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Review

Molecular Pharmacology of K_{2P} Potassium Channels

Niels Decher[a] Susanne Rinné[a] Mauricio Bedoya[b,c] Wendy Gonzalez[b,c] Aytug K. Kiper[a]

[a]Vegetative Physiology, Institute for Physiology and Pathophysiology, Philipps-University Marburg, Marburg, Germany, [b]Centro de Bioinformática y Simulación Molecular, Universidad de Talca, Talca, Chile, [c]Millennium Nucleus of Ion Channels-Associated Diseases (MiNICAD), Universidad de Talca, Talca, Chile

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Drug binding sites • K_{2P} potassium channels • Ion channels • Molecular pharmacology

Abstract
Potassium channels of the tandem of two-pore-domain (K_{2P}) family were among the last potassium channels cloned. However, recent progress in understanding their physiological relevance and molecular pharmacology revealed their therapeutic potential and thus these channels evolved as major drug targets against a large variety of diseases. However, after the initial cloning of the fifteen family members there was a lack of potent and/or selective modulators. By now a large variety of K_{2P} channel modulators (activators and blockers) have been described, especially for TASK-1, TASK-3, TREK-1, TREK2, TRAAK and TRESK channels. Recently obtained crystal structures of K_{2P} channels, alanine scanning approaches to map drug binding sites, *in silico* experiments with molecular dynamics simulations (MDs) combined with electrophysiological studies to reveal the mechanism of channel inhibition/activation, yielded a good understanding of the molecular pharmacology of these channels. Besides summarizing drugs that were identified to modulate K_{2P} channels, the main focus of this article is on describing the differential binding sites and mechanisms of channel modulation that are utilized by the different K_{2P} channel blockers and activators.

Introduction

Tandem of two-pore-domain potassium (K_{2P}) channels belong to the latest family of potassium channels cloned, with TOK1 from *Saccharomyces cerevisiae* as the first channel discovered in 1995 [1]. The mammalian K_{2P} potassium channel family contains 15 members with different subfamilies, characterized by mechanistic hallmarks like acid inhibition, stretch activation, alkaline activation and halothane inhibition (shown in Fig. 1a). K_{2P} channels have

N. Decher and S. Rinné contributed equally to this work.

Prof. Dr. Niels Decher
Vegetative Physiology
Institute for Physiology and Pathophysiology, Philipps-University Marburg
Deutschhausstraße 1-2, 35037 Marburg (Germany)
Tel. +49 6421-28-62148, E-Mail decher@staff.uni-marburg.de

four transmembrane domains and contain two pore loops and thus assemble as dimer in order that four pore loops form the potassium selectivity filter, similar as in other potassium channel families (shown in Fig. 1b, 1c). The channels have a large extracellular M1-P1 loop which has been identified in early studies as self-interacting domain ('SID') and is relevant for the dimerization of the channels (shown in Fig. 1b) [2]. In fact, the extracellular M1-P1 loop forms the so called 'cap' structure which is a structural hallmark of the K_{2P} channels (shown in Fig. 1b, 1c) [3-9]. It has been postulated that the extracellular 'cap' prevents classical toxins from binding to the pore region [4]. However, whether there are other physiological functions that can be assigned to this unique structure has not been addressed yet. Although K_{2P} channels were initially described as leak channels with an outward rectification that appeared to solely obey the Goldman-Hodgkin-Katz equation for a potassium selective hole, we know in the meantime that these channels are highly regulated by a plethora of different stimuli (shown in Fig. 1a). Moreover, these channels are in fact voltage sensitive with potassium acting as the actual voltage sensor, meaning similar to the CLC chloride channel family [10], the permeating ion actually also gates the channel [11]. Thus, K_{2P} channels are potassium gated potassium channels with a potassium efflux increasing the open probability of the channel at depolarized potentials.

Initially, K_{2P} channels were described to have a unique pharmacology compared to other potassium channel families, as they were less sensitive to the classical potassium channel blockers like TEA, 4-AP, Cs^+ or Ba^{2+}. Channels of the K_{2P} family appeared to be drug resistant. However, classical open channel blockers of ion channels share a conserved binding site scheme with about two or more interacting residues of the pore forming helices and an additional binding to residues of the pore signature sequence (shown in Fig. 1d). This binding

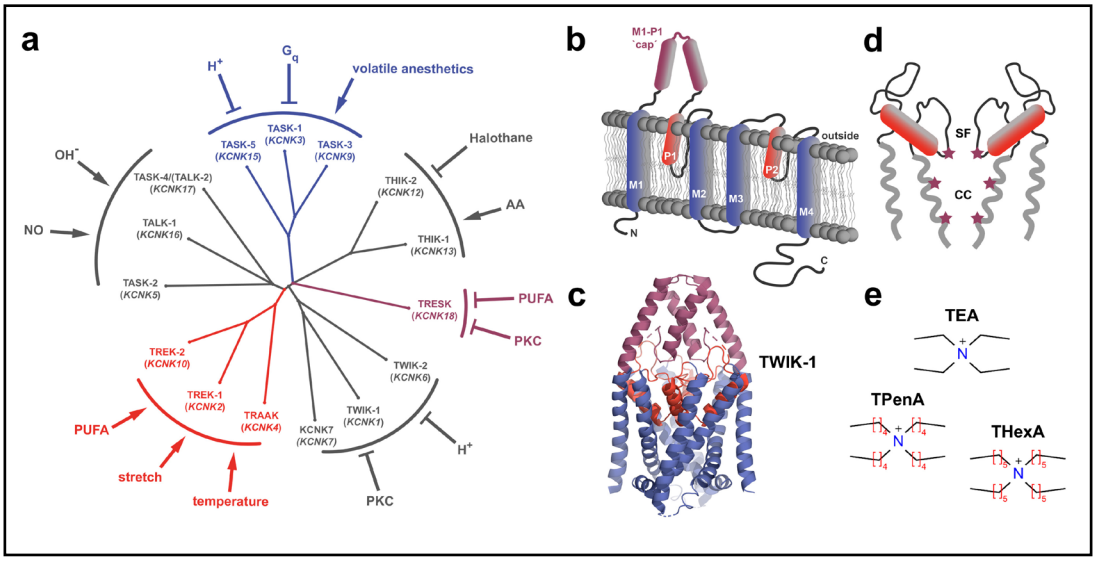

Fig. 1. The K_{2P} channel family. (a) Dendrogram of K_{2P} channels with their physiological or pharmacological key modulators. TWIK: Tandem of P-domains in a weak inward rectifying K^+ channel, TREK: TWIK-related K^+ channel, TRAAK: TWIK-related arachidonic acid activated K^+ channel, TASK: TWIK-related acid-sensitive K^+ channel, TALK: TWIK-related alkaline activated K^+ channel, THIK: TWIK-related halothane inhibited K^+ channel, TRESK: TWIK-related spinal cord K^+ channel. (b) Membrane topology of K_{2P} channels depicting transmembrane domains in blue, the pore helices in red and the extracellular 'cap' structure in purple. (c) Illustration of the crystal structure of the K_{2P} channel TWIK-1 (PDB ID: 3UKM). Transmembrane domains are illustrated in blue, the pore helices in red and the extracellular 'cap' structure in purple. (d) Illustration of the conserved scheme of drug binding sites in voltage gated ion channels. The typical binding sites of classical pore blockers are indicated with purple stars. CC: central cavity; SF: selectivity filter (e) Chemical formula of quaternary ammonium compounds, which are classical pore blockers with different alkyl side chain lengths.

site pattern can be found in different potassium channel families, but also in voltage-gated sodium and calcium channels [12-20]. Thus, it appears unlikely that K_{2P} channels in general should be resistant to classical pore blockers. In the meantime, we know that K_{2P} channels are also highly sensitive to classical pore block by quaternary ammonium compounds (QA) (shown in Fig. 1e), however, presumably due to differences in the architecture of the central cavity providing more lateral space underneath the selectivity filter, the QAs need longer alkyl side chains, like TPenA or THexA (shown in Fig. 1e), to be stabilized underneath the selectivity filter by interact with the pore forming helices [21]. While the sensitivity of TASK-3 channels to extracellular polyamines and ruthenium red (RR) has been described very early after cloning of the TASK channels [22-29], there was for a longer time a lack of potent small compound inhibitors. A decade after cloning of the first K_{2P} channels and TASK-1 [23, 30, 31], we described the first potent K_{2P} channel blocker A293 [32]. Strikingly, the development of a potent and selective TASK-1 channel blocker opened the door for functional studies of the channel in native tissue [33], leading for instance to the isolation of a whole cell TASK-1 current in ventricular myocytes [32]. However, for most of the K_{2P} channels there are still no highly potent and selective blockers or activators available (shown in Table 1-8). Thus, we eagerly anticipate the development of small compound modulators for all K_{2P} channels as this will, besides the generation and study of transgenic mouse models, definitely foster our research aiming to understand the physiological role of K_{2P} channels in different organs.

However, while we still have a poor understanding of the pharmacology of TWIK, THIK and TALK channels, there are in the meantime many potent activators and blockers described for the channels of the TASK and TREK-subfamily, which we would like to summarize in separate sections, especially by focusing on the mechanistic models of channel modulation and the differential binding sites utilized by the drugs.

Table 1. List of inhibitors of the TWIK, THIK and TALK K_{2P} channel subfamilies

channel family	channel	substance category	inhibitor	IC_{50} (μM)	reference	PMID
TWIK						
	TWIK-1					
		class I antiarrhythmics	quinidine	≈95	Lesage et al., 1996	8605869
		local anesthetics	bupivacaine		Kindler et al., 1999	10201682
		malaria treatments	quinine	≈50	Lesage et al., 1996	8605869
	TWIK-2					
		class I antiarrhythmics	quinidine		Patel et al., 2000	10887187
		malaria treatments	quinine		Patel et al., 2000	10887187
		small molecules	RU-TRAAK-1		Su et al., 2016	27091997
		TK inhibitors	genistein		Gierten et al., 2008	18516069
		volatile anesthetics	chloroform		Patel et al., 2000	10887187
		volatile anesthetics	halothane		Patel et al., 2000	10887187
THIK						
	THIK-1					
		small molecules	A1899	≈2	Streit et al., 2011	21362619
		TK inhibitors	genistein		Gierten et al., 2008	18516069
		volatile anesthetics	halothane	≈2800	Rajan et al., 2001	11060316
	THIK-2					
		class I antiarrhythmics	quinidine		Renigunta et al., 2014	24297522
		volatile anesthetics	halothane		Renigunta et al., 2014	24297522
TALK						
	TALK-1					
		cardiac glycosides	digitoxin		Schmidt et al., 2018	29643254
		class I antiarrhythmics	quinidine		Han et al., 2003	12724142
		cloxyquin analogs	A2764		Lengyel et al., 2019	30979812
		malaria treatments	quinine		Girard et al., 2001	11263999
		volatile anesthetics	chloroform		Girard et al., 2001	11263999
		volatile anesthetics	halothane		Girard et al., 2001	11263999
	TASK-2					
		antipsychotics	fluoxetine	≈17	Bustos et al., 2020	31947679
		class I antiarrhythmics	quinidine		Reyes et al., 1998	9812978
		class III antiarrhythmics	clofilium	≈25	Niemeyer et al., 2001	11560934
		local anesthetics	bupivacaine		Kindler et al., 2003	12660311
		local anesthetics	lidocaine		Kindler et al., 2003	12660311
		local anesthetics	ropivacaine		Kindler et al., 2003	12660311
		malaria treatments	quinine	≈22	Reyes et al., 1998	9812978
		small molecules	A1899	≈12	Streit et al., 2011	21362619
		small molecules	A293	≈8	Putzke et al., 2007	17389142
	TASK-4/ (TALK-2)					
		beta blockers	sotalol		Staudacher et al., 2018	30008082
		calcium channel blockers	verapamil		Staudacher et al., 2018	30008082
		class III antiarrhythmics	amiodarone		Staudacher et al., 2018	30008082
		small molecules	A1899	≈8	Streit et al., 2011	21362619
		small molecules	A293	≈20	Putzke et al., 2007	17389142
		sodium channel blockers	ranolazine		Staudacher et al., 2018	30008082
		volatile anesthetics	chloroform		Girard et al., 2001	11263999
		volatile anesthetics	halothane		Girard et al., 2001	11263999
		volatile anesthetics	isoflurane		Girard et al., 2001	11263999

Table 2. List of activators of the TWIK, THIK and TALK K_{2P} channel subfamilies

channel family	channel	substance category	activator	EC_{50} (μM)	no activation	reference	PMID
TWIK							
	TWIK-1						
			n.a.				
	TWIK-2						
			n.a.				
THIK							
	THIK-1						
		fenamate derivates	BL-1249	≈450		Schewe et al., 2019	30792303
		lipids	arachidonic acid	≈1000		Rajan et al., 2001	11060316
	THIK-2						
		small molecules	BL-1249	≈300		Schewe et al., 2019	30792303
TALK							
	TALK-1						
		fenamate derivates	BL-1249	≈200		Schewe et al., 2019	30792303
		gaseous anesthetics	nitrous oxide			Duprat et al., 2005	15513946
	TASK-2						
		fenamates	flufenamic acid			Veale et al., 2016	26914156
		plant extracts	aristoholic acid			Veale et al., 2016	26914156
		volatile anesthetics	halothane			Gray et al., 2000	10839924
		volatile anesthetics	isoflurane			Gray et al., 2000	10839924
		volatile anesthetics	chloroform			Gray et al., 2000	10839924
	TASK-4/ (TALK-2)						
		beta blockers	metoprolol			Staudacher et al., 2018	30008082
		beta blockers	propranolol			Staudacher et al., 2018	30008082
		class I antiarrhythmics	mexiletine			Staudacher et al., 2018	30008082
		class I antiarrhythmics	propafenone			Staudacher et al., 2018	30008082
		class I antiarrhythmics	quinidine			Staudacher et al., 2018	30008082
		class III antiarrhythmics	vernakalant	≈40		Seyler et al., 2014	25108155
		fenamate derivates	BL-1249	≈60		Schewe et al., 2019	30792303
		gaseous anesthetics	nitrous oxide			Duprat et al., 2005	15513946
		volatile anesthetics	isoflurane		TALK-1	Girard et al., 2001	11263999

Pharmacology of K_{2P} channels of the TWIK, THIK and TALK subfamilies

For K_{2P} channels of the TWIK, THIK and TALK subfamilies a low potency block was observed for instance by local anesthetics or quinidine (shown in Table 1). Similar as for other K_{2P} channels these subfamilies are also sensitive to volatile anesthetics (shown in Table 1, 2). In addition, many anti-arrhythmic drugs were reported to block TASK-4/(TALK-2) channels, albeit also with a very low potency (shown in Table 1). Thus, for the K_{2P} channels of the TWIK, THIK and TALK subfamilies there are to our best knowledge no small compound activators or blockers reported that are active in the submicromolar range.

Pharmacology of the TRESK channel

TRESK is a K_{2P} channel that was reported to be primarily or almost exclusively expressed in the spinal cord and DRG neurons [34, 35] which posed this channel on the list of novel promising drugs targeting pain sensation. Noteworthy, TRESK was also found in other tissue like the heart and lung [36]. As a channel sharing 65% identity with human TRESK was isolated from mouse testis, the authors termed this as TRESK-2 [34], however later on it became clear, that this channel is the mouse orthologue of TRESK and that there is only one channel within this subfamily of K_{2P} channels. In contrast to the channels of the TWIK, THIK and TALK subfamilies, TRESK appears to be more 'druggable' since many blockers and/or activators were already described (shown in Table 3, 4). TRESK is blocked by many small compound inhibitors and also with fairly low IC_{50} values (shown in Table 3), for instance by the antihistaminic drug loratadine, with an IC_{50} of about 1 μM [37]. Also many activators have been described (shown in Table 4), here some compounds exhibit even EC_{50} values in the submicromolar range. For instance, acetyl-B-methylcholine, oxotremorine and OXA-22 activate TRESK with an EC_{50} of about 700 nM, 300 nM and 100 nM, respectively [37] (shown in Table 4). Why it appears more feasible to identify modulators of TRESK channels than for members of the TWIK, THIK and TALK subfamilies is an open question.

Table 3. List of inhibitors of the TRESK K_{2P} channel subfamily

channel	substance category	inhibitor	IC_{50} (μM)	no inhibition	reference	PMID
TRESK						
	antibiotics	mevastatin	≈9		Bruner et al., 2014	24972239
	antibiotics	oligomycin A	≈4		Bruner et al., 2014	24972239
	anticonvulsants	gabapentin			Liu et al., 2004	15562060
	anticonvulsants	lamotrigine			Kang et al., 2008	18190784
	antidiabetics	glyburide			Sano et al., 2003	12754259
	antimitotics	podophyllotoxin	≈7		Bruner et al., 2014	24972239
	antipsychotics	fluoxetine			Kang et al., 2008	18190784
	antipsychotics	vanoxerine	≈15		Bruner et al., 2014	24972239
	AT1 angiotensin IIR agonists	L-162,313	≈12		Bruner et al., 2014	24972239
	barbiturates	pentobarbital			Liu et al., 2004	15562060
	calcium channel blockers	caffeine	≈22		Bruner et al., 2014	24972239
	calcium channel blockers	calmidazolium	≈28		Bruner et al., 2014	24972239
	calcium channel blockers	gallopamil			Park et al., 2018	29973548
	calcium channel blockers	GW2974	≈27		Bruner et al., 2014	24972239
	calcium channel blockers	mibefradil	≈3		Bruner et al., 2014	24972239
	calcium channel blockers	nicardipine	≈22		Bruner et al., 2014	24972239
	calcium channel blockers	nimodipine	≈17		Bruner et al., 2014	24972239
	calcium channel blockers	nitrendipine	≈20		Bruner et al., 2014	24972239
	calcium channel blockers	verapamil	≈5	THIK-1	Park et al., 2018	29973548
	cannabinoid receptor agonists	JWH-015	≈6		Bruner et al., 2014	24972239
	cannabinoid receptor agonists	WIN 55,212-2	≈18		Bruner et al., 2014	24972239
	class I antiarrhythmics	propafenone			Sano et al., 2003	12754259
	class I antiarrhythmics	quinidine			Kang et al., 2004	15123670
	cloxyquin analogs	A2764	≈12		Lengyel et al., 2019	30979812
	cloxyquin analogs	A2793	≈7		Lengyel et al., 2019	30979812
	D2R antagonists	octoclothepin	≈7		Bruner et al., 2014	24972239
	calcium channel blockers	nifedipine	≈64		Park et al., 2018	29973548
	FRX antagonists	guggleserone	≈12		Bruner et al., 2014	24972239
	general anesthetics	alphaxalone			Liu et al., 2004	15562060
	general anesthetics	etomidate			Liu et al., 2004	15562060
	general anesthetics	ketamine			Liu et al., 2004	15562060
	guanylyl cyclase activators	YC-1	≈17		Bruner et al., 2014	24972239
	H1R antagonists	loratadine	≈0.7		Bruner et al., 2014	24972239
	leukotriene antagonists	MK-886	≈10		Bruner et al., 2014	24972239
	lipids	arachidonic acid	≈7		Kang et al., 2004	15123670
	local anesthetics	bupivacaine	≈80		Liu et al., 2004	15562060
	local anesthetics	lidocaine	≈3400		Liu et al., 2004	15562060
	local anesthetics	mepivacaine	≈1300		Liu et al., 2004	15562060
	local anesthetics	ropivacaine	≈610		Liu et al., 2004	15562060
	local anesthetics	tetracaine	≈500		Liu et al., 2004	15562060
	malaria treatments	quinine			Liu et al., 2004	15562060
	MEK inhibitors	PD 98,059	≈15		Bruner et al., 2014	24972239
	melatonin Receptor blockers	K 185	≈22		Bruner et al., 2014	24972239
	nAChR antagonists	DHBetaE	≈19		Bruner et al., 2014	24972239
	neuroprotective agents	sipatrigine			Meadows et al., 2001	11172753
	NK1 antagonists	L-703,606	≈9		Bruner et al., 2014	24972239
	PDE III inhibitors	trequinsin	≈8		Bruner et al., 2014	24972239
	PKC&CaMKIII inhibitors	rottlerin	≈16		Bruner et al., 2014	24972239
	plant extracts	6-Gingerol	≈155	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	aristoholic acid			Veale et al., 2016	26914156
	plant extracts	capsaicin	≈70	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	IBA			Tulleuda et al., 2011	21527011
	plant extracts	piperine	≈230	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	polygodial		TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	sanshool	≈50		Bautista et al., 2008	18568022
	pyrethroids	tetramethrin			Castellanos et al., 2018	28937579
	quaternary ammonium ions	TButA	≈7		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TEA	≈800		Piechotta et al., 2011	21822218
	quaternary ammonium ions	THepA	≈0.5		Piechotta et al., 2011	21822218
	quaternary ammonium ions	THexA	≈0.5		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TOctA	≈6		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TPenA	≈0.3		Piechotta et al., 2011	21822218
	small molecules	A1899	≈1		Streit et al., 2011	21362619
	small molecules	RU-TRAAK-1			Su et al., 2016	27091997
	small molecules	RU-TRAAK-2			Su et al., 2016	27091997
	triols	triethanolamine			Sano et al., 2003	12754259

Table 4. List of activators of the TRESK K_{2P} channel subfamily

channel	substance category	activator	EC_{50} (μM)	no activation	reference	PMID
TRESK						
	AChR agonists	arecoline	≈3		Bruner et al., 2014	24972239
	AChR agonists	carbachol	≈2		Bruner et al., 2014	24972239
	alcohols	ethanol			Liu et al., 2004	15562060
	amphetamines	4-MA	≈9	TASK-2, TASK-3, THIK-1, TREK-1, TREK-2	Wright et al., 2019	31564414
	antituberculosics	cloxyquin	≈4		Wright et al., 2013	24383077
	D2R antagonists	spiperone	≈3		Bruner et al., 2014	24972239
	fenamate derivates	BL-1249	≈6	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	fenamates	flufenamic acid	≈474		Monteillier et al., 2016	27641472
	gaseous anesthetics	nitrous oxide			Liu et al., 2004	15562060
	LTRA antagonists	montelukast		TASK-3, TREK-1, TREK-2	Wright et al., 2019	31564414
	LTRA antagonists	pranlukast	≈6	TASK-2, THIK-1	Wright et al., 2019	31564414
	LTRA antagonists	zafirlukast		TREK-1	Wright et al., 2019	31564414
	mAChR agonists	acetyl-B-methylcholine	≈0.7		Bruner et al., 2014	24972239
	mAChR agonists	OXA-22	≈0.1		Bruner et al., 2014	24972239
	mAChR agonists	oxotremorine	≈0.3		Bruner et al., 2014	24972239
	phosphatases	calcineurin			Czirják et al., 2004	14981085
	PKC activators	PMA	≈0.03		Bruner et al., 2014	24972239
	small molecules	GI-530159	≈5	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	LA-TREK2-1		TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	LA-TREK2-2		TASK-2, TASK-3, TREK-1, THIK-1	Wright et al., 2019	31564414
	volatile anesthetics	desflurane	≈660		Liu et al., 2004	15562060
	volatile anesthetics	halothane	≈300		Liu et al., 2004	15562060
	volatile anesthetics	isoflurane	≈160		Liu et al., 2004	15562060
	volatile anesthetics	sevoflurane	≈225		Liu et al., 2004	15562060

Pharmacology of channels from the TREK/TRAAK subfamily

TREK/TRAAK activators

A hallmark of the members of the TREK/TRAAK subfamily of K_{2P} channels is the channel modulation by polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) [38, 39] (shown in Fig. 2a and Table 5). Analyses of deletion constructs demonstrated that the C-terminus of TREK-1 is crucial for the response to AA [39]. In addition, replacing the C-terminus of TREK-2 with the C-terminus of TASK-3 abolished the sensitivity to AA. However, replacing the C-terminus of TRAAK with that of TASK-1 or TASK-3 did not affect the response to AA. These results show that the mechanism of activation of TRAAK and TREK by fatty acids may be different [40].

Volatile anesthetics, including diethyl ether, halothane, isoflurane or chloroform, activate TREK-1 and TREK-2 (but not TRAAK) channels which requires the C-terminus of the channels [41] (shown in Table 5). Chloroform specifically and reversibly activates TREK channels [41], whereas halothane or isoflurane activates both, TASK and TREK channels [41] (shown in Fig. 2a and Table 5, 6). On the contrary, diethyl ether increased TREK-1 whereas it decreased TASK-1 currents [41]. In addition, anesthetic gases, as nitrous oxide, xenon and cyclopropane activate TREK-1 channels in clinically relevant concentrations, whereas TASK-3 is insensitive [42] (shown in Table 5). The Glu306 residue, also critical for TREK-1 modulation by AA, stretch or internal pH, was shown to be important for TREK-1 channel activation by these anesthetic gases [42].

In 2000 it was shown by Duprat *et al.* that the neuroprotective agent riluzole activates TREK-1 and TRAAK channels (shown in Fig. 2a and Table 5). As TREK-1 is inhibited by increased cAMP levels via PKA phosphorylation and riluzole has the capacity of increasing cAMP levels, the activation of TREK-1 is only transient. In contrast, TRAAK channels, which lack a PKA inhibition, are permanently activated [43].

TREK-1 channels are discussed as novel drug targets against pain [44, 45]. Devilliers *et al*. demonstrated, that TREK-1 contributes to morphine-induced analgesia in mice. The channel was directly activated (independent of μ opioid receptor activation) leading to analgesia without adverse effects [46].

Another TREK-1 activator with therapeutic potential is BL-1249 [47] (shown in Fig. 2a and Table 5). Using a whole exome sequencing approach in a patient with right ventricular outflow tract tachycardia (RVOT-VT), Decher *et al*. identified the TREK-1^{I267T} mutation, an amino acid exchange located directly before the selectivity filter of the second pore loop [47].

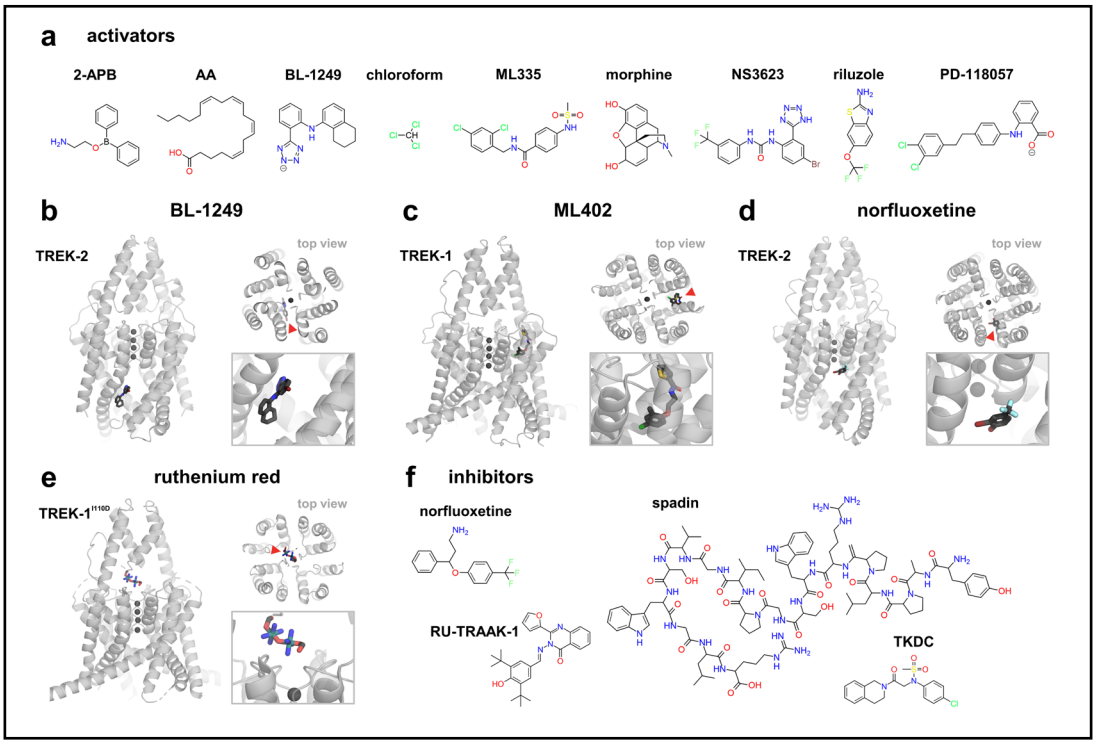

Fig. 2. Drugs that modulate channels of the TREK subfamily of K_{2P} channels and different binding sites identified. (a) Chemical formula of the most important activators for channels of the TREK/(TRAAK) subfamily. (b) Binding site of the 'NCA' BL-1249 mapped in the TREK-2 crystal structure template (PDB ID: 4XDJ) [48]. (c) ML402 bound in the 'cryptic' binding site of TREK-1 determined by a co-crystallization study (PDB ID: 6CQ9) [6]. (d) Binding site of norfluoxetine in TREK-2, see co-crystal structure PDB ID: 4XDL [5]. (e) Binding site of ruthenium red at the 'keystone' binding site, determined by co-crystallization with TREK-1^{I110D} (PDB ID: 6V3C) [58]. (f) Chemical formula of the most important inhibitors for channels of the TREK/TRAAK subfamily. (b-e) Potassium ions are represented by black spheres. Red arrows indicate the positions of the respective drugs in top view.

The mutation almost completely abolished outward currents through the channel and introduced a strong sodium permeability to the potassium channel [47]. Interestingly, application of BL-1249 rescued the potassium selectivity and loss-of-function of the TREK-1^{I267T} channel. The fact that this fenamate-like compound was able to rescue the selectivity filter defect of TREK-1^{I267T} indicated that this drug or maybe other similar activators directly stabilize the selectivity filter. Consequently, Schewe *et al*. identified negatively charged activators (NCAs) harboring a negatively charged tetrazole or carboxylate group (such as BL-1249, PD-118057 and NS11021) as activators of the mechano-gated K_{2P} channels TREK-1 and TREK-2 [48] (shown in Fig. 2a and Table 5). Strikingly, these NCAs activate most of the K_{2P} channels (shown in Table 2, 4, 5) and also activate other selectivity filter gated ion channels like hERG or BK channels with equal efficiency [48]. Thus, NCAs act with a common mechanism on selectivity filter gated channels, providing a universal 'master key' to unlock the selectivity filter gate by binding below the selectivity filter where their negative charge promotes K^+ binding to the pore cavity (shown in Fig. 2b). This in turn alters the ion occupancy in the selectivity filter in a way that is known to promote activation of the filter gate [11, 48].

In contrast, 2-aminoethoxydiphenyl borate (2-APB) is a non NCA that activates channels of the TREK/TRAAK subfamily (shown in Fig. 2a and Table 5). TREK-2 is much more sensitive to modulation by 2-APB compared with TREK-1 or TRAAK [49] (shown in Table 5). 2-APB does not bind to either the binding site of NCAs or the 'cryptic' binding site described

Table 5. List of activators of the TREK/(TRAAK) K_{2P} channel subfamily

channel	substance category	activator	EC_{50} (μM)	no activation	reference	PMID
TREK-1						
	ALS therapeutics	riluzole (early effect)			Duprat et al., 2000	10779373
	caffeic acid esters	CAPE			Danthi et al., 2004	14978238
	caffeic acid esters	CDC			Danthi et al., 2004	14978238
	ethacrynic acid derivates	DCPIB			Minieri et al., 2013	23072356
	fenamate derivates	BL-1249	≈7	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	fenamates	diclofenac			Veale et al., 2014	24509840
	fenamates	flufenamic acid	≈100		Takahira et al., 2005	16075240
	fenamates	mefenamic acid	≈100		Takahira et al., 2005	16075240
	fenamates	niflumic acid	≈100		Takahira et al., 2005	16075240
	furyl derivates	compound 36			Vivier et al., 2017	28051863
	gaseous anesthetics	cyclopropane		TASK-3	Gruss et al., 2004	14742687
	gaseous anesthetics	nitrous oxide		TASK-3	Gruss et al., 2004	14742687
	general anesthetics	trichloroethanol			Harinath and Sikdar, 2004	14996553
	hydroxycoumarin derivates	ostruthin	≈5	TASK-2, THIK-1	Joseph et al., 2018	30110354
	lipids	arachidonic acid			Patel et al., 1998	9687497
	lipids	lysophospholipids			Chemin et al., 2005	15572365
	LTRA antagonists	pranlukast	≈6	TASK-2, THIK-1, TRAAK	Wright et al., 2019	31564414
	opiates	morphine			Devilliers et al., 2013	24346231
	plant extracts	aristoholic acid		TRESK	Veale et al., 2016	26914156
	small molecules	2-APB	≈500	TASK-1, TASK-3, TRESK	Beltrán et al., 2013	23720627
	small molecules	C3001a	≈13	TASK-1, TASK-3, THIK-1, TRESK	Qiu et al., 2020	32162512
	small molecules	GI-530159	≈1	TRAAK	Loucif et al., 2018	29150838
	small molecules	GoSlo-SR-5-6			Schewe et al., 2019	30792303
	small molecules	LA-TREK2-1		TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	ML335	≈14	TRAAK	Lolicato et al., 2012	28693035
	small molecules	ML402	≈14	TRAAK	Lolicato et al., 2012	28693035
	small molecules	ML67-33	≈36		Bagriantsev et al., 2013	23738709
	small molecules	NS11021			Schewe et al., 2019	30792303
	small molecules	PD-118057			Schewe et al., 2019	30792303
	small molecules	PD-307243			Schewe et al., 2019	30792303
	small molecules	SR 2640		TASK-2, TASK-3, THIK-1, TREK-2,TRESK	Wright et al., 2019	31564414
	volatile anesthetics	chloroform		TASK-1, TRAAK	Patel et al., 1999	10321245
	volatile anesthetics	diethyl ether		TASK-1, TRAAK	Patel et al., 1999	10321245
	volatile anesthetics	halothane		TRAAK	Patel et al., 1999	10321245
	volatile anesthetics	isoflurane		TRAAK	Patel et al., 1999	10321245
TREK-2						
	ethacrynic acid derivates	DCPIB			Schewe et al., 2019	30792303
	fenamate derivates	BL-1249	≈6	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	fenamates	flufenamic acid			Takahira et al., 2005	16075240
	fenamates	mefenamic acid			Takahira et al., 2005	16075240
	fenamates	niflumic acid			Takahira et al., 2005	16075240
	hydroxycoumarin derivates	ostruthin	≈4000	TASK-2, THIK-1	Joseph et al., 2018	30110354
	lipids	arachidonic acid			Kim et al., 2001	11680629
	lipids	lysophospholipids			Chemin et al., 2005	15572365
	LTRA antagonists	pranlukast	≈6	TASK-2, THIK-1, TRAAK	Wright et al., 2019	31564414
	LTRA antagonists	zafirlukast		TREK-1	Wright et al., 2019	31564414
	plant extracts	aristoholic acid		TRESK	Veale et al., 2016	26914156
	plant extracts	baicalein			Kim et al., 2011	21306568
	plant extracts	wogonin			Kim et al., 2011	21306568
	prostaglandins	11-Deoxy PGF2a	≈5	TASK-2, TASK-3, THIK-1, TREK-1, TRESK	Wright et al., 2019	31564414
	small molecules	2-APB	≈1200	TASK-1, TASK-3, TRESK	Beltrán et al., 2013	23720627
	small molecules	C3001a	≈11	TASK-1, TASK-3, THIK-1, TRESK	Qiu et al., 2020	32162512
	small molecules	GI-530159	≈6	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	GoSlo-SR-5-6			Schewe et al., 2019	30792303
	small molecules	LA-TREK2-1		TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	LA-TREK2-2		TREK-1, TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	small molecules	ML67-33	≈30	TASK-1, TASK-2, TASK-3, TRESK	Bagriantsev et al., 2013	23738709
	small molecules	NS11021			Schewe et al., 2019	30792303
	small molecules	PD-118057			Schewe et al., 2019	30792303
	small molecules	T2A3		TREK-1	Dadi et al., 2017	27805811
	volatile anesthetics	chloroform			Lesage et al., 2000	10880510
	volatile anesthetics	halothane			Lesage et al., 2000	10880510
	volatile anesthetics	isoflurane			Lesage et al., 2000	10880510
TRAAK						
	ALS therapeutics	riluzole			Fink et al., 1998	9628867
	fenamate derivates	BL-1249	≈6	TASK-2, TASK-3, THIK-1	Wright et al., 2019	31564414
	fenamates	flufenamic acid			Takahira et al., 2005	16075240
	fenamates	mefenamic acid			Takahira et al., 2005	16075240
	fenamates	niflumic acid			Takahira et al., 2005	16075240
	general anesthetics	trichloroethanol			Harinath and Sikdar, 2004	14996553
	lipids	arachidonic acid			Fink et al., 1998	9628867
	lipids	lysophospholipids			Chemin et al., 2005	15572365
	small molecules	2-APB		TASK-1, TASK-3, TRESK	Beltrán et al., 2013	23720627
	small molecules	C3001a	≈15	TASK-1, TASK-3, THIK-1, TRESK	Qiu et al., 2020	32162512
	small molecules	ML67-33	≈27		Bagriantsev et al., 2013	23738709

Table 6. List of activators of the TASK K_{2P} channel subfamily

channel	substance category	activator	EC_{50} (μM)	no activation	reference	PMID
TASK-1						
	guanylate cyclase activators	riociguat			Cunningham et al., 2019	30365877
	phospholipase inhibitors	ONO-RS-082			Ma et al., 2013	23883380
	prostacyclin analogs	treprostinil			Olschewski et al., 2006	16574908
	volatile anesthetics	halothane		TRAAK	Patel et al., 1999	10321245
	volatile anesthetics	isoflurane		TRAAK	Patel et al., 1999	10321245
	volatile anesthetics	sevoflurane			Putzke et al., 2007	17699638
TASK-3						
	antifungals	terbinafine		TASK-2, THIK-1, TREK-2, TRESK, TWIK-1	Wright et al., 2017	28882594
	biguanide derivates	CHET3	≈1.4	THIK-1, TREK subfamily, TRESK	Liao et al., 2019	31748231
	fenamates	flufenamic acid			Veale et al., 2014	24342771
	gaseous anesthetics	cyclopropane			Gruss et al., 2004	14742687
	gaseous anesthetics	nitrous oxide			Gruss et al., 2004	14742687
	gaseous anesthetics	xenon			Gruss et al., 2004	14742687
	LTRA antagonists	cinalukast		TASK-2, THIK-1, TREK-1, TREK-2, TRESK	Wright et al., 2019	31564414
	LTRA antagonists	pranlukast		TASK-2, THIK-1	Wright et al., 2019	31564414
	LTRA antagonists	zafirlukast	≈6	TREK-1	Wright et al., 2019	31564414
	small molecules	NPBA	≈7	TASK-1, THIK-1, TREK-1, TRESK	Tian et al., 2019	31015283
	volatile anesthetics	halothane			Gruss et al., 2004	14742687
	volatile anesthetics	isoflurane			Berg et al., 2004	15282272
TASK-5						
		n.a.				

below. Zhuo *et al.* described that for TREK-2 channels the cytosolic C-terminus plays a role in controlling the stimulatory effects of the compound. In particular the proximal C-terminus, including His368 as a key residue, was required for channel activation by 2-APB [50]. In addition, specific mutations in the M4 segment reduced the 2-APB efficiency and thus the authors proposed an allosteric coupling between the proximal C-terminus and the selectivity filter induced by 2-APB [51]. This allosteric coupling is facilitated by the movement of M4 and thus mutations that reduce the flexibility of the M4 movements impair 2-APB activation [51].

Very recently a novel class of small molecule activators has been identified that utilizes a completely different, the 'cryptic', binding site, which is clearly distinct to that of NCAs [6] (shown in Fig. 2c). ML335 and ML402 bind to an L-shaped pocket behind the selectivity filter formed by the P1 pore helix and M4 transmembrane helix intrasubunit interface. The drugs activate the channels by acting as 'molecular wedges', restricting the interdomain interface movement behind the selectivity filter [6]. Mechanistically, binding to the 'cryptic' binding site stabilizes the C-type gate in a more conductive 'leak mode' through a common set of hydrogen bonds, π-π, and cation-π interactions of ML335 and ML402 with the 'P1 face' and an 'M4 face' reducing P1/TM4 interface dynamics [6].

A high-throughput fluorescence-based thallium flux screen identified small molecules that selectively activated TREK-2 [52]. These novel compounds were subsequently proven to directly activate TREK-2 channels, using single channel measurements in excised membrane patches [52]. Strikingly, 11-deoxy PGF2α or T2A3 which were described in this study, activated TREK-2 while they blocked TREK-1 channels [52] (shown in Table 5, 7). For these compounds a region connecting the second pore loop to the M4 segment was proposed to determine the observed activation or inhibition [52].

TREK/TRAAK inhibitors

In terms of blockers for channels of the TREK/TRAAK subfamily, spadin is presumably the best known blocker of TREK channels described (shown in Fig. 2f). Spadin is a 17 amino acid sortilin-derived peptide targeting TREK-1 channels with an IC_{50} of 70 nM [53] (shown in Fig. 2f and Table 7). It is discussed as a putative antidepressant [53, 54], although the binding site and mechanism of inhibition are not known so far. However, it has been postulated that spadin should bind to the 'down state' of the channel to specifically antagonize activation of TREK-1 by AA, utilizing an allosteric mechanism of inhibition [55].

Dong *et al.* described the crystal structure of TREK-2 in complex with norfluoxetine [5] (shown in Fig. 2d, 2f). Here several residues in the side fenestrations, including Ile194 and Pro198 of the M2 segment, Cys249 and Val253 in M3, Phe316 and Leu320 in M4, as well

Table 7. List of inhibitors of the TREK/(TRAAK) K_{2P} channel subfamily

channel	substance category	inhibitor	IC_{50} (μM)	no inhibition	reference	PMID
TREK-1						
	ALS therapeutics	riluzole (late effect)		TRAAK	Duprat et al., 2000	10779373
	antipsychotics	chlorpromazine	≈3	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	flupentixol	≈2	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	fluphenazine	≈5	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	haloperidol	≈6	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	loxapine	≈20	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	pimozide	≈2	TRAAK	Thümmler et al., 2007	17222806
	antipsychotics	fluoxetine	≈19		Kennard et al., 2005	15685212
	antipsychotics	norfluoxetine	≈9		Kennard et al., 2005	15685212
	beta blockers	carvediol	≈20		Kisselbach et al., 2014	25168769
	calcium channel blockers	diltiazem	≈180	TRAAK	Takahira et al., 2005	16075240
	calcium channel blockers	mibefradil			Enyeart et al., 2002	12368289
	calcium channel blockers	penfluridol			Enyeart et al., 2002	12368289
	cannabinoids	anandamide	≈5		Liu et al., 2007	17622574
	class I antiarrhythmics	mexiletine	≈170		Schmidt et al., 2013	24070813
	class I antiarrhythmics	propafenone	≈8		Schmidt et al., 2013	24070813
	class III antiarrhythmics	dronedarone	≈27		Schmidt et al., 2012	22790794
	class III antiarrhythmics	vernakalant	≈13	TASK-1	Seyler et al., 2014	24374008
	cloxyquin analogs	A2764			Lengyel et al., 2019	30979812
	DHP antagonists	amlodipine	≈0.5		Liu et al., 2007	17622574
	DHP antagonists	nifedipine	≈8		Liu et al., 2007	17622574
	DHP antagonists	niguldipine	≈0.8		Liu et al., 2007	17622574
	diphenlydiperazines	flunarazine	≈3		Liu et al., 2007	17622574
	local anesthetics	bupivacaine	≈100		Shin et al., 2014	23797625
	local anesthetics	levobupivacaine	≈130		Shin et al., 2014	23797625
	local anesthetics	lidocaine	≈180		Nayak et al., 2009	19622790
	local anesthetics	ropivacaine	≈400		Shin et al., 2014	23797625
	LTRA antagonists	zafirlukast			Wright et al., 2019	31564414
	neuroprotective agents	NBP	≈0.1		Ji et al., 2011	21293470
	neuroprotective agents	sipatrigine	≈4		Meadows et al., 2001	11172753
	prostaglandins	11-Deoxy PGF2a		TREK-2	Dadi et al., 2017	27805811
	pyrethroids	tetramethrin			Castellanos et al., 2018	28937579
	pyrimidines	ML45	≈20		Bagriantsev et al., 2013	23738709
	quaternary ammonium ions	TButA	≈2000		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TEA	≈90000		Piechotta et al., 2011	21822218
	quaternary ammonium ions	THexA	≈1		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TPenA	≈11		Piechotta et al., 2011	21822218
	small molecules	A1899	≈24		Streit et al., 2011	21362619
	small molecules	A293	≈10		Putzke et al., 2007	17389142
	small molecules	T2A3		TREK-2	Dadi et al., 2017	27805811
	small molecules	TKDC	≈5		Luo et al., 2017	28851868
	sortilin-derivates	spadin	≈0.1		Mazella et al., 2010	20405001
	TLR7 agonists	imiquimod	≈80		Lee et al., 2012	22233604
TREK-2						
	antipsychotics	norfluoxetine			Dong et al., 2015	25766236
	beta blockers	carvediol	≈25		Kisselbach et al., 2014	25168769
	calcium channel blockers	diltiazem	≈330	TRAAK	Takahira et al., 2005	16075240
	calcium channel blockers	methoxyverapamil			Park et al., 2018	29973548
	calcium channel blockers	verapamil		THIK-1	Park et al., 2018	29973548
	cationic dyes	ruthenium Red	≈0.2	TREK-1	Braun et al., 2015	25409575
	class III antiarrhythmics	vernakalant			Seyler et al., 2014	25108155
	pyrethroids	tetramethrin			Castallenos et al., 2018	28937579
	small molecules	A1899	≈8		Streit et al., 2011	21362619
	small molecules	TKDC	≈5		Luo et al., 2017	28851868
TRAAK						
	cationic dyes	ruthenium Red		TREK-1	Braun et al., 2015	25409575
	cationic dyes	ruthenium Violet	≈0.1		Braun et al., 2015	25409575
	class III antiarrhythmics	vernakalant			Seyler et al., 2014	25108155
	neuroprotective agents	sipatrigine			Meadows et al., 2001	11172753
	pyrethroids	tetramethrin			Castallenos et al., 2018	28937579
	small molecules	A1899			Streit et al., 2011	21362619
	small molecules	RU-TRAAK-1	≈0.4	TALK-1, THIK-1	Su et al., 2016	27091997
	small molecules	RU-TRAAK-2	≈0.7		Su et al., 2016	27091997
	small molecules	TKDC	≈66		Luo et al., 2017	28851868
	TLR7 agonists	imiquimod	≈180		Lee et al., 2012	22233604

as Val276, Leu279 and Thr280 of the second pore helix, close to the selectivity filter, were proposed to interact with the compound [5]. Norfluoxetine binds to the channel in the 'down state', presumably impairing the transition to the 'up state' from which the channel opening preferentially occurs [56]. Surprisingly, although norfluoxetine appears to preferentially bind to the 'down state' [5] this state dependence is not reflected by a voltage-dependent inhibition of TREK channels [57].

RR inhibits a number of ion channels including members of the K_{2P} channel family [28, 29] (shown in Table 7, 8) with E70 in TASK-3 [29] and D135 in TREK-2 [28] as key residue for RR action. In contrast, TREK-1 is not sensitive to RR [28]. Using X-ray crystal structures of a RR sensitive TREK-1 mutant (TREK-1^{I110D}) alone or complexed with RR revealed that

a negatively charged residue at this specific site provides the key inhibitor site in the extracellular ion pathway ('EIP') above the selectivity filter which is formed by the 'cap' structure (shown in Fig. 2e). Binding of RR to this site occludes the 'EIP' and thus the current flux [58].

The methanesulfonamide TKDC was described as another small molecule inhibitor of the TREK/TRAAK subfamily (shown in Fig. 2f and Table 7). However, TKDC blocks with a novel and unusual allosteric mechanism. Luo *et al.* identified an allosteric ligand-binding site located in the extracellular 'cap' of the channels. From this site the ligands are supposed to induce an allosteric conformational transition which ultimately leads to an obstruction of the 'EIP' [59].

Pharmacology of TASK channel subfamily members

TASK-1 and TASK-3 blockers

TASK-1 transcripts and currents are upregulated under atrial fibrillation (AF) [60, 61], variants of *KCNK3*, encoding TASK-1, are associated with AF [62] and genetic ablation of *KCNK3* by a dominant negative viral approach suppresses AF in a pacemaker-induced AF model of the pig [63]. Furthermore, both, TASK-1 and TASK-3, are expressed in the carotid body and brain stem regions associated with respiratory control, and mice lacking these channels have impaired carotid body function [64]. Thus, TASK channel inhibition is for instance a promising therapeutic approach for the treatment of AF (DOCTOS Trial) or breathing disorders like obstructive sleep apnea (OSA) (SANDMAN Trial) [65-67].

In 2007 Putzke *et al*. described A293 (shown in Fig. 3a), the first potent K_{2P} channel blocker [32] with an IC_{50} of 222 nM on TASK-1 expressed in *Xenopus* oocytes (shown in Table 8), enabling the isolation of the first native whole cell current of a K_{2P} channel, the I_{TASK-1} in rat, mouse and human cardiomyocytes [32, 68, 69]. A few years later we described the first highly potent and selective TASK-1 channel blocker being active in the one digit nanomolar range. The IC_{50} of A1899 on TASK-1 expressed in CHO cell was 7 nM and in oocytes 35.1 nM [70] (shown in Fig. 3a and Table 8). A1899 was in the submicromolar range not active on a plethora of ion channels tested [70] and thus A1899 is an even more promising tool than A293 in terms of specificity. Subsequently, using this compound we described the first drug binding site of a K_{2P} channel which helped understanding the pore structure of these channels, as these were not crystallized at this time [70]. Using an alanine scanning mutagenesis approach we found that the M2 and M4 segments form the inner pore of K_{2P} channels and which residues actually face into the central cavity [70]. The A1899 binding site is formed by Thr93 of the first pore loop, Ile118 and Leu122 of the M2 segment, Thr199 of the second pore loop, Ile235, Gly236, Leu239 and Asn240 of the M4 segment and Val243 and Met247 of the halothane response element ('HRE') [70]. Noteworthy, the IC_{50} of A1899 for TASK-3 is 10-fold higher than that of TASK-1 [70] (shown in Table 8). The binding site of A1899 in TASK-1 is fully conserved in the TASK-1/3/5 subfamily except for one residue. This amino acid variation is located in the 'HRE' of TASK-3, which is ^{243}VLRF*M*T^{248} for TASK-1 and ^{243}VLRF*L*T^{248} for TASK-3. This sequence variation might contribute to the different drug affinities of TASK-1 and TASK-3, since the TASK-1^{M247L} mutation causes a 3.3-fold increase in IC_{50} for A1899 [70].

A few years later we found that blockers of the Kv1.5 channel which were developed as antiarrhythmic compounds to treat or prevent AF, are much more potent inhibitors of TASK-1 than Kv1.5 [65]. Note that A1899 was initially developed by Sanofi as a blocker of Kv1.5 (S0200951) and A293 was initially described as the Kv1.5 blocker AVE1231 which was under clinical investigation against AF [65]. However, both compounds were about 70-fold more potent on TASK-1, making them TASK selective when low doses of the compounds are applied [65]. These data suggest that the real channel, effectively targeted against AF by Kv1.5 blockers was TASK-1 and not Kv1.5, further supporting the notion that TASK-1 might be a promising drug target against AF and OSA [65]. However, it also raised the question

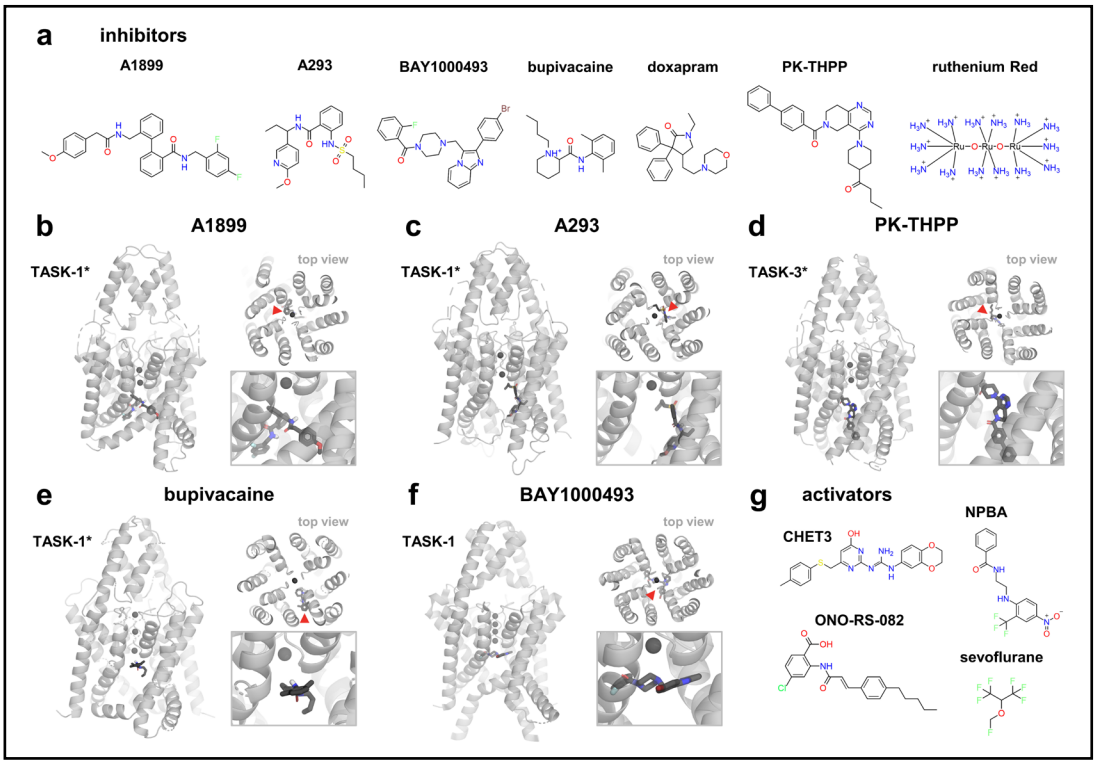

Fig. 3. Drugs that modulate channels of the TASK subfamily of K_{2P} channels and different binding sites identified. (a) Chemical formula of the most important inhibitors of channels of the TASK subfamily. (b) Binding site of A1899 in a TASK-1 homology model [70, 71]. (c) Binding site of A293 in a TASK-1 homology model [72]. (d) Binding site of PK-THPP in a TASK-3 homology model [74]. (e) Illustration of the allosteric bupivacaine binding site in the side fenestration of a TASK-1 homology model [79]. (f) Binding site of BAY1000493 in TASK-1 determined by co-crystallization (PDB ID: 6RV3) [7]. (g) Chemical formula of the most important activators of channels of the TASK subfamily. (b-f) Potassium ions are represented by black spheres. Red arrows indicate the positions of the respective drugs in top view. *: homology model.

how different compounds can efficiently block both, TASK-1 and Kv1.5 channels, albeit they belong to only very remotely related families of potassium channel. Surprisingly, *in silico* analyses comparing the binding sites in TASK-1 and Kv1.5 revealed important similarities. 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 [65]. We proposed that the accessibility of the drug to the pore and the more lipophilic environment of TASK-1 might be the reason why most of the Kv1.5 blockers are even more potent on TASK-1 [65].

The binding mode of A1899 to the TASK-1 channel pore was initially modelled on a KvAP-based homology model that has a fourfold symmetric pore [70]. However, since K_{2P} channels do not have such a fourfold symmetry in the central cavity, Ramirez *et al.* re-evaluated the A1899 binding site in TASK-1 using a pore homology model of TASK-1 based on TWIK-1 (shown in Fig 3b). TWIK-1 was, together with TRAAK, one of the first K_{2P} channels crystallized and TWIK-1 is more closely related to TASK-1 than TRAAK. The TWIK-1 based homology model, combined with docking experiments and MD simulations revealed that A1899 binds to residues located in the side fenestrations providing a physical 'anchor', reflecting an energetically favorable binding mode that after pore occlusion stabilizes the closed state of the channel [71] (shown in Fig. 3b).

Table 8. List of inhibitors of the TASK K_{2P} channel subfamily

channel	substance category	inhibitor	IC_{50} (μM)	no inhibition	reference	PMID
TASK-1						
	alpha&beta blockers	carvedilol	≈4		Staudacher et al., 2011	21410455
	anticonvulsants	phenytoin			Leonoudakis et al., 1998	9437008
	antipsychotics	fluoxetine	≈226		Hajdú et al., 2003	12667945
	cannabinoids	anandamide		TASK-2, TASK-3, TWIK subfamily, TREK subfamily	Maingret et al., 2001	11226154
	cannabinoids	methandamide	≈0.7		Maingret et al., 2001	11226154
	cardiac glycosides	digitoxin	≈8	TASK-2, TASK-3, TASK-4, THIK-1, TREK subfamily, TRESK	Schmidt et al., 2018	29643254
	cardiac glycosides	digoxin	≈8	TASK-2, TASK-3, TASK-4, THIK-1, TREK subfamily, TRESK	Schmidt et al., 2018	29643254
	class I antiarrhythmics	mexiletine	≈97		Schmidt et al., 2013	24070813
	class I antiarrhythmics	propafenone	≈5		Schmidt et al., 2013	24070813
	class I antiarrhythmics	quinidine			Leonoudakis et al., 1998	9437008
	class III antiarrhythmics	amiodarone	≈0.4		Gierten et al., 2010	19777211
	class III antiarrhythmics	dronedarone	≈19	TALK-1, TRAAK	Schmidt et al., 2012	22790794
	cloxyquin analogs	A2764			Lengyel et al., 2019	30979812
	general anesthetics	etomidate	≈120		Putzke et al., 2007	17699638
	local anesthetics	bupivacaine	≈70		Leonoudakis et al., 1998	9437008
	local anesthetics	etidocaine	≈40		Kindler et al., 1999	10201682
	local anesthetics	lidocaine			Leonoudakis et al., 1998	9437008
	local anesthetics	mepivacaine	≈700		Kindler et al., 1999	10201682
	local anesthetics	ropivacaine	≈50		Kindler et al., 1999	10201682
	local anesthetics	tetracaine	≈670		Kindler et al., 1999	10201682
	opioids	D-norpropoxyphene	≈170		Hajdú et al., 2003	12667945
	plant extracts	6-Gingerol	≈230	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	capsaicin	≈60	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	piperine	≈45	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	sanshool	≈30		Bautista et al., 2008	18568022
	quaternary ammonium ions	TEA			Leonoudakis et al., 1998	9437008
	respiratory stimulants	doxapram	≈0.4		Cotton et al., 2006	16492828
	small molecules	A1899	≈0.04		Streit et al., 2011	21362619
	small molecules	A293	≈0.2		Putzke et al., 2007	17389142
	small molecules	BAY1000493	≈0.001		Rödström et al., 2020	32499642
	small molecules	BAY2341237	≈0.001		Rödström et al., 2020	32499642
	small molecules	DR16.1	≈21		Ramírez et al., 2019	31426491
	small molecules	ML365	≈0.02		Flaherty et al., 2014	25017033
	small molecules	NPBA	≈8	TASK-3	Tian et al., 2019	31015283
	small molecules	PK-THPP	0.3		Coburn et al., 2012	21916012
	TK inhibitors	genistein	≈10		Gierten et al., 2008	18516069
TASK-3						
	antibiotics	mevastatin	≈160		Bruner et al., 2014	24972239
	antibiotics	oligomycin A	≈48		Bruner et al., 2014	24972239
	calcium channel blockers	mibefradil			Bruner et al., 2014	24972239
	cannabinoids	anandamide			Berg et al., 2004	15282272
	cannabinoids	methandamide			Berg et al., 2004	15282272
	cationic dyes	ruthenium Red	≈0.7	TASK-1	Czirják and Enyedi, 2003	12606773
	class I antiarrhythmics	quinidine			Kim et al., 2000	10734076
	D2 antagonists	octoclothepin	≈74		Bruner et al., 2014	24972239
	H1 antagonists	loratadine	≈64		Bruner et al., 2014	24972239
	general anesthetics	etomidate	≈130		Putzke et al., 2007	17699638
	lipids	arachidonic acid			Kim et al., 2000	10734076
	nAChR antagonists	DHBetaE	≈74		Bruner et al., 2014	24972239
	NK1 antagonists	L-703,606	≈46		Bruner et al., 2014	24972239
	plant extracts	6-Gingerol	≈385	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	capsaicin	≈42	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	piperine	≈135	TREK subfamily	Beltrán et al., 2013	24302912
	plant extracts	sanshool	≈450		Bautista et al., 2008	18568022
	quaternary ammonium ions	TButA	≈200		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TEA	≈3000		Piechotta et al., 2011	21822218
	quaternary ammonium ions	THepA	≈0.4		Piechotta et al., 2011	21822218
	quaternary ammonium ions	THexA	≈0.3		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TOctA	≈3		Piechotta et al., 2011	21822218
	quaternary ammonium ions	TPenA	≈9		Piechotta et al., 2011	21822218
	respiratory stimulants	doxapram	≈0.04		Cotton et al., 2006	16492828
	small molecules	A1899	≈0.3		Streit et al., 2011	21362619
	small molecules	A293	≈1		Putzke et al., 2007	17389142
	small molecules	DR16	≈60		Ramírez et al., 2019	31426491
	small molecules	DR16.1	≈14		Ramírez et al., 2019	31426491
	small molecules	GW2974	≈50		Bruner et al., 2014	24972239
	small molecules	ML365	≈1		Flaherty et al., 2014	25017033
	small molecules	PK-THPP	≈0.04		Coburn et al., 2012	21916012
	small molecules	RU-TRAAK-1			Su et al., 2016	27091997
	small molecules	RU-TRAAK-2			Su et al., 2016	27091997
	TK inhibitors	genistein			Gierten et al., 2008	18516069
TASK-5		n.a.				

Subsequently we reported the binding site of the antiarrhythmic compounds A293 in TASK-1 which partially overlaps with the A1899 binding site [72] (shown in Fig. 3c). Although, the A293 binding site has not been studied as detailed as that of A1899, it appears that A293 binds at a lower position within the central cavity, nearby the opening of the lateral fenestrations, however without parts of the drugs extending downwards to the halothane response element ('T' shaped binding mode of A1899) (compare Fig. 3b versus Fig. 3c).

A1899 and PK-THPP are effective breathing stimulants in rats and thus both compounds may have therapeutic potential for treating breathing disorders [67]. PK-THPP which more potently blocks TASK-3 than TASK-1 channels (shown in Fig. 3a and Table 8), also shared

several residues of the A1899 binding site in the central cavity [73-76] (shown in Fig. 3d). While L122, L239, G236 and L247 (M247 in TASK-1) were identified to be part of both binding sites, there were also some novel residues identified as relevant for PK-THPP inhibition (Q126, G231, A237, V242, L244 and T248) [73, 74]. On the other hand, other residues were found to be important exclusively for A1899 binding (I118, I235, N240 and V243) [70]. The binding of PK-THPP to TASK-3 depends on the state of the fenestration, as PK-THPP exclusively binds to the open state [74]. Whether differences in the state dependence and affinity towards TASK-1 and TASK-3 (shown in Table 8) depends on the different binding mode of PK-THPP and A1899, especially the different set of residues identified in the late M4 segment and halothane response element, remains an open question.

Following a high throughput fluorescent screen and structure activity relationship analysis of active compounds, ML365, a bisamide, was identified as another promising TASK channel blocker [76] (shown in Table 8). ML365 has an IC_{50} of 4 nM in a thallium flux fluorescent assay and an IC_{50} of 16 nM in an automated electrophysiology assay [76]. The small molecule inhibitor displayed little or no effect on more distantly related potassium channels like Kir2.1, KCNQ2 or hERG after application of 30 μM of the compound [76]. ML365 showed a 62-fold more potent IC_{50} for TASK-1, than for the closely related TASK-3 channel, thus displaying the strongest 'split' in pharmacology between TASK-1 and TASK-3 channels that was described so far [76] (shown in Table 8). However, the molecular explanation for this phenomenon was not addressed so far.

Interestingly, channel inhibition by low-affinity antiarrhythmic compounds, such as carvedilol, propafenone and amiodarone was also affected by mutations of the A1899 and A293 binding site [70, 72]. Also, the respiratory stimulant doxapram is a blocker of TASK channels [77, 78] (shown in Fig. 3a and Table 8) that acts at this common intracellular binding site, located in the inner vestibule of TASK-1 [76] and TASK-3 [73]. Hence, there is an overlap for residues in the TM2 and TM4 regions for different compounds arguing for a conserved common site of action, however with substance specific variations in the binding mode that appear to modulate affinity and/or specificity.

Rinné *et al*. described a novel binding site for the local anesthetic bupivacaine, differing from those described above which results in an allosteric and voltage-dependent inhibition of TASK-1 and TASK-3 channels [79] (shown in Fig. 3a, 3e). A large alanine scanning mutagenesis approach identified residues that include I118 in M2 and I235, G236, L239 and N240 in M4, which were also part of the A1899 binding site, however there were several novel 'hits' in the M2 segment (C110, M111, A114, Q126, S127) as well as in the M4 segment (V234A and F238A). Bupivacaine was located laterally underneath the pore helices, in the side fenestrations, previously described for other K_{2P} channels (shown in Fig. 3e, top view). Thus, bupivacaine was proposed to act by an allosteric mechanism disrupting the voltage-dependent K^+-flux gating [11] at the selectivity filter [79].

Recently, the TASK-1 channel crystal structure was reported revealing an unexpected second gate located at the entrance to the inner vestibule which was not observed in the structures of other K_{2P} channels crystallized so far [7] (shown in Fig. 3f). This observation was very unexpected as K_{2P} channels were thought to be exclusively gated at the selectivity filter. This lower gate was created by interaction of two M4 helices for which the C-terminal ends crossed underneath the central cavity, prompting us to term it 'X-gate' [7]. Strikingly, the 'X-gate' is actually formed by amino acid residues of the 'HRE' motif, V243 to T248, which was previously described to be essential for the regulation of the channel by G_q pathways, volatile anesthetics and drugs [70, 80]. Two bends can be observed in the M4 segment, one before the 'X-gate' which is supposedly a gating 'hinge' relevant for the positioning of the extended alpha helix that actually forms the 'X-gate' and a second bend observed following the 'X-gate' at residue Asn250, allowing the distal end of M4 to adopt an α-helical structure which forms extensive interactions with the early M1 and late M2 segment [7]. This region which stabilizes the 'X-gate' and thus the closed state of the channel was named 'latch'. In the progress of this study our co-workers at Bayer identified by ultra-high throughput screening (uHTS) novel highly potent TASK blockers, namely BAY1000493 (shown in Fig. 3a, 3f and

Table 8) and BAY2341237 (shown in Table 8), which were subsequently co-crystallized with TASK-1 [7]. Both drugs were bound in a planar orientation directly below the selectivity filter, interacting with several key residues previously described by Streit *et al.* for the binding of A1899 [7, 70]. The planar orientation of the compound is stabilized by an interaction of the drugs with L122 of both subunits. Rödström *et al.* proposed, that the blockers get trapped by the 'X-gate' within the inner vestibule, explaining the slow wash-out rates and almost irreversible inhibition of TASK-1 by these blockers [7]. Strikingly, mutations that are thought to destabilize the closed 'X-gate' did not only cause an increased open probability of the channel, but also impaired the ability of the 'X-gate' to trap BAY1000493 [7]. The higher potency of BAY1000493 in comparison to A1899 (shown in Table 8) might be reflected by the T-shaped binding mode of the A1899 (shown in Fig. 3b) for which parts of the compound extend all the way down to span the narrowest restriction point of the 'X-gate' (at residue L244) [7] and to interact with M247 of the 'HRE' motif [70, 71]. This binding mode might prevent an efficient trapping of A1899. Compound trapping in the central cavity combined with a complementarity between the shapes of the inhibitor and the upper vestibule appear to be important for high-affinity drug binding in TASK-1 channels.

TASK-1 and TASK-3 activators

In contrast to blockers, TASK channels activators would be of therapeutic interest against pulmonary arterial hypertension (PAH), Birk-Barel mental retardation syndrome and some manifestations of pain [81-83]. However, only a few TASK-1 or TASK-3 channel activators are described so far (shown in Fig. 3g and Table 6). For TASK channels molecular modeling studies suggested an anesthetic binding pocket [84], including the HRE [41, 80] in the late M4 and M159 [85] in the late M3 segment [84]. Halogenated ether, alcohol, and alkane anesthetics were reported to be positioned near the side fenestrations of TASK-3 channels, in proximity to L239. However, mutating the pore facing L122 residue completely eliminated the activation by isoflurane [86]. The authors suggest that these effects are caused by altered channel gating by the L122 mutant that is in close proximity to L239 and the side fenestrations. This hypothesis is supported by mutations at an equivalent residue in TWIK-1 (Leu146) that activate TWIK-1 and by molecular dynamic studies which suggest that this region is important for pore hydration and/or the lipid access into the pore [87]. Alternatively, it could be also possible that volatile anesthetics are also bound to residues of the central cavity and act from the pore on the selectivity filter, similar as described for the 'master key' mechanism [48].

However, there are also examples of synthetic small molecule activators already: The guanylate cyclase stimulator riociguat, licensed for the treatment of PAH, enhances TASK-1 currents [82]. Albeit, this activation occurs after incubation of transiently transfected tsA201 cells with the compound and a direct channel modulation has not been demonstrated yet [82]. In addition, TASK-3 for instance is activated by flufenamic acid [83], terbinafine [88], CHET3 [81] or NPBA [89] (shown in Fig. 3g and Table 6). Interestingly, the TASK-3^{G236R} mutation which causes Birk-Barel mental retardation conducts only very little currents [90]. This electrophysiological phenotype could be partially rescued by the TASK activators flufenamic acid [83] or terbinafine [88]. Moreover, Garcia *et al.* demonstrated that intrathecal pre-treatment with terbinafine, reduced the formalin-induced flinching and allodynia/hyperalgesia in rat, further supporting the putative future clinical relevance of TASK activators [91].

In terms of the binding sites, amino acid residues in the early M2 and late M3 segment, which are not conserved in TASK-1, were important for TASK-3 channel activation by NPBA [89]. In contrast for CHET3 a binding site in TASK-3 was found underneath the selectivity filter close to the M2 and M4 segments altering channel gating by affecting the selectivity filter conformation [81].

Outlook - K_{2P} channel modulators in human diseases

As briefly discussed above, K_{2P} channel modulators carry a huge therapeutic potential, in many diseases, as became evident from their involvement in inherited 'channelopathies' [47, 90, 92-94] and by the fact that ion channels are known to be very good 'druggable'. However, despite the multitude of K_{2P} channel modulators known by now, we clearly require more K_{2P} modulating compounds, not only to further increase potency and selectivity to avoid toxicity and/or specific side effects, but also to have compounds with the right pharmacokinetics of Liberation, Absorption, Distribution, Metabolism and Excretion (LADME). Thus, given the development of further compounds from distinct chemical structural classes, presumably primarily possible by pharmaceutical industry, one can think of many future applications of K_{2P} channel modulators in human diseases. TASK-1 activators might rescue PAH and be even beneficial in those cases of PAH in which the disease was not caused by a *KCNK3* loss-of-function mutation as described by Ma *et al.* [94, 95]. TASK-3 activators might be able to rescue some aspects of the Birk-Barel mental retardation [90], when applied early in juvenile development. TASK-1 blockers are already under clinical investigations against OSA (DOCTOS Trial) and AF (SANDMAN Trial). Also, as a lesson learned from a *KCNK17* mutation causing progressive cardiac conduction disorder (PCCD) [93], TASK-4 blockers might be advantageous for the treatment of specific forms of PCCD. Furthermore, TRESK modulators might be beneficial in migraine [92, 96] and novel potent TREK-1 modulators could be effective against pain without the classical side effects of opioids [46]. These putative future therapeutic applications are, as we think, a strong motivation to further study the molecular pharmacology of K_{2P} channels.

Conclusion

K_{2P} channels notoriously suffered from a poor pharmacologic profile which was a drawback for studies aiming to address the physiological role of these channels. However, recent advances in the understanding of the molecular pharmacology of K_{2P} channels, provided an understanding about a large variety of complex mechanisms that can cause modulation of these potassium channels. While the gating and molecular pharmacology of TREK, TRESK and TASK channel subfamily members was the subject of many excellent studies, we eagerly anticipate new drugs and more mechanistic insights for the molecular modulation of channels of the TWIK, THIK and TALK subfamilies.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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