract

T-type Ca^{2+} current has been recorded in smooth muscle myocytes, and associated interstitial cells, from smooth muscle cells isolated from the gastro-intestinal tract, urinary bladder, urethra, prostate gland, myometrium, vas deferens, lymphatic vessels and airways smooth muscle. By contrast, current through such channels has not been recorded from other tissues, such as the ureter. Whilst the properties of this Ca^{2+} current are similar in most of these cells, with respect to their voltage-dependence, ion selectivity and response to channel modulators, some differences have been recorded, most notably in the gastro-intestinal tract, and may demand a reappraisal of how a T-type Ca^{2+} current is characterised. The functions of such a current in different tissues remains uncertain. In most of smooth muscles discussed in this review, it is hypothesised that it underlies rhythmic or spontaneous electrical activity, especially in concert with other current-carrying systems, such as Ca^{2+} -activated outward currents. Of equal interest is that the T-type Ca^{2+} channel may be a target for agents that modulate tissue function, especially in pathological conditions, or are the site of secondary effects of agents used in clinical medicine. For example, T-type Ca^{2+} channel modulators have been proposed to reduce overactive muscular activity in the gastro-intestinal or urinary tract, or function as tocolytic agents: and the action of volatile anaesthetics on them in airways smooth muscle requires consideration in their overall action.

Introduction.

T-type Ca^{2+} channels have been described in smooth muscle cells from a number of internal organs, including the G-I tract, the genito-urinary tract, airways and the lymphatic system. In all these tissues the biophysical characteristics of the current are similar, but there remains much uncertainty as to the physiological functions it carries out. The current activates at more negative membrane potentials than other Ca^{2+} currents, and displays generally a considerable window current due to an overlap of the steady-state activation and inactivation curves around a range of potentials near to the resting potential of these smooth muscle cells. In consequence, it is often argued that the current generates spontaneous or pacemaking activity and permits Ca^{2+} influx that is then utilized by other cellular systems. An extrapolation of these observations, made usually with isolated cells, to tissue function is more difficult however, especially as there are few very selective modulators of channel function. Many functional conclusions have derived from the differential use of low concentrations of Ni^{2+} salts [1] or the agent mibefradil (Ro 40-9567) [2,3] as selective blockers of the channel, with respect to the effect of 'classical' blockers of other Ca^{2+} channels. Whilst these agents do reduce T-type Ca^{2+} current, they also have a lesser, but sometimes significant, potency on other ion channels and exchangers in a variety of cells, including the L-type Ca^{2+} channel [4], the N-type Ca^{2+} channel [5], and a TTX-resistant Na^+ channel that have also been described in smooth muscles [3-6]. Thus, a caveat must be inserted in the interpretation of data, especially with channel modulators. However, there is gathering evidence that T-type Ca^{2+} channels are found in many smooth muscles. This review will discuss the distribution of T-type channels in these various smooth muscles in relation to various physiological functions that they might perform.

The urinary tract.

The lower urinary tract functions to store urine, and then periodically to void the contents of the bladder. This is a complex control mechanism that requires relaxation of the bladder (detrusor) smooth muscle and maintenance of a closed outlet (bladder neck and urethra) during filling, and the opposite situation during micturition. Urine is delivered to the bladder by the ureters by peristaltic contractions of the muscular wall. Associated with the urinary tract are accessory organs such as the prostate gland. This organ has secretory activity, but frequently undergoes enlargement by hyperplasia of the stromal and epithelial components and can impose a large outflow resistance on the bladder. The regulation of the contractile state of all these component tissues is vital to maintain proper urinary continence and the role of T-type Ca^{2+} channels has received considerable recent interest.

T-type Ca^{2+} current has been recorded in isolated smooth muscle cells from the detrusor layer of human and animal bladders [7,8], from human and animal urethra [9,10] and from human prostate samples [11]. In each case T-type channels co-exist with their L-type counterparts, and their separation has been achieved by using different holding potentials and the sensitivity of ionic current to micromolar concentrations of Ni^{2+} salts or nifedipine respectively. An example of Ni^{2+} -sensitive inward current from an isolated human detrusor myocyte upon depolarization from -100 mV to -30 mV is shown in figure 1A. In each tissue T-type current contributes 20-30% of the total Ca^{2+} current and represents a peak inward current of about 1 pA.pF⁻¹ in detrusor cells. There are however, qualitative differences in current characteristics from the different tissues, for example Ni^{2+} sensitivity is greater with current from urethral compared to detrusor myocytes [7,9]. This may reflect different $\alpha 1$ subunits that comprise the channel pore, as Ni^{2+} has variable potency on channels composed of these various subunits [1].

Urinary tract smooth muscles exhibit spontaneous electrical and mechanical activity to varying degrees, and one hypothesis regarding the function of T-type channels is that they contribute to the generation of such activity. This is given weight because the resting membrane potential of these smooth muscle is about -50 to -60 mV from which spontaneous fluctuations of potential are often superimposed (figure 1B); similar to the window current of Ca^{2+} influx via this channel, due to the significant overlap of steady-state activation and inactivation curves – see figure 1C [7-11]. Furthermore, the action potential threshold voltage becomes more negative, as the membrane potential is hyperpolarised [7]. Hyperpolarisation increases T-type Ca^{2+} channel availability, so that at more negative potentials it will contribute more to the net inward current that supports the action potential upstroke.

