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Pleiotropic effects of prokineticin 2 in the control of energy metabolism

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Abstract

Prokineticins are family of small proteins involved in many important biological processes including food intake and control of energy balance. The prokineticin 2 (PROK2) is expressed in several peripheral tissues and areas in the central nervous system. PROK2 activates G protein-coupled receptors, namely, prokineticin receptor 1 (PROKR1) and prokineticin receptor 2 (PROKR2). Preclinical models exhibiting disturbances of the PROK2 pathway (at the level of PROK2 or its receptors) are characterized by changes in food intake, feeding behavior and insulin sensitivity related to a dysfunction of the energy balance control. In Humans, mutations of *PROK2* and *PROKR2* genes are associated to the Kallmann syndrome (KS) that affects both the hormonal reproductive axis and the sense of smell and may also lead to obesity. Moreover, plasma PROK2 concentration has been correlated with various cardiometabolic risk factors and type 2 diabetes (T2D). The present review summarizes knowledge on PROK2 structure, signaling and function focusing on its role in control of food intake and energy homeostasis.

1. Introduction

The first members of the prokineticin family were described in the 1980s in reptiles, then amphibians and finally mammals and humans. The role of prokineticins including prokineticin 2 (PROK2, the subject of this review) has been increasingly studied since the beginning of the 2000s. Physiological or pharmacological effects of PROK2 and its function in mechanisms as diverse as nociception, circadian rhythms, angiogenesis, neurogenesis, reproduction, etc. have begun to be well characterized as well as its role in energy metabolism including food intake, dietary behavior and insulin action. In the human species, a disturbance of the PROK2 signaling pathway has been identified as responsible for Kallmann syndrome disorder, whose affected patients present a hypogonadism and anosmia that is sometimes accompanied by a deregulation of the energy balance. With these pleiotropic effects, PROK2 and more broadly the prokineticin family provides a relevant endocrine study model of interorgan dialogue that takes place in a multicellular organism throughout its development and life. Finally, from a pharmacological point of view, the prokineticin system could also be of therapeutic interest in the context of metabolic diseases, such as obesity and type 2 diabetes (T2D). This review is an update on the scientific knowledge on PROK2, focusing on its structure, signaling and function in energy balance, glucose homeostasis and feeding behavior.

2. PROK2 and PROK2 receptors

2.1. PROK2

2.1.1. The prokineticin family

The first member of the prokineticin family to be identified was Mamba Intestinal Toxin 1 (MIT1) isolated from the venom of the black mamba snake [1] that showed the ability to stimulate contraction of the guinea pig ileum [2,3]. The Bombina variegata (Bv8) was then isolated from the skin secretion of a frog and was found to stimulate the contraction of gastrointestinal (GI) smooth muscle with high potency. Bv8 has 58% identity with MIT1 and was reported to induce hyperalgesia and colon relaxation in rats in addition to its contracting effects on guinea pig, rat, and mouse ileum. Homologues of Bv8 were identified in other amphibians, lizards, fishes, and orthologues were described in mammals : they were named prokineticin because of

1 their ability to contract GI smooth muscle, with no effect on vascular or respiratory
2 smooth muscle.

3 The prokineticin family comprises prokineticin 1 (PROK1 also called EGVEGF for
4 endocrine gland vascular endothelial growth factor [4]) and PROK2, that are codified
5 by two different genes, *Prok1* (mouse chromosome 3 syntenic with human
6 chromosome 1p13.1) and *Prok2* (mouse chromosome 6 and human 3p21). These
7 small protein contain 10 conserved cysteines residues with identical spacing forming
8 five disulfide bonds in the carboxy-terminal region. Their mature forms consist of 86
9 and 81 amino acids, respectively for PROK1 and PROK2, and show a highly
10 conserved N-terminal AVITGA sequence essential for their biological activity [5].
11 Substitutions, deletions, or insertions to this hexapeptide result in the loss of agonist
12 activity on prokineticin receptors [6,7]. PROK1 will not be discussed in the present
13 review.

