Research article

Effects of modulators of TASK potassium channels on rat pulmonary artery tone

Sajni Dipak Shah*

Faculty of Life Sciences, University of Manchester, Core Technology Facility, Manchester, UK.

*Corresponding author: 15 Crundale Avenue, Kingsbury, London NW9 9PJ, UK. Tel: +44 (0)7828 974984. Email: sajds@hotmail.co.uk

Supervisor: Professor A. M. Gurney, Faculty of Life Sciences, University of Manchester, Core Technology Facility, 46 Grafton Street, Manchester M13 9NT, UK.

TWIK-related acid-sensitive potassium (TASK) channels have been implicated as having a role in maintaining and mediating the tone of pulmonary arteries by influencing the membrane potential of the smooth muscle cells. Inhibition of these channels would be expected to promote depolarization, calcium influx and contraction. The purpose of this study was to investigate the effects of TASK modulators on rat intrapulmonary artery tone. The modulators included pH, an important physiological TASK modulator, and drugs that inhibit TASK channels, such as bupivacaine, methanandamide and zinc. Small vessel myography was used to measure the tone of both conduit and resistance pulmonary arteries. Cumulative bupivacaine and methanandamide dose–response curves were compared with phenylephrine (a sympathomimetic vasoconstrictor). The effects of pH were investigated on vessel tone and responses to bupivacaine, methanandamide or zinc chloride. Bupivacaine and methanandamide (>10 μM) resulted in increased artery tone, with similar effects seen in conduit and resistance vessels. Zinc had no effect, possibly reflecting an inhibitory action on calcium channels. In the presence of endothelial blockers, methanandamide (100 μM) still resulted in an increase in artery tone, implying an action on smooth muscle. The application of nifedipine resulted in the inhibition of the response seen with bupivacaine (100 μM), implying that voltage-gated calcium entry was involved. Changing the pH from 7.3 to 8.3 resulted in vasoconstriction, and a relaxation was seen in acidic conditions. This is opposite to the result expected for TASK channel modulation, but may reflect the multiple effects of pH on smooth muscle. The contractions seen with bupivacaine and methanandamide were increased at pH 8.3 but inhibited at pH 6.3, consistent with an effect on TASK channels. Responses to bupivacaine and methanandamide were, however, very small, suggesting that currents produced by TASK channels may not be a major factor in contracting intrapulmonary arteries, but may still have a minor role.

Key words: arterial tone, TASK channels, rat, bupivacaine, methanandamide, pH.

Introduction

The TWIK-related acid-sensitive potassium (TASK) channels, a subtype of the two-pore domain family of potassium channels, have been implicated as having a role in maintaining and mediating the tone of pulmonary arteries. Thus, the inhibition of TASK channels prevents potassium efflux and results in depolarization, and activation leads to hyperpolarization of the pulmonary artery smooth muscle cells (PASMCs).\textsuperscript{1–6} Depolarization of the cell membrane is expected to enhance the open probability of 1-type calcium channels, allowing calcium entry into the cell which leads to constriction of the artery. Conversely, hyperpolarization would result in the closure of voltage-dependent calcium channels, thus decreasing calcium entry and subsequent vasodilation.\textsuperscript{4}

The α-subunit of the two-pore domain channels, which are mainly expressed as homodimers, comprised four transmembrane segments, two pore-forming domains and a short N-terminus and a long C-terminus, both of which are intracellular.\textsuperscript{6,7} Five TASK channels, TASK-1, TASK-2, TASK-3, TASK-4 and TASK-5, have been cloned thus far. Studies have found the presence of TASK-1 proteins and mRNA in rat, rabbit and human PASMCs as well as in rat endothelial cells,\textsuperscript{2–5} suggesting a role for TASK channels, particularly TASK-1, in setting resting membrane potential. A small role has also been suggested for TASK-2 by the finding that small interfering RNA directed against the TASK-2 mRNA reduced the smooth muscle resting membrane potential and the effects of pH on membrane potential in intact intrapulmonary arteries (IPAs).\textsuperscript{5}
Three known modulators of TASK channels have been investigated in rat, rabbit and human PASMCs: bupivacaine, a local anaesthetic, the endogenous cannabinoid anandamide and Zn\(^{2+}\). In electrophysiological studies, a voltage-insensitive potassium current was found to be inhibited by these three drugs, suggesting that they might cause an increase in IPA tone.\(^{2-4}\) Gardener et al.\(^{5}\) looked at the effect of anandamide and bupivacaine on vessel tone and found a gradual increase, which was concentration-dependent. Whether it was due to an effect on TASK and voltage-gated calcium channels was not investigated.