FIGURE 1 NEAR HERE

The use of channel blockers also supports the involvement of T-type Ca^{2+} channels in modulating electromechanical activity. With detrusor muscle, submillimolar NiCl_2 concentrations reduce the frequency of spontaneous action potentials and contractions, although not the amplitude of the latter [12,13]. Thus, it may be suggested that Ca^{2+} influx through T-type channels may be sufficient to initiate individual transient depolarisations and associated contractions. However, their complete development might also involve activation of L-type Ca^{2+} channels to explain the sensitivity of such spontaneous activity to L-type Ca-antagonists [14]. Paradoxically T-type Ca^{2+} channels may also stabilize the detrusor cell by reducing the tendency of action potential bursts to develop after the initiation of a single event, as Ca^{2+} influx through the channel may couple to activation of Ca^{2+} -activated K^+ -channels. Transient outward currents have been recorded immediately after the generation of T-type inward current, in isolated cells [7,8] – see figure 1D. Furthermore, Ni^{2+} and apamin (a blocker of small conductance Ca^{2+} -activated K^+ -channels) generate bursts of action potentials from preparations that previously elicited single events [13]. In the urethra similar functional conclusions have been reached. NiCl_2 and mibefradil reduce the frequency of spontaneous action potentials, but neither their amplitude

nor the resting membrane potential [7]. However, in contrast to detrusor, Ni^{2+} did not alter the number of action potentials in a burst [10,13] and this may indicate that Ca^{2+} influx through T-channels may not be linked in the same way to other Ca^{2+} -dependent channels.

FIGURE 2 NEAR HERE

Figure 2 shows a hypothetical scheme that incorporates some of the above observations. Spontaneous intracellular Ca^{2+} transients are observed in isolated cells, that can be of significant size when compared for example to a caffeine-mediated transient that will release Ca^{2+} from intracellular stores. Because of the involvement of both low-concentrations of Ni^{2+} and L-type Ca^{2+} antagonists in suppressing these phenomena, it may be postulated that Ca^{2+} influx through T-type channels may also locally activate L-type channels to raise, at least locally the intracellular Ca^{2+} concentration. This may evoke further release from intracellular stores and the net effect will be to activate Ca^{2+} dependent outward current (see also fig 1D) to terminate the electrical and Ca^{2+} transient. The involvement of intracellular stores in this scheme is supported by the fact that ryanodine will suppress spontaneous transient outward currents (figure 2 inset).

Of interest is a report of T-type Ca^{2+} channel activity in cells cultured from the rhabdosphincter of the male urethra [15]. Although this is a skeletal muscle, postulated to provide a major continence mechanism in this part of the urinary tract [16], it responds by contracting to exogenous agonists and has many features intermediate between skeletal and smooth muscle [17]. However, active tone of the muscle is important to maintain a closed bladder outlet during bladder filling, and we may hypothesise that T-channel activity contributes to the myogenic component of the maintenance of this sphincter mechanism.

There is also evidence that the functional expression of T-type Ca^{2+} channels can vary in urinary tract tissues at different stages of tissue development and during pathological conditions. In human detrusor cells from confluent cultures T-type Ca^{2+} current was absent, in contrast to freshly isolated cells from the same biopsy sample [7], whereas L-type Ca^{2+} current was still recorded. With prostate smooth muscle cells [11], T-type Ca^{2+} current density was less in samples from organs in a state of benign prostatic hypertrophy, compared to more normal tissue (the latter samples were taken from excised prostates because of carcinoma, but distant from the tumour). Finally, we have unpublished data that in detrusor myocytes from overactive bladders, compared to those from stable bladders, the T-type component of total inward voltage-activated Ca^{2+} current is increased, although total current density is not significantly altered. These results suggest that T-channel expression is plastic and does not coincide with the expression of other channel types. It would be of interest to monitor the progression of channel expression during fetal and neonatal development, and to compare this with the maturation of other electromechanical properties of urinary tract tissues.

Ca^{2+} currents have also been characterised in smooth muscle from the ureter [18-20]. However, in this tissue either only an L-type Ca^{2+} -current was identified (guinea-pig cells; [18,19]), or in rat cells a second, slower component, attributed to a Ca^{2+} -sensitive Cl^- channel [20]. The absence of a T-type current in the above experiments was in spite of a sufficiently negative holding potential (-80 to -100 mV). However, the guinea-pig studies were carried out at room temperature and it has subsequently been shown that Ca^{2+} currents have a significant temperature sensitivity [21], so that a small current would have been more difficult to identify. However, the experiments with rat myocytes were carried out at 37°C and thus it may be concluded that there is no evidence for the presence of a significant T-type Ca^{2+} current in this tissue. This conclusion is corroborated by a pharmacological study that showed

L-type Ca-antagonists were effective at abolishing completely contractions induced by membrane depolarization with high-KCl solutions [22].

Interstitial cells, similar to those in the G-I tract (below), have also been described at various locations, including the muscular layers of the bladder [23], urethra [24] and prostate [25] and the sub-urothelial layers of the bladder [26]. In all cases they exhibit spontaneous electrical activity and rises of intracellular Ca^{2+} . Various functions have been proposed, such as initiation of smooth muscle activity in the urethra, co-ordination of activity between different detrusor muscle bundles, or regulation on bladder wall sensations. T-type Ca^{2+} channel activity has not been described in these cells, and focused investigations remain to be done.

Overall, T-type Ca^{2+} channels are well-distributed around the different smooth muscles of the urinary tract, and their associated cells, but not are ubiquitous. The Ca^{2+} current seems to play a role in the modulation of spontaneous electrical (and contractile) activity, but the variability of this phenomenon implies that channel activity may be linked to other modulators such as Ca^{2+} activated K^{+} channels. The importance T-type channels in the urinary tract is increasingly being recognized in view of the significance of spontaneous activity, in its ability to maintain normal muscle function and to generate pathological responses. The plasticity of channel function in different pathological conditions is of especial interest in the latter respect.

The gastro-intestinal tract.

The gastrointestinal tract undergoes a number of co-coordinated movements that convey their contents from one region to another, or allow separation and mixing of contents to facilitate digestion and absorption. The muscular layers are intimately associated with an enteric nervous system and a network of interstitial cells (e.g. interstitial cells of Cajal, ICC) that are understood to regulate and co-ordinate smooth muscle contraction [27]. In particular phasic mechanical activity in the GI tract is associated with electrical activity that may show as slow regular depolarisations, overlain in some cases by action potential bursts. There has thus been considerable interest in the basis of electrical activity in the GI tract, and the role of ICC cells in acting as pacemakers to drive smooth muscle activity; hence the role of T-type Ca^{2+} channels in both cell types will be considered.