14 15 **2.1.2. Tissue expression (Table 1)**

16 *Prok2* primary transcript has two alternative splice variants : *Prok2* mRNA, that
17 encodes the 81 AA protein PROK2 and *Prok2 long form (Prok2L)* mRNA, that
18 encodes a protein of 102 amino acids, PROK2L, consisting of an insert of 21 basic
19 amino acids (encoded by an exon) in PROK2 sequence. In mammalian cells,
20 secreted PROK2L is processed by proteolytic cleavage into a smaller peptide, called
21 PROK2beta, that retains the receptor binding and activation domain [8,9].

22 *Prok2* mRNA is present in GI tract, enteric plexus, myocardial tissue [5,8,10,11],
23 uterus, testis [10,12], adrenal glands [8], Kupffer cells in the liver [8,10] as well as in
24 progenitors and mature blood cells in peripheral blood and bone marrow [4,13]. Its
25 expression is associated with the infiltrating cells, predominantly neutrophils, at sites
26 of inflammation.

27 In the brain, *Prok2* mRNA is expressed in the hypothalamus, mainly in the
28 suprachiasmatic nucleus (SCN) [14–17] and dense clusters are also found in medial
29 preoptic area (MPA) and in the hippocampus [10,17–19]. Moderate expression of
30 *Prok2 mRNA* was evidenced in nucleus accumbens shell and hypothalamic arcuate
31 nucleus (ARC) [17]. *Prok2* mRNA was also detected in amygdala, cerebral cortex
32 (high levels being detected in layer II) and cerebellum, in mice dorsal and ventral
33 horns of the spinal cord (mainly in astrocytes), in nucleus of solitary tract (NTS)
34 [10,17,18] and in some dorsal root ganglion (DRG) neurons coexpressing the

vanilloid receptors TRPV1 (transient receptor potential cation channel subfamily V member 1) [10,20,21].

Immunofluorescence studies from cortical and hippocampal cultures of embryonic day 17–18 brain rats embryos localized PROK2 in body neurons (NeuN + cells) and in astrocytes processes (GFAP+ cells) [22]. *Prok2* mRNA shows also an intense expression in the olfactory bulb (OB), mainly in the glomerular, granular and mitral cell layers, in a subset of granule cells and tufted cells [10,17,23,24]. A few PROK2-positive cells that are not neural progenitors or migrating neuroblasts were found in the medial part of the rostral migratory stream (RMS). No staining was detected in the subventricular zone (SVZ) of the lateral ventricle [25].

Prok2L mRNA was detected in murine and human testis, in macrophages [10,13], OB and hypothalamus of rodents [23] as well as in lung and spleen where its expression was higher than that of *Prok2* mRNA [8].

2.1.3. Gene regulation

The *Bv8/Prok2* human and mouse promoter sequences are highly conserved, indicating a related transcriptional regulation in these species. The *Prok2* promoter is replete with multiple E-box sequences (CACGTG) that bind basic helix-loop-helix (bHLH) transcription factors such as Hypoxia-inducible factor 1-alpha (HIF1 α), which regulates *Prok2* expression during angiogenesis [26]. In the OB, two proneural basic helix-loop-helix (bHLH) factors, Neurogenin1 (NGN1) and mammalian achaete scute homolog-1 (MASH1), were found to upregulate *Prok2* transcription by binding to E-box motifs. On the contrary, Distal-Less Homeobox 1 and 2 (DLX 1 and 2) was identified as a repressor [27]. In the SCN, *Prok2* gene expression is controlled by clock genes *Circadian Locomotor Output Cycles Kaput* (*Clock*) and *Brain and Muscle ARNT-Like 1* (*Bmal1*) acting on E-box enhancers in the gene's promoter. In the immune system cells, as well as in sensitive neurons and in central nervous system (CNS) after nerve damage or neuroinflammation [28], *Prok2* is positively regulated by granulocyte colony stimulating factor (GCSF) (see review in [10]).

