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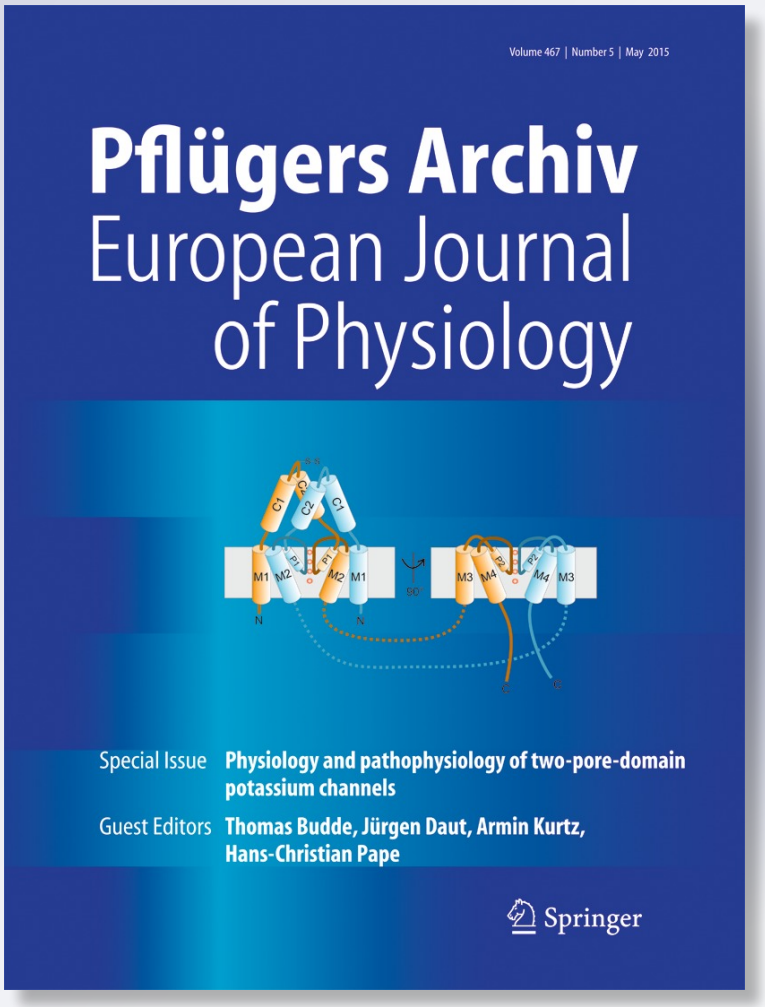

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ION CHANNELS, RECEPTORS AND TRANSPORTERS

Kv1.5 blockers preferentially inhibit TASK-1 channels: TASK-1 as a target against atrial fibrillation and obstructive sleep apnea?

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Abstract Atrial fibrillation and obstructive sleep apnea are responsible for significant morbidity and mortality in the industrialized world. There is a high medical need for novel drugs against both diseases, and here, Kv1.5 channels have emerged as promising drug targets. In humans, TASK-1 has an atrium-specific expression and TASK-1 is also abundantly expressed in the hypoglossal motor nucleus. We asked whether known Kv1.5 channel blockers, effective against atrial fibrillation and/or obstructive sleep apnea, modulate TASK-1 channels. Therefore, we tested Kv1.5 blockers with different chemical structures for their TASK-1 affinity, utilizing two-electrode voltage clamp (TEVC) recordings in *Xenopus* oocytes. Despite the low structural conservation of Kv1.5 and TASK-1 channels, we found all Kv1.5 blockers analyzed to be even more effective on TASK-1 than on Kv1.5. For instance, the half-maximal inhibitory concentration (IC_{50}) values of AVE0118 and AVE1231 (A293) were 10- and 43-fold lower on TASK-1. Also for MSD-D, ICAGEN-4, S20951 (A1899), and S9947, the IC_{50} values were 1.4- to 70-fold lower than for Kv1.5. To describe this phenomenon on a molecular level, we used in silico models and identified unexpected structural similarities between the two drug binding sites. Kv1.5 blockers, like AVE0118 and AVE1231, which are promising drugs against atrial fibrillation or obstructive sleep apnea, are in fact potent TASK-1 blockers. Accordingly, block of TASK-1 channels by these compounds might contribute to the clinical effectiveness of these drugs. The higher affinity of these blockers for TASK-1 channels suggests that TASK-1 might be an unrecognized molecular target of Kv1.5 blockers effective in atrial fibrillation or obstructive sleep apnea.

Keywords Atrial fibrillation · K_{2P} channel · Kv1.5 · Two-pore domain potassium channel · TASK-1 · Obstructive sleep apnea

Aytug K. Kiper, Susanne Rinné, and Caroline Rolfes contributed equally.

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Introduction

Atrial fibrillation and obstructive sleep apnea are among the most prominent diseases in the elderly and responsible for significant morbidity, health care costs, and mortality in the industrialized world [33, 38]. Drugs currently available for the treatment of these two diseases lack effectiveness and/or specificity. Accordingly, there is a high medical need for novel drugs targeting atrial fibrillation or obstructive sleep apnea. For both diseases, Kv1.5 channels have emerged as promising drug targets.

To avoid ventricular side effects, complicating the therapy of atrial fibrillation, current drug design focuses on atrial specific targets, including the atrial sodium channel [3], G protein-gated potassium channel [11], and voltage-gated potassium channel Kv1.5 [8, 9]. Kv1.5 channels are highly expressed in human atria, but not ventricle, and are the major

constituents of the delayed rectifier sustained outward current (I_{Ksus}) that contributes to action potential repolarization of human atrial myocytes [12, 35]. The two-pore domain potassium (K_{2P}) channel TASK-1 also contributes to human atrial I_{Ksus} albeit to a lesser extent than Kv1.5 [20]. TASK-1 was also discussed as a target for the treatment of atrial fibrillation due to its atrial specific expression [20]. In a recent study, the Kv1.5 blocker AVE0118 was reported to be highly effective against obstructive sleep apnea. Here, Wirth et al. utilized an anesthetized pig model to propose that a sensitization of upper airway mechanoreceptors by topical, nasal administration of a potassium channel blocker is a new pharmacologic principle to treat obstructive sleep apnea [36]. The idea of using AVE0118 was to block K^+ channels in order to depolarize superficial mechanoreceptors of the upper airways and thereby modulate the negative pressure reflex; however, the molecular target for the effectiveness of AVE0118 in this apnea model is not yet known. Although TASK-1 is strongly expressed in the hypoglossal motor neurons, forming a nerve that is, when activated, involved in keeping the upper airways open [2], TASK-1 or Kv1.5 expression in the superficial mechanoreceptors of the upper airways or in the sensible and sensory parts of glossopharyngeal and vagus nerves has not yet been reported.

We have previously found that the Kv1.5 blockers S20951 and AVE1231 are in fact highly potent TASK-1 blockers. Due to our observation that these blockers have a strong preference for TASK-1 channels and as the structures of S20951 and AVE1231 were not yet released by Sanofi-Aventis, we published these compound as TASK-1 blockers using the synonyms A1899 [30] for S20951 [16, 17] and A293 [27] for AVE1231 [27]. Both compounds were previously described as being effective in animal models against atrial fibrillation [16, 17, 37], and AVE1231 (A293) had entered clinical development (phase I trial) against atrial fibrillation.

In our current manuscript, we analyze the abovementioned preferential block of TASK-1 by the Kv1.5 blockers AVE1231 (A293) and S20951 (A1899). In addition, we asked whether AVE0118, which had entered clinical phase IIa trials for acute cardioversion of atrial fibrillation and was recently described as a putative drug for obstructive sleep apnea, is also a potent modulator of TASK-1 channels, which turned out to be the case. In addition, we found that other known antiarrhythmic Kv1.5 blockers like S9947, ICAGEN-4, and MSD-D [1, 31] are also potent TASK-1 blockers. As Kv1.5 belongs to the family of tetrameric voltage-gated potassium (Kv) channels with six transmembrane domains per subunit and TASK-1 is a member of the dimeric family of potassium (K_{2P}) channels, we additionally focused on the analysis why these structurally unrelated channels are frequently blocked by the same blockers and why those blockers preferentially inhibit TASK-1.

Methods

Oocyte preparation, cRNA synthesis, and injection

Xenopus laevis oocytes were isolated as previously described [30] and incubated in OR2 solution containing the following in millimolar: NaCl 82.5, KCl 2, $MgCl_2$ 1, and HEPES 5 (pH 7.5) substituted with 2 mg/ml collagenase II (Sigma) to remove residual connective tissue. Subsequently, oocytes were stored at 18 °C in ND96 solution supplemented with 50 mg/l gentamicin, 274 mg/l sodium pyruvate, and 88 mg/l theophylline. Human TASK-1 complementary DNA (cDNA) was subcloned into pSGEM or pBF1 vectors and linearized with NheI or MluI, respectively. Kv1.5 cDNA was subcloned into pSGEM vector, and the construct was linearized with NheI. Complementary RNA (cRNA) was synthesized with mMESSAGE mMACHINE Kit (Ambion). The quality of cRNA was tested using agarose gel electrophoresis. Oocytes were injected with 50 nl of cRNA.

TEVC recordings

All two-electrode voltage clamp (TEVC) recordings were performed at room temperature (20–22 °C) with a Turbo TEC-10CD (npi) amplifier and a Digidata 1200 Series (Axon Instruments) as A/D converter. Micropipettes were made from borosilicate glass capillaries GB 150TF-8P (Science Products) and pulled with a DMZ-Universal Puller (Zeitz). Recording pipettes had a resistance of 0.5–1.5 MΩ and were filled with 3 M KCl solution. Recording solution, ND96, contained the following in millimolar: NaCl 96, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, and HEPES 5 (pH 7.5). Block was analyzed with 1 s voltage steps to +40 mV from a holding potential of -80 mV with a sweep time interval of 10 s. The frequency dependence of peak current block was analyzed by stepping from −80 to 0 mV for 250 ms at a pulse frequency that ranged from 0.5 to 4 Hz. At 4 Hz, a pulse duration of 200 ms was used. Data were acquired with Clampex 10 (Molecular Devices) and analyzed with Clampfit 10 (Molecular Devices) and Origin 7 (OriginLab Corporation).

Drugs and IC_{50} values

The compounds AVE1231 (A293), S20951 (A1899), and AVE0118 were synthesized by Sanofi-Aventis GmbH, Germany, and MSD-D, S9947, ICAGEN-4 were provided by Prof. G. Seebohm [31]. All drugs were dissolved in DMSO, aliquoted, stored at −20 °C, and added to the external solution (ND96) on the day of experimentation. The concentration required for 50 % block of current (half-maximal inhibitory concentration (IC_{50})) was determined from Hill plots. The concentrations of the drugs used to describe the 12 different dose-response curves and IC_{50} values varied

depending on the IC_{50} of the drug on TASK-1 or Kv1.5, but at least four concentrations were used (n=4–6), and the number of experiments ranged from three to 12 experiments per concentration (n=3–12). For all drugs using the lowest stimulation frequency of 0.5 Hz, we applied concentrations to achieve a block of at least 90 %; however, higher concentrations to reach a full inhibition were not applied. For all the TASK-1 channel blockers, a dose-response curve with a Hill factor near 1 was observed. Final DMSO concentration did not exceed 0.1 %. All fitting procedures were based on the simplex algorithm.

Molecular modeling

Modeller 9v5 [29] was used to create the different pore homology models. The TASK-1 homology model was based on the crystal structure of TWIK-1 (Protein Data Bank (PDB) code 3UKM) [22]. The open-state Kv1.5 homology model was based on Kv1.2/Kv2.1 chimera (PDB code 3UKM), and the corresponding closed-state model was based on the computational closed-state model of the *Shaker* channel by Pathak et al. [24]. All models were compared to the original crystal structure template to verify that the modeling step had not altered backbone and side-chain conformations.

Results

Kv1.5 blockers preferentially inhibit TASK-1 channels

In our current study, we describe and carefully analyze the preference of known Kv1.5 blockers for TASK-1 channels. In order to confirm our theory that the effectiveness of known Kv1.5 blockers is biased by an additional or even preferential TASK-1 inhibition, we tested several substances targeting Kv1.5 for their TASK-1 affinity. For this purpose, Kv1.5 or TASK-1 channels were expressed in *Xenopus* oocytes, and IC_{50} values of the substances were determined. First, we probed the inhibition of Kv1.5 and TASK-1 channels by the biphenyl derivates AVE0118, S20951 (A1899), and S9947 (Fig. 1a–c). All these drugs inhibited TASK-1 in a nanomolar range and were more effective on TASK-1 than on Kv1.5 (Fig. 1a–c). The IC_{50} values on TASK-1 were 603 nM for AVE0118, 35 nM for S20951 (A1899) [30], and 200 nM for S9947, respectively. Strikingly, S20951 (A1899) was about 68-fold more effective on TASK-1 than on Kv1.5 (Fig. 1b). Here, it is of particular interest that AVE0118 which entered clinical trials against atrial fibrillation and is very effective in a disease model against obstructive sleep apnea is about 10-fold more affine for TASK-1.

Next, we also tested Kv1.5 blockers with different chemical structures and/or from different pharmaceutical companies. Similar as described for the biphenyl compounds, the anthranilic acid derivative AVE1231 (A293) had an IC_{50} on TASK-1 which was in the nanomolar range. The IC_{50} was 222 nM [27], and the drug was about 43-fold more effective on TASK-1 (Fig. 1d). The IC_{50} of ICAGEN-4 on TASK-1 expressed in oocytes was 1.05 μM (versus 1.6 μM on Kv1.5) and 350 nM for MSD-D (versus 505 nM on Kv1.5). Thus, also for these compounds, we found a TASK-1 inhibition which was more pronounced for TASK-1 than for Kv1.5 channels (Fig. 1e, f).

Similarities in the drug binding site of Kv1.5 and TASK-1

Next, we tried to understand the molecular reason for the similarities in the pharmacology of Kv1.5 and TASK-1 channels and why TASK-1 channels are blocked with similar or even much higher affinity. This conservation of drug affinities between the two unrelated channels is somewhat surprising, as Kv1.5 belongs to the family of Kv channels with six transmembrane domains and TASK-1 to the K_{2P} family with four transmembrane domains per subunit. Drug binding of potassium channel blockers occurs at the selectivity filter and at the inner pore-forming helices, meaning the S6 segment in Kv1.5 [9] or the M2 and M4 segments in K_{2P} channels [30]. Next, we made a protein alignment of the S6 segment of Kv1.5 with the M2 and M4 domains of TASK-1 (Fig. 2a), using a glycine residue (discussed as "gating hinge") which is conserved in most potassium channels [15]. Figure 2a illustrates the most important residues of the previously identified drug binding site in TASK-1 [30] and Kv1.5 channels [9] highlighted in bold and color. Not unexpected, the S6 segment of Kv1.5 and the M2 or M4 segment of TASK-1 are almost entirely different in their sequence, and on first glance, one cannot identify any putative conservation of drug binding sites between these two channels (Fig. 2a). However, at a closer look, considering the dimeric nature of K_{2P} channels, the drug binding sites are conserved and in both channels formed by threonine residues of the signature sequence and by isoleucine and valine/leucine residues of the inner pore-forming helix [30, 31] (Fig. 2b, c). Therefore, the binding sites might be more similar than expected from the visual inspection of the S6, M2, and M4 segments. The cartoons in Fig. 2b, c show that the drug binding sites are conserved, as they include in a similar way four threonine residues of the pore signature sequence (4× T480 in Kv1.5 and 2× T93 plus 2× T199 in TASK-1), and at the inner helices, the binding sites are formed by a "ring" of four isoleucine residues (4× I508 in Kv1.5 and 2× I118 plus 2× I235 in TASK-1). However, closer to the cytosol, the residues involved in drug binding are not identical, and while Kv1.5 has valine moieties (4× V512) facing to the central cavity, TASK-1 has larger and more aliphatic leucine residues contributing to drug binding (2× L122 plus 2× L239).

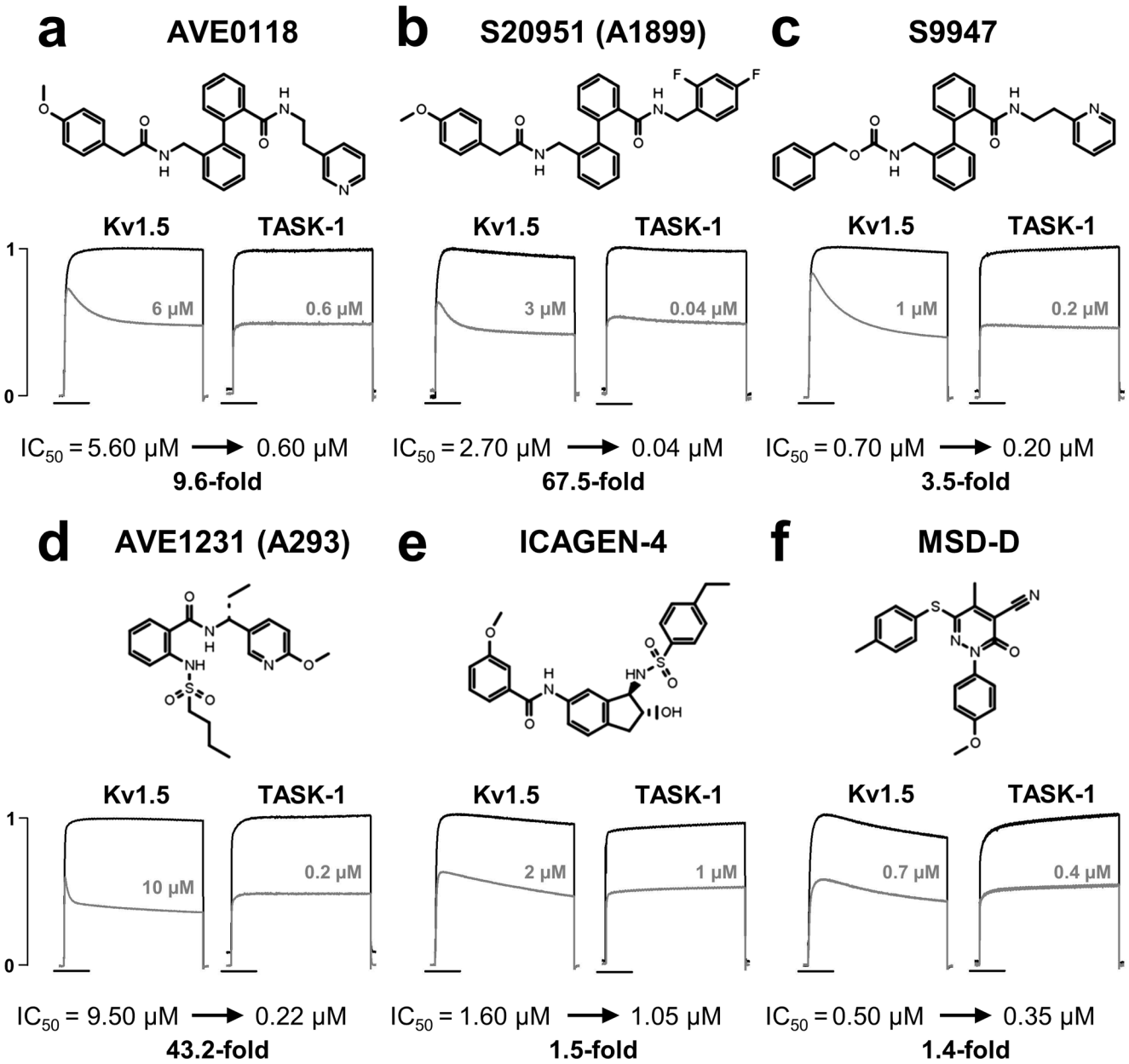

Fig. 1 Chemical structures of Kv1.5 blockers and their affinity for Kv1.5 and TASK-1. Chemical structures for the biphenyl derivates **a** AVE0118, **b** S20951 (A1899), and **c** S9947. Chemical structures for **d** AVE1231 (A293), **e** ICAGEN-4, and **f** MSD-D. **a**–**f** Normalized currents of Kv1.5 or TASK-1 recorded in *Xenopus* oocytes by voltage steps to 0 mV before (*black*) and after (*gray*) half-maximal inhibition by different blockers are illustrated. The drug concentrations used in the recordings are indicated in *gray letters*. The IC_{50} values for Kv1.5 and TASK-1 are depicted under the current traces, and the fold decrease in IC_{50} for TASK-1 compared to Kv1.5 is indicated at the *bottom*. *Scale bars at the left* indicate the normalized Kv1.5 or TASK-1 current amplitudes, and the *scale bars of the x-axis* indicate 250 ms

Structural comparison of the lipophilic TASK-1 and Kv1.5 binding site

Drug binding to TASK-1 and Kv1.5 is caused by binding to threonine residues of the signature sequence and to lipophilic residues facing the central cavity. Next, we generated pore homology models to more closely compare the structures of the two previously described binding sites [9, 30]. The pore homology model of Kv1.5 was based on the open pore structure of the rat Kv1.2 channel [21], and the TASK-1 pore model was based on the TWIK-1 crystal structure [22] (Fig. 3). We displayed the side view of these homology models and highlighted the residues previously described as drug binding sites in the central cavity [9, 30] (Fig. 3a–d). The drug binding sites in the central cavities of both channels consist of a ring of threonine residues and layers of isoleucine

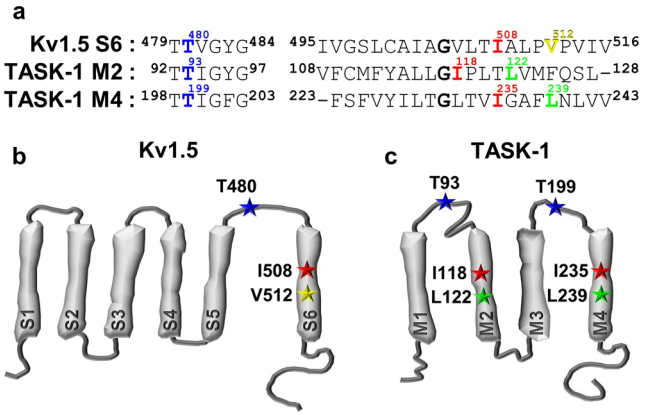

Fig. 2 Sequence alignment and cartoon illustrating the drug binding site of Kv1.5 and TASK-1. **a** Sequence alignment of the S6 segment of Kv1.5 and the transmembrane domains M2 and M4 of TASK-1. The glycine residue (putative "gating hinge") conserved in most potassium channels is highlighted in *bold*. Residues of the previously described drug binding sites in TASK-1 and Kv1.5 are labeled and also highlighted in *bold* and *colored letters*. Cartoon of a **b** Kv1.5 and **c** TASK-1 subunit illustrating that Kv1.5 and TASK-1 share similar residues at their drug binding sites and a similar pattern of residues that interact with drugs in the central cavity. Threonine (*blue*), isoleucine (*red*), and valine (*yellow*)/leucine (*green*)

and valine/leucine residues underneath the selectivity filter (zoomed box in Fig. 3a, c), confirming the conserved lipophilic nature of the two binding sites.

Despite these physicochemical and sterical similarities in the two drug binding sites, there are clear differences in their geometry, primarily arising from the dimeric structure and the lack of a 4-fold symmetry in K_{2P} channels. The rings of the four threonine residues (T480 versus T93/T199) are highly similar for the Kv1.5 and TASK-1 drug binding sites and do not differ in geometry (Fig. 3b, d). Considering the drug binding site V505 in Kv1.5, the channel has three rings of lipophilic residues, forming a binding site with a 4-fold rotational symmetry (4× V505, 4× I508, and 4× V512). In contrast to the expectations derived from the cartoon in Fig. 2c, the TASK-1 drug binding site is not formed by a ring of four isoleucine residues (2× I118 plus 2× I235) plus a ring of four leucine residues (2× L122 plus 2× L239), as we have previously proposed using a KvAP-based TASK-1 pore homology model with a 4-fold symmetry [30]. Here, using a K_{2P} channel (TWIK-1)-based TASK-1 pore homology model with a rhombic 2-fold symmetry, it becomes evident that there are three layers of drug binding residues similar as in Kv1.5 (Fig. 3b, d). However, due to the dimeric nature of the channel, the uppermost, first lipophilic layer of drug binding residues is not formed by a ring, but by only two isoleucine residues (I118) of the M2 segments. Similarly, the lowermost, third layer of drug binding residues is formed by only two leucine residues (L239) of the M4 segments. Only in the middle, second layer of drug binding residues, the binding site is formed by a ring of four lipophilic residues, albeit it is a "mixed ring" containing two leucine residues of M2 (L122) and two isoleucine residues of M4 (I235). In addition, this second "layer" does not have a rotational symmetry, reflecting the rhombic structure of the dimeric K_{2P} channel also in the middle of the cavity (Fig. 3d).

Thus, despite the strong similarities in the lipophilic drug binding sites between TASK-1 and Kv1.5, there are differences in the shape and symmetry of the residues that can complex drugs in the central cavity. For instance, in TASK-1, the first layer of drug binding residues seems to be wider and the second "mixed layer" is narrower than those in Kv1.5 (Fig. 3b, d). Two major differences might account to the fact that some lipophilic drugs prefer TASK-1 channels: (1) the different symmetry of the pore and the binding site and (2) the presence of a mix of lipophilic leucine and isoleucine residues at the lower two layers of the drug binding site. In contrast, in Kv1.5 channels, the lowermost third layer is formed by a ring of smaller and less lipophilic valine residues (V512). In this context, it is noteworthy that even such a small change at a lipophilic residue of the Kv1 drug binding site can have profound effects on the drug affinity [10]. For instance, an isoleucine to valine exchange in Kv1.1, at the site corresponding to I508 in Kv1.5, reduced the affinity of S20951 (A1899), AVE0118, and Psora-4 by 4- to 70-fold [10]. In addition, this third layer only gains a diameter of about 13 Å, when the channel is in the open state, while the pore of TASK-1 is constitutively open, "presenting" the lipophilic isoleucine and leucine residues of the second and third layers for drug binding.

Increased accessibility to the constitutively open TASK-1 channel pore might contribute to the increased affinity of Kv1.5 blockers to TASK-1

Using a pore homology model of Kv1.5 in the open state, the size of the entrance to the central cavity is very similar for TASK-1 and Kv1.5 (Figs. 3b, d and 4a, b). However, using a closed-state model of Kv1.5, the entry pathway to the central cavity is largely diminished, as expected (Fig. 4b, c). This phenomenon is the molecular reason for the use dependence observed for Kv1.5 open-channel blockers [9, 31]. Applying S20951 (A1899) or AVE0118 at their respective IC_{50} concentrations and after achieving half-maximal inhibition, increasing the pulsing frequency (ranging from 0.5 to 4 Hz) caused an additional, frequency-dependent block of Kv1.5 channels (Fig. 4d, e). In contrast, no additional or use-dependent inhibition of TASK-1 was observed for S20951 (A1899) or AVE0118 at all frequencies tested (Fig. 4d, e).

This additional, use-dependent inhibition of Kv1.5 channels by open-channel blockers is caused by an increased accessibility of the blockers to the open pore. As TASK-1 channels are expected to be constitutively open at the inner gate [26, 28], the relative increase in affinity of some blockers for TASK-1 compared to Kv1.5 might be caused by a facilitated accessibility of the blockers to the more lipophilic binding site at the lower end of the central cavity.

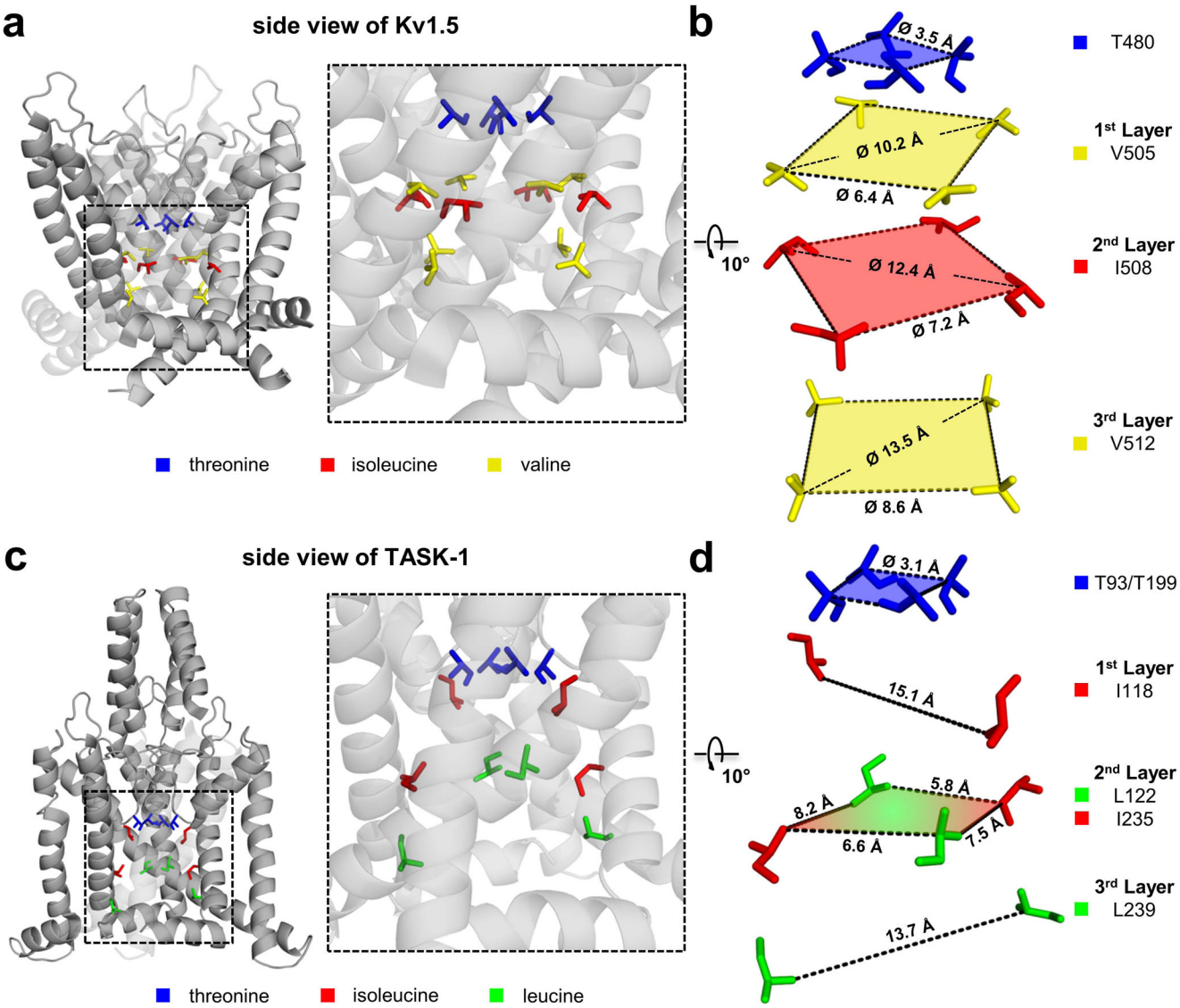

Fig. 3 The drug binding sites of Kv1.5 and TASK-1 illustrated in pore homology models. Side view of the pore homology models for **a** Kv1.5 based on the open-state Kv1.2 crystal structure and **c** TASK-1 based on the TWIK-1 crystal structure. Residues previously described as drug binding sites [9-30] are highlighted. **b**, **d** Layers or "rings" formed by lipophilic residues of the drug binding site. Shortest distances between the residues are indicated

A common pharmacophore model for the TASK-1 blockers AVE0118, S20951 (A1899), S9947, AVE1231 (A293), and MSD-D

With respect to future drug design based on the common blockers of Kv1.5 and TASK-1 channels, it is an important first to map a shared pharmacophore between these six structures. The pharmacophore could be used then as query for designing molecules with specific desired attributes (lead optimization) for each channel. Using e-Pharmacophore [5], three common features were identified within five of the six structures: two hydrogen bond acceptor atoms ("A") and one aromatic ring ("R") (Fig. 5). The A atoms could bind to the ring of threonine residues at the signature sequence of the selectivity filter as it was observed for A1 atom of S20951 (A1899), a carbonyl oxygen interacting with T93/T199 residues of TASK-1 channel [30]. Meanwhile, the R part of the structures could establish hydrophobic contacts with the second layer of the drug binding site (Fig. 3). Such an interaction was also observed between R of S20951 (A1899) and I118 of TASK-1 trough molecular docking [30]. The compound ICAGEN-4 was not within the structures that share this common pharmacophore, although it presents the A atoms and R ring (Fig. 5a). Presumably, ICAGEN-4 was not included within the structures sharing the detected pharmacophore, as the A atoms and R ring were not in the common distances and angles shown in Fig. 5b.

From the present study, it is too early to conclude about how to modify the known common blockers of Kv1.5 and

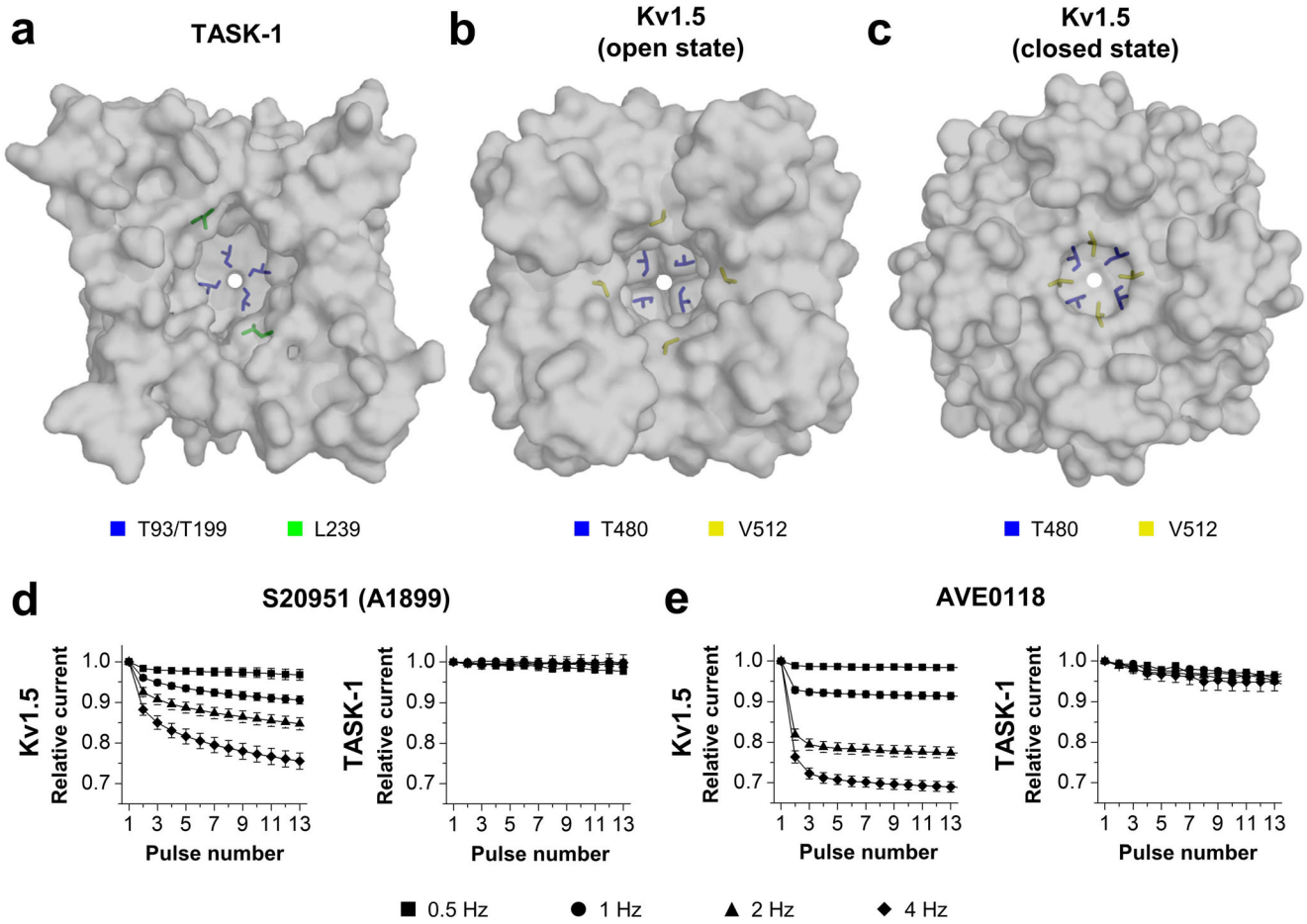

Fig. 4 Diameter of the cytosolic opening to the central cavity and analysis of the frequency-dependent inhibition of Kv1.5 and TASK-1. *Bottom view* of the entry to the central cavity of **a** TASK-1 based on the TWIK-1 crystal structure, **b** Kv1.5 in the open state based on the Kv1.2 crystal structure, and **c** Kv1.5 based on a closed-state model of Pathak et al. [24]. The diameter of the cytosolic entry to the central cavity is similar for the open state of Kv1.5 and the constitutively open TASK-1 channels. **d**–**e** Analysis of the frequency-dependent inhibition of Kv1.5 and TASK-1. After half-maximal inhibition of Kv1.5 or TASK-1, the currents were normalized to 1 and the additional inhibition by pulsing with 0.5, 1, 2, and 4 Hz was analyzed. Frequency-dependent inhibition of Kv1.5 and TASK-1 was analyzed for **d** S20951 (A1899) or **e** AVE0118. Note that only for Kv1.5 inhibition, an additional use-dependent inhibition was observed

TASK-1 channels. However, the shared pharmacophore shown here could be used not only for lead optimization, but also as query for retrieving potential leads from structural databases (lead discovery).

Discussion

In order to identify more effective and safer anti-arrhythmic drugs, recent research focuses on the identification of compounds that target cardiac ion channels that are exclusively or predominantly expressed in the human atria. The major repolarizing current in human atria is the ultra-rapid activating delayed rectifier K^+ current (I_{Kur}) carried by Kv1.5 channels which seems to play a minor role in the ventricles [19, 20, 35]. Surprisingly, we found in our study that several Kv1.5 blockers are more effectively inhibiting TASK-1 than Kv1.5 channels. For instance, AVE1231 (A293) and S20951 (A1899) block TASK-1 43- and 68-fold more efficient than Kv1.5 channels. AVE0118 was described as an atrium-specific blocker of the early action potential repolarization, as it blocks the potassium currents I_{Kur} and I_{to} carried by Kv1.5 and Kv4.3, respectively [9, 14] as well as I_{KACh} [6]. Subsequently, Burashnikov et al. reported a SCN5A affinity for this compound [4]. Therefore, AVE0118 might be a low potent "multichannel blocker" which could be the key to effectiveness, acting as an "amiodarone-like" compound. However, we show that AVE0118 blocks TASK-1 channels in the submicromolar range, with an IC_{50} of 603 nM, while the IC_{50} on Kv1.5, for instance, is only 5.6 μM. This suggests that TASK-1 is the primary molecular target of AVE0118, especially, when considering that the IC_{50} value of AVE0118 was recorded in *Xenopus* oocytes and the potency of drug block is usually underestimated, when currents are measured in intact oocytes. While relative comparisons of affinities, as we have

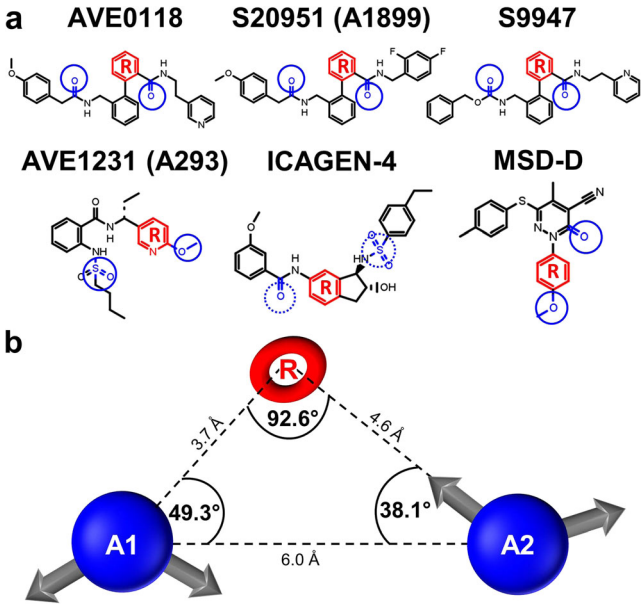

Fig. 5 Common pharmacophore for the TASK-1 blockers AVE0118, S20951 (A1899), S9947, AVE1231 (A293), and MSD-D. **a** Chemical structures of TASK-1 blockers. *Circles* indicate the two hydrogen bond acceptor atoms (*blue*) determined for the pharmacophore model by e-Pharmacophore, and "*R*" indicates the aromatic ring (*red*) of the pharmacophore model. *Dotted lines* used for the *blue circles* of ICAGEN-4 indicate that the drug has the two necessary hydrogen bond acceptor atoms, but not in the right distances and angles. **b** Pharmacophore model in which the two hydrogen bond acceptor groups are denoted as "*A1*" and "*A2*" and the aromatic ring as *R*. The average distances and angles between the groups are indicated. The *arrows* in the hydrogen bond acceptors A1 and A2 indicate the direction in which the hydrogen bond interactions occur

observed here for TASK-1 and Kv1.5 with 1.4- to 68-fold changes, are very accurate using the oocyte expression system, it is well known that overall lower apparent potencies (2–10-fold) are observed, especially for lipophilic compounds. We have observed IC_{50} values for TASK-1 inhibition of 603 nM (AVE0118), 35 nM (S20951/A1899), 200 nM (S9947), 222 nM (AVE1231/A293), 1.05 μM (ICAGEN-4), and 350 nM (MSD-D). Accordingly, the IC_{50} values are expected to be for most of these anti-arrhythmic compounds in the two-digit or even one-digit nanomolar range, when recording from mammalian cells which do not act as a "lipophilic sink" for drugs.

It was also our aim to elucidate how different compounds can block both, TASK-1 and Kv1.5 channels, albeit they belong to very remotely related potassium channel families. Surprisingly, our molecular modeling experiments showed a similar topology of the drug binding sites. For both channels, the drug binding sites are formed by a ring of threonine residues at the signature sequence of the selectivity filter plus three layers of lipophilic residues facing the central cavity underneath the selectivity filter. Despite the strong similarities in the drug binding sites between TASK-1 and Kv1.5, there are differences in the nature of the lipophilic residues and the symmetry of the drug binding site that might explain why we observed a preferential TASK-1 block for most of the Kv1.5 blockers. The fact that TASK-1 channels are constitutively open at the intracellular gate and have more lipophilic isoleucine residues at the entry of the pore (versus a ring of valine in Kv1.5), might in part explain the relative preference of many of the Kv1.5 blockers for TASK-1 channels. These unexpected pharmacological similarities, but also the differences in the molecular architecture of the drug binding sites, should be considered in future drug design of specific TASK-1 or Kv1.5 blockers. Another question arising is whether these Kv1.5 blockers also cause an inhibition of TASK-3, the closest relative of TASK-1. Although we did not investigate the affinity for TASK-3, it is very likely that these channels have a similar affinity, since the drug binding site, which we have previously determined using S20951 (A1899), only differs by a single residue in the M4 segment [30]. This residue in the halothane response element of M4, M247, of TASK-1 is at the homologue position of TASK-3 replaced by a leucine. On the other hand, as we could previously show that an M247L mutation in TASK-1 can cause an about 3.3-fold change in the IC_{50} for S20951 (A1899) [30], it is also possible that the conservation of drug affinities does only comprise Kv1.5 and TASK-1 channels.

In the past years, the preference of Kv1.5 blockers for TASK-1 channels was not known, and thus, the data obtained using those compounds and the conclusions may need to be revisited. For example, Kun et al. used AVE0118 to propose that blocking of neuronal Kv1.5-type potassium channels in the medio-adventitial layer of rat small mesenteric artery [18] mediates a vascular contraction. Expression of TASK-1 channels has been also shown in rat mesenteric artery [13], and therefore, TASK-1 may participate in this effect. Wirth et al. used AVE0118 and found that topical, nasal administration of AVE0118 to the upper airway sensitized and amplified the negative pressure reflex, suggesting that this is a promising pharmacologic approach for the treatment of obstructive sleep apnea [36]. Although the idea of using AVE0118 was to block K^+ channels in order to depolarize superficial mechanoreceptors of the upper airways and thereby modulate the negative pressure reflex, the molecular target for the effectiveness of AVE0118 in this apnea model is not yet known. Interestingly, in the hypoglossal motor neurons, forming a nerve that is, when activated, involved in keeping the upper airways open, TASK-1 is strongly expressed [2]. In addition, TASK-1 plays an important role in motoneuron excitability [32] and thus is a major candidate to mediate the observed effects. However, TASK-1 expression in the superficial mechanoreceptors of the upper airways or in the sensible and sensory parts of glossopharyngeal and vagus nerves has not yet been reported. Although we have obtained preliminary results detecting TASK-1 in the ganglia of these nerves (data not shown), the analyses of the TASK-1 expression, especially in the mechanoreceptors and the nerves of the upper airways, are clearly

hindered by the lack of specific TASK-1 antibodies. As there are currently no reliable tools to detect TASK-1 in these tissue or mechanoreceptors, the use of highly specific TASK-1 blockers in the recently described obstructive sleep apnea model might be the most reliable experiment to show that some or even most of the anti apnea effects are mediated by TASK-1 inhibition. Currently, it seems most reasonable to assume that TASK-1 inhibition in the mechanoreceptors of the upper airways or the glossopharyngeal and vagus nerves might result in a membrane depolarization which modulates the negative pressure reflex. In this context, it is noteworthy that the drug doxapram which is used to treat drug-induced ventilator depression, chronic obstructive pulmonary disease, and apnea in premature infants was shown to be a very potent TASK-1 channel blocker [7]. Taken together, it appears very likely that TASK-1 is a promising target for sleep disorders, especially obstructive sleep apnea.

Due to the atrium-specific expression of TASK-1 in the human heart, TASK-1 channels were previously proposed as drug targets against atrial fibrillation [20]. This becomes more likely, taking our data into account, that Kv1.5 blockers effective in animal models against atrial fibrillation are in fact more potent TASK-1 blockers. It is noteworthy that in mice, after repeated atrial and ventricular burst stimulation with different cycle lengths, a fraction of wild-type mice develops atrial fibrillation whereas in TASK-1 knock-out animals, it appears that they are protected against provoked atrial fibrillation [25]. Also, these data suggest that TASK-1 may indeed be a drug target against atrial fibrillation. Future studies would do well in examining what is the best atrium-specific channel to target for the prevention or conversion of atrial fibrillation. Subject of future research might be, for instance, whether a specific TASK-1 inhibition is as beneficial as a specific inhibition of Kv1.5 or whether a combined inhibition of the two channels is a more superior pharmacological approach to treat or prevent atrial fibrillation. TASK-1 inhibition might be more efficient for the treatment of chronic atrial fibrillation as Kv1.5 inhibition, since patients with chronic atrial fibrillation undergo a strong electrical remodeling, including a TASK-1 upregulation, whereas Kv1.5 is downregulated [23, 34]. In contrast, an advantage of targeting Kv1.5 channels is the positive use dependence of open-channel blockers [8, 9, 31] which we did not observe for TASK-1 channel inhibition, as K_{2P} channels are constitutively in the open state [26, 28]. Thus, Kv1.5 blockers become more efficient/potent under atrial fibrillation which is an excellent prerequisite for the development of drugs for the prevention of atrial fibrillation, as under "attacks" of atrial fibrillation, Kv1.5 channels are more efficiently blocked as under "normal" conditions. In addition, while the close correlation between the reduction in I_{Ksus} current density and the reduction in Kv1.5 expression in atrial fibrillation supports the hypothesis that the Kv1.5 α-subunit is the major component of I_{Ksus} current in human atria [12, 34], we found that $I_{TASK\text{-}1}$ contributes only about 15 % to the sustained potassium current in right human auricular cardiomyocytes [20].

To identify whether the inhibition of TASK-1 or Kv1.5 channels or the combined block is the most superior principle of action it requires the development of novel and more selective blockers and the subsequent testing in disease models of atrial fibrillation or chronic sleep apnea. However, the fact that most of the Kv1.5 blockers that we have analyzed are very potent TASK-1 blockers already indicates that TASK-1 channel block contributes to the effectiveness of these drugs against atrial fibrillation or obstructive sleep apnea.

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