T-type Ca^{2+} current has been recorded from myocytes isolated from several parts of the G-I tract including the small intestine [28, 29], colon [30, 31] and stomach [32] from several species. Its presence has also been inferred in oesophageal smooth muscle from the actions of Ni^{2+} salts [33]. However, this is not a ubiquitous observation, as several reports have documented no current even when cells were depolarized from potentials that should be sufficiently negative to activate T-type channels [34, 35].

Several explanations may be put forward for the ability or inability of T-type current to be recorded. Firstly, in rat colon myocytes T-type current appeared as a greater proportion of total inward Ca^{2+} current during ageing [30]. It was virtually absent in neonatal cells, but increased during development, until in aged rats the proportion was about one-third of the net current density, especially as the L-type component also declined in later age. Of interest is an earlier observation in rat ileal myocytes, where two components of inward current were also measured [36], a high-voltage Ca^{2+} current, susceptible to

nifedipine; and a low-voltage, Ni^{2+} -sensitive fraction. The latter was also a negligible fraction of total inward Ca^{2+} current in cells from adults. A second confounding issue is that nifedipine is not effective as an L-type Ca^{2+} channel blocker at all potentials, being less effective at more negative values [37]. Thus, the isolation of a nifedipine-insensitive current as a possible T-type Ca^{2+} current must be interpreted with caution. A final issue was a description of a current from mouse colon myocytes that had some attributes of a T-type current, e.g. activation at negative potentials, inhibition by low Ni^{2+} concentrations and mibefradil. However, other properties were inconsistent with this interpretation, for example, Na^+ , but not Ba^{2+} , was a charge carrier, and the current was blocked by external Cs^+ and Gd^{3+} [38].

Interstitial cells of Cajal (ICC) are considered to generate slow waves of membrane potential that function as pacemaker potentials to drive smooth muscle cells via gap junctions. The generation of slow waves may be controlled by transmembrane Ca^{2+} influx, that is in part independent of L-type Ca^{2+} channels, as they are unaffected by dihydropyridines [39]. Such a current has been identified in ICC from dog and mouse G-I tract [40, 41]. As above, the current was not characterized as a conventional T-type Ca^{2+} current but was activated at negative potentials, and was blocked by low concentrations of Ni^{2+} , as well as mibefradil.

The exact molecular relationship of these ion channels to T-type channels identified in other tissues remains to be established, but they possess in principle the attributes to allow them to entrain regular electrical activity at resting potentials measured in smooth muscle and interstitial cells. Whether there are species and regional differences in the distribution of this current, and if its functional characteristics show similar variation remains to be finally resolved.

One model proposed for pacing potentials generated by ICC is that intracellular $[Ca^{2+}]$ waxes and wanes including an interaction of Ca^{2+} influx through nifedipine-resistant Ca^{2+} channels and mitochondrial Ca^{2+} uptake. The reduced phase of intracellular $[Ca^{2+}]$ stimulates a non-specific cation channel that actually generates a pacemaker potential that spreads by gap junctions between a functional syncytium of cells [41]. The model explains a number of features, including a role for the inward Ca^{2+} current activated at negative potentials, the dependence of pacemaking on extracellular and on intracellular metabolic activity [42, 43].

Multicellular recordings of slow wave activity also support a role for Ni^{2+} -sensitive electrical activity. Slow wave activity in mouse colon preparations consists of a depolarization for several seconds with a rapid upstroke and subsequent plateau phase [44], with a similar pattern in cultured cell clusters on intestinal cells [45]. Ni^{2+} (10 μ M) reduced the upstroke rate, but not the amplitude or frequency in the colon preparation, and higher concentrations (120 μ M) virtually abolished the oscillations in the cultured system. Nifedipine also affected the responses and the data suggest that at least two current components are responsible for the slow wave that may have a basis in the currents described above.

The available data show that inward Ca^{2+} current activated at negative membrane potentials is also present in G-I tract smooth muscle and interstitial cells, and that it could contribute to pacemaking activity, possibly through a complex interplay with intracellular cycling mechanisms. Mathematical models corroborate the role of T-type Ca^{2+} channels by playing an important role in the generation of rhythmic activity [46].

Myometrium.

The uterus is a muscular organ, the myometrium, which is lined with stromal cells and a mucous membrane, the endometrium that in pregnancy provides nutrition and support for the foetus. During foetal growth the uterus also enlarges and increases its contractility, about one month before term uncoordinated contractions develop. It is important to understand the precise controls that prevent premature labour, and co-ordinate parturition itself and so there is interest in the physiological control of myometrial contractility, and the development of tocolytic agents that prevent pre-term labour.

L- and T-type Ca^{2+} currents have also been recorded in smooth muscle cells from myometrium [47-49], and T-type current can contribute up to one-half of the overall inward Ca^{2+} current. These recordings were made using human myocytes from women undergoing either Caesarian section (non-labouring myometrium) or spontaneous vaginal delivery (labouring myometrium), showing that their presence was not only associated with tissue from the terminal stage of pregnancy. However, other groups report a TTX-sensitive Na^+ current, rather than a T-type Ca^{2+} current, that accompanies L-type Ca^{2+} current using pregnant and non-pregnant rat myometrium, as well as human cultured human cells [50-52]. This observation has been strengthened by measurement of the Na^+ channel gene product $\text{mNa}_v2.3$ in mouse myometrium [53].

It has been suggested that, as in other smooth muscles, that T-type Ca^{2+} channels may influence the excitability of myometrium and the frequency of contractions [48]. Because myometrial activity increases towards term one question is whether channel expression varies during pregnancy. L-type Ca^{2+} channel does indeed increase over this period [54], and may contribute to the associated enhanced myometrial contractility. There is also a generalized increase of T-type Ca^{2+} channel mRNA in human myometrium during this period, in particular the $\alpha 1\text{G}$ and $\alpha 1\text{H}$ isoforms of the channel and their many

splice variants [54, 55]. Whether there is more than association between increased T-type channel transcription and contractile function remains to be established.

If T-type Ca^{2+} channels are present, at least in human myometrium, their modulation should provide an important approach to the development of tocolytic agents. For example, administration of magnesium sulphate is an important procedure to reduce preterm labour, especially in the US [56], and it has been shown *in vitro* using uterine myocytes that a high Mg^{2+} concentration (8 mM) reduces the magnitude of T-type, but not L-type, Ca^{2+} channels [48]. Mibefradil has been studied for similar reasons. With rat myometrial preparations from pregnant and non-pregnant animals, mibefradil (3 μM) attenuated spontaneous contractions [57]. In addition, mibefradil reduced contractions elicited by high-K solutions (and hence via Ca^{2+} influx), as well as by oxytocin (via IP_3 generation and release of intracellular Ca^{2+}) with muscle from pregnant rats, although it was less effective in their non-pregnant counterparts. The action was attributed to a decrease of L-type Ca^{2+} channel activity, as mibefradil has a less potent action on these channels [58], and the authors noted the previous findings in rat tissue that T-type Ca^{2+} channels could not be demonstrated [50-52]. However, using human myometrium, mibefradil also attenuated electrical and mechanical function by an action that could be separated from that of nifedipine. It was proposed that T-type channel activity could modulate contractile activity [59] and that mibefradil had the potential as a useful tocolytic agent.

Another target for T-type channel modulation may be the endometrium, which in pregnancy develops predominantly from stromal cells. These cells have the characteristics of myofibroblasts, including contractile activity [60], and thus may influence normal or abnormal delivery of the foetus. Isolated stromal cells exhibit inward Ca^{2+} current that is predominantly T-type [61] as judged by a high sensitivity to Ni^{2+} , but not to nifedipine, that furthermore is attenuated by oxytocin. In addition,

oxytocin reduced the inward current, but the effect was greater in cells from women in labour, compared to those obtained at Caesarean section. It was proposed that T-type channel activity may co-ordinate pathways in the endometrium that are associated with the onset of labour, and provides a further route of control over uterine function that may be exploited to develop tocolytic agents.

The male genitary tract

The smooth muscles of the vas deferens, corpus cavernosum and seminal vesicle have received relatively little attention with respect to the characterization of their ionic currents. K^+ channels that may relax corpus cavernosum have been recorded, in part motivated to understand more about the aetiology of erectile dysfunction and the only depolarising current studied is a Ca^{2+} -activated Cl^- current [62]. Vas deferens smooth muscle has received most attention and in human myocytes an L-type and T-type current have been recorded, with the T-type current contributing about 20% of the total inward current [63]. The vas deferens undergoes rhythmic activity when stimulated by adrenoreceptor agonists such as noradrenaline, but the role that T-type channels may play is not known. However, a synthetic agent, 2,6-dibutylbenzylamine, does induce such activity, that in turn is inhibited by removal of extracellular Ca^{2+} , as well as by addition of Ni^{2+} (but not nifedipine) [64].

The vas deferens is predominantly under the control of sympathetic fibres that release not just noradrenaline, but also ATP. The purine generates excitatory junction potentials (EJPs) by binding to ionotropic $P2X_1$ receptors [65], that in turn can generate intracellular Ca^{2+} sparks which may coalesce to cause larger intracellular transients [66]. A role for transmembrane Ca^{2+} influx has been implicated in this overall scheme, but the route is not known. Both Ni^{2+} (50 μM) and mibefradil were ineffective in attenuating EJPs or intracellular Ca^{2+} transients, suggesting that the T-type channel is not involved [67,

68]. This contrasts to a study with mesenteric blood vessels, which showed the Ni^{2+} did attenuate EJPs [68] and shows that similar phenomena are regulated by alternative processes in different cells.

The role of Ca^{2+} currents, in particular the T-type current, in male genitry tract tissues remains unclear. However, in view of the increasing interest in these tissues with respect to various manifestations of sexual dysfunction, their investigation will be of increasing importance.

Lymphatics.

Rhythmical contractions of smooth muscle in lymphatic vessels generate regular movements to convey lymph back towards the circulation, but little is known about the basis of such a rhythm. Several depolarizing currents have been identified, and their attenuation reduces the rate of spontaneous contractions: these include T-type Ca^{2+} current [69,70], Ca^{2+} -activated Cl^- current [71] and a hyperpolarizing-activated inward current [72]. However, the relative contribution of each component, and whether there are regional or species differences, has not been systematically investigated.

Airway smooth muscle.

The tone of airway smooth muscle is a key determinant of airways resistance and the modulation of such tone will therefore be an important factor in regulating airflow. The site of maximum airways resistance is between the third and fifth subdivision of bronchioles, rather than the trachea, and so the properties of smooth muscle in this region is especially interesting.

Many studies have recorded inward Ca^{2+} currents using myocytes from both tracheal and bronchial smooth muscle, using tissue from a variety of species. Whilst almost all record L-type Ca^{2+} currents [73-76] (a more complete reference list is given in [75]) the presence of a T-type Ca^{2+} current is not ubiquitous [75]. One hypothesis is that T-type Ca^{2+} channels are not uniformly distributed in the respiratory tree and this was tested in an elegant study [77] whereby pig myocytes from the trachea and from third to fifth generation bronchi were compared. Only one current component was present (L-type) in tracheal myocytes; whereas in a subpopulation (about 30%) of bronchial myocytes T-type, as well as L-type, Ca^{2+} current was recorded. The earlier study [75] in which L- and T-type Ca^{2+} current were recorded was also carried out with bronchial myocytes. Reviewing previous studies, the majority of which have been carried out using tracheal myocytes, only one current component is generally recorded and attributed to L-type Ca^{2+} current. However, in one study using tracheal myocytes the one inward Ca^{2+} current component was attributed to that through a T-type Ca^{2+} channel [78]. However, in some respect its characteristics, such as the current-voltage-relationship, voltage-dependent kinetics and an occasional component of non-inactivating current were not entirely typical of T-channel properties.

The significance of T-type channels lies in two aspects of airways activity: the maintenance of smooth muscle tone; and the physiological and pathological action of airway modulators. Spontaneous oscillations of membrane potential occur in bronchial smooth muscle cells in the range of the T-type window current [79] and thus would support a route for Ca^{2+} influx and maintenance of tone. The role of T-type Ca^{2+} channels and their interaction with a Ca^{2+} -activated outward current in this activity remains however to be established.

The vagus nerve exerts control over the airways, as cholinergic agonists contract bronchial smooth muscle. The muscle also possesses relaxatory adrenergic receptors, even though there is probably little

direct control by sympathetic fibres in humans. Finally, a number of agents can either constrict (e.g. histamine, prostaglandin $F_{2\alpha}$) or dilate (e.g. β -agonists, volatile anaesthetics) the airways. The involvement of T-type Ca^{2+} channel activity in these effects has been explored in some, but not all, cases.

Acetylcholine elicits release of Ca^{2+} from intracellular stores and hence contraction, and there is considerable convergence with the histamine induced pathways [80]. However, acetylcholine actually suppresses L-type Ca^{2+} current in airways smooth muscle [81], and this may impose a negative feedback control on the system. However, acetylcholine has no effect on T-type Ca^{2+} current [75, 79] and thus in airways resistance (bronchial) vessels the contractile response may proceed more completely. This observation is corroborated by the fact that dihydropyridines are less effective in bronchial than in tracheal preparations in attenuating carbachol-induced contractions [82]. Thus, T-type Ca^{2+} channels do appear to exert a role over the contractile state of bronchial smooth muscle, possibly by regulating the filling of intracellular Ca^{2+} stores.

Volatile anaesthetics, such as isoflurane and halothane, dilate the airways and have a greater effect on more distal regions [83]. Experiments with isolated cells showed that local anaesthetics had a greater potency on depression of T-type, compared to L-type Ca^{2+} channel activity. This was associated with a larger depression of the carbachol-induced contracture in bronchial compared to tracheal preparations by these anaesthetics, in contrast to the action of L-type Ca -antagonists [77]. Because T-type Ca^{2+} channels are localized in the bronchial regions of the airways, this supports the hypothesis that these channels support the filling of intracellular Ca^{2+} stores that can be released by agonists such as carbachol.

Conclusions

T-type Ca^{2+} channels are distributed throughout smooth muscles, although not as ubiquitous as their L-type counterpart. In general they co-exist with L-type channels and form the smaller component of net inward Ca^{2+} current. However, their voltage-range of activation and inactivation, and the presence of a significant window current near to the resting membrane potential of most smooth muscle cells, mean that they could play a role in generating a significant Ca^{2+} influx and, if coupled to other current components, could underlie spontaneous and rhythmic activity. The ability to manipulate T-channel activity in different tissues means that they could be the targets for agents designed to modulate tissue function under normal and pathological conditions. Perhaps an outstanding issue is to characterise the channels subtypes [1, 84] present in various tissues, to enable better tissue specificity to be generated when developing targeted channel modulators.

References

- 1 J.H. Lee, J.C. Gomora, L.L. Cribbs, E. Perez-Reyes. Nickel block of three cloned T-type calcium channels: low concentrations selectively block $\alpha 1H$. *Biophys J*, 77 (1999) 3034-3042.
- 2 J.P. Clozel, E.A. Ertel, S.I. Ertel. Discovery and main pharmacological properties of mibefradil (Ro 40-5967), the first selective T-type calcium channel blocker. *J. Hypertens*, 15 (1997) Suppl S17–S25.
- 3 P.R. Strege, C.E. Bernard, Y. Ou, S.J. Gibbons, G. Farrugia. Effect of mibefradil on sodium and calcium currents. *Am J Physiol*, 289 (2005) G249-253.
- 4 C.M. Santi, A. Darszon, A. Hernandez-Cruz. A dihydropyridine-sensitive T-type Ca^{2+} current is the main Ca^{2+} current carrier in mouse primary spermatocytes. *Am J Physiol*. 271 (1996) C1583-1593.
- 5 I. Bezprozvanny, R.W. Tsien. Voltage-dependent blockade of diverse types of voltage-gated Ca^{2+} channels expressed in *Xenopus* oocytes by the Ca^{2+} channel antagonist mibefradil (Ro 40-5967). *Mol Pharmacol*. 48 (1995) 540-549.
- 6 I.A. Hobai, J.C. Hancox, A.J. Levi, Inhibition by nickel of the L-type Ca channel in guinea pig ventricular myocytes and effect of internal cAMP. *Am J Physiol*. 279 (2000) H692-701.
- 7 G.P. Sui, C. Wu, C.H. Fry. Inward calcium currents in cultured and freshly isolated detrusor muscle cells: evidence of a T-type calcium current. *J Urol*. 165 (2001) 621–626.
- 8 G.P. Sui, C. Wu, C.H. Fry. A description of Ca^{2+} channels in human detrusor smooth muscle. *BJU Int* 92 (2003) 476–482.
- 9 M.A. Hollywood, S. Woolsey, I.K. Walsh, P.F. Keane, N.G. McHale, K.D. Thornbury. T- and L-type Ca^{2+} currents in freshly dispersed smooth muscle cells from the human proximal urethra. *J Physiol*. 550 (2003) 753-764.

- 10 J.E. Bradley, U.A. Anderson, S.M. Woolsey, K.D. Thornbury, N.G. McHale, M.A. Hollywood. Characterization of T-type calcium current and its contribution to electrical activity in rabbit urethra. *Am J Physiol.* 286 (2004) C1078–C1088.
- 11 G.P. Sui, C. Wu, C.H. Fry. Ca^{2+} currents in smooth muscle cells isolated from human prostate. *Prostate.* 15 (2004) 275-281.
- 12 K.W. Chow, C. Wu, G.P. Sui, C.H. Fry. Role of the T-type Ca^{2+} current on the contractile performance of guinea pig detrusor smooth muscle. *Neurourol Urodyn.* 22 (2003) 77-82
- 13 Y. Yanai, H. Hashitani, Y. Kubota, S. Sasaki, K. Kohri, H. Suzuki. The role of Ni^{2+} -sensitive T-type Ca^{2+} channels in the regulation of spontaneous excitation in detrusor smooth muscles of the guinea-pig bladder. *BJU Int.* 97 (2006) 182-189.
- 14 H. Hashitani, H. Fukuta, H. Takano, M.F. Klemm, H. Suzuki. Origin and propagation of spontaneous excitation in smooth muscle of the guinea-pig urinary bladder. *J Physiol.* 530 (2001) 273–286.
- 15 S. Berjukow, E. Margreiter, R. Marksteiner, H. Strasser, G. Bartsch, S. Hering. Membrane properties of single muscle cells of the rhabdosphincter of the male urethra. *Prostate.* 58 (2004) 238-247.
- 16 A. Elbadawi, R. Mathews, J.K. Light, T.M. Wheeler. Immunohistochemical and ultrastructural study of rhabdosphincter component of the prostatic capsule. *J Urol.* 158 (1997) 1819-1828.
- 17 C.T. Chien, H.J. Yu, T.B. Lin, C.F. Chen. Neural mechanisms of impaired micturition reflex in rats with acute partial bladder outlet obstruction. *Neuroscience.* 96 (2000) 221–230.
- 18 R.J. Lang. Identification of the major membrane currents in freshly dispersed single smooth muscle cells of guinea-pig ureter. *J Physiol.* 412 (1989) 375-395.
- 19 R.J. Lang. The whole-cell Ca^{2+} channel current in single smooth muscle cells of the guinea-pig ureter. *J Physiol.* 423 (1990) 453-473.

- 20 R.D. Smith, L. Borisova, S. Wray, T. Burdya. Characterisation of the ionic currents in freshly isolated rat ureter smooth muscle cells: evidence for species-dependent currents. *Pflugers Arch.* 445 (2002) 444-453.
- 21 M. Nobile, E. Carbone, H.D. Lux, H. Zucker. Temperature sensitivity of Ca currents in chick sensory neurones. *Pflugers Arch.* 415 (1990) 658-663.
- 22 C.A. Maggi, S. Giuliani. A pharmacological analysis of calcium channels involved in phasic and tonic responses of the guinea-pig ureter to high potassium. *J Auton Pharmacol.* 15 (1995) 55-64.
- 23 K.D. McCloskey, A.M. Gurney. Kit positive cells in the guinea-pig bladder. *J Urol.* 168 (2002) 832-836.
- 24 G.P. Sergeant, M.A. Hollywood, K.D. McCloskey, K.D. Thornbury, N.G. McHale. Specialised pacemaking cells in the rabbit urethra. *J Physiol.* 526 (2000) 359-366.
- 25 F. van der Aa, T. Roskams, W. Blyweert, D. de Ridder. Interstitial cells in the human prostate: a new therapeutic target? *Prostate.* 56 (2003) 250-255.
- 26 G.P. Sui, S. Rothery, E. Dupont, C.H. Fry, N.J. Severs. Gap junctions and connexin expression in human sub-urothelial interstitial cells. *Br J Urol Int.* 90 (2002) 118-129.
- 27 G.D. Hirst, F.R. Edwards. Role of interstitial cells of Cajal in the control of gastric motility. *J Pharmacol Sci.* 96 (2004) 1-10.
- 28 M. Yoshino, T. Someya, A. Nishi, K. Yazawa, T. Usikii, H. Yabu. Multiple types of voltage-dependent Ca channels in mammalian intestinal smooth muscle cells. *Pflügers Archiv.* 414 (1989) 401-409.
- 29 Y. Yamamoto, S.L. Hu, C. Y. Kao. Inward current in single smooth muscle cells of the guinea-pig taenia coli. *J Gen Physiol.* 93 (1989) 521-550.
- 30 Z. Xiong, N. Sperelakis, A. Noffsinger, C. Fenoglio-Preiser. Changes in calcium channel current densities in rat colonic smooth muscle cells during development and aging. *Am J Physiol.* 265

(1993) C617-625.

- 31 Z. Xiong, N. Sperelakis, A. Noffsinger, C. Fenoglio-Preiser. Ca^{2+} currents in human colonic smooth muscle cells. *Am J Physiol.* 269 (1995) G378-385.
- 32 M.B. Vivaudou, L.H. Clapp, J.V. Walh, J.J. Singer. Regulation by one type of Ca^{2+} current in smooth muscle cells by diacylglycerol and acetylcholine. *Faseb J.* 2 (1988) 2497-2504.
- 33 K. Uchida, R. Yuzuki, Y. Kamikawa. Pharmacological characterization of endothelin-induced contraction in the guinea-pig muscularis mucosae. *Br J Pharmacol.* 125 (1998) 849-857.
- 34 E.A. Mayer, D.D. Loo, W.J. Snape Jr, G. Sachs. The activation of calcium and calcium-activated potassium channels in mammalian colonic smooth muscle by substance P. *J Physiol.* 429 (1990) 47-71.
- 35 Y. Inoue, Z.L. Xiong, K. Kitamura, H. Kuriyama. Modulation produced by nifedipine of the unitary Ba current of dispersed smooth muscle cells of the rabbit ileum. *Pflügers Arch.* 414 (1989) 534-542.
- 36 S.V. Smirnov, A.V. Zholos, M.F. Shuba. Potential-dependent inward currents in single isolated smooth muscle cells of the rat ileum. *J Physiol.* 454 (1992) 549-571.
- 37 S.M. Ward, K.M. Sanders. Upstroke component of electrical slow waves in canine colonic smooth muscle due to nifedipine-resistant calcium current. *J Physiol.* 455 (1992) 321-337.
- 38 S.D. Koh, K. Monaghan, S. Ro, H.S. Mason, J.L. Kenyon, K.M. Sanders. Novel voltage-dependent non-selective cation conductance in murine colonic myocytes. *J Physiol.* 533 (2001) 341-355.
- 39 J. Malysz, D. Richardson, L. Farraway, M.O. Christen, J.D. Huizinga. Generation of slow wave type action potentials in the mouse small intestine involves a non-L-type calcium channel. *Can J Physiol Pharmacol.* 73 (1995) 1502-1511.
- 40 H.K. Lee, K.M. Sanders. Comparison of ionic currents from interstitial cells and smooth muscle cells of canine colon. *J Physiol.* 460 (1993) 135-152.
- 41 Y.C. Kim, S.D. Koh, K.M. Sanders. Voltage-dependent inward currents of interstitial cells of Cajal

- from murine colon and small intestine. *J Physiol.* 541 (2002) 797-810.
- 42 J.D. Huizinga, L. Farraway L, A. den Hertog. Generation of slow-wave-type action potentials in canine colon smooth muscle involves a non-L-type Ca^{2+} conductance. *J Physiol.* 442 (1991) 15-29.
 - 43 J.D. Huizinga, L. Farraway L, A. den Hertog. Effect of voltage and cyclic AMP on frequency of slow-wave-type action potentials in canine colon smooth muscle. *J Physiol.* 442 (1991) 31-45.
 - 44 S. Yoneda, H. Takano, M. Takai, H. Suzuki. Effects of nifedipine and nickel on plateau potentials generated in submucosal interstitial cells distributed in the mouse proximal colon. *J Smooth Muscle Res.* 39 (2003) 55-65.
 - 45 S. Nakayama, S. Torihashi. Spontaneous rhythmicity in cultured cell clusters isolated from mouse small intestine. *Jap J Physiol.* 52 (2002) 217-227.
 - 46 R.N. Miktakhov, G.R. Abdusheva, J. Christensen. Numerical simulation of motility patterns of the small bowel. II Comparative pharmacological validation of a mathematical model. *J Theor Biol.* 200 (1999) 261-269.
 - 47 Y. Inoue, K. Nakao, K. Okabe et al. Some electrical properties of human pregnant myometrium. *Am J Obstet Gynecol.* 162 (1990) 1090-1098.
 - 48 R.C. Young, L.H. Smith, M.D. McLaren. T-type and L-type calcium currents in freshly dispersed human uterine smooth muscle cells. *Am J Obstet Gynecol.* 169 (1993) 785-792.
 - 49 G.A. Knock, P.I. Aaronson. Calcium antagonistic properties of the cyclooxygenase-2 inhibitor nimesulide in human myometrial myocytes. *Br J Pharmacol.* 127 (1999) 1470-1478.
 - 50 Y. Ohya, N. Sperelakis. Fast Na^{+} and slow Ca^{2+} channels in single uterine muscle cells from pregnant rats. *Am J Physiol.* 257 (1989) C408-C412.
 - 51 Y. Inoue, N. Sperelakis. Gestational change in Na^{+} and Ca^{2+} channel current densities in rat myometrial smooth muscle cells. *Am J Physiol.* 260 (1991) C658-663.
 - 52 M. Yoshino, S.Y. Wang, C.Y. Kao. Sodium and calcium inward currents in freshly dissociated

- smooth myocytes of rat uterus. *J Gen Physiol.* 110 (1997) 565-577.
- 53 T.J. Knittle, K.L. Doyle , M.M. Tamkun. Immunolocalization of the $mNa_v2.3$ Na^+ channel in mouse heart: upregulation in myometrium during pregnancy. *Am J Physiol.* 270 (1996) C688-C696.
 - 54 T. Ohkubo, T. Kawarabayashi, Y. Inoue, K. Kitamara. Differential expression of L- and T-type calcium channels between longitudinal and circular muscles of the rat myometrium during pregnancy. *Gynec Obstet Invest.* 59 (2005) 80-85.
 - 55 T. Ohkubo, Y. Inoue, T. Kawarabayashi, K. Kitamara. Identification and electrophysiological characteristics of isoforms of T-type calcium channel $Ca_v3.2$ expressed in pregnant human uterus. *Cell Physiol Biochem.* 16 (2005) 245-254.
 - 56 D.F. Lewis. Magnesium sulfate: the first-line tocolytic. *Obstet Gynecol Clin North Am.* 32 (2005) 485-500.
 - 57 K.T. Asokan, S.N. Sarkar, S.K. Mishra, V. Raviprakash. Effects of mibefradil on uterine contractility. *Eur J Pharmacol.* 455 (2002) 65-71.
 - 58 S.K. Mishra, K. Hermsmeyer. Selective inhibition of T-type Ca^{2+} channels by Ro 40-5967. *Circ Res.* 75 (1994) 144-148.
 - 59 R.C. Young, P.S. Zhang. Inhibition of in vitro contractions of human myometrium by mibefradil, a T-type calcium channel blocker. *J Soc Gynec Invest.* 12 (2005) e7-12.
 - 60 C. Oliver, M.J. Montes, J.A. Galindo, C. Ruiz, E.G. Olivares. Human decidual stromal cells express α -smooth muscle actin and show ultrastructural similarities with myofibroblasts. *Hum Reprod.* 14 (1999) 1599-1605.
 - 61 B. Liu, S.J. Hill, R.N. Khan. Oxytocin inhibits T-type calcium current of human decidual stromal cells. *J Clin Endocrin Metab.* 90 (2005) 4191-4197.
 - 62 T. Karkanis, L. DeYoung, G.B. Brock, S.M. Sims. Ca^{2+} -activated Cl^- channels in corpus cavernosum smooth muscle: a novel mechanism for control of penile erection. *J Appl Physiol.* 94

(2003) 301-313.

- 63 S.Y. Park, M.Y. Lee, E.M. Keum, S.C. Myung, S.C Kim. Ionic currents in single smooth muscle cells of the human vas deferens. *J Urol.* 172 (2004) 628-633.
- 64 R. Pirisino, G. Banchelli, G. Ignesti, et al. Calcium modulatory properties of 2,6-dibutylbenzylamine (B25) in rat isolated vas deferens, cardiac and smooth muscle preparations. *Br J Pharmacol.* 109 (1993) 1038-1045.
- 65 P. Sneddon, G. Burnstock. ATP as a co-transmitter in rat tail artery. *Eur J Pharmacol.* 106 (1984) 149-152.
- 66 K.L. Brain, V.M, Jackson, S.J. Trout, T.C. Cunnane. Intermittent ATP release from nerve terminals elicits focal smooth muscle Ca^{2+} transients in mouse vas deferens. *J Physiol.* 541 (2002) 849-862.
- 67 K.L. Brain, A.M. Cuprian, D.J. Williams, T.C. Cunnane. The sources and sequestration of Ca^{2+} contributing to neuroeffector Ca^{2+} transients in the mouse vas deferens. *J Physiol.* 553 (2003) 627-635.
- 68 Q. Xi, J. Ziogas, J.A. Roberts, R.J. Evans, J.A. Angus. Involvement of T-type calcium channels in excitatory junction potentials in rat resistance mesenteric arteries. *Br J Pharmacol.* 137 (2002) 805-812.
- 69 M.A. Hollywood, K.D. Cotton, K.D. Thornbury, N.G. McHale. Isolated sheep mesenteric smooth muscle cells possess both T- and L-type calcium currents. *J Physiol.* 501P (1997) 109.
- 70 M. Convery, M.A. Hollywood, K.D. Cotton, K.D. Thornbury, N.G. McHale. Role of inward currents in pumping activity of isolated sheep. *J Physiol.* 501P (1997) 110-111.
- 71 H.M. Toland, K.D. McCloskey, K.D. Thornbury, N.G. McHale, M.A. Hollywood. Ca^{2+} -activated Cl^- current in sheep lymphatic smooth muscle. *Am J Physiol.* 279 (2000) C1327-C1335.

- 72 K.D. McCloskey, H.M. Toland, M.A. Hollywood, K.D. Thornbury, N.G. McHale. Hyperpolarisation-activated inward current in isolated sheep mesenteric lymphatic smooth muscle. *J Physiol.* 521 (1999) 201-211.
- 73 T. Hisada, Y. Kurachi, T. Sugimoto. Properties of membrane currents in isolated smooth muscle cells from guinea-pig trachea. *Pflügers Archiv.* 416 (1990) 151-161.
- 74 K.A. Green, R.C. Small, R.W. Foster. The properties of voltage-operated Ca^{2+} -channels in bovine isolated trachealis cells. *Pulm Pharmacol.* 6 (1993) 49-62.
- 75 L.J. Janssen. T-type and L-type Ca^{2+} currents in canine bronchial smooth muscle: characterization and physiological roles. *Am J Physiol.* 272 (1997) C1757-C1765.
- 76 R. Marthan, C. Martin, T. Amédée, J. Mironneau. Calcium channel currents in isolated smooth muscle cells from human bronchus. *J Appl Physiol.* 66 (1989) 1706-1714.
- 77 M. Yamakage, X. Chan, N. Tsujiguchi, Y. Kamada, A. Namiki. Different inhibitory effects of volatile anesthetics on T- and L-type voltage-dependent Ca^{2+} channels in porcine tracheal and bronchial smooth muscles. *Anesthesiology.* 94 (2001)683-693.
- 78 M.I. Kotlikoff. Calcium currents in isolated canine airway smooth muscle cells. *Am J Physiol.* 254 (1988) C793-C801.
- 79 L.J. Janssen, E.E. Daniel. Depolarising agents induce oscillations in canine bronchial smooth muscle membrane potential: possible mechanisms. *J Pharm Exp Therap.* 259 (1991)110-117.
- 80 L.J. Janssen, S.M. Sims. Histamine activates Cl^- and K^+ currents in guinea-pig tracheal myocytes: convergence with muscarinic signalling pathway. *J Physiol.* 465 (1993) 661-677.
- 81 G.R. Wade, J. Barbera, S.M. Sims. Cholinergic inhibition of Ca^{2+} current in guinea-pig gastric and tracheal muscle cells. *J Physiol.* 491 (1996) 307-319.
- 82 T.L. Croxton, C. Fleming, C.A. Hirshman. Expression of dihydropyridine resistance differs in porcine bronchial and tracheal smooth muscle. *Am J Physiol.* 267 (1994) L106-L112.

A

A.J. Mazzeo, E.Y.
Z.J. Bosnjak, R.L.
J.P. Kampine.
graphical differences
effects of isoflurane on
l) 948-954.

B

el member of the low-
ium channel family. J
Neurosci 19 (1999)

C

D

Figure 1. T-type Ca^{2+} current in human detrusor smooth muscle.

A: Fluctuations of resting membrane potential in an isolated detrusor myocyte – K^+ -filled patch pipette.

B: Inward current elicited from a holding potential of -100 mV to -30 mV (brown trace); the current is abolished by 100 μM NiCl_2 (green trace) - Cs^+ -filled patch pipette. C: Steady-state activation (d_∞ , brown symbols) and inactivation (f_∞ , green symbols) for Ni^{2+} -sensitive T-type Ca^{2+} current. Mean data $\pm$ s.d. – the curves are fitted to Boltzmann curves $d_\infty = 1/[1+\exp((V_{0.5} - V_m)/k)]$ and $f_\infty = 1/[1+\exp((V_m - V_{0.5})/k)]$. D: Ionic current elicited from a depolarising step from -80 mV to -40 mV. Spontaneous outward transient currents, K^+ -filled patch pipette, follow inward current. Data in parts B and C modified from ref [8]

Figure 2. T-type Ca^{2+} channels and rhythmic activity, a proposed mechanism for detrusor smooth muscle

Spontaneous intracellular Ca^{2+} transients can be measured in isolated detrusor myocytes, a particularly active example is shown in the upper left inset. It is proposed that Ca^{2+} influx through T-type Ca^{2+} channels around the 'window current' range of membrane potentials can elicit further influx through L-type Ca^{2+} channels. In turn this may evoke further Ca^{2+} release from intracellular Ca-stores and the consequent rise of intracellular $[\text{Ca}^{2+}]$, at least near the inner face of the surface membrane will open Ca^{2+} activated K^+ channels, to terminate the transient. The involvement of intracellular Ca-stores is indicated by the fact the application of ryanodine will reduce the frequency of spontaneous transient outward currents (inset top right).