An important modulator of membrane potential in these studies was pH,\(^{2-5}\) which also affects the TASK channels. Increasing and decreasing the extracellular pH of PASMCs had the effect of activating a potassium current, leading to hyperpolarization, or inhibition of the current, leading to depolarization of the membrane, respectively.\(^{2-5,13}\) In theory, this suggests that an alkaline environment would increase in baseline arterial tone would be seen with a more acidic pH. Surprisingly, few studies have addressed the effects of pH on isolated pulmonary arteries;\(^{2-5}\) therefore, it is not yet clear if this prediction holds true.

The aim of this study was to investigate the effects of TASK channel modulator drugs and pH on rat IPA tone. Both large conduit and small resistance vessels were used, because arteries from different levels of the pulmonary arterial tree are known to respond differentially to a variety of physiological stimuli,\(^{14}\) and the size and source of arteries are thought to be important factors in determining responsiveness to pH.\(^{15}\)

**Materials and methods**

**Preparation of vessels**

Sprague Dawley rats (250–300 g) were killed by cervical dislocation in accordance with Schedule 1 of the UK Animals (Scientific procedures) Act and the lungs and heart removed. Both large (conduit) and small (resistance) IPAs were required. Large vessels were taken from the middle of the main conduit artery running the length of the lung lobe with an approximate diameter of 300 μm. The small vessels, with an approximate diameter of 200 μm, were taken from the second- and third-order branches. Vessels were cut into segments 1–2 mm long and suspended horizontally in 5 ml baths of a multi-channel myograph (Danish Myotechnology, Aarhus, Denmark). The larger vessels were mounted on pins attached to a force transducer; the smaller vessels were gently threaded onto steel wires attached to the force transducer. The baths contained physiological salt solution (PSS) of composition (in millimolar): NaCl 122, KCl 5, HEPES 10, KH\(_2\)PO\(_4\) 0.5, NaH\(_2\)PO\(_4\) 0.5, MgCl\(_2\) 1, glucose 5 and CaCl\(_2\) 1. The pH was adjusted to 7.3 with NaOH, bubbled with air and maintained at 37°C.

**Myography**

Chart software (J. Dempster, Strathclyde University) was used to record the isometric tensions of the vessels. After the vessels were mounted, resting tensions of 5 and 4 mN were applied to the large and small vessels, respectively, to provide some initial tone, followed by an equilibration period of 30 min. At the start of every experiment, vessels were contracted three times using 50 mM KCl, with relaxation back to baseline occurring between each contraction in response to being washed with PSS. The final potassium contraction was used as the reference against which all measurements were made.

**Experimental procedures**

Individual phenylephrine, bupivacaine and methanandamide dose–response curves were created by applying increasing and cumulative concentrations (10\(^{-7}\) M–10\(^{-3}\) M) of the drugs to the 5 ml bath of PSS surrounding the vessels. The interval between each application was ∼5 min or after the response was seen to reach its peak.

After creating the dose–response curve for methanandamide followed by washing, the endothelial inhibitors, N-nitro-L-arginine methyl ester (L-NAME) (100 μM) and indomethacin (10 μM), were added to the myograph chamber and left to equilibrate for 40 min. A second dose–response curve was then created in the same manner as the first in order to compare the two conditions.

Nifedipine, a calcium channel blocker, was used to investigate the mechanism of the bupivacaine contraction. The response to 100 μM bupivacaine was recorded. After washing off the bupivacaine with PSS, 1 μM nifedipine was administered for 10 min followed by 100 μM bupivacaine.

The effect of pH was examined by adjusting the PSS to pH 6.3 (acid) or pH 8.3 (alkaline) using 1 M HCl or NaOH. After equilibration at each pH, either bupivacaine or methanandamide (10 and 100 μM) or zinc chloride (100 and 200 μM) was applied individually and the responses recorded.

**Data analysis**

Cursor measurements of the amplitudes of responses were entered into Excel where the drug responses were calculated as a percentage of maximal 50 mM KCl-induced tension. Data are displayed as a means ± standard error of the mean. Data were analysed using the paired t-test or one-way analysis of variance (ANOVA) where appropriate. Significance was assumed if P < 0.05.
Reagents used

Stock solutions (10 mM) of phenylephrine (Sigma) and bupivacaine hydrochloride (Sigma) were prepared with distilled water, methanandamide (TOCRIS) was dissolved in ethanol and stored in aliquots in the freezer. A 100 mM stock solution of zinc chloride (Fluka) was prepared and stored in the fridge. These solutions were serially diluted in PSS as required.

Results

Vessels were used for experiments only if they contracted in response to the application of 50 mM KCl. In each case, three reproducible responses, averaging ~2–3 mN in amplitude for both large and small IPAs, were obtained to 50 mM KCl before the experiments began.

The effect of TASK channel inhibitors on vessel tone

The effect of applying increasing concentrations of bupivacaine (n = 5) on IPA tone can be seen in Fig. 1A and C. Both large and small IPAs were noted to have increased tone with increasing concentrations of bupivacaine; however, significant changes in tone were only seen at 10 and 100 μM for the large IPA and 100 μM for the small IPA. The tensions produced were much lower than (<30%) those achieved by 50 mM KCl or phenylephrine. The maximum tensions produced by 100 μM bupivacaine in small and large vessels were analysed by a paired t-test and not found to be significantly different.

Methanandamide, another TASK channel inhibitor, also caused a concentration-dependent increase in vessel tension; however, the response was very small (Fig. 1B and D). The methanandamide (n = 5) response was much smaller than (<20%) that induced by KCl or phenylephrine. As can be seen in Fig. 1D, arterial tension did not increase until 100 μM methanandamide was administered. A paired t-test showed that the maximum tensions recorded in response to 100 μM methanandamide for the large and small IPAs were not significantly different.

The Influence of the endothelium on the effects of methanandamide

The influence of the endothelium was inhibited with the use of the endothelial blockers l-NAME and indomethacin, which inhibit the release of the vasodilators nitric oxide and prostacyclin, respectively. Physical removal of the endothelium was not undertaken due to the difficulty in removing the endothelium from the small vessels. The response to 100 μM methanandamide seen following the addition of endothelial blockers (n = 4) was similar to that seen with methanandamide alone (Fig. 2). A paired t-test comparing the tensions produced at 100 μM methanandamide in the presence and absence of endothelial blockers for both types of vessels suggests that the responses to methanandamide were not affected by the endothelial blockers.

Figure 1. Effects of bupivacaine (BUP) and methanandamide (ANA) on pulmonary artery tone. Raw traces show the tension produced in a large IPA in response to increasing concentrations (1 nM–100 μM) of bupivacaine (A) or a single application of 100 μM methanandamide (B). Cumulative concentration–response curves for bupivacaine (C, n = 3) and methanandamide (D, n = 5) show the mean ± SEM. Increase in tone expressed as a percentage of that induced by 50 mM KCl in the same tissue. The concentration–effect curve for phenylephrine is shown for comparison.
Nifedipine, a calcium channel antagonist, inhibits depolarization-induced calcium entry, thus indicating that activation of voltage-gated calcium channels has occurred. Such a step was proposed as part of the mechanism involving TASK channel inhibition in pulmonary arteries.2, 3 Thus, if the contraction caused by TASK channel inhibitors is due to voltage-gated calcium entry, it should be blocked by nifedipine. The addition of 100 mM bupivacaine produced an increase in IPA tone. However, when bupivacaine was added after the administration of 1 μM nifedipine, this response was inhibited (Fig. 3). The raw trace of this response can be seen in Fig. 3A and the mean results from both large and small IPAs in Fig. 3B. This pattern of block was seen with both the large and small IPAs. A paired t-test comparing the mean bupivacaine-induced tensions in the absence and presence of nifedipine showed that the results for the large vessel were significantly different (Fig. 3B, P < 0.01).

The effect of pH on artery tone

The effect of changing the pH of the bath solution was to cause an increase or decrease in the vessel tone, although the effect was usually small and variable. Changing pH from 7.3 to 8.3, thus making it more alkaline, produced an increase in the IPA tone, whereas changing pH from 7.3 to the more acidic pH 6.3 caused a decrease in tension. The mean percentage increases in tone (relative to the 50 mM KCl-induced contraction) on changing pH from 7.3 to 8.3 (n = 6) for the large and small vessels were 16 ± 5% and 56 ± 19%, respectively. On changing from pH 7.3 to 6.3 (n = 6), the percentage decreases in tone were −5 ± 2% and −11 ± 6% for the large and small arteries, respectively. The change in tension induced by pH 6.3 compared with that induced at pH 8.3 was found to be significantly different using a paired t-test for both types of vessels (large IPA: P < 0.005 and small IPA: P < 0.01).

The effect of pH on the responsiveness of IPA to TASK channel inhibitors

The concentrations of 10 and 100 μM bupivacaine (n = 5) and 100 μM methanandamide (n = 3) were chosen to be applied in the presence of PSS adjusted to pH 8.3 and 6.3, as it was shown by the dose–response curves in Fig. 1 that these concentrations produced adequate increases in tone to allow the effects of pH on their activity to be demonstrated.

The effect of pH on bupivacaine and methanandamide responses can be seen in Fig. 4. In the raw traces shown (Fig. 4A and B), changing pH had little effect by itself, but it did influence the response to bupivacaine and methanandamide. The bar charts (Fig. 4C and D) show that the responses to these drugs were inhibited at pH 6.3, whereas the responses were enhanced at pH 8.3. This pattern is clear at 100 μM bupivacaine and methanandamide, which produced substantial contractions at pH 8.3, but had no effect at pH 6.3. A similar response pattern was seen with
the lower concentration of 10 μM bupivacaine; however, this concentration produced small effects when compared with those recorded for 100 μM bupivacaine, which, in this experiment, were only measurable at pH 8.3. Using ANOVA, it was found that the differences in tension seen with changing the pH were statistically significant for bupivacaine at 100 μM on the large artery (P, 0.01).

Zinc chloride, at concentrations blocking TASK channels in PASMCs (100 and 200 μM), was found to produce no changes in tension at pH 6.3, 7.3 or 8.3.

**Discussion**

Bupivacaine was found to increase vessel tone. Gardener et al. also found this when investigating the effect of bupivacaine on pulmonary arteries, with the increase in tone being concentration-dependent. Bupivacaine is a local anaesthetic drug that is believed to inhibit TASK channels. It has been suggested that TASK channel inhibition leads to depolarization due to a decrease in the potassium efflux. The resulting depolarization enhances the open probability of L-type calcium channels. Subsequently, calcium enters the smooth muscle cells, resulting in muscle contraction and vasoconstriction. By using nifedipine to block these calcium channels, it was possible to test whether this is the mechanism by which bupivacaine exerts its effects. The fact that bupivacaine did not increase vessel tone after the application of nifedipine indicates that bupivacaine probably does cause the opening of L-type calcium channels via TASK channel inhibition. In the presence of nifedipine, calcium was unable to enter the cell and cause vasoconstriction, despite bupivacaine causing depolarization. As pulmonary arteries do not express sodium channels, a local anaesthetic action on Na⁺ channels cannot contribute to the result, although inhibition of another K⁺ channel cannot be excluded.

The increase in the vessel tone at the higher concentrations of 10 and 100 μM bupivacaine in large and small vessels may reflect the point at which the numbers of inhibited TASK channels reach the level required to cause increased tension. This is possibly the concentrations at which sufficient numbers of TASK channels are closed to bring the membrane potential to the calcium channel activation threshold, after which calcium enters and results in contraction. Gardener et al. found similar concentration dependence with an increase in the vessel tone occurring at a concentration slightly higher than 10 μM.

Methanandamide, another putative inhibitor of TASK-1 channels, also increased arterial muscle tension at concentrations above 10 μM. Gardener et al. reported similar findings. This contraction may have been due to depolarization as a result of TASK channel inhibition; however, this was not studied further. Methanandamide is a cannabinoid, so an action on cannabinoid receptors cannot be ruled out. Two cannabinoid receptors, CB1 and CB2, were found to
be present in the endothelium upon which anandamide may exert relaxant effects. This would counteract and limit the amplitude of any direct contractile action of methanandamide on the smooth muscle. TASK channels have been found to be present in the endothelium as well as in the smooth muscle cells of pulmonary arteries, and the inhibition of endothelial TASK channels could also influence the constrictor effect of methanandamide. To investigate the possible influence of the endothelium on responses to methanandamide, the effect of blocking the release of endothelium-derived relaxing factors on its effects was, therefore, investigated. The endothelial blockers used, l-NAME and indomethacin, are nitric oxide synthase and cyclooxygenase inhibitors, respectively. They inhibit the production of nitric oxide and prostacyclin, both of which are relaxing factors produced by the endothelium. If the constrictor effect of methanandamide was limited by concurrent endothelium-dependent relaxation, it would be expected that the addition of endothelial blockers would cause it to be increased. This was not observed. Methanandamide produced a response in the presence of the endothelial blockers that was not significantly different from that in their absence. However, it cannot be ruled out that TASK channel inhibition or activation of cannabinoid receptors in the endothelium by methanandamide may result in the release of factors not blocked by l-NAME and indomethacin. One such factor that may be involved is the endothelium-derived hyperpolarizing factor (EDHF), which has been implicated in cannabinoid-induced mesenteric vasodilation in the rat. Thus, an EDHF-dependent relaxation may be masking the full methanandamide contraction.

Although both bupivacaine and methanandamide caused constriction of IPA, the tensions produced were very small. Phenylephrine, an α-adrenergic agonist, is known to cause constriction of IPA and its effects showed the vessels used to be viable, as did the constriction to 50 mM KCl. The responses to bupivacaine and methanandamide were far smaller than those produced by phenylephrine, even at 100 μM, which is well above the concentrations producing 50% block of TASK channels. This indicates that TASK channel block does not induce the maximal IPA tension and suggests that TASK currents are not of major importance in contracting IPA, but may still have a minor role.

**The effect of pH on tone and responsiveness of IPA to TASK channel inhibitors**

In the intact rat lung, acidosis causes vasoconstriction and alkalosis causes vasodilation in the presence of elevated basal tone. Surprisingly, however, there is very little in the literature about the effects of extracellular pH on tone in isolated IPA. In this study, changing pH to create an acidic environment had the effect of relaxing IPA, whereas in more alkaline conditions, the IPA constricted. This is opposite to what is found in the intact lung and is hypothesized from the effects of pH on TASK channels. Findings from studies investigating TASK channel currents and their influence on the membrane potential showed that an acidic pH causes depolarization and an alkaline pH causes hyperpolarization due to inhibition or activation of TASK currents, respectively. Thus, it would be expected that acidic conditions would result in vasoconstriction and alkaline conditions would cause vasodilation. The present finding is consistent with one other study, in which relaxation of pulmonary artery in the presence of extracellular acidosis was seen, as well as with the known characteristics of systemic vessels.

The effects of pH that was observed on IPA tone cannot be due to its effect on TASK channels. As pH can affect the activities of many pathways involved in regulating vascular tone, it is possible that TASK channel inhibition does contribute, but is masked by opposing effects. Vascular tone is mainly determined by the intracellular concentration of calcium ions, and the influence of extracellular and intracellular acidosis and alkalosis on the calcium ion concentration has been investigated with conflicting results. Wakabayashi and Groschner found that a decrease in the extracellular, but not intracellular, proton concentration (i.e. extracellular alkalosis) caused calcium entry into endothelial cells, which resulted in the release of vasoactive mediators and thus a decrease in the vascular tone. It has also been suggested that the vasodilator effects seen in acid pH may be due to its action on the endothelium; however, two studies, one in which the endothelium was removed and one using endothelial blockers, found that the relaxation was endothelium-independent. Klockner and Isenberg suggested that extracellular protons exert their effects in the same way as intracellular acidification as they can permeate the membrane of vascular smooth muscle cells. Kramper and Rhoaes found that intracellular alkalosis caused direct contraction of PASMCs through the release of calcium from intracellular sites; however, they also found that intracellular acidosis caused contraction via voltage-activated calcium channels. pH has been found to affect the activity of l-type calcium channels. Intracellular acidification decreases, and intracellular alkalosis increases, calcium influx through these channels. It must be noted, however, that the aforementioned effects of pH on vascular smooth muscle cells do not necessarily occur in IPA.

