Pharmacology & Therapeutics 194 (2019) 185–198

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Fighting against depression with TREK-1 blockers: Past and future. A focus on spadin

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ARTICLE INFO

Available online 3 October 2018

Keywords:
TREK-1
Depression
Blockers
Spadin
Peptide
Analogs

ABSTRACT

Depression is a devastating mood disorder and a leading cause of disability worldwide. Depression affects approximately one in five individuals in the world and represents heavy economic and social burdens. The neurobiological mechanisms of depression are not fully understood, but evidence highlights the role of monoamine neurotransmitter balance. Several antidepressants (ADs) are marketed to treat depression and related mood disorders. However, despite their efficacy, they remain nonspecific and unsafe because they trigger serious adverse effects. Therefore, developing new molecules for new targets in depression has become a real necessity. Eight years ago, spadin was described as a natural peptide with AD properties. This 17-amino acid peptide blocks TREK-1 channels, an original target in depression. Compared to the classical AD drugs such as fluoxetine, which requires 3–4 weeks for the AD effect to manifest, spadin acts rapidly within only 4 days of treatment. The AD properties are associated with increased neurogenesis and synaptogenesis in the brain. Despite the advantages of this fast-acting AD, the *in vivo* stability is weak and does not last for >7 h. The present review summarizes different strategies such as retro-inverso strategy, cyclization, and shortening the spadin sequence that has led to the development and optimization of spadin as an AD. Shortened spadin analogs present increased inhibition potency for TREK-1, an improved AD activity, and prolonged *in vivo* bioavailability. Finally, we also discuss about other inhibitors of TREK-1 channels with a proven efficacy in treating depression in the clinic, such as fluoxetine.

Contents

Abbreviations: 5-HT, serotonin; AA, arachidonic acid; AD, antidepressant; AKAP, A-kinase-anchoring protein; BBB, blood–brain barrier; BDNF, Brain-derived neurotrophic factor; BrdU, 5-Bromo-2′-Deoxyuridine; CNS, central nervous system; CREB, cAMP response element-binding protein; CSMT, conditioned suppression of motility test; DA, dopamine; DAT, dopamine transporter; DRN, dorsal raphe nucleus; FST, forced swim test; GABA, γ-Aminobutyric acid; GLP-1, glucagon-like peptide-1; IC_{50}, half-maximal inhibitory concentration; i.c.v., intracerebroventricular route; i.p., intraperitoneal route; i.v., intravenous route; K_{2P}, two-pore domain K^+ channel; K_{ATP}, ATP-sensitive potassium channels; LHT, learned helplessness test; MAO, monoamine oxidase; MDD, major depression disorder; mGluR, metabotropic glutamate receptor; MMP, matrix metalloproteinase; Mtap2, microtubule-associated protein; NA, norepinephrine; NAT, norepinephrine transporter; NBP, 3-*n*-Butylphthalide; NSF, novelty-suppressed feeding test; NT, neurotensin; NTSR-3, neurotensin receptor-3; PE, propeptide; PKA, protein kinase A; ProNGF, precursor of the nerve growth factor; PSD-95, postsynaptic density protein-95; PTZ, pentylenetetrazol; RI, retro-inverso; SERT, serotonin transporter; SNRI, serotonin–norepinephrine reuptake inhibitor; Spadin, sortilin-derived peptide with antidepressant activity; SSRI, selective serotonin reuptake inhibitor; TdP, Torsades de pointes; TRAAK, TWIK-related arachidonic acid-activated K^+ channel; TREK-1, TWIK-related K^+ channel-1; TWIK-1, Tandem of P domains in weak inward rectifying K^+ channel-1; TST, tail suspension test.

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https://doi.org/10.1016/j.pharmthera.2018.10.003

1. Introduction

Depression is a devastating mental disorder that affects one in five individuals and is considered as a leading cause of disability worldwide (Nestler et al., 2002; Otte et al., 2016). Thus, the economic burden is huge for governments and particularly for patients suffering from depression (Kessler, 2012). Depression is a product of several and complex molecular and cellular mechanisms that are difficult to identify. One of the main common hypotheses that attempt to explain the neurobiology of depression is the imbalance in monoamine levels in the brain known as the "monoaminergic theory of depression" (Delgado, 2000). Presently, the majority of antidepressant (AD) drugs aim to restore the physiological amount of three central monoamines (serotonin (5-HT), norepinephrine (NA), and dopamine (DA)) in the synaptic cleft. Indeed, this is achievable by blocking the serotonin transporter (SERT), norepinephrine transporter (NAT), and dopamine transporter (DAT) responsible for the reuptake of 5-HT, NA, and DA, respectively, in the brain (Tatsumi, Groshan, Blakely, & Richelson, 1997). Inhibiting 5-HT and NA degradation through monoamine oxidase (MAO) enzyme increases the synaptic concentrations of monoamines (Brandon, 1982). However, with their proven efficacy, these AD drugs present a delay of action that remains very long (Cipriani et al., 2018; Stassen, Angst, & Delini-Stula, 1997). The late onset of action of current AD drugs takes several weeks to manifest. Given the complexity of the physiopathology of depression, many mechanisms and factors could be responsible for monoaminergic transmission, stress, inflammation, neurogenesis, and neuroplasticity (Dean & Keshavan, 2017). Moreover, AD drugs are frequently associated with various adverse effects such as fatigue, anxiety, sexual dysfunction, headache, and nausea. Often, these adverse effects lead patients to discontinue their AD treatment and in some cases can worsen or even cause increased suicide risk (Bull et al., 2002; Nischal, Tripathi, Nischal, & Trivedi, 2012; Sharma, Guski, Freund, & Gotzsche, 2016; Sicouri & Antzelevitch, 2008). This increased risk could be attributed to either the drug itself, a delayed onset of action, a wrong dosage, or a discontinuity in the treatment (Fergusson et al., 2005; Otte et al., 2016). To date, the main goals of the new AD strategy in drug design and development are to shorten the latency time for AD activity to manifest and substantially reduce adverse effects (Ramaker & Dulawa, 2017). The most common AD drugs in use currently as a first-line treatment are the selective serotonin reuptake inhibitors (SSRIs) and the serotonin–norepinephrine reuptake inhibitors (SNRIs). Their safety is seriously questioned in a series of studies (Cascade, Kalali, & Kennedy, 2009; Ferguson, 2001; Masand & Gupta, 2002; Montgomery, 2008; Read & Williams, 2018). As a result, the biggest challenge currently is to prescribe efficient AD drugs but with an acceptable tolerance in depressive patients. Newer AD classes have been discovered, such as multimodal AD drugs (Richelson, 2013). In addition to SERT inhibition, these drugs also antagonize $5HT_3$ and $5HT_7$ receptors and behave as partial agonists of $5HT_{1B}$ and full agonists of $5HT_{1A}$ receptors (Katona & Katona, 2014). Recently, the fast-acting AD drug ketamine has been identified and might represent a new generation of AD drugs able to counteract depression with a fast onset of action (Caraci, Leggio, Salomone, & Drago, 2017; Kavalali & Monteggia, 2015). However, despite the advanced level of clinical development accomplished by ketamine, this drug licensed as an anesthetic and painkiller has to be handled with extreme caution. A long list of serious adverse effects caused by ketamine was reported in many studies as described in the review of Short et al. (Short, Fong, Galvez, Shelker, & Loo, 2017). More recently, a study has identified ELK-1 transcription factor, which is an ERK downstream module as a potential target for treating depression. ELK-1 is upregulated in depressive patients. In mice, selective inhibition of ELK-1 phosphorylation using a 31-amino acid peptide, called TDE, produces AD-like behavior in different tests (Apazoglou et al., 2018). To improve AD efficacy, or suppress unwanted adverse effects, new strategies should be considered involving endogenous molecules. This includes essentially peptides that are naturally synthesized in the human body to exert specific actions. Indeed, peptides are involved in numerous biological functions in the cell, mainly as signaling molecules and also as ligands for several types of receptors. Considering their attractive pharmacological properties, peptides constitute an excellent starting point for designing novel therapeutic molecules. Their specificity results in excellent efficacy, safety, and tolerability in humans (Fosgerau & Hoffmann, 2015).

Growing evidence places therapeutic peptides as a very promising market, and the number of candidate peptides has shown an important increase in clinical trials (Uhlig et al., 2014). To date, approximately 100 therapeutic peptides are marketed in the United States, Europe, and Japan (Kaspar & Reichert, 2013). In a pharmaceutical industry that lacks efficient innovations, peptides constitute a potential alternative in the treatment of numerous diseases and disorders. Furthermore, new targets in depression need to be discovered and validated for innovative molecules. The goals are to improve AD therapy, eliminate adverse effects, and treat patients who are resistant to classical AD drugs. Ion channels represent almost 20% of all the human protein targets (Santos et al., 2017). Until 2015, 177 small molecules and biologic effectors of ion channels had been approved as treatments for several pathologies (Santos et al., 2017). In the present review, we discuss the spadin (or its analogs)–TREK-1 channel interaction that leads to channel blocking, one of the most promising drug–target interactions in term of CNS disorder treatments, mainly in the depression process.

2. TREK-1 in depression

2.1. TREK-1 channel

The TWIK-related K^+ channel-1 (TREK-1) is a member of the two-pore domain K^+ channel family (K_{2P}) (Honore, 2007; Lesage & Lazdunski, 1998). As with other K_{2P} channels, TREK-1 channels are responsible for maintaining the neuronal resting membrane potential and controlling action potential duration and also participate in neurotransmitter release (Honore, 2007). TREK-1, TREK-2, and TRAAK are part of lipid and mechanosensitive K_{2P} channel subfamily. TREK-1 and TREK-2 share >78% of homology (Lesage & Lazdunski, 2000). In the cell, TREK-1 is regulated by a variety of physical and chemical stimuli (Honore, 2007). Activation of TREK-1 channels can be mediated by membrane stretch (Maingret, Patel, Lesage, Lazdunski, & Honore, 1999; Patel et al., 1998), internal acidosis (Honore, Maingret, Lazdunski, & Patel, 2002; Maingret et al., 1999), heat (Maingret et al., 2000), lipids (Kim, 2003; Patel & Honore, 2001), and polyunsaturated fatty acids such as arachidonic acid (AA) (Patel et al., 1998). Pharmacological opening of the channel is mediated by volatile general anesthetics (Patel et al., 1999) and analgesics such as morphine through the activation of μ-opioid receptors (Devilliers et al., 2013). TREK-1 is downregulated upon stimulation of Gs- and Gq-coupled receptors. In fact, stimulation of $5\text{-}HT_4$ receptors by 5-HT (Fink et al., 1996; Patel et al., 1998) or the metabotropic glutamate receptors mGluR1 and mGluR5 by glutamate inhibits the TREK-1 channel (Chemin et al., 2003; Lopes et al., 2005). TREK-1 is also inhibited by SSRIs such as fluoxetine (Heurteaux et al., 2006b; Kennard et al., 2005) and the endogenous peptide spadin; this is described in detail in this review (Mazella et al., 2010).

2.2. Screening of TREK-1 modulators in vitro

Since its discovery, TREK-1 was shown to play a major role in several physiopathological processes (Honore, 2007). Its widespread presence in a multitude of functions varying from neurologic brain disorders to arrhythmia in the heart confirms the highly attractive target that TREK-1 represents currently. Through the generation of TREK-1 knockout ($kcnk2^{-/-}$) mice, it was clearly demonstrated that TREK-1 channels are involved in several pathologies. TREK-1 activation is known to have neuroprotective properties against ischemia or epilepsy (Blondeau

et al., 2007; Heurteaux et al., 2004; Lauritzen et al., 2000). More interestingly, *kcnk2*$^{-/-}$ mice demonstrated that TREK-1 channels are involved in the depression process. Deletion of TREK-1 channels resulted in a depression-resistant phenotype. This particular phenotype was identified by different animal assays for depressive-like behaviors (such as FST, TST, CMST, NSF, or LHT), *i.e.*, an increase in 5-HT neurotransmission and a reduction in elevated corticosterone levels under stress (Heurteaux, Lucas, et al., 2006b). Together, these data indicate that TREK-1 modulators are of great pharmacological interest. With the aim to efficiently and easily screen TREK-1 modulators, a HEK293 cell line that stably expresses the human TREK-1 channel was generated and named as the hTREK-1/HEK cell line (Moha ou Maati et al., 2011). Thus, screening TREK-1 activators for characterizing new neuroprotective molecules could be made easier using the hTREK-1/HEK cell line. The hTREK-1/HEK cell line was validated as an efficient pharmacological tool for screening TREK-1 effectors because it responded to various chemical and physical stimuli that modulate TREK-1 activity. In addition, TREK-1 was associated with pain perception (Alloui et al., 2006). Consequently, activators of TREK-1 channels displaying *in vivo* analgesic activity were also screened using the hTREK-1/HEK cell line (Vivier et al., 2017). Another stable TREK-1 transfected HEK cell line was used to study the role of TREK-1 channels in maintaining uterine quiescence during pregnancy (Heyman et al., 2013). Recently, a CHO cell line stably expressing hTREK-1, named as CHO/hTREK-1 cells, was generated to study the effect of overexpression of TREK-1 on cell proliferation (Zhang, Yin, Wang, Li, & Wang, 2016). The design of the hTREK-1/HEK cell line was also of great importance for research and screening of new and specific TREK-1 inhibitors with AD properties (Borsotto et al., 2015; Djillani et al., 2017; Moha Ou Maati et al., 2012; Veyssiere et al., 2015).

2.3. Small molecule inhibitors of TREK-1

2.3.1. Selective serotonin reuptake inhibitors

The monoaminergic hypothesis of depression has driven a number of research laboratories to search for new molecules that block 5-HT reuptake. Fluoxetine was approved as an AD in 1986, and clinical trials showed a clear improvement in adverse effects previously observed with tricyclics (Perez-Caballero, Torres-Sanchez, Bravo, Mico, & Berrocoso, 2014). The AD activity was attributed to the high-affinity blockade of SERT, thereby leading to an increase in the amount of 5-HT in the synapses (Wong, Horng, Bymaster, Hauser, & Molloy, 1974).

At clinical concentrations, TREK-1 channels were significantly blocked by fluoxetine and its active metabolite norfluoxetine in a concentration-dependent manner, and the IC_{50}s were 19 μM and 9 μM, respectively (Heurteaux, Lucas, et al., 2006b; Kennard et al., 2005) (Table 1). TREK-1 inhibition seems to be state dependent because norfluoxetine binds to the fenestration in TREK-1 that is only available in the down state (when the lower sections of M2, M3, and M4 transmembrane domains project into the cytoplasm) corresponding to the channel lower activity (Dong et al., 2015). However, when TREK-1 is activated by stretch or AA, the channel conformation changes to the up state (when lower sections of M2, M3, and M4 transmembrane domains project into the membrane), which suppresses the fenestration and does not allow norfluoxetine to bind (Dong et al., 2015; Kennard et al., 2005). In a recent study, other SSRIs have been shown to be potent blockers of TREK-1 and TREK-2 channels in HEK-293 cells and HT-22 neuronal cells. In addition to fluoxetine, TREK-1 inhibition was also observed with paroxetine and citalopram, two other commonly prescribed AD drugs (Kim, Lee, Hong, Han, & Kang, 2017) (Table 1).

2.3.2. Other inhibitors of TREK-1 channels

Few molecules were identified in the literature as potent TREK-1 channel blockers (Table 1). SID1900, a small molecule screened from a large library of 487 compounds, has been shown to block TREK-1 channels in a manner similar to that of spadin and to induce an AD-like behavior in a rat model of chronic unpredictable mild stress (Ye et al., 2015). SID1900 inhibits TREK-1 with an IC_{50} of 29.72 μM, which largely exceeds that obtained with spadin (peptide #1 Table 2) (IC_{50} = 40 nM) (Djillani et al., 2017). Furthermore, antipsychotic drugs have been shown to be modulators of TREK-1 channels (Thummler, Duprat, & Lazdunski, 2007). Typical and atypical antipsychotics such as fluphenazine, chlorpromazine, haloperidol, loxapine, and clozapine inhibit TREK-1 and TREK-2 channels in a dose-dependent manner without affecting TRAAK channels (Kim et al., 2017; Thummler et al., 2007) (Table 1). In contrast to antipsychotic drugs, mood stabilizers such as lithium chloride, valproate, gabapentin, and carbamazepine activate TREK-1 channels but have no effect on TREK-2 channels (Kim et al., 2017). Dihydropyridine Ca^{2+} channel antagonists such as amlodipine and niguldipine are nonspecific blockers of TREK-1 channels (Table 1). They inhibit TREK-1 channels with an IC_{50} of 0.43 and 0.75 μM, respectively (Liu, Enyeart, & Enyeart, 2007). *In vitro*, they potently block calcium-induced vascular smooth muscle contractions with IC_{50} of 1.9 and 4.1 nM for amlodipine and nifedipine, respectively (Burges et al., 1987). In addition, L-methionine was reported to decrease the probability of TREK-1 opening in a cell-attached patch-clamp configuration (Baker et al., 2008; Lei et al., 2014) (Table 1). This amino acid was used as a tool to investigate the role of TREK-1 in uterine contraction (Yin et al., 2018) and in controlling bladder smooth muscle cell excitability following contraction (Baker et al., 2008). However, L-methionine inhibition on TREK-1 has been shown to have contrast in another study where no TREK-1 inhibitory effect was observed in rat colon smooth muscle cells after activation with AA (Gil et al., 2012). The neuroprotective compound 3-*n*-Butylphthalide (NBP) and its racemic form *dl*-NBP were reported to block TREK-1 (Ji, Zhao, Cao, Shi, & Wang, 2011). Recently, another NBP analog, named as lig4-4, was described as a specific blocker of TREK-1 (Wang et al., 2018). The authors presumed that the neuroprotective effects of lig4-4 observed in ischemic stroke might be related to TREK-1 inhibition (Wang et al., 2018). This hypothesis shows total contradiction to data published >10 years ago that showed that the opening of TREK-1 is neuroprotective (Heurteaux, Laigle, Blondeau, Jarretou, & Lazdunski, 2006a). However, neither NBP nor lig4-4 can be considered as specific blockers for TREK-1 because lig4-4 also inhibits hERG, K_v1.5, K_v2.1, K_v3.1, and neuronal Na^+ and Ca^{2+} channels (Wang et al., 2018).

2.4. Birth of an idea…

Approximately 10 years ago, TREK-1 was described as a new target in depression (Heurteaux, Lucas, et al., 2006b). This K_{2P} mechanosensitive channel is mainly expressed in the prefrontal cortex and the hippocampus (Heurteaux, Lucas, et al., 2006b; Medhurst et al., 2001). These regions are known to mediate cognitive aspects of depression, such as memory impairment, feeling of worthlessness, guilt, and suicidality (Nestler et al., 2002; Otte et al., 2016). TREK-1 is also expressed in the amygdala, hypothalamus, and in the striatum, particularly in the nucleus accumbens to mediate memory of emotional events. Finally, TREK-1 is abundant in GABA (γ-aminobutyric acid)-containing neurons of the caudate nucleus and putamen (Hervieu et al., 2001) and in hippocampal glutamatergic neurons (Medhurst et al., 2001). Investigation of the role of TREK-1 channel in the physiopathology of depression by using the knockout of *Kcnk2* (the gene coding for TREK-1 channel) in mice demonstrated a depression-resistant phenotype (Heurteaux, Lucas, et al., 2006b) in five assays for depression-like behaviors (Cryan & Holmes, 2005): Tail Suspension Test (TST), Forced Swim Test (FST), Conditioned Suppression of Motility Test (CSMT), Learned Helplessness Test (LHT), and Novelty-Suppressed Feeding Test (NSF). In TREK-1-deficient mice, neurogenesis induced by the well-known SSRI fluoxetine was significantly increased compared to the wild-type mice (Heurteaux, Lucas, et al., 2006b). However, the proliferation of newborn cells manifested only after 21 days of treatment.

Table 1
Molecules described as TREK-1 blockers.

TREK-1 blockers		Cell types	IC_{50}	Specificity	Antidepressant activity	Onset of action	Reference
SSRIs	Fluoxetine	HEK 293	19 μM 37.9 ± 7.7 μM	Blocks TREK-2 (IC_{50} = 28.7 ± 7.6 μM), SERT and TASK-3	FST (acute and chronic – 14-21 days) No effect after 3 day subchronic treatment	Slow 2–4 weeks	(Detke, Rickels, & Lucki, 1995; Kennard et al., 2005; Kim et al., 2017; Wong et al., 1974)
	Norfluoxetine	HEK 293	9 μM	Blocks TREK-2 (IC_{50} = 4.9 ± 0.5 μM)	nd	nd	(Kim et al., 2017; Kobayashi, Washiyama, & Ikeda, 2006; Lee, Chai, Hahn, & Choi, 2018; McClenaghan et al., 2016; Thummler et al., 2007)
	Paroxetine	COS-7	20 μM tested	Blocks TREK-2 (at 20 μM tested), SERT, GIRK and Kv3.1	FST (acute)	Slow 2–4 weeks	
	Citalopram	HEK 293	100 μM tested	Blocks TREK-2 (at 100 μM tested), SERT, Kv1.5 (IC_{50} = 2.8 ± 1.1 μM) and L-type Ca^{2+} channels (IC_{50} = 60.3 ± 8.5 μM)	nd	Slow 2–4 weeks	(Hamplova-Peichlova et al., 2002; Kim et al., 2017; Lee, Hahn, & Choi, 2010)
Spadin and analogs	Spadin (PE 12–28)	**Cos-7, HEK293**	**40 nM**	Specific to TREK-1 No effect on TREK-2, TRAAK, TRESK, TASK-1, and hERG channels	FST (acute and subchronic), TST, LHT, and NSF (subchronic)	**Fast only after 4 days**	(Mazella et al., 2010; Moha Ou Maati et al., 2012) (Djillani et al., 2017)
	PE 22–28	**HEK 293**	**0.12 nM**				
	G/A-PE 22–28	**HEK 293**	**0.10 nM**				
	Biotin-G/A-PE 22–28	**HEK 293**	**1.2 nM**				
Antipsychotics	Fluphenazine	Cos-7	4.7 μM	Block TREK-2 (at 10 μM tested), no effect on TRAAK at 10 μM	nd	nd	(Thummler et al., 2007)
	Chlorpromazine	Cos-7	2.7 μM				
	Haloperidol	Cos-7	5.5 μM				
	Flupenthixol	Cos-7	2.0 μM				
	Loxapine	Cos-7	19.7 μM				
	Pimozide	Cos-7	1.8 μM				
	Clozapine	Cos-7	10 μM tested				
Dihydropyridine Ca^{2+} channel antagonists	Amlodipine	AZF cells (adrenal gland)	0.43 μM	Block L-type Ca^{2+} channels	nd	nd	(Liu et al., 2007)
	Niguldipine	AZF cells (adrenal gland)	0.75 μM		nd	nd	
Other TREK-1 blockers	SID1900	HEK 293	29.72 μM	nd	Rat model of CUMS, 14 day and 21 day FST, Sucrose preference (14 d and 28 d)	2 weeks	(Ye et al., 2015)
	L-methionine	Bladder smooth muscle cells	1 mM (controversial)	nd	nd	nd	(Lei et al., 2014)
	l-NBP, *d*-NBP, *ld*-NBP	CHO	0.06 ± 0.03 μM	nd	nd	nd	(Ji et al., 2011)
	lig4–4	CHO	2.06 μM	Blocks K_v2.1, K_v1.5, K_v3.1, hERG and neuronal Na^+ and Ca^{2+} channels (IC_{50} = 30 μM)	nd	nd	(Wang et al., 2018)

On the other hand, deletion of the *Kcnk2* gene enhanced the firing of 5-HT neurons in the dorsal raphe nucleus (Heurteaux, Lucas, et al., 2006b). Knowing the importance of 5-HT in the neurobiology of depression "Monoaminergic Theory of Depression" (Duman, Heninger, & Nestler, 1997) and because TREK-1 is inhibited by SSRIs such as fluoxetine, this K_{2P} channel has been considered as a serious candidate to play a key role in the physiopathology of depression (Heurteaux, Lucas, et al., 2006b).

In humans, Star*D study has identified an association between the existence of four genetic variants (single nucleotide polymorphisms [SNPs]) in the TREK-1 locus and resistance to multiple AD classes (Perlis et al., 2008). Another study showed that an SNP at the 3′-untranslated region on exon 7 of the kcnk2 gene could be associated with both depression incidence and poor treatment efficacy (Liou et al., 2009). Although no brain imaging studies in depressive patients have been published yet, a study suggested that some TREK-1 genotypes in humans can be associated with a depression-resistant phenotype (Dillon et al., 2010). Taken together, these studies in humans strengthen the idea that TREK-1 represents a crucial target in the field of depression and the search for selective blockers of TREK-1 might potentially lead to a new generation of AD drugs.

2.5. SORTing TREK-1...

Within the neuron, the TREK-1 channel forms a complex made of the A-kinase-anchoring protein AKAP150 (Sandoz et al., 2006) and the microtubule-associated protein Mtap2 (Sandoz et al., 2008). Both proteins regulate the sorting of TREK-1 to the plasma membrane. However, these partner proteins are not unique. In 2010, another crucial interacting protein, named as sortilin, was discovered. Sortilin regulates, transports, and targets the TREK-1 channel to the plasma membrane where it exerts its role as a background potassium channel (Mazella et al., 2010). Two decades ago, sortilin, a 95 kDa protein, was identified as a sorting molecule in the human brain (Petersen et al., 1997). It was also described shortly later in another study as the neurotensin receptor-3 (NTSR-3) (Mazella et al., 1998). Sortilin/NTSR-3 binds a number of ligands such as neurotensin (NT), precursor of the nerve growth factor (proNGF) (Nykjaer et al., 2004), lipoprotein lipase (Nielsen, Jacobsen, Olivecrona, Gliemann, & Petersen, 1999), and propeptide (PE) (Munck Petersen et al., 1999). In the Golgi network, the post-translational cleavage of prosortilin (precursor of sortilin) by the protein convertase furin results in mature sortilin and the release

Table 2
Summary of effects of spadin analogs on TREK-1 inhibition and FST immobility time.

Peptide number	Peptide names	Modifications	% of TREK-1 inhibition	Significance	FST in acute (s)	Significance
1	**PE 12-28 (Spadin)**	**No modification**	**87.08 ± 7.32**	***	**107.40 ± 5.05**	***
2	Ac-PE 12-28	*N*-acetylation	83.46 ± 8.33	***	nd	nd
3	Ac-RI-PE 12-28	retro-inverso of spadin	94.43 ± 10.87	***	135.10 ± 8.11	**
4	Ac-PE-22-28	*N*-acetylation	19.29 ± 25.7	ns	nd	nd
5	Ac-PE-21-28	*N*-acetylation	26.89 ± 14.9	ns	nd	nd
6	Ac-RI-PE-21-28	*N*-acetylation + retro-inverso	31.19 ± 17.8	ns	nd	nd
7	Ac-PE 1-28	*N*-acetylation	10.43 ± 28.5	ns	nd	nd
8	Ac-RI-PE 1-28	*N*-acetylation + retro-inverso	108.59 ± 10	***	83.60 ± 9.01	***
9	Ac-PE 6-28	*N*-acetylation	71.85 ± 28.12	**	nd	nd
10	Ac-RI-PE 6-28	*N*-acetylation + retro-inverso	56.47 ± 24.13	*	nd	nd
11	Ac-PE 1-44	*N*-acetylation	26.97 ± 6.25	ns	nd	nd
12	Ac-RI-PE 1-44	*N*-acetylation + retro-inverso	37.73 ± 8.77	ns	nd	nd
13	G/A-PE 12-28	aa substitution	83.5 ± 9.76	***	120.4 ± 9.7	**
14	RI-G/A-PE 12-28	retro-inverso +aa substitution	72.15 ± 11.75	***	127.7 ± 8.32	**
15	c(RI-PE 12-28)	cyclization	67.24 ± 3.41	***	157 ± 8.09	ns
16	$c(RI\text{-}PE\ 12\text{-}28)_2$	cyclization	91.25 ± 8.14	***	146.7 ± 12.99	ns
17	PE 12-27	No modification	28.39 ± 9.916	**	100.2 ± 5.0	***
18	PE 14-25	No modification	0	ns	112.2 ± 7.1	**
19	PE 22-27	No modification	25.7 ± 20.01	ns	168.2 ± 4.2	ns
20	PE 22-25	No modification	36.02 ± 17.47	*	100.2 ± 5.0	***
21	**PE 22-28**	**No modification**	**55.46 ± 4.555**	***	**91.8 ± 6.1**	***
22	Biotin-PE 22-28	N-biotinylation	53.03 ± 6.416	***	112.1 ± 4.3	***
23	Dansyl-PE 22-28	N-dansylation	48.78 ± 14.52	**	104.6 ± 11.8	***
24	PE 22-28-O-Methyl	C-methoxylation	42.98 ± 13.47	**	137.1 ± 8.1	*
25	PE 22-28-O-Ethyl	C-ethoxylation	41.39 ± 11.52	**	113.2 ± 8.5	***
26	Formyl-PE 22-28	N-formylation	32.45 ± 12.22	*	nd	nd
27	**G/A-PE 22-28**	**aa substitution**	**50.61 ± 7.935**	***	**110.2 ± 3.6**	***
28	**Biotin-G/A-PE 22-28**	**aa substitution + N-biotinylation**	**46.11 ± 7.743**	***	**140.7 ± 7.1**	*
29	PI-PE 22-28	No modification	46.19 ± 7.565	***	119.7 ± 11.8	**
30	Biotin-PI-PE 22-28	N-biotinylation	49.11 ± 7.454	***	124.1 ± 11.7	**
31	Palmitoyl-PE 22-28	N-palmitoylation	26.69 ± 16.45	ns	nd	nd
32	FITC-PE 22-28	N-FITC group	22.1 ± 12.63	ns	nd	nd
33	Acetyl-PE 22-28	*N*-acetylation	20.49 ± 8.777	*	nd	nd
34	Myristoyl-PE 22-28	N-myristoylation	18.04 ± 17.77	ns	nd	nd
35	LC biotin-PE 22-28	N-long chain biotinylation	15.86 ± 11.21	ns	nd	nd
36	5'FAM-PE 22-28	N-5'FAM group	6.633 ± 7.065	ns	nd	nd
37	FMoc-PE 22-28	N-Fmoc group	5.826 ± 10.91	ns	nd	nd
38	Stearic acid-PE 22-28	N-stearic acid group	5.412 ± 5.496	ns	nd	nd

of a 44-amino acid peptide named as PE (Fig. 1) (Munck Petersen et al., 1999).

Sortilin/NTSR-3 co-localizes with TREK-1 in numerous brain areas involved in mood, such as the prefrontal cortex, hippocampus, striatum, amygdala, and hypothalamus (Mazella et al., 2010). In addition, physical interaction between TREK-1 and sortilin/NTSR-3 was characterized by pull down experiments. It has been showed that TREK-1 expression at the plasma membrane is greatly enhanced in the presence of sortilin/NTSR-3 (Mazella et al., 2010). Although present in low amounts at the plasma membrane in the absence of sortilin, TREK-1 channels can be modulated by the different effectors described above.

Furthermore, an interesting observation from our team showed that sortilin/NTSR-3-deficient mice represent a depression-resistant phenotype similar to the behavior of *kcnk2*$^{-/-}$ mice (Moreno et al., 2018). Arguments cited above support the involvement of both TREK-1 and sortilin/NTSR-3 in the physiopathology of depression.

3. Discovery of SPADIN

3.1. Sortilin-derived peptide with antidepressant properties

PE is a 44-amino acid peptide that binds with high affinity (Kd ~20–30 nM) to the mature sortilin/NTSR-3 (Munck Petersen et al., 1999). The peptide sequence required for the binding of PE to sortilin/NTSR-3 was identified as Gln^1-Arg^{28} (Fig. 1). Moreover, the PE fragment corresponding to Gln^1-Arg^{16} had lower affinity in binding the mature sortilin (Westergaard et al., 2004). In this study, a 17-amino acid-containing peptide was designed and named as spadin, acronym generated from Sortilin-derived Peptide with Antidepressant properties. Spadin contains the main fragment of PE, Trp^{17}-Arg^{28}, capable of binding to sortilin/NTSR-3 and stabilized by the sequence Ala^{12}-Pro^{13}-Leu^{14}-Pro^{15}-Arg^{16} added upstream to finally generate a 17-amino acid peptide corresponding to the sequence Ala^{12}-Arg^{28} or PE 12-28 (Fig. 1, Table 2, **peptide #1**) (Mazella et al., 2010).

PE has been shown to antagonize the effects of NT on cell migration of the human microglia cell line C13NJ that expresses the NTSR-3 subtype, exclusively (Martin, Vincent, & Mazella, 2003). Similarly to PE, spadin (PE 12-28) is able to bind with an identical affinity (Kd = 8 nM) to sortilin/NTSR-3 (Mazella et al., 2010). The similarity between PE and spadin to bind to sortilin/NTSR-3 is extended to their functional properties. For example, spadin totally blocks cell migration by displacing NT from the binding site of sortilin/NTSR3 (Mazella et al., 2010).

Given the role of TREK-1 channels in depression (Heurteaux, Lucas, et al., 2006b), the question that arose was to determine whether PE or spadin could regulate TREK-1 channels and consequently generate a depression-resistant phenotype in mice. To answer this question, spadin was tested on TREK-1-expressing COS-7 cells in electrophysiological experiments. In the patch-clamp technique using whole-cell configuration, TREK-1 was first activated by 10 μM of AA, and then, when spadin was applied extracellularly, TREK-1 was potently blocked by spadin with an IC_{50} of 70.7 nM (Tables 1 and 2) (Mazella et al., 2010). TREK-1 blockade was confirmed on other type of cell lines such as the hTREK-1/HEK (IC_{50} = 40 nM) or the pancreatic β-TC3 cells (Djillani et al., 2017; Mazella et al., 2010). The direct blocking of TREK-1 channel activity was demonstrated on the h-TREK-1/HEK cell line by using excised patches in an inside-out configuration (Gil et al., 2012). On brain slices, endogenous TREK-1 currents recorded in the hippocampal CA3

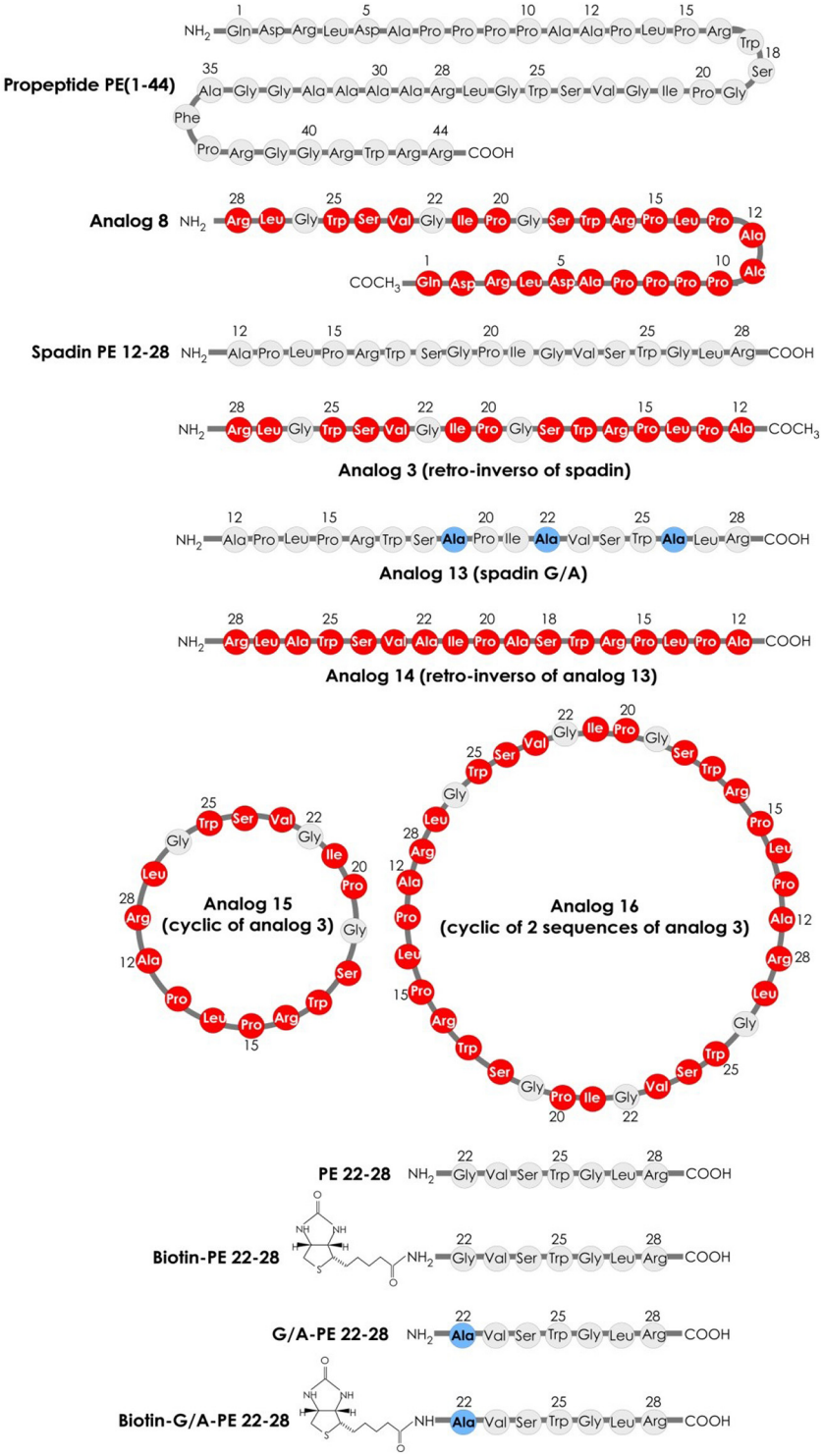

Fig. 1. Spadin analog sequences. Peptide sequences designed from the parent molecule spadin using the RI strategy, peptide cyclization and shortening sequence. The peptidic sequences are shown as three-letter nomenclature and numbered according to the sequence of PE (1–44). Amino-acids in L-configuration are shown in grey circles while amino-acids in D-configuration (inverso aa) are represented as red circles. Replacement of glycine residue by an alanine is shown by blue circles.

pyramidal cells were also activated by AA and strongly blocked by spadin (Mazella et al., 2010). More interestingly, on hippocampal brain slices, the spadin effect totally disappeared in TREK-1-deficient mice, which confirms TREK-1 as a target for spadin (Mazella et al., 2010).

TREK-1 is known to be highly expressed in the dorsal raphe nucleus (DRN) (Medhurst et al., 2001) and *kcnk2*$^{-/-}$ mice showed an enhanced serotonergic neurotransmission (Heurteaux, Lucas, et al., 2006b). To address the question whether pharmacological blockade of TREK-1 by spadin affects 5-HT neurotransmission, mice were given a 10 μM dose of spadin as an i.p injection and the unitary extracellular activity of serotonergic neurons was recorded in anesthetized animals. Data showed that spadin potentiates 5-HT neurotransmission firing *in vivo* (Mazella et al., 2010), similar to the activity of the 5-HT neurons in *kcnk2*$^{-/-}$

mice (Heurteaux, Lucas, et al., 2006b). To investigate further, we addressed the question whether blocking TREK-1 and increasing 5-HT firing in the DRN by spadin could have an impact on mouse behavior. Spadin was administered to mice in acute and subchronic treatments and AD activity was assessed using several mouse models of depression. Spadin displays AD properties in mice in depression-like behavior tests (FST, TST, CSMT, LHT, and NSF). Moreover, spadin treatments generate two canonical effects of AD drugs: the increase in 5-HT neurotransmission and the induction of neurogenesis (Mazella et al., 2010). These results confirmed the previous data obtained with *Kcnk2*$^{-/-}$ mice, whose main phenotype is resistance to depression (Heurteaux, Lucas, et al., 2006b). In addition, subchronic treatment with spadin results in increasing CREB activation and hippocampal neurogenesis. This effect is outstanding, as it occurs only after a 4 day treatment in contrast to SSRIs that need 3–4 weeks of treatment to increase hippocampal neurogenesis (Mazella et al., 2010).

Moreover, *in vitro* (in cortical neurons) and *in vivo* (in the hippocampus), studies showed that spadin increases both mRNA and protein expression of markers of synaptogenesis, such as the post-synaptic density protein-95 (PSD-95) or synapsin (Devader et al., 2015). These data indicated that spadin increases the number of functional neurons. This observation was supported by the fact that spadin treatment significantly increases the number of mature spines on axons (Devader et al., 2015).

Taken together, these data confirmed that the newly generated neurons by spadin are indeed functional and able to interact within the neuronal complex network.

3.2. Spadin is a specific and safe blocker of TREK-1

Despite the high homology in structure, function, and regulation between TREK-1, TREK-2, and TRAAK channels (Lesage & Lazdunski, 2000), spadin specifically blocks TREK-1, as no effect is observed on the other stretch-activated K_{2P} channels such as TREK-2 and TRAAK. TASK-1 and TRESK, two other members of the K_{2P} channel family, are also insensitive to spadin (Table 1) (Moha Ou Maati et al., 2012). As TASK-1 is involved in inflammation (Bittner et al., 2009) and apoptosis (Lauritzen et al., 2003; Leithner et al., 2016) and TRESK in migraine (Lafreniere & Rouleau, 2011) and pain (Marsh, Acosta, Djouhri, & Lawson, 2012; Tulleuda et al., 2011), the absence of spadin effect on these channels might be beneficial.

TREK-1 was also shown to play an important role in pain perception (Alloui et al., 2006). *Kcnk2*$^{-/-}$ mice are more sensitive to thermal pain (Alloui et al., 2006). Here again, spadin is unable to modify pain perception in tail flick and hot plate tests (Moha Ou Maati et al., 2012).

Undoubtedly, TREK-1 plays a crucial role in the regulation of neuronal excitability (Heurteaux et al., 2004). TREK-1-mediated neuroprotection against epilepsy and cerebrovascular diseases is not affected by spadin treatment. Spadin does not increase pentylenetetrazol (PTZ) or kainate-induced seizures (Moha Ou Maati et al., 2012). Furthermore, a 3-week spadin treatment does not increase the infarct size after focal ischemia (Moha Ou Maati et al., 2012).

Finally, at the cardiovascular level, long-term treatment by spadin has no effect on systolic blood pressure and heart pulses (Moha Ou Maati et al., 2012).

One of the major challenges in the development of safe drugs is the early detection of prolongation of the QT interval, which causes Torsades de pointes (TdP). Long QT-inducing drugs block two types of potassium currents: the rapid (I_{Kr}) and the slow (I_{Ks}) potassium currents (Cheng & Kodama, 2004; Sanguinetti & Jurkiewicz, 1990). I_{Kr} current is carried by the hERG channel. However, I_{Ks} activity requires the association of KCNQ1 with KCNE1 (Barhanin et al., 1996; Sanguinetti et al., 1996). As I_{Kr} and I_{Ks} are essential components for normal cardiac function, drug-acquired QT interval prolongation due to hERG inhibition causes an increase in sudden death (Brown, 2004). Consequently, several cardiovascular and non-cardiovascular drugs have been withdrawn from the market (Finlayson, Witchel, McCulloch, & Sharkey, 2004). Presently, all drug candidates should be tested *in vitro* to check whether or not they inhibit hERG channels (Chen, Sampson, & Kass, 2016). With the aim to develop spadin as an approved drug in the treatment of depression, this peptide was tested for a possible hERG channel inhibition. Spadin does not affect hERG channel biophysical properties, as the use of 10 μM or higher concentrations of spadin do not show any modifications of I_{Kr} or I_{Ks} (Djillani et al., 2017; Moha Ou Maati et al., 2012). Taken together, these observations confirm spadin as a safe molecule for further use in clinic. The potent AD properties of spadin have given rise to the patent published under the no. US8252748B2 (Mazella, Petrault, Borsotto, Heurteaux, & Widmann, 2012).

3.3. Increasing spadin efficacy and in vivo bioavailability

In FST, spadin AD activity lasts for 7 h after a single i.p injection (Veyssiere et al., 2015). To improve *in vivo* stability of spadin and prolong AD activity beyond 7 h after an acute treatment, different strategies have been thoroughly considered, such as the retro-inverso (RI) strategy, peptide cyclization, amino acid replacement, protection of C– and N-terminal ends of the peptides, and finally shortening of the spadin sequence. The RI strategy consists of changing the amino acid configuration from L to D. At the same time, the amino acid sequences are inverted. RI technology was shown in many studies to increase the resistance of peptides to proteolysis and thus improve their bioavailability in the blood (Chorev & Goodman, 1995; Chorev, Shavitz, Goodman, Minick, & Guillemin, 1979). Eleven spadin analogs including RI spadin analogs were synthesized and screened on the hTREK-1/HEK cell line by the patch-clamp technique (Table 2, **peptides #2 to #12**) Two RI analogs were identified: analog 3 (Ac-RI-PE 12-28, peptide # 3) and 8 (Ac-RI-PE 1-28, peptide # 8) (Fig. 1, Table 2). They display a better inhibition potency for TREK-1 channel activity, IC_{50} were 11.5 ± 0.59 nM and 9.95 ± 0.85 nM for analog 3 and analog 8, respectively, compared to 56.39 ± 0.01 nM for spadin (Veyssiere et al., 2015).

The analogs 3 and 8 share the same AD properties with spadin after acute or subchronic treatments. Similar to spadin, analogs 3 and 8 significantly reduce the immobility time in the FST (Veyssiere et al., 2015). Moreover, in the NSF, the two RI analogs shorten the latency time for eating in mice. More interestingly, these analogs induce hippocampal neurogenesis.

One of the challenges in terms of development is to improve the *in vivo* stability of spadin. RI analogs prolong the AD activity by three times compared to spadin (Veyssiere et al., 2015). Specifically, analog 3 does not produce any adverse effects on pain, epilepsy, or arrhythmia. RI analogs including analog 3 and analog 8 were patented for the treatment of depression under the number WO2015110915A2 (Gaudriault et al., 2015).

Other analogs were designed by the RI strategy, such as analog 13 (G/A-PE 12-28, Table 2 peptide #13), analog 14 (RI-G/A-PE 12-28, Table 2 peptide #14), analog 15 (c(RI-PE 12-28), Table 2 peptide #15), and analog 16 (a tandem of c(RI-PE 12-28), Table 2 peptide #16). The peptide sequences are depicted in Fig. 1.

Analog 13 contains the same sequence as spadin, but the three glycine residues were replaced by three alanine residues. Analog 14 is the RI of analog 13. Both analogs 13 and 14 are able to strongly inhibit TREK-1 channels (83.50% ± 9.76%, $n = 8$, $p = 0.94$), (72.15% ± 11.75%, $n = 9$, $p = 0.36$), respectively, compared to spadin (87.08% ± 7.32%, n = 8) (Fig. 2 **a, b**). More interestingly, both analogs conserve their AD activity in FST when injected intravenously (i.v.). The immobility time is significantly decreased (120.4 ± 9.7 s, $n = 10$, $p = 0.0049$ and 127.7 ± 8.32 s, n = 10, $p = 0.0105$, respectively) for analogs 13 and 14 compared to saline (160.6 ± 7.97 s, n = 10) (Fig. 3a).

Peptide cyclization is one of the common strategies used to increase peptide stability and efficacy (Adessi & Soto, 2002). It has been shown that cyclization could increase peptide resistance against proteolytic degradation that subsequently enhances the peptide bioavailability

(Wang et al., 2014). Therefore, the strategy consisted of cyclizing analog 3 alone (analog 15, Table 2 peptide #15) or in tandem (analog 16, Table 2 peptide #16) sequence (Fig. 1) to study the impact of the cyclization on peptides crossing the blood–brain barrier (BBB). Using the whole-cell configuration of the patch-clamp technique, both analogs 15 and 16 inhibit TREK-1 current with different potencies (67.24% ± 3.41%, n = 9, $p = 0.046$ and 91.25% ± 8.14%, n = 10, $p = 0.67$), respectively (Fig. 2). However, in the FST, when both analogs are administered through the i.v. route, neither analog 15 nor analog 16 is able to produce a significant decrease in immobility time (157 ± 8.09 s, n = 10, $p = 0.755$ and 146.7 ± 12.99 s, n = 10, $p = 0.374$, respectively) compared to saline (160.6 ± 7.97 s, n = 10) (Fig. 3a). It appears that the lack of activity of analog 15 is due to its inability to cross the BBB, as it displays AD activity only when injected directly in the brain by an intracerebroventricular route (i.c.v.) (Fig. 3b).

3.4. Shortened spadin analogs with antidepressant activity

Spadin blood degradation products revealed by high-pressure liquid chromatography (HPLC) consist of at least two short peptides PE 12–27 (Table 2, peptide #17) and PE 14–25 (Table 2, peptide #18) (Djillani et al., 2017). On the basis of this analysis, several short analogs were designed and screened on the hTREK-1/HEK cell line. PE 22–28 (Table 2, peptide #21) is the shortest, most efficient sequence capable of blocking the TREK-1 channel with higher potency ($IC_{50} = 0.12$ nM *versus* $IC_{50} =$ 40 nM for spadin (PE 12–28)) (Fig. 1). Then, PE 22–28 (peptide #21) was used as the core peptide to design other analogs with different N- and C-terminal end modifications. These PE 22–28 analogs abolish or maintain TREK-1 inhibition on the basis of the nature of the chemical group attached to C or N-terminus (Table 2) (Djillani et al., 2017).

With PE 22–28, two other analogs were retained for further studies, as they inhibit the TREK-1 channel with a higher potency. They correspond to the PE 22–28, where Gly^{22} is replaced by an Ala^{22} (G/A-PE 22–28, Table 2 peptide #27) and its biotinylated derivative (biotin-G/A-PE 22–28, Table 2 peptide #28) (Fig. 1). Their TREK-1-inhibiting potencies are $IC_{50} = 0.10$ nM and $IC_{50} = 1.2$ nM for G/A-PE 22–28 and biotin-G/A-PE 22–28, respectively (Djillani et al., 2017). This represents, respectively, a 400- and 33-fold increase in TREK-1 inhibition potency in comparison to spadin.

Current blockade is specific to TREK-1, as TREK-2, TRAAK, TRESK, and TASK-1 channels are not inhibited by these shortened peptides. How about their ability to counteract depression behavior? Does shortening spadin sequence and replacing Gly^{22} by Ala^{22} have any consequences on mice behavior? To answer these questions, a series of behavioral studies on mice treated with short spadin analogs were performed (Djillani et al., 2017).

These candidate peptides display AD properties after acute and 4 day subchronic treatments in FST, NSF, and LHT. More interestingly, similar to spadin, they produce an AD-like behavior regardless of the route of administration. They efficiently reduce the immobility time in the FST after i.p, i.v., and gavage administration (Djillani et al., 2017).

Similar to spadin, hippocampal neurogenesis is increased as revealed by BrdU (5-Bromo-2′-Deoxyuridine) labeling with a prominent effect of G/A-PE 22–28 (Table 2, peptide #27). At the same time, neurogenesis induced by spadin short analogs is consistent with the increase in PSD-95 expression, a marker of synaptogenesis (Djillani et al., 2017).

One of the most important effects observed with short spadin analogs is the improvement in terms of *in vivo* stability compared to spadin. Indeed, G/A-PE 22–28 (21*h*) and biotin-G/A-PE 22–28 (23*h*) prolong significantly the action duration of spadin (only 7 h) as revealed by FST (Djillani et al., 2017). This is extremely promising and interesting, as the ultimate goal in the near future is to test them in clinical trials and eventually commercialize these long-lasting peptides as ADs of a new generation (Fig. 4).

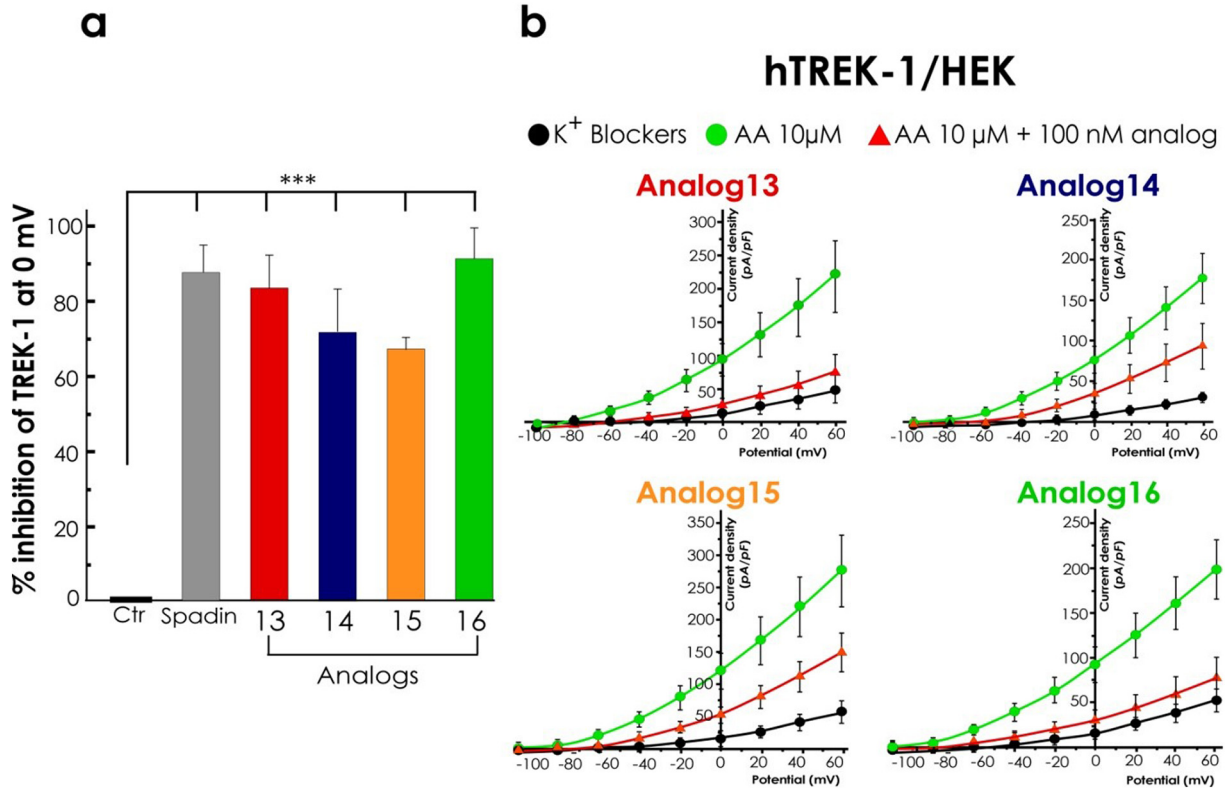

Fig. 2. Spadin analogs display strong TREK-1 inhibition. Spadin analogs named analog 13, analog 14, analog 15 and analog 16 were screened on the hTREK-1/HEK cell line for their ability to block TREK-1 channels. **a.** Following activation by 10 µM AA, TREK-1 inhibition by 100 nM spadin analogs was measured at 0 mV using the whole-cell configuration of the patch-clamp technique in voltage-clamp mode. **b.** I/V curves obtained with spadin analogs and generated by a ramp protocol from −100 mV to +60 mV with a holding potential at −80 mV. *, $p < 0.01$, ***, $p < 0.001$.

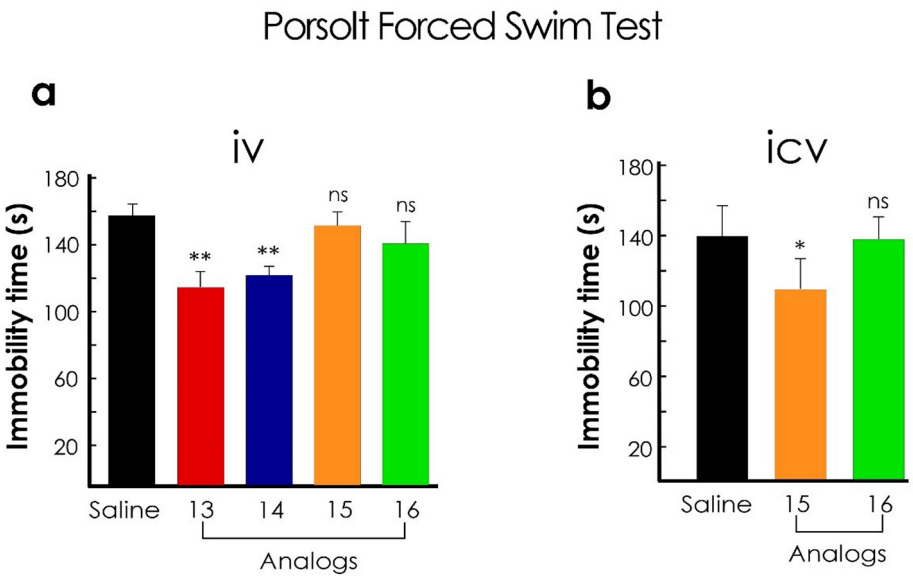

Fig. 3. Antidepressant activity of spadin analogs in the Forced Swim Test. a. In the FST, when administered intravenously, analog 13 and analog 14 significantly decreased the immobility time in mice compared to saline injected mice (control). However, analog 15 and analog 16 were unable to display AD-like behavior when i.v injected. **b.** Intracerebroventricular route (i.c.v) demonstrated that analog 15 did not cross the BBB while analog 16 remains inefficient in reducing the immobility time in mice. *, $p < 0.05$; **, $p < 0.01$; ns, not significant. *Methods*. 30 min before the test, animals were injected with the different spadin's analogs by using either the intraveinous route (iv) or the intracerebroventricular route (icv). Mice were individually placed for 6 min in a non-escapable cylinder (30 cm height and 15 cm diameter) half-filled with water at 22 °C ± 1 °C. The immobility time was manually measured only during the last 4 min. A mouse was considered immobile when it remained immobile with only slight movements in order to keep its head above water (Porsolt, Le Pichon, & Jalfre, 1977). Statistical comparisons were performed using ANOVA one-way.

However, for the clinical development of any molecule, whether a small drug or a therapeutic peptide, it should be screened to detect any change in the QT interval. PE 22–28 and analogs do not modify hERG channel activity, which render them more specific and safer for a possible further pharmaceutical development (Djillani et al., 2017).

3.5. Spadin and analogs as biomarkers of major depressive disorder

To clinically predict patients with major depressive disorder (MDD) who respond to classical AD treatment and routinely monitor AD activity, there is a pressing need to validate biomarkers for MDD. This will allow an inexpensive diagnosis and predictive test for an MDD response to facilitate patient follow-up (Woods, Iosifescu, & Darie, 2014). Thus far, there has been no validated biomarker for MDD, and clinical diagnosis is only symptomatic. Recently, various candidates as biomarkers for MDD were described in the literature, such as inflammatory mediators, growth factors, neurotransmitters, neurotrophic factors, and metabolic biomarkers (Gururajan, Clarke, Dinan, & Cryan, 2016; Strawbridge, Young, & Cleare, 2017). Many preclinical studies have pointed out the crucial role of BDNF in the physiopathology of MDD, as BDNF serum levels are lower in patients with MDD than in healthy volunteers or MDD-medicated patients (Aydemir et al., 2006; Aydemir, Deveci, Taskin, Taneli, & Esen-Danaci, 2007; Gervasoni et al., 2005; Gonul et al., 2005; Huang, Lee, & Liu, 2008; Karege et al., 2005; Monteleone, Serritella, Martiadis, & Maj, 2008; Piccinni et al., 2008; Shimizu et al., 2003; Yoshimura et al., 2007).

It was already demonstrated that sortilin is an important regulator of BDNF sorting and trafficking to the secretory pathways (Chen et al., 2005). A clinical study confirmed the correlation between circulating levels of sortilin, BDNF, and MDD (Buttenschon et al., 2015). A recent study was conducted with a cohort of 37 patients suffering from MDD and treated with an AD drug for 12 weeks. They were compared to 49 healthy volunteers (Devader et al., 2017). Using the dosing alpha-screen method validated for PE detection in the serum (Mazella et al., 2010), the concentrations of PE, spadin (PE 12–28, Table 2 peptide #1), PE 12–27 (Table 2 peptide #17), and PE 14–25 (Table 2 peptide #18) were measured with the aim to find a correlation between the serum levels of PE peptides in healthy and patients with MDD whether or not treated with AD drugs. Serum concentrations of the PE peptides are significantly decreased in patients with MDD. Interestingly, PE peptide levels are partially but significantly restored after a 12 week treatment with an AD drug, thus indicating a correlation between the serum levels of PE-like activity and the mood of patients.

Recently, Buttenschon et al. conducted a new study, and they concluded that sortilin could not serve as a biomarker to follow AD treatment in patients with MDD (Buttenschon, Nielsen, Glerup, & Mors, 2017). At this stage, it is important to underline that the release of soluble sortilin (as measured in the Buttenschon's work) depends on matrix metalloproteinases (MMPs) (Navarro, Vincent, & Mazella, 2002), whereas PE release depends on the intracellular maturation of sortilin by furin (Devader et al., 2017). To validate whether or not PE and sortilin could be used as biomarkers in MDD, further complete clinical studies, which include larger cohorts of patients with MDD whether or not treated with AD drugs, need to be performed in the future. Moreover, advances in developing specific antibodies able to recognize the short peptide sequences with higher sensitivity could guide research toward new biomarkers of MDD.

4. Conclusions and perspectives

The main advantages that peptides present compared to xenobiotics are their high specificity and low toxicity owing to their high binding affinity to their specific targets. Two categories of currently available drugs exist, small molecules <500 Da active *per os*, and large molecules >5000 Da that are inefficient orally and need to be administered in the parenteral form as injections (Craik, Fairlie, Liras, & Price, 2013). If small molecules are orally bioavailable, they could be designed with a lesser cost. However, owing to their size, they could lack specificity and potentially could lead to off-target adverse effects. Large biologic drugs show high affinity for their targets but need to be injected into patients (Craik et al., 2013). Spadin and its analogs belong to a class of molecules that range between small molecules and large polypeptides that represent a nonnegligible advantage for both drug administration and specificity for targets. Advantages of spadin and its analogs as promising AD drugs are summarized in Box 1. In addition to the central effect of spadin on depression through targeting TREK-1 channels in the brain,

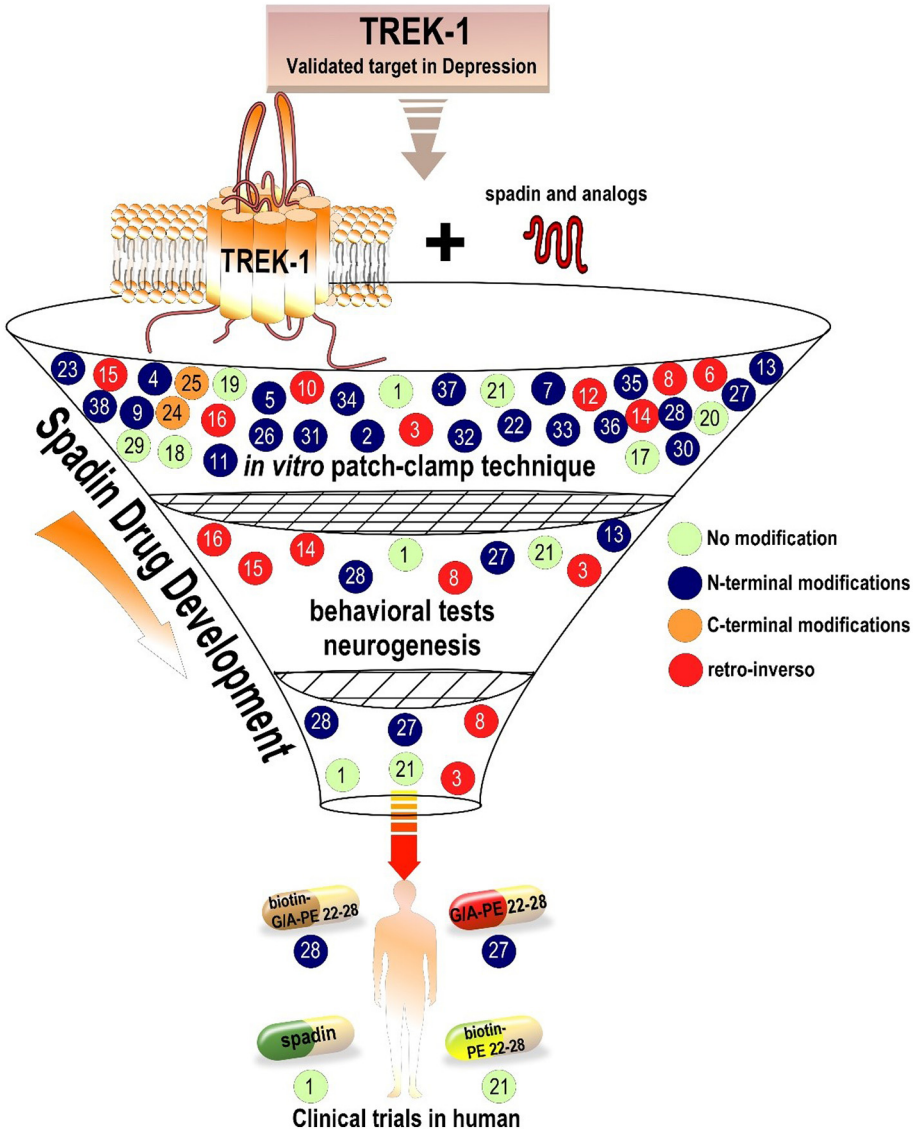

Fig. 4. Schematic representation of spadin development as new concept in AD drug discovery. Since the validation of TREK-1 channel as a novel target for AD drugs, spadin was discovered to be a specific blocker of TREK-1 with high affinity. Different optimization processes were conducted in order to improve the affinity, efficacy and *in vivo* stability while maintaining the AD properties. The strategies used led to the identification as AD drug promising candidates: G/A-PE 22–28, biotin-G/A-PE 22–28 in addition to spadin. The final goal is to complete preclinical development before launching clinical trials in human and finally develop spadin and/or its analogs as AD drugs. The number in circle corresponds to the analog number indicated in Table 2.

spadin diffuses in the blood circulation and reaches a number of organs including the pancreas and prostate. Indeed, as described above, in the pancreatic β-cells, spadin plays a crucial role as an insulin-releasing peptide upon hyperglycemia (Hivelin et al., 2016).

>60 peptides have been approved by the US *Food and Drug Administration* (FDA), and this is expected to greatly increase in the near future, as >140 molecules are currently being processed and tested in clinical trials. In addition, at least 500 peptides are under investigation in preclinical studies (Fosgerau & Hoffmann, 2015; Kaspar & Reichert, 2013) and are quite ready to be tested in humans.

Spadin and its analogs undoubtedly constitute important candidate peptides intended to treat depression in clinic. As natural peptides, they irrefutably represent a huge advantage compared to small classical ADs, as the risk–benefit ratio will be sharply improved. The next step consists of validating their efficacy and tolerability processes in healthy volunteers during clinical trials. Many peptides have been designed for various pathologies such as cancer, diabetes, and infectious diseases. However, only few peptides are used in the treatment of neurological disorders; this is mainly due to their weak BBB crossing and their rapid degradation *in vivo* (McGowan, Bidwell 3rd, & Vig, 2015). These observations are not applicable to spadin because the effects of peripheral i.p. injections of spadin and its analogs (at doses as low as 100 μg/kg) can be measured in the brain (Mazella et al., 2010). Additionally, the *in vivo* stability of spadin ranges between 7 and 23 h, a duration that is compatible with the use in human clinic. As the validation of TREK-1 as a potential target in the treatment of depression, TREK-1 druggability was made possible after the discovery of spadin and its analogs (Fig. 4). Thereafter, spadin efficacy was gradually optimized through different strategies such as RI strategy, cyclization, sequence shortening, and modifications of N- and C-terminal ends. Ultimately, efficiency, inhibition potency, and *in vivo* stability of these analogs are significantly improved compared to spadin. Moreover, AD activity is maintained despite shortening and modifying the peptide sequence. Interestingly, the AD phenotype is associated with an enhancement of both *in vivo* hippocampal neurogenesis and cortical synaptogenesis. It will be challenging to overcome obstacles and therefore lead spadin analogs to the finish line, *i.e.*, marketing spadin analogs as novel AD drugs with an original mechanism of action. However, all these observations give

Box 1
Potential advantages of spadin and its analogs as antidepressant molecules

Fast acting
Psychiatrists, physicians, and patients want drugs that act more rapidly than those that are prescribed presently. Current marketed AD drugs often take several weeks to be efficient. Spadin or its analogs only need 4 days to exert their AD effects (Mazella et al., 2010). Additionally, spadin and its analogs are effective regardless of the route they are administered: intravenous, intraperitoneal, intracerebroventricular, subcutaneous, or *per os*.

Specificity
Spadin and its analogs are highly specific for TREK-1 channels. They do not modify the activity of other K_{2P} channels such as TREK-2, TRAAK, TRESK, and TASK 1 channels (Djillani et al., 2017; Moha Ou Maati et al., 2012). This is particularly remarkable because TREK-2 and TRAAK belong to the same subfamily and share almost 80% homologies with TREK-1 (Noel, Sandoz, & Lesage, 2011).

Cardiac safety
In rodent, spadin and its analogs do not affect hERG or KCNQ1/KCNE1 channels and do not modify heart rates. These data indicate that spadin and its analogs have no deleterious effects at the cardiac level (Djillani et al., 2017; Moha Ou Maati et al., 2012).

No side effects on TREK-1-controlled functions
In addition to the absence of effects on the heart, preclinical studies have shown that spadin and its analogs have no deleterious side effects on pathologies that are under the control of TREK-1 channels, such as pain, epilepsy, or ischemia (Moha Ou Maati et al., 2012).

High efficiency
The development of spadin analogs has increased the AD potency of the original peptide. Analogs of concentration 3 μg/kg are adequate to produce an AD effect same as that obtained with a concentration of 100 μg/kg (Djillani et al., 2017).

Natural peptides
In addition to the fact that they are natural peptides, we can reasonably expect a high level of specificity and safety for these peptides. Converse to the majority of actual ADs that provoke withdrawal behavior, the probability to observe such a phenomenon with a natural peptide is low.

Duration effects
The relatively short time (7 h) of *in vivo* effects of spadin could have been a problem, but it was solved with the development of spadin analogs. Therefore, the *in vivo* stability has become very close to 24 h (Djillani et al., 2017). This sustained effect is important for use in clinics.

Biomarker
Spadin-like activity in the serum, mainly corresponding to PE and its degradation products, is significantly decreased in patients suffering from MDD in comparison to healthy controls (Devader et al., 2017).

Comparison with other putative drugs
Other TREK-1 blockers such as SID1900 or lig4–4 have been recently identified. Although these molecules were described to be specific blockers of TREK-1 channels, they probably affect other types of ion channels as indicated in Table 1. Furthermore, they show very low affinities for TREK-1 channels (Wang et al., 2018; Ye et al., 2015) in comparison to spadin or its analogs (several μM *vs.* nM) (Djillani et al., 2017). Many other molecules are able to block TREK-1 channels, but none of them is specific for TREK-1. Nevertheless, these molecules were not extensively studied in the field of depression, and their properties are detailed in the paragraph 2.3.2 of this review.
Some microRNAs were also supposed to be involved in the depression process, but it is very soon to really define a therapeutic strategy using microRNAs (Yuan, Mischoulon, Fava, & Otto, 2017).
Recently, ketamine was also described (Ramaker & Dulawa, 2017) as a fast-acting molecule, but ketamine needs to be frequently administered (sometimes in hours) to obtain a sustained effect (Schwartz, Murrough, & Iosifescu, 2016). This point is an important drawback in the use of ketamine. Additionally, ketamine displays numerous adverse effects (Li & Vlisides, 2016).

rise to a very positive signal for the use of spadin and/or its derivatives as new ADs. Nevertheless, we have to keep in mind that all these preclinical studies were performed on animal models described to mimic a part of a clinical symptom of depression. Definitive answers can be obtained from clinical trials.

To our knowledge, spadin and its analogs are the first therapeutic peptides with a high potential to successfully go through clinical trials and thus be marketed for the treatment of mood disorders.

Conflict of interest statement

The authors declare that they are no conflicts of interest.

Funding sources

This work was supported by the Centre National de la Recherche Scientifique and the Agence Nationale de la Recherche (ANR-13-SAMA-0001 and 0002 and ANR-13-RPIB-0001 and 0002). We also thank the French Government for the "Investments for the Future" LabEx ICST #ANR-11 LabEx 0015 and the Fondation de l'Avenir No. AP-RMA-2015-021. M. Pietri was supported by a CIFRE fellowship.

Methods. hTREK-1/HEK cells were seeded at a density of 20,000 cells/35 mm dish. Electrophysiological recordings were performed 24–48 h after plating using the whole-cell configuration of patch-clamp technique. In order to measure TREK-1 current (I_{TREK-1}), a cocktail of potassium channel blockers was added to the bath solution. This cocktail contained: 3 mM 4-AP (4-aminopyridine), 10 mM TEA (tetraethylammonium), 10 μM Glibenclamide, 100 nM Apamin and 50 nM Charybdotoxin (for more experimental details see (Djillani et al., 2017)). Whole-cell I_{TREK-1} currents were generated by running a pulse or ramp protocol every 5 s from −100 to +60 mV with a holding potential maintained at −80 mV. The inhibitory effect on TREK-1 channels of 100 nM of spadin analogs were compared with the inhibitory effect of spadin, on cells pre- activated with 10 μM of arachidonic acid (AA). Patch-clamp recording data were analyzed using Clampfit (Molecular Devices, USA). I = f(V) curves were obtained from −100 to +60 mV ramp. Data were presented as mean ± SEM from at least 3 independent experiments. In GraphPad Prism (GraphPad software, La Jolla, USA), statistical comparisons were performed using Student's *t*-test or ANOVA one-way. A result is considered as statistically significant when $p < 0.05$.

Acknowledgments

We thank 3P3D startup, and particularly its director Dr. Serge Richard for his friendly support, confidence in the spadin project, and fruitful discussions. We thank Franck Aguila for artwork contribution. We particularly thank Dr. A. Patel and S. Morgan for careful reading of the manuscript.

Authorship contributions

AD wrote the manuscript. CH, JM, and MB corrected and improved the manuscript. AD conducted patch-clamp experiments, and MP and MB performed behavioral tests. CH, JM, and MB contributed to reagents/materials/analysis tools.

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