2.2. Prokineticin receptors

PROK2 acts on the two plasma membrane G protein-coupled receptors (GPCR), namely, prokineticin receptor 1 (PROKR1, *ProkR1* gene : 2p13.3 in humans and chromosome 6 in mouse) and prokineticin receptor 2 (PROKR2 ; *ProkR2* gene :

20p13 in humans and chromosome 2 in mouse). They have about 85% sequence identity [29] and show ~80% identity to the previously described mouse orphan receptor GPR73 [30].

2.2.1. Tissue expression

In the periphery, both PROKR are detected in immune and endothelial cells and have a tissue-specific expression pattern. For example PROKR1 is the only isoform detected in adipose tissue [31] and it is more abundantly expressed than PROKR2 in the gut, with its expression varying all along the length of the intestine. PROKR1 is also detected in endocrine glands and organs of the reproductive system, the gastrointestinal tract, lungs, and the circulatory system [32,33].

Both PROKR are also expressed in the CNS. In adult rodents, *ProkR2* mRNA was evidenced in several brain areas including the SCN, the cortex, the paraventricular hypothalamic nucleus (PVN), the dorso-median nucleus (DMN), the paraventricular (PVT) and paratenial thalamic nuclei, the paracentral thalamic nucleus, the lateral habenular nucleus, the lateral septal nucleus, the lateral globus pallidus and the amygdala [15]. *ProkR2* mRNA also showed prominent expression in hippocampus, NTS, DRG neurons and circumventricular organs [17,29,34]. Interestingly, studies of *ProkR2-Cre* mouse evidenced a sexually dimorphic pattern of *ProkR2* expression in brain [35]. Indeed, in females, higher Cre activity was found in the MPA, ventromedial nucleus of the hypothalamus (VMN), ARC, medial amygdala and lateral parabrachial nucleus, whereas in males, Cre was higher in the amygdalo-hippocampal area. This suggests differential roles of PROKR2 signaling in reproductive function and, potentially, in other physiological systems. In the OB of rodents, intense expression of both transcripts was observed, in the granular and subependymal cell layers as well as in the ependyma and olfactory ventricle, an embryologic cavity inside the OB that persists in some species [36,37]. *ProkR2* transcript and protein were also evidenced in the SVZ –where *Prok2* is not expressed-, in almost all migrating neuroblasts as well as in a large population of their precursors. Along the RMS and in the OB, *ProkR2* expression was absent in astrocytes. *ProkR1* mRNA was found in fewer brain regions including SVZ, RMS, olfactory ventricle in the OB, hippocampus and dorsal motor nucleus of the vagus (DMV) [17,24].

2.2.2. Signaling pathways

PROKRs have been reported to couple to i) Gq proteins [11,29,33,34,38–43] to mediate the intracellular calcium mobilization or mitogen-activated protein kinase (MAPK)/Akt pathways activation, ii) Gi proteins [4,29,44], leading to phosphorylation of p44/p42 MAPK, or iii) Gs proteins [8,40], leading to adenylate cyclase activation and cAMP accumulation (Table 2). However it seems that Gq-signaling pathways are the most common ones associated with PROKRs stimulation. For instance, addition of PROK2 to mouse cultured astrocytes causes increases in astrocytic cytosolic Ca^{2+} levels through PROKR1 activation and astrocytic proliferation [38]. PROK2 also induced calcium mobilization in a dose-dependent manner in PROKR1-overexpressed HEK293T cells. In skeletal muscle cells, PROKR1 signaling activated Gq-mediated PI3K/AKT and MAPK/ERK signaling pathways and also promoted the translocation of glucose transporter 4 (GLUT4) into the plasma membrane that influences insulin sensitivity [39]. Interestingly, PROKR1 signaling is significantly and specifically inhibited by the Melanocortin Receptor Accessory Protein 2 (MRAP2) an important regulator of energy homeostasis [40].

As *Prok2*, *Prokr2* expression may be directly or indirectly regulated by MASH1 since *Mash1*^{-/-} mutant mice show a highly marked decrease of *Prokr2* expression [37].

Prokineticins exhibit different affinities for their receptors : PROK2 binds PROKRs with higher affinity than does PROK1 [8] and PROK2beta displays clear selectivity for PROKR1 [9]. However, all the natural prokineticins bind and activate both receptors in nanomolar range. Functional studies showed that PROK2 and PROK2beta stimulate intracellular Ca^{2+} responses in PROKR1-expressing cells with similar potencies. However, the PROK2beta stimulus of Ca^{2+} responses in PROKR2-expressing cells is at least 10-fold less potent than that of PROK2.

PROK2 has been shown to modulate the electrical activity of neurons in the area postrema (AP), subfornical organ (SFO), PVN and SCN [45–48]. Current-clamp recordings, slow voltage ramps and ion-substitution experiments evidenced that focal application of PROK2 produced depolarizations (38%) and hyperpolarizations (28%) of dissociated AP cells, i) in a concentration-dependent manner and ii) that were the result of PROK2-induced Cl^- current and inhibition of a nonselective cation current respectively [46]. Similarly, PROK2 induced depolarizations in 40% of dissociated SFO neurons, that were accompanied by an increase in action potential frequency in responding cells. This excitatory effect appeared to be, in part, attributable to a

PROK2-induced decrease in the delayed rectifier potassium current ($I(K)$) [45]. PROK2 was also evidenced to depolarize the majority (76%) of magnocellular (MNC) neurons of the PVN through the activation of glutamate interneurons within the hypothalamic slice. Although parvocellular preautonomic (PA), and parvocellular neuroendocrine (NE) of the PVN also depolarized in response to PROK2 (respectively 68 % and 67%), this effect was directly induced by activation of PROK2R and a MAPK signalling mechanism [48]. Finally, whole cell patch-clamp recording in SCN slices evidenced that PROK2 dose-dependently increased spontaneous firing rates in most neurons from the dorsal SCN. Moreover, PROK2 acted postsynaptically to reduce γ -aminobutyric acid (GABA)-ergic function within the SCN and reduced the amplitude but not frequency of miniature inhibitory postsynaptic currents (mIPSCs). PROK2 also suppressed exogenous GABA-induced currents. The inhibitory effect of PROK2 required PKC activation in the postsynaptic cells [47].

In vitro studies indicated that both PROKRs undergo rapidly (in 1 min) agonist-induced endocytosis. For the PROK2-PROK2R couple, this process is G-protein-coupled receptor kinase 2 (GRK2) - and clathrin-dependent, but beta-arrestin-independent. Internalization is maximal at 30–60 min and the receptor recycles back to the plasma membrane after the removal of ligand. Protein kinase C activation also induced PROK2R endocytosis, however, it was not necessary for the PROK2-induced PROK2R endocytosis [49]. Receptors desensitization was also evidenced *in vivo* after persistent intracerebroventricular (ICV) infusion of Bv8/PROK2 [15].

3. Physiological role of PROK2 in control of energy balance There is now growing amount of evidences that PROK2 is involved in regulation of energy metabolism through pleiotropic effects as depicted in Figure 1. PROK2 has a major role in regulation of circadian cycles that rhythm the day/night metabolic and biological behaviors. It is also involved in the regulation of energy balance and eating pattern, acting in the CNS and/or in peripheral organs. It may participate to the regulation of cell growth and differentiation of metabolic tissues such as the adipose tissue.

3.1 Role in the circadian clock of physiologic functions

In mammals, the pacemaker that drives circadian rhythms of physiology and behavior such as body temperature, sleep, locomotor activity, energy metabolism resides in

1 the SCN of the anterior hypothalamus [50]. Light exposure is the main input for
2 entraining and resetting of the SCN clock. *Prok2* mRNA is expressed in the SCN with
3 a circadian profile, with high levels during the day and low or undetectable levels at
4 night [14]. PROK2 has been suggested as a candidate that would link SCN and its
5 efferent targets since projections of neurons expressing *Prok2* were evidenced in
6 brain area including the ventral lateral septum, medial preoptic area,
7 subparaventricular zone, PVN, DMH, lateral hypothalamic area and PVT [16].
8 *Prok2* oscillation in the SCN is abolished in mutant mice lacking the functional
9 clockwork, meaning that *Prok2* is predominantly controlled by the endogenous
10 circadian clock. This suggests that light plays a modulatory role, but light can also
11 induce *Prok2* expression independently of the circadian oscillator [14].
12 *ProkR2* is also expressed in the SCN -with an non-oscillating pathway- as well as in
13 virtually all known primary SCN targets [15] leading to the idea that the
14 PROK2/PROKR2 system may have a function in synchronizing SCN output.
15 ICV injection of PROK2 suppressed the high level of wheel running behaviour
16 associated with dark phase in rats, suggesting that PROK2, which is normally at high
17 levels during subjective day, inhibits wheel-running activity. During subjective night,
18 when endogenous *Prok2* mRNA levels are low, the wheel running activity is
19 disinhibited [15]. *Prok2* null mice evidenced reduction of locomotor rhythms and
20 displayed significantly reduced rhythmicity for a variety of other physiological and
21 behavioral parameters, including sleep-wake cycle, body temperature, circulating
22 glucocorticoid and glucose levels, as well as the expression of peripheral clock
23 genes. In addition, *Prok2*-null mice showed accelerated acquisition of food
24 anticipatory activity during a daytime food restriction which suggests a weaker SCN
25 control in *Prok2* null mice for the locomotor activity and a higher control of food
26 entrained oscillators [51].
27 The PVN is a primary SCN efferent nucleus involved in the direct regulation of
28 endocrine rhythms. Because *ProkR2* is expressed in the PVN, SCN-derived PROK2
29 may regulate physiological parameters through this pathway, exciting or inhibiting
30 PVN neurons (as magnocellular, parvocellular preautonomic, and parvocellular
31 neuroendocrine neurons). *Prok2* is also detected in the PVN and its expression is
32 increased by fasting while it falls to the basal undetectable level after refeeding.
33 Administration of 2-deoxy-D-glucose (2-DG), an inhibitor of glucose utilization, also
34 rapidly induced *Prok2* expression in the PVN implying that the induction was a

1 response to the dwindling energy reserves. Thermosensitive neurons in the PVN can
2 detect variations of body temperature and activate the sympathetic nervous system
3 which regulates the heat production through general activity, shivering, and brown fat
4 thermogenesis. Interestingly, *Prokr2* null mice showed sporadic bouts of torpor with
5 decreased locomotor activities, oxygen consumption and respiratory quotient, under
6 *ad libitum* feeding and constant temperature. The remove of food for a period
7 induced a deeper and longer hypothermia in *Prokr2* null mice compared to their wild
8 type littermates, suggesting that PROKR2 pathway is involved in energy balance and
9 thermoregulation [52]. In the same way, *Prok2* null mice entered torpor after fasting
10 with no difference in activity compared to wild type mice, but with lower energy
11 expenditure and lower body weight loss. Wild type animals responded to reduced
12 food availability by becoming more wakeful and active -a phenomenon called fasting-
13 induced arousal (FIA) - so as to maintain body temperature in a normal range and be
14 ready for food supply. Once food was applied, they ingested the food and utilized it to
15 compensate the body weight loss. Contrary to wild type mice, *Prok2* null mice
16 exhibited no appreciable increase in arousal during the entire fasting period. Supply
17 of limited food daily during fasting rescued the body weight loss and hypothermal
18 phenotype in wild type mice, but not in *Prok2* null mice, suggesting a deficit of food
19 utilization in transgenic mice during refeeding period. The assumption is that fasting
20 increases *Prok2* level in the PVN to facilitate the sensing of hypothermia and
21 activation of the sympathetic nerve system which in turn leads to increasing activity,
22 expanding the wake periods, and using body energy storage [53].

23 Recently, the role of PROK2 as a SCN input signal was also investigated. Mice with
24 *Prok2* overexpression displayed reduced oscillation of *Prok2* expression in the SCN
25 because of increased *Prok2* expression during the dark phase. They also had
26 decreased amplitude of circadian locomotