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REVIEW

Targeting two-pore domain K^+ channels TREK-1 and TASK-3 for the treatment of depression: a new therapeutic concept

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Depression is a disease that is particularly frequent, affecting up to 20% of the population in Western countries. The origins of this pathology involve multiple genes as well as environmental and developmental factors leading to a disorder that remains difficult to treat. Several therapies for depression have been developed and these mainly target monoamine neurotransmitters. However, these treatments are not only associated with numerous adverse effects, but they are also ineffective for more than one-third of patients. Therefore, the need to develop new concepts to treat depression is crucial. Recently, studies using knockout mouse models have provided evidence for a crucial role of two members of the two-pore domain potassium channel (K_{2P}) family, tandem P-domain weak inward rectifying K^+ (TWIK)-related K^+ channel 1 (TREK-1) and TWIK-related acid-sensitive K^+ channel 3 (TASK-3) in the pathophysiology of depression. It is believed that TREK-1 and TASK-3 antagonists could lead to the development of new antidepressants. Herein, we describe the discovery of spadin, a natural peptide released from the maturation of the neurotensin receptor-3 (also known as sortilin), which specifically blocks the activity of the TREK-1 channel and displays particular antidepressant properties, with a rapid onset of action and the absence of adverse effects. The development of such molecules may open a new era in the field of psychiatry.

Abbreviations

BDNF, brain-derived neurotrophic factor; COP, coatomer protein; FST, forced swimming test; K_{2P}, two-pore domain potassium channel; MDD, major depressive disorder; NSC, neural stem cell; NSF, novelty-suppressed feeding task; NTS_3 receptor, neurotensin receptor 3 (also known as NTR3 and sortilin); REM, rapid eye movement; SSRI, selective 5-HT re-uptake inhibitor; TASK-3, TWIK-related acid-sensitive K^+ channel 3 (also known as $K_{2P}9.1$); THPP, 6,7,8-tetrahydropyridol[4,3-*d*]pyrimidine; TREK-1, TWIK-related K^+ channel 1(also known as $K_{2P}2.1$); TST, tail suspension test

Tables of Links

TARGETS	
GPCRs[a]	**Ion channels**[b]
5-HT_{1A} receptor	GIRK (K_{ir}3.x)
5-HT_2 receptor	TASK-1 (K_{2P}3.1)
5-HT_4 receptor	TASK-3 (K_{2P}9.1)
5-HT_6 receptor	TASK-5 (K_{2P}15.1)
5-HT_7 receptor	TRAAK (K_{2P}4.1)
NTS_3 receptor (sortilin)	TREK-1 (K_{2P}2.1)
Transporters[c]	TREK-2 (K_{2P}10.1)
SERT	**Enzymes**[d]
Other protein targets	Furin
CREB binding protein	MAP kinase
	PI3 kinase

LIGANDS	
2-AG	GABA
4-aminopyridine	Glibenclamide
5-HT	Halothane
Aldosterone	Isoflurane
Anandamide	Methanandamide
Apamin	Muscarine
BDNF	Neurotensin
Charybdotoxin	Paroxetine
Dexamethasone	Riluzole
Endothelin-1	Staurosporine
Fluoxetine	Tetraethylammonium

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d]Alexander *et al.*, 2013a,b,c,d).

Introduction

Depression is a polygenic and highly complex psychiatric disorder that is currently a major burden on society. It is highly heterogeneous in presentation and frequently exhibits high comorbidity with other psychiatric and somatic deficits. Depression is highly prevalent. It is thought to be the fourth most common cause of a worldwide of reduction in disability-adjusted life years and is predicted to become the second by 2020 (Kessler *et al.*, 2005). The economic burden of this disease is estimated to be $53 billion per year in the United States, and more than $100 billion in Europe. The treatments available for depression are suboptimal and still unsatisfactory; less than 50% of depressed patients when treated achieve full remission. The antidepressants (ADs) currently available still have tolerability issues such as insomnia, weight gain, nausea and sexual dysfunction, which commonly lead to treatment discontinuation. All current ADs also have a slow onset of action, which may take several weeks to months to produce a therapeutic response and are only moderately effective, leaving more than one-third of depressed individuals resistant to drug treatments (Wong and Licinio, 2001). In addition, the reasons why some individuals respond to ADs while others do not, are unknown. Understanding the neurobiological basis of depression and improving the therapeutic strategies remains one of the foremost challenges for modern psychiatry (Nestler *et al.*, 2002). The industry has so far struggled to improve on the current therapeutic options and develop new concepts for the treatment of depression.

Recently, preclinical studies using experimental mouse models have provided compelling evidence implicating two members of the two-pore domain potassium channel family (K_{2P}), TWIK-related K^+ channel 1 (TREK-1, also known as K_{2P}2.1) (Heurteaux *et al.*, 2006) and TWIK-related acid-sensitive K^+ channel 3 (TASK-3; also known as K_{2P}9.1) (Gotter *et al.*, 2011; Coburn *et al.*, 2012) in the pathophysiology of depression and in the biology of response to ADs. The K_{2P}, that are made up of four transmembrane segments and two-pore domains in tandem are thought to be the molecular basis of leak or background K^+ currents in excitable cells (Lesage and Lazdunski, 2000) (Figure 1A). These channels are modulated by many endogenous signalling molecules, clinical compounds and environmental factors. This review focuses on the recent evidence indicating TREK-1 and TASK-3 as new targets in the field of depression, and provides an overview of the recent advances in their pharmacological properties, which link these two K_{2P} channels to their role in depression. The therapeutic potential of TREK-1 and TASK-3 modulators is demonstrated by the discovery of spadin, an endogenous sortilin-derived peptide and selective blocker of TREK-1 (Mazella *et al.*, 2010) as well as selective small-molecule TASK-3 channel inhibitors (Coburn *et al.*, 2012).

Molecular organization and regulations of TREK and TASK channels

Since TWIK-1, the first member of the K_{2P} family (KCNK) was cloned 18 years ago (Lesage *et al.*, 1996), 14 others members have been identified. This family is divided into six distinct subfamilies, according to their electrophysiological and/or pharmacological properties and designed by acronyms such as: TWIK, TREK, TASK, TALK (TWIK-related alkaline pH-activated K^+ channels), THIK (tandem-pore domain halothane-inhibited K^+ channels) and TRESK (TWIK-related spinal cord K^+ channels) (Figure 1B). The membrane topology of each K_{2P} subunit consists of two–pore-forming loops, P1 and P2, arranged in tandem with four transmembrane domains (Figure 1A). The proteins mainly form functional

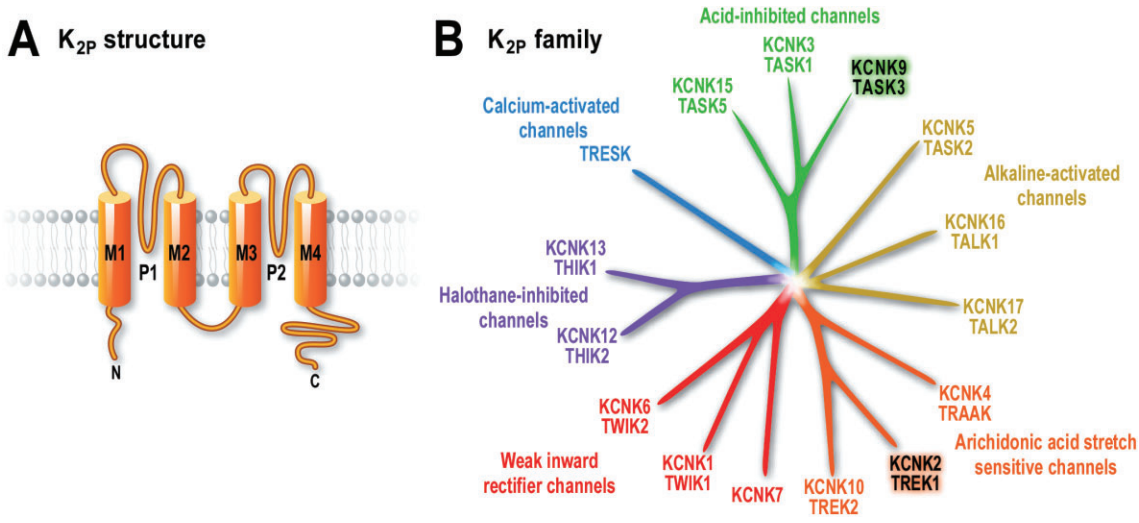

Figure 1

Two-pore domain K^+ (K_{2P}) channels. (A) Structure of K_{2P} channels: these channels are constituted by four transmembrane segments (M1 to M4) and two pore domains (P1 and P2) that constitute the wall of the channel pore. (B) K_{2P} channel family: these channels are classified into six different subfamilies as symbolized by the branch colour code.

homodimers, although heterodimers combining different subunits have been reported in the TASK family (Lesage and Lazdunski, 2000; Enyedi and Czirjak, 2010). The K^+ selectivity, voltage-independent gating and Goldman–Hodgkin–Katz rectification of K_{2P} currents are characteristics that identify them as strong candidates for mediating background K^+ currents. Importantly, the sensitivity of K_{2P} channels to numerous physical and chemical stimuli, including stretch, pH, oxygen, phospholipids, neurotransmitters, volatile anaesthetics and GPCR-mediated pathways, strongly suggests their involvement in the regulation of membrane potential and excitability in various cell types in a range of physiological and pathological situations.

The TASK family, including TASK-1, -3 and -5, share sensitivity to variations in extracellular pH over a narrow physiological range (Duprat *et al.*, 1997) (Figure 1B). TASK-1 (*kcnk3*, K_{2P}3.1) and TASK-3 (*kcnk9*, K_{2P}9.1) subunits are functional when associated as homodimers or heterodimers (Bayliss *et al.*, 2003). TASK-5 (*kcnk15*, K_{2P}15.1) is not functional when expressed in heterologous expression systems (Chemin *et al.*, 2003; Lesage, 2003). TASK-1 and TASK-3 are activated by alkalization and inhibited by acidification as well as by the endocannabinoid, anandamide (Maingret *et al.*, 2001). Each subunit presents, in the whole-cell configuration of patch-clamp currents, voltage-dependent and kinetic properties of instantaneous open rectifiers. The TASK channels have been implicated in the background conductance of neuronal cells, including the oxygen-sensitive background current in carotid body cells (Buckler *et al.*, 2000; Kim, 2005) and in the standing outward current, IK_{SO}, in cerebellar neurons (Aller *et al.*, 2005).

TASK-1 and TASK-3 channels contribute to the muscarine- and halothane-sensitive conductance in thalamocortical relay neurons, thereby being responsible for the change in the activity mode of thalamocortical networks observed during the sleep–wake cycle and on application of inhalational anaesthetics (Meuth *et al.*, 2003) (Figure 2). TASK-1 has been shown to be a molecular substrate for the neuronal effects of inhalation anaesthetics in hypoglossal motoneurons and locus coeruleus neurons (Sirois *et al.*, 2000). TASK-1 channels are also involved in pulmonary arterial hypertension (Olschewski *et al.*, 2006). These channels, that are regulated by the vasoconstrictor peptide, endothelin-1, have a role in maintaining the membrane potential of human

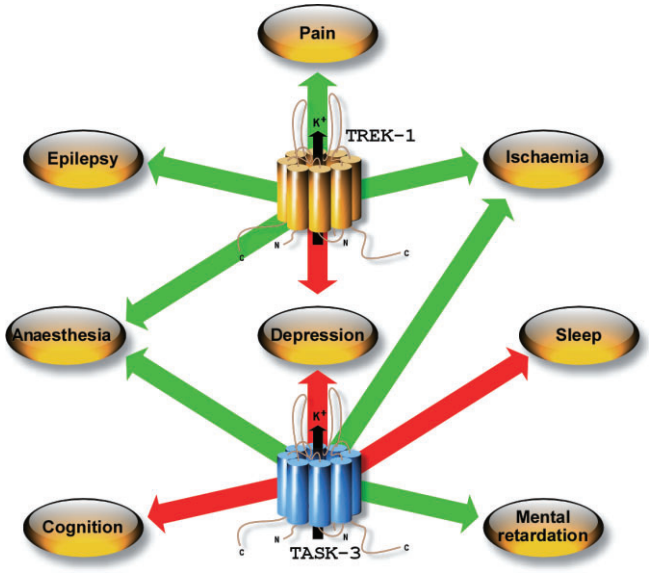

Figure 2

Pathologies where TREK-1 and TASK-3 are involved. Green and red arrows indicate that channels have to be respectively opened or closed for exerting a positive influence on the physiopathological process.

pulmonary artery smooth muscle cells (Tang *et al.*, 2009). TASK-1 and TASK-3 channels have also been implicated in the protection against ischaemia/hypoxia. Both are inhibited by pH reduction and O_2 deprivation in mouse brain slices (Bittner *et al.*, 2010). Pharmacological blockade or genetic deletion of TASK-1 channels leads to an increase in the infarct size compared with control in a model of focal ischaemia, probably through a combination of direct neuronal action and effects on BP/aldosterone (Meuth *et al.*, 2009; Muhammad *et al.*, 2010). Interestingly, genetic and pharmacological inactivation of endogenous TASK channels induce protection against apoptotic K^+-dependent cell death in granule cells in culture (Lauritzen *et al.*, 2003). These results highly suggest an important role for the TASK channel subunits in the control of cell death. Their dysregulation, which may occur in disease states, may have functional implications for both cellular excitability and survival. The expression and activity levels of a number of their chaperone protein partners, such as p11 and 14-3-3, which regulate the trafficking of TASK channels to the plasma membrane can also influence cell survival by modulating TASK-1/TASK-3 expression (Girard *et al.*, 2002; Rajan *et al.*, 2002). Other proteins, notably GPCRs also interact with p11 and are involved in the mechanisms of action of ADs (Svenningsson *et al.*, 2013). Figure 2 summarizes the involvement of TASK-3 in different physiological and pathological situations.

The TREK family, which includes TREK-1 (*kcnk2*, $K_{2P}2.1$), TREK-2 (*kcnk10*, $K_{2P}10.1$) and TWIK-related arachidonic acid K^+ (TRAAK; *kcnk4*, $K_{2P}4.1$) display low basal activity, but are naturally stimulated by membrane stretch, cell swelling, polyunsaturated fatty acids, lysophospholipids, phosphatidinositol 4,5-biphosphate, volatile anaesthetics, temperature and internal acidosis. In contrast, they are inhibited by hypoosmolarity as well as by PKA- and PKC-induced phosphorylation (Lesage, 2003). TREK-like currents are also regulated through GPCR pathways, being inhibited by $G\alpha_q$ and $G\alpha_s$ activation, but activated by $G\alpha_i$ activation (Lesage and Lazdunski, 2000; Chemin *et al.*, 2003). TREK-1 and TREK-2 are opened by clinical concentrations of volatile anaesthetics (Patel *et al.*, 1999) and by riluzole, a neuroprotective drug used in amyotrophic lateral sclerosis (Duprat *et al.*, 2000). TREK and TRAAK channels are predominantly expressed in the CNS. TREK-1 plays an essential role in anaesthesia, neuroprotection, depression and pain (Heurteaux *et al.*, 2004; 2006; Alloui *et al.*, 2006; Noel *et al.*, 2009) (Figure 2). The deletion of *kcnk2* gene leads to an increased vulnerability to epileptic seizures and brain ischaemia (Heurteaux *et al.*, 2004). Neuroprotection induced by polyunsaturated fatty acids or lysophospholipids is lost in mutant mice, which also display a resistance to volatile anaesthetics (Heurteaux *et al.*, 2004). TREK-1 and TRAAK channels are present in sensory neurons, particularly in nociceptors, and are involved in pain perception (Alloui *et al.*, 2006; Noel *et al.*, 2009). TREK-1 may also have a critical role in mediating the vasodilator response of resistance arteries to polyunsaturated fatty acids, thus contributing to their protective effects on the CVS (Blondeau *et al.*, 2007). TREK-1 is expressed in endothelium of mesenteric arteries as well as in cutaneous microvessels, and also plays a major role in endothelial-derived pressure-induced vasorelaxation (Garry *et al.*, 2007). These channels interact with partner proteins, which influence their activity and their plasma membrane expression. A proteomic approach has identified the A-kinase-anchoring protein, AKAP150, as a constituent of native TREK-1 channels (Sandoz *et al.*, 2006) and microtubule-associated protein 2 as another partner of TREK-1 and TREK-2 channels (Sandoz *et al.*, 2008). β-coatomer protein (COP), a subunit of COP1 has been shown to interact directly with the TREK-1 channel by increasing TREK-1 surface expression and current density (Kim *et al.*, 2010). Interestingly, the neurotensin receptor 3 (NTS_3 receptor; also called gp95/sortilin) (Petersen *et al.*, 1997; Mazella *et al.*, 1998) has been recently identified as another TREK-1 partner involved in TREK-1 trafficking (Mazella *et al.*, 1998; 2010). The important role of TREK-1/NTS_3 receptor association in depression will be described in detail later.

TASK-3 channel and depression

The cerebral expression of the TASK-3 channel in the CNS strengthens the hypothesis that TASK-3 could have potential roles in mood disorders, sleep–wake control and cognition. The TASK-3 channels are particularly abundant in the hippocampus, cortex and cerebellum as well as in specific nuclei including paraventricular nuclei of thalamus, locus coeruleus and the dorsal raphe (Talley *et al.*, 2001). The TASK-3 channels regulate neurotransmitter release and mediate the effects of neurotransmitter activation. 5-HT increases GABA release in rat entorhinal cortex by inhibiting interneuron TASK-3 channels, resulting in decreased pyramidal cell activity and inhibition of low-Mg^{2+}-induced seizure activity (Deng and Lei, 2008). These channels also regulate the activity of 5–HT-releasing neurons of the dorsal raphe (Washburn *et al.*, 2002). Likewise, they contribute to the control of activity modes in thalamocortical relay neurons, known to be involved in the sleep–wake cycle, anaesthetic action and absence epilepsy (Meuth *et al.*, 2003). Therefore, *kcnk9* has been regarded as a promising candidate gene for absence epilepsy in humans. A mutation analysis of the *kcnk9* gene in absence epilepsy patients reveals one exon-2 polymorphism, which, however, is not associated with the disease (Kananura *et al.*, 2002).

Behavioural alterations induced by the disruption of the kcnk9 *gene encoding TASK-3*

The *kcnk9* gene has been localized to the chromosomal region 8q24. A human gene mutation responsible for mental retardation has been identified and reported to be associated with a maternally transmitted dysmorphism syndrome (Barel *et al.*, 2008). Consistent with these findings, TASK-3 KO mice show deficits in cognitive functions. The mutant mice display impaired working memory in the T-maze spontaneous alternation test. Both male and female KO mice visit the same arm as on the previous trial more often than the wild-type mice, leading to a significantly lower percentage of spontaneous alternation (Linden *et al.*, 2007). Likewise, TASK-3 mutant mice show a significant impairment in the spatial memory as measured in the Morris water-maze. During training, KO mice are slower to find the hidden platform, and in the probe trial, female KO mice visit the platform quadrant fewer times

than male KO and wild-type mice (Linden *et al.*, 2007). Altered cognitive performances of TASK-3 mutant mice have been confirmed in the Y-maze spontaneous alternation task (Gotter *et al.*, 2011). TASK-3 KO mice also exhibit a reduced sensitivity to the inhalation anaesthetics (halothane and isoflurane) and to the cannabinoid receptor agonist WIN55212-2 mesylate (Linden *et al.*, 2007; Pang *et al.*, 2009). Because TASK-3 channels have been suggested to control the activity of the hypothalamic orexin neurons (Burdakov *et al.*, 2006), the locomotor activity and circadian rhythm of TASK-3 mutant mice have been analysed. Interestingly, their amplitude of nocturnal locomotor activity is significantly enhanced compared with wild-type littermate controls, light phase activity being similar (Linden *et al.*, 2007; Gotter *et al.*, 2011). Continuous EEG/EMG recordings reveal that TASK-3 KO mice exhibit a slower progression from their waking to sleeping states and, during their sleeping period, their sleep episodes as well as their rapid eye movement (REM) θ oscillations are more fragmented (Pang *et al.*, 2009; Gotter *et al.*, 2011). These findings are highly suggestive that TASK-3 plays a key role in the regulation of sleep and suggest that TASK-3 could be a therapeutic target for ADs. These findings are also consistent with the observations that despair-related animal models and patients with major depressive disorder (MDD) exhibit decreased periods of diurnal to nocturnal sleep as well as an elevated propensity towards REM sleep, that is reversed by AD treatments (Steiger and Kimura, 2010).

TASK-3 knock-out mice and AD-like phenotype

The inhibition of the TASK-3 channel has the potential to induce AD effects (Gotter *et al.*, 2011). TASK-3 KO mice display resistance to despair behaviour, which is evaluated in both Porsolt forced swimming test (FST) and tail suspension test (TST). The mutant mice show a significant decrease in immobility relative to wild-type controls in both tests (Gotter *et al.*, 2011). A hallmark of AD treatment is the suppression of REM sleep (Steiger and Kimura, 2010). Fluoxetine administered to wild-type animals induces a marked reduction in REM sleep, which is prevented in mice lacking TASK-3. These observations confirm the potential of this channel as a target for ADs. The development of compounds that can specifically block TASK-3 channels is a field of active interest. Cannabinoid agonists (anandamide, methanandamide, WIN212-2) have been described to inhibit these channels (Maingret *et al.*, 2001; Berg *et al.*, 2004; Veale *et al.*, 2007). A number of studies have shown that these cannabinoid agonists and indirect agonists (inhibitors of degradation of endocannabinoid ligands anadamide and 2-arachidonoylglycerol) produce AD-like effects in mouse models (Gobbi *et al.*, 2005; Jiang *et al.*, 2005; Zhong *et al.*, 2014). However, the block of these channels by anandamide and other cannabinoids may induce side effects including ataxia and movement disorders that can be observed following treatment with these agents. Very recently, a novel class of TASK-3 channel antagonists based on a 5,6,7,8-tetrahydropyridol[4,3-*d*]pyrimidine (THPP) high-throughput screening has been discovered. One THPP analogue has been identified as a specific TASK-3 inhibitor at a concentration around 1 μM, and exhibits good selectivity over other K_{2P} channels and a good ability to modulate sleep patterns in rodent EEG telemetry models (Coburn *et al.*, 2012). The proof of concept, for this interesting compound, in the context of depression still needs to be validated.

TREK-1 channel and depression

Association of genes with depression

A series of gene expression studies on humans have provided evidence for a role of *kcnk2* (the gene of TREK-1) in MDD. Primary analysis performed by Perlis *et al.* (2008) examined genetic variants of *kcnk2* in terms of their association with treatment response in non-psychotic subjects with depression in the levels 2 and 3 phases of the Sequenced Treatment Alternatives to Relieve Depression study (Perlis *et al.*, 2008). It appears that an allelic variation in four *kcnk2* single nucleotide polymorphisms (SNPs) is associated with a positive response to ADs (A allele of rs 10494996, G allele of rs12136349, C allele of rs2841608 and G allele of rs2841616, all variants located at the 5′ end of the *kcnk2* gene). Another study in a Han Chinese population reported that the genotype frequency of rs6686529, located in the 3′-UTR of the *kcnk2* gene differs significantly among the MDD patients and controls. Individuals with homozygous genotypes (CC or GG) show greater susceptibility to MDD than those with heterozygous genotypes, indicating a possible heterosis effect of the polymorphism on MDD. This polymorphism also affects the efficacy of AD treatment: the CC carriers have a greater probability of achieving remission after 8 weeks of treatment than the G allele carriers (Liou *et al.*, 2009). The disparity observed between the two studies with regard to the genetic variations in different regions of the *kcnk2* gene can be explained by different treatment response histories and by ethnicity. Nevertheless, these data probably mean that distinctive regions in the *kcnk2* gene contribute to particular clinical responses to the first or sequential AD treatment. The genetic variation in the human *kcnk2* gene is also associated with individual differences in neural responses to rewards. Individuals possessing rs10494996, rs2841608 and rs2841616 *Kcnk2* genotypes, previously linked to a better response to ADs show stronger basal ganglia responses to gains relative to individuals in the at-risk groups (Dillon *et al.*, 2010). A greater number of protective alleles across these SNPs is positively correlated with neural responses to gains in several regions of the reward network, including the right caudate-putamen, orbitofrontal cortex, medial prefrontal cortex and dorsal anterior cingulate cortex (Dillon *et al.*, 2010). These results strongly suggest that certain *Kcnk2* genotypes may promote remission from depression *via* their association with potentiated neural responses to gains.

*TREK1 (*kcnk2*)-knock-out mice and AD-like phenotype*

Deletion of the *kcnk2* gene results in a resistance to several inducible depression-like states (Heurteaux *et al.*, 2006), as revealed by the use of current behavioural tests (Table 1) (Porsolt *et al.*, 1977; Kameyama *et al.*, 1985; Caldarone *et al.*, 2000; Porsolt, 2000; Cryan *et al.*, 2002; Santarelli *et al.*, 2003; Cryan and Mombereau, 2004). *Kcnk2*$^{-/-}$ mice exhibit greater mobility in the FST (Figure 3A) and TST and fail to suppress

Table 1

Widely used rodent models sensitive to the effects of AD drugs

Behavioural test	DSM V criterion modelized	Measured features	Advantage/disadvantage	Reliability	Reference
FST	Despair	Mobility/ immobility	Rapid, easy, sensitive to acute and chronic treatments	High	Porsolt *et al.*, 1977; Porsolt, 2000; Cryan and Mombereau, 2004
TST	Despair	Mobility/ immobility	Rapid, easy, sensitive to acute and chronic treatments	High	Porsolt, 2000; Cryan *et al.*, 2002; Cryan and Mombereau, 2004
Conditioned motility suppression test	Despair, anxiety	Freezing	Efficient, sensitive to acute treatments	High	Kameyama *et al.*, 1985
Learned helplessness test	Despair, anxiety	Time to escape	Sensitive to acute and subchronic treatments	Medium	Caldarone *et al.*, 2000; Porsolt, 2000; Cryan *et al.*, 2002; Cryan and Mombereau, 2004
Novelty suppression of feeding	Despair, anxiety, neurogenesis	Time to feed	Needs subchronic or chronic treatments	Medium	Santarelli *et al.*, 2003; Cryan and Mombereau, 2004

DSM V, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

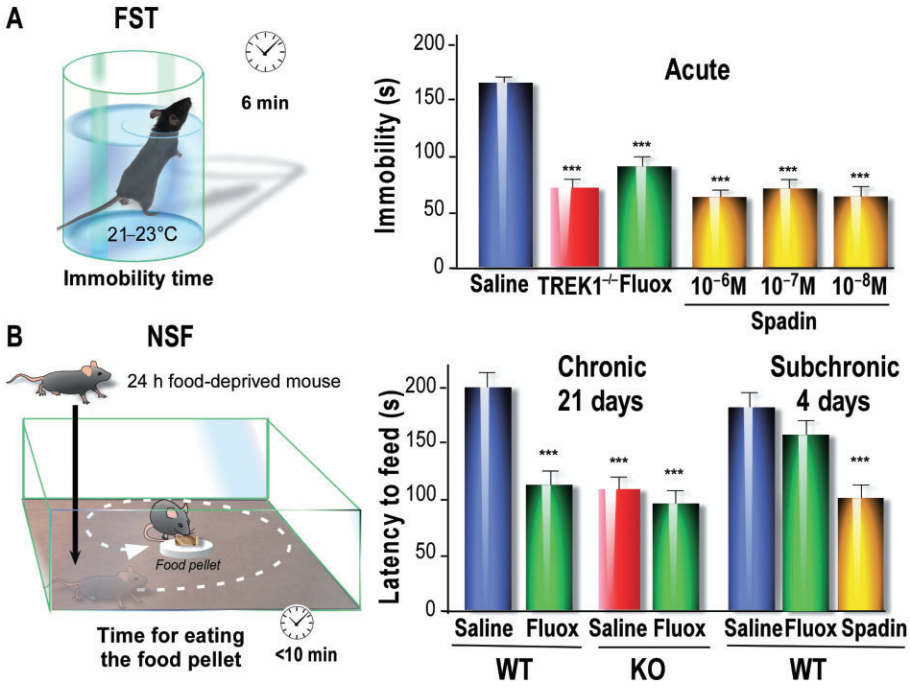

Figure 3

Effects of the deletion of TREK-1 channels or spadin in animal models of depression. (A) FST: mice were introduced into a beaker half-filled with 21–23°C water for 6 min. and the immobility time was measured during the last 4 min. Saline, mice injected with NaCl 0.9%, TREK1 (*kcnk2*)$^{-/-}$, TREK-1 knock-out mice, Fluox, mice injected i.p. with fluoxetine (3 mg·kg^{-1}), spadin (10^{-6}, 10^{-7} and 10^{-8} M), mice injected i.v. at 10 μg·kg^{-1}, 1.0 and 0.1 μg·kg^{-1}, respectively. (B) NSF: mice were deprived of food for 24 h. Then, a food pellet was placed in a white circle at the centre of a brightly lit area (45 × 45 × 20 cm), and the mouse was placed in a corner of the box. The time needed to go and eat the food pellet was measured. In the chronic treatment, fluoxetine (Fluox) was given through the drinking water (80 mg·mL^{-1}) for 21 days. For the subchronic treatment, saline (0.9% NaCl) and Fluox (3 mg·kg^{-1}) were administered by i.p. injections (once a day) and spadin (10 μg·kg^{-1}) was administered by i.v. injections (once a day). ***$P < 0.001$.

locomotion in the conditioned suppression of motility test. They also show shorter escape latencies after exposure to uncontrolled aversive electric foot shocks (learned helplessness protocol, LH). In the novelty-suppressed feeding task (NSF) they eat food more readily in a threatening environment (Figure 3B). When treated with ADs or not, these mutant mice display behavioural expressions similar to wild-type mice treated with current ADs such as fluoxetine or paroxetine (Figure 3B). The TREK-1 channel is expressed in 5-hydoroxytryptaminergic neurons of the midbrain dorsal raphe nucleus (DRN). Interestingly, combined treatment with fluoxetine and p-chlorophenylalanine methyl ester, a tryptophan hydroxylase inhibitor, which depletes 5-HT from the nerve terminals completely prevent the AD phenotype of the *kcnk2*$^{-/-}$ mice (Heurteaux *et al.*, 2006). Moreover, 5-hydoroxytryptaminergic neuron activity is increased in mutant mice, just as it is in animals treated with selective 5-HT re-uptake inhibitors (SSRIs). The firing rate of 5-hydoroxytryptaminergic neurons from the DRN, where the TREK-1 channels are highly expressed is more than twofold higher in *kcnk2*$^{-/-}$ mice compared with wild-type mice. This increase in 5-hydoroxytryptaminergic neuronal activity increases the 5-HT released in target structures such as the hippocampus. Indeed, firing rates of hippocampal neurons are increased by a 5-HT$_{1A}$ receptor antagonist in the knock-out mice, as they are in wild-type mice treated with ADs (Haddjeri *et al.*, 1998). Moreover, *kcnk2*$^{-/-}$ mice are insensitive to the administration of SSRIs, suggesting that the therapeutic effects of SSRIs may be related to TREK-1 inhibition. Consistent with this notion, SSRIs have been shown to inhibit TREK-1 in a concentration-dependent manner (Kennard *et al.*, 2005; Heurteaux *et al.*, 2006). A recent report suggested that fluoxetine-induced inhibition of TREK-1 activity is correlated with the dissociation of its C-terminal domain from the plasma membrane (Sandoz *et al.*, 2011). It remains to be seen whether this mechanism may play a role in genetically conferred elevations in 5-HT. These results indicate that TREK-1 has a major role in mood regulation *via* the 5-hydoroxytryptaminergic system. This effect is specific to the TREK-1 channel, as the TRAAK channel, which is closely related to TREK-1, but not modulated by the cAMP/PKA pathway (linking TREK-1 to the 5-HT$_{1A}$ receptor), does not display an AD phenotype (Heurteaux *et al.*, 2006). It is likely that the TREK-1 channels are involved in the 5-HT$_{1A}$ receptor-dependent feedback inhibition of 5-hydoroxytryptaminergic dorsal raphe neurons (Gordon and Hen, 2006). 5-HT released back onto 5-hydoroxytryptaminergic neurons would activate 5-HT$_{1A}$ receptors, decreasing their firing rate and thereby decreasing 5-HT release *via* the activation of G–protein-gated inwardly rectifying K+ channels and the inhibition of cAMP production leading to an increase in TREK-1 channel activity. Additionally, the direct inhibition of TREK-1 channels by SSRIs probably also contributes to increased excitability of dorsal raphe neurons and 5-HT release (Figure 4).

Not all effects of SSRIs are absent in TREK-1 knock-out mice. SSRI-induced increases in neurogenesis are paradoxically enhanced (Heurteaux *et al.*, 2006). Nevertheless, SSRIs could potentially inhibit TREK-1 in two ways: directly and *via* increasing 5-HT release onto cAMP-inhibiting 5-HT$_{1A}$ receptors. More work is clearly necessary to understand the nature of the interaction between SSRIs and the channel, and to rule

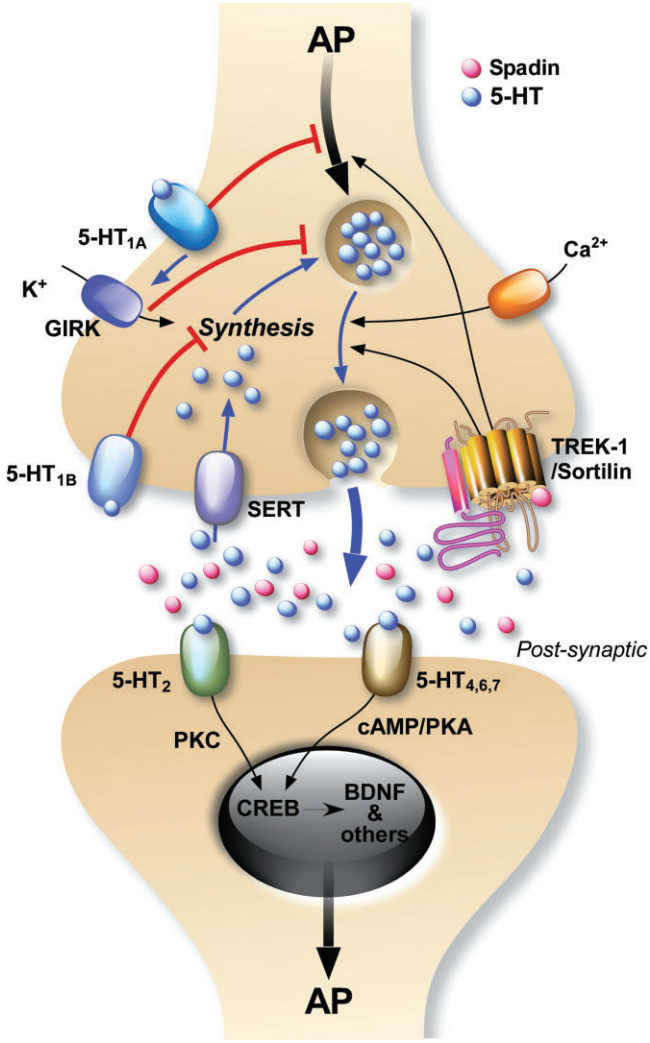

Figure 4

Schematic involvement of TREK-1/sortilin complex in the 5-HT transmission. Red arrows represent the natural pathway of synthesis, secretion re-uptake of 5-HT (red circles). Black bars represent the pathway that slows down or inhibits the release of 5-HT. Black arrows represent pathways that are beneficial for 5-HT release. TREK-1/sortilin complex is beneficial when spadin is bound. AP, action potential; SERT, 5-HT transporter.

out indirect influences. The direct effect of SSRIs on TREK-1 channels leads also to a diminished stress response, suggesting that TREK-1 antagonists might evoke a faster AD response than conventional AD drugs. Numerous studies have reported that stress decreases the proliferation of progenitor cells in the dentate gyrus of the hippocampus (Duman *et al.*, 1999), which is in part ascribed to an increased activity of the hypothalamic–pituitary–adrenal axis with higher levels of glucocorticoids (Brown *et al.*, 1999). The stress-mediated effect on neurogenesis is suggested to be indirect, occurring primarily through effects on the activity of the 5-hydoroxytryptaminergic system by glucocorticoids (Gould, 1999), which have been reported to directly inhibit the proliferation of embryonic neural stem cells (NSCs) both

in vitro and *in vivo* (Sundberg *et al.*, 2006). Interestingly, dexamethasone (a glucocorticoid hormone receptor agonist) up-regulates both mRNA and protein TREK-1 levels leading to a decreased proliferation of NSCs. Fluoxetine, which induces a down-regulation of TREK-1 expression in NSC attenuates the inhibitory effect of glucocorticoids on neurogenesis *via* the TREK-1 channel (Xi *et al.*, 2011). However, unlike the common functional polymorphism in the 5-HT transporter-linked promoter region (5-HTTLPR), chronic fluoxetine treatment for 39 weeks is not associated with a reduced expression of TREK-1 protein in the cortex of Rhesus macaques (Bogdan *et al.*, 2011). Given that the short allele rh5-HTTLPR confers elevated synaptic 5-HT (Bennett *et al.*, 2002) these data suggest that elevated 5-HT levels may reduce cortical TREK-1 expression. The reasons why chronic SSRI administration fails to affect TREK-1 expression in the cortex are unknown. The therapeutic effects of SSRIs on TREK-1 inhibition may be regionally specific, transient and dependent on the length of the SSRI treatment.

Sortilin, a protein partner of TREK-1 channels

Sortilin, also called NTS_3 receptor (Petersen *et al.*, 1997; Mazella *et al.*, 1998), was recently identified as a partner of TREK-1 channels and is shown to regulate the plasma membrane level of the channel (Mazella *et al.*, 2010) (Figure 5). Sortilin is a 95 kDa type-1 membrane protein, consisting of a large luminal domain, a single transmembrane segment and a short C-terminal cytoplasmic tail, which is expressed in the CNS and peripheral nervous systems (Figure 5A). Studies in mammalian cells demonstrated that sortilin moves from the trans-Golgi network to late endosomal compartments where the protein triggers intracellular functions of trafficking. The roles of sortilin are already known to be multiple and complex and concern its functions as receptor or co-receptor and its involvement in the sorting of proteins to lysosomes or to the plasma membrane (Coutinho *et al.*, 2012; Nykjaer and Willnow, 2012). At the cellular level, sortilin binds neurotensin, the precursor of the nerve growth factor, the receptor-associated protein and the lipoprotein lipase (Petersen *et al.*, 1997; Mazella *et al.*, 1998; Nielsen *et al.*, 1999; Nykjaer *et al.*, 2004). Recent studies reported a pivotal role for sortilin in control of pro-brain-derived neurotrophic factor (BDNF) secretion (Evans *et al.*, 2011; Yang *et al.*, 2011). At the physiological level, sortilin is involved in the regulation of a series of neurological and cardiovascular functions. Therefore, the function or the dysfunction of sortilin may be responsible for hypercholesterolaemia and myocardial infarction as well as in neurodegenerative disorders (for a review, see Carlo *et al.*, 2014). Sortilin may be also involved in several other diseases including diabetes and obesity (Kaddai *et al.*, 2009; Mazella

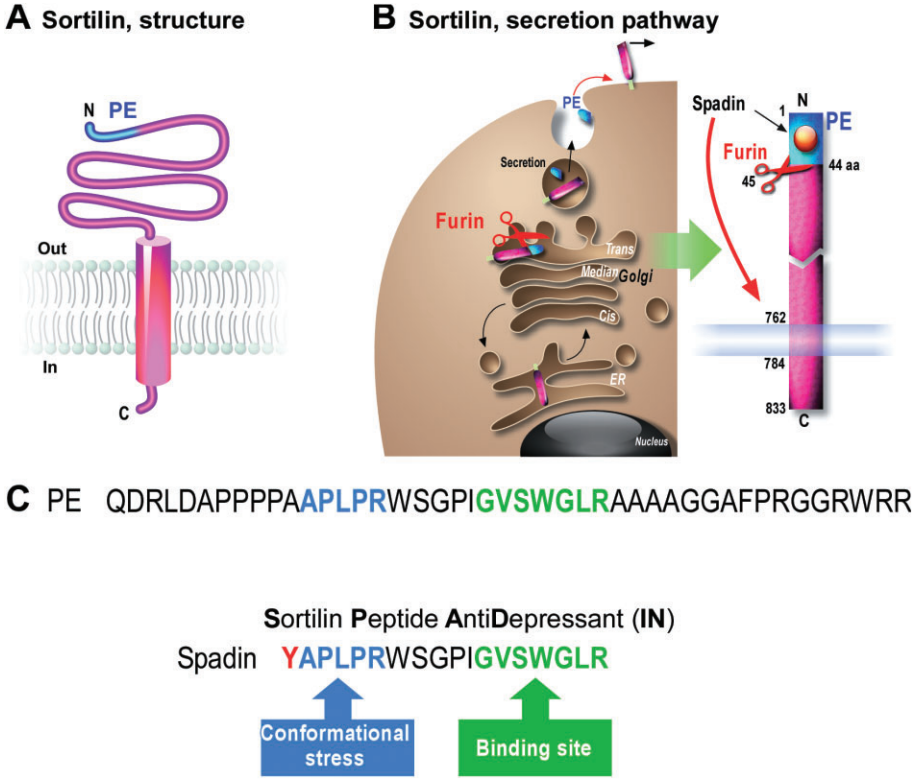

Figure 5

Sortilin and spadin. (A) Structure of the sortilin receptor: sortilin is constituted by a unique transmembrane segment, a short cytoplasmic C-terminal moiety and a large external N-terminal sequence. (B) Sortilin secretion pathway: Sortilin is processed in the Golgi network where the enzyme named furin releases the last 44 amino acids (aa) from the N-terminus; this released peptide is called propeptide (PE). (C) Spadin sequence designed from the PE sequence.

et al., 2012), and cancer (Wilson *et al.*, 2014). Sortilin is known to be shedded at the cell surface by MMPs (Navarro *et al.*, 2002). The resulting soluble form of sortilin has been shown to be functional (Massa *et al.*, 2013) and to trigger impairment of cancer cell cohesion that could be responsible for cancer cell dissemination (Massa *et al.*, 2014).

Sortilin is synthesized as a precursor protein (prosortilin) harboring a 44-amino acid propeptide (Gln^{1}-Arg^{44}) that acts as an intrinsic chaperone for the correct folding and transport of the receptor. Prosortilin is converted to the functional ligand-binding receptor by cleavage and release of the propeptide by furin (Munck Petersen *et al.*, 1999) (Figure 5B). Propeptide binds to the mature receptor with a high affinity (K_D ~ 5 nM). Sortilin as well as TREK-1 are highly expressed in several cerebral structures involved in the pathophysiology of depression, such as the prefrontal and cingulate cortice, amygdala, hippocampus, nucleus accumbens, dorsal raphe and hypothalamus. A partial propeptide (Ala^{12}-Arg^{28}) called spadin has been designed and described to regulate the TREK-1 channel (Mazella *et al.*, 2010). Spadin binds to TREK-1/sortilin complexes at the cell surface (Figure 5B), inducing endocytosis and lysosomal degradation. The internalization of TREK-1 channels results in depolarization of the plasma membrane.

Therapeutic potential of spadin, a specific blocker of the TREK-1 channel

Competition experiments showed that spadin binds specifically to TREK-1 with an affinity of about 10 nM (Figure 6A). Electrophysiological studies have identified spadin as a peptidic antagonist of the TREK-1 channel (Figure 6B,C,D) (Mazella *et al.*, 2010). TREK-1 basal channel activity is

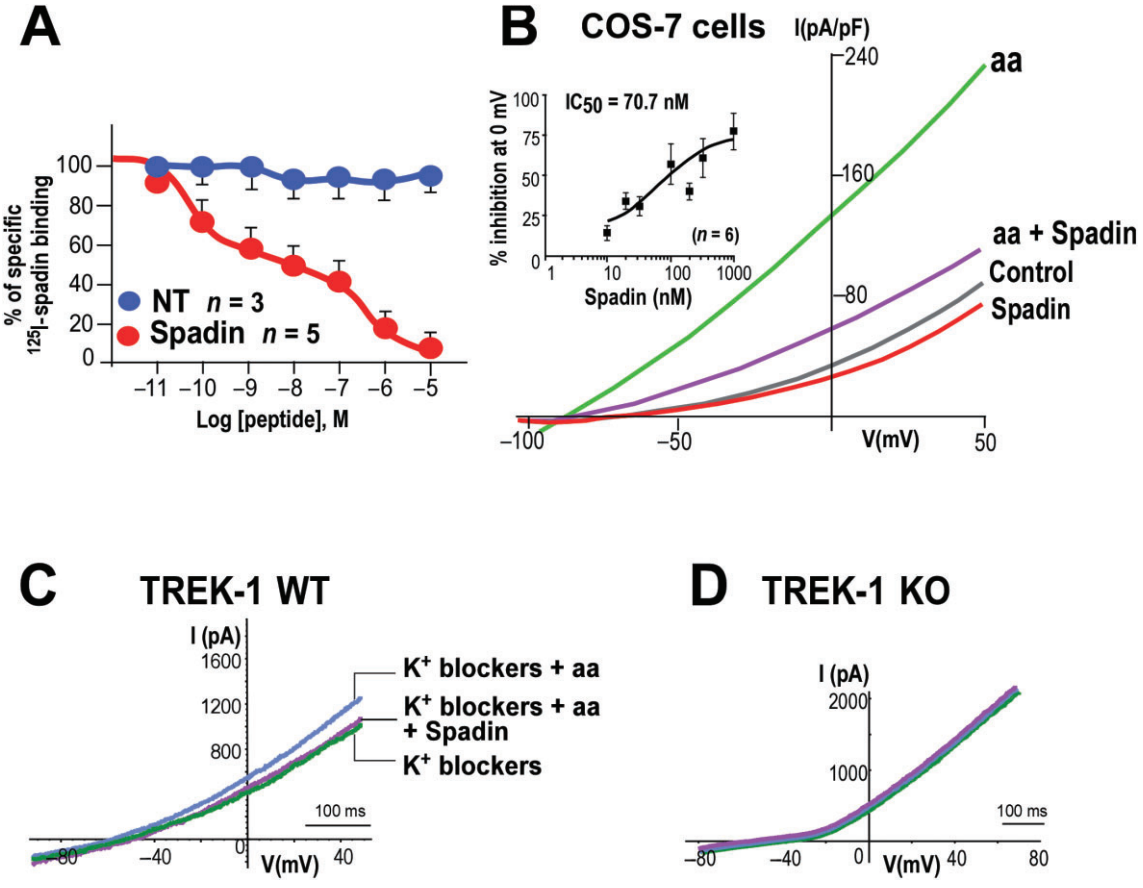

Figure 6

Spadin, a specific TREK-1 channel blocker. (A) Binding experiments with [^{125}I]-spadin performed on COS-7 cells transfected with the TREK-1 channel. Unlike neurotensine (NT), unlabelled spadin was able to compete with radiolabelled spadin. (B, C and D) All the experiments were performed in the whole-cell configuration of the patch-clamp protocol and in the presence of a cocktail of potassium channel inhibitors (K^+ blockers: 3 mM 4-aminopyridine, 10 mM tetraethylamonium, 10 μM glibenclamide, 100 nM apamin and 50 nM charybdotoxin). aa indicates that currents were recorded in the presence of 10 μM arachidonic acid. As previously described (Patel *et al.*, 1998; Mazella *et al.*, 2010), the amplitude of the basal whole-cell TREK-1 current (control) was currently low. It is the reason why aa was applied before spadin to first activate the TREK-1 channel and then observed the blocking of these channels by spadin. (B) Effects of spadin on the TREK-1 channel current expressed in transfected COS-7 cells. *Inset.* Dose–response curves of the percentage of current inhibition measured at 0 mV as function of spadin concentration, current values were obtained in the presence of arachidonic acid. IC_{50} = 70.7 nM. (C and D) Effects of spadin on neurons from hippocampus slices. Currents increased by aa application were inhibited by spadin on the wild-type neurons (C), aa had no effect on currents generated by neurons from TREK-1 knock-out mice; consequently, spadin had no effect on these currents (D).

strongly and reversibly activated by arachidonic acid (10 μM), which induces a typical TREK-1 background current, characterized by outward rectification reversed at the predicted value for E_{K+}. Using the whole-cell patch-clamp technique on TREK-1 transfected COS-7 cells, it has been reported that 100 nM of spadin are able to block 63% of the TREK-1 current stimulated by arachidonic acid (Figure 6B). A spadin dose–response experiment indicates an IC_{50} value of 70.7 nM at 0 mV (Figure 6B inset). Spadin also blocks the arachidonic acid-sensitive potassium current in CA3 hippocampal neurons on brain slices of wild-type mice and not in *kcnk2*$^{-/-}$ mice, indicating a specific inhibitory effect of spadin on the TREK-1 channel (Figure 6C–D).

Very interestingly, spadin is described as the first peptidic and fast-acting AD (Mazella *et al.*, 2010). Spadin AD efficacy was assessed in the FST and the TST tasks, where a spadin treatment (at doses of 10^{-6} to 10^{-8} M) reduces the immobility times compared with that observed in saline-treated counterparts. The magnitude of the AD behaviour is similar to that observed in fluoxetine-treated wild-type and saline-injected *kcnk2*$^{-/-}$ mice (Figure 3A). In both tests, the injection of spadin in *kcnk2*$^{-/-}$ mice did not induce any change, indicating that there is no additional effect of spadin in the absence of the TREK-1 channel and strengthens the hypothesis that TREK-1 mediates the effects of spadin on mood (Mazella *et al.*, 2010). It has been proposed that a direct facilitation of 5-hydoroxytryptaminergic firing rate in the DRN, which has been shown in *kcnk2*$^{-/-}$ mice should be a requirement for a faster onset of AD action (Blier, 2001). Interestingly, an increase in the firing activity of DRN 5-hydoroxytryptaminergic neurons is observed after spadin treatment (Mazella *et al.*, 2010). Obviously, such results raise the possibility that spadin could have a rapid onset of action. Unlike fluoxetine a 4 day treatment with spadin (i.v., 10^{-6} M) significantly reduces the time spent immobile by 43% in FST. In the NSF, which is usually carried out for demonstrating AD efficacy after chronic, but not acute treatment (Santarelli *et al.*, 2003), mice treated with spadin (i.v., 10^{-6} M) for 4 days showed a significant decrease in latency to feed relative to saline-injected animals. A 4 day regimen with fluoxetine had no effect in the same conditions (Mazella *et al.*, 2010) (Figure 3B).

The fast-acting AD potential of spadin was confirmed by its ability to activate two specific markers of AD action, currently observed after 2 weeks of classical AD treatment: the transcription factor cAMP response element-binding protein (CREB) and neurogenesis (Nibuya *et al.*, 1996; Santarelli *et al.*, 2003). Unlike fluoxetine, a 4 day chronic treatment with spadin is able to enhance the pCREB/CREB ratio and consequently increases cell division and proliferation in the subgranular zone of dentate gyrus. Spadin also enhances the hippocampal expression of BDNF and of two proteins involved in synaptogenesis, PSD-95 and synapsin (Figure 4). This peptide significantly increases the proportion of mature spines in cortical neurons, suggesting a role for the peptide in neurogenesis and in the maturation of neurons.

Deletion of the TREK-1 channel is known to increase sensitivity to pain, seizures and ischaemia. Blocking these channels could result in deleterious effects. Spadin does not display TREK–1-related side effects and does not induce cardiac dysfunctions (Moha ou Maati *et al.*, 2012). *In vitro* studies on cortical neurons in culture demonstrated that spadin increases the neuronal membrane potential and activates the MAP and PI3 kinase pathways in a time- and concentration-dependent manner, leading to a strong protective effect against staurosporine-induced apoptosis. To summarize, spadin can be considered as a natural endogenous AD and constitutes the first peptide identified as an AD with a rapid onset of action. Spadin is able to rapidly stimulate neurogenesis and dendritic spine maturation as well as synaptogenesis by increasing the expression of synaptic proteins and of the BDNF. Because of these peculiar properties, spadin brings a new concept to address the treatment of depression. Recently, retroinverso analogues of spadin, which display increased AD effects with a better affinity for TREK-1 channels and an increased stability have been identified (Veyssiere *et al.*, 2014).

The heterogeneous nature of depression makes the development of practical biomarkers very complex. In a clinical setting, at present there is no established biological marker that can predict treatment response for an individual patient before the initiation or during the early course of AD treatment (Tadic *et al.*, 2011). Because it is possible to administer spadin in the serum of depressed patients by the AlphaScreen technology and because spadin is an endogenous and natural peptide (Mazella *et al.*, 2010), spadin could also become a specific and an endogenous marker of depression in humans. This hypothesis is strengthened by the fact that the sortilin receptor has been already identified as a candidate gene. The observation that *SORT1* (the gene encoding sortilin), whose mRNA expression in peripheral blood mononuclear cells is altered during MDD and varied in the opposite direction 8 weeks after AD treatment further confirms this hypothesis (Belzeaux *et al.*, 2010).

To date, spadin is also the first identified blocker of TREK-1 channel, which is not only of relevance in the field of depression, but also constitutes a useful tool to further understand the role of TREK-1 channels in other neurological pathologies (Bittner *et al.*, 2013). Given the present state of understanding, it may well be that not all of the effects of spadin and sortilin will turn out to be mediated by TREK-1 channels, and that the effects of blocking or knocking out these channels will perhaps have wide-ranging effects on neurophysiology and not only a narrow effect on mood regulation.

Conclusion

Depression is one of the most common psychological problems and remains a devastating mood disorder, affecting nearly everyone through either personal experience or through a family member. It is the reason why the discovery of new targets and new AD molecules is challenging. Since the cloning of the first member of the particular two-pore domain K^+ channel family in 1996 (Lesage *et al.*, 1996), major findings have been reported and have demonstrated the wide-ranging importance of this channel family and its implications in multiple diseases. The use of knock-out mice, along with electrophysiological, genetic and behaviour experiments, has indicated that TASK-3 and TREK-1 channels are promising and attractive targets for mood disorders. Addi-

tional studies are clearly necessary to provide definite evidence that the TASK-3 channel is a suitable target for the actions of ADs. The TASK-3 KO mouse model provides a novel tool that may be critical in understanding brain mechanisms associated with several drug effects and physiopathological functions. While TASK-3 channel modulation does have therapeutic potential, there are only a few documented examples of potent and selective small-molecule channel blockers. The recent discovery and lead optimization efforts for a novel series of TASK-3 channels antagonists based on a THPP high-throughput screening may identify interesting prospects for the development of new ADs.

For the TREK-1 channel, the proof of concept in AD drug design is more advanced. The discovery of spadin, a natural peptide and specific blocker of TREK-1 channels with a rapid onset of action and no adverse effects is an important step for the development of a novel treatment for depression. In fact, intense work, performed in compliance with Good Laboratory Practice is in progress to test spadin and its analogues for adverse effects, mutagenicity and toxicity, a crucial step towards its clinical application. With the aim of validating spadin and sortilin as reliable and specific biomarkers of depression seric dosages of these proteins have been administered to patients with depression. Additionally, with regard to the particular properties of spadin, possible clinical applications of this new drug will be studied in the context of stroke, epilepsy and neurodegenerative disorders.

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Conflict of interest

The authors declare that there is no conflict of interest.

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