Although pH did not affect basal tone in the way expected of TASK channel modulation, it did influence the responses to bupivacaine and methanandamide. There was a very slight or no response from both the drugs at pH 6.3. This may be due to the acid conditions inhibiting TASK channels so that no further modulation by bupivacaine and methanandamide can take place. The inhibitory effect of an acid pH is believed to occur due to protonation of the histidine residue.
at position 98 on the TASK subunit, which leads to channel block, thus preventing the efflux of potassium ions.\(^{10,26}\) With the channel already blocked, further interaction with modulator drugs would have no effect.

Alkaline conditions caused an enhanced response to both drugs. Patch clamp studies have shown that an alkaline pH results in hyperpolarization, which is reversed by both drugs and to a membrane potential that is close to that reached when the drugs are applied at pH 7.3.\(^2\)–\(^4\) From these effects of pH, it might be expected that, in terms of vessel tone, the alkaline pH would have little effect on responsiveness to bupivacaine and methanandamide. The enhanced effect of the drugs at pH 8.3 can be explained, however, if we consider the initial contraction seen with the change of pH from 7.3 to 8.3. This caused the tension to be slightly higher than normal, so that the subsequent administration of TASK channel blockers may have more easily caused vasoconstriction as the efflux of potassium ions was inhibited. The initially higher baseline tone at pH 8.3, compared with 7.3 or 6.3, may therefore have had a synergistic effect with the actions of bupivacaine and methanandamide.

Although zinc chloride is also an inhibitor of TASK channels and blocks TASK-like currents in pulmonary artery myocytes,\(^2\) it had no effect on vessel tone at any pH. One explanation for the lack of effect could be the finding by Busselberg et al.\(^{27}\) that zinc can block voltage-gated calcium channels. As activation of L-type calcium channels is essential for contraction mediated by TASK channel inhibition, block of these channels by zinc would inhibit any contraction that could potentially occur.

**Comparison of the large and small IPA?**

Responses generally seemed smaller in the small IPA, but no significant differences were measured. This may reflect the small number of experiments performed, so more studies are required for clarification.

**Conclusions**

The TASK channel inhibitor drugs, bupivacaine and methanandamide, increase IPA tone; however, zinc had no effect. The hypothesis that vasoconstriction would occur in acid conditions and vasodilation in alkaline conditions was disproved; thus TASK channels do not appear to be involved in the tissue response to pH. The bupivacaine- and methanandamide-induced contractions were enhanced at alkaline pH and inhibited in acidosis, supporting the presence and function of TASK channels in rat IPA. It must be stressed, however, that the effects of TASK inhibitors were small, meaning that either TASK channels have a small role in mediating IPA tone or the drugs used are non-selective and have additional actions that mask the effect of TASK inhibition. With the already strong evidence for the presence and role of TASK channels in IPA, it is hoped that further research into the development of TASK modulator drugs will help, in the future, to clarify the role of TASK channels in regulating pulmonary artery tone.

**Acknowledgements**

Obtaining rats and microdissection of the pulmonary vessels were carried out by Professor A. Gurney (Supervisor), Boris Manoury (Research Fellow), Shreena Joshi (PhD Student) and Sarah Etheridge (Technician). All other work in this project was carried out by the student: Sajni Dipak Shah. I would like to thank Professor A. M. Gurney for guidance throughout.

**Funding**

Biotechnology and Biological Sciences Research Council (grant BBS/B/11761/2).

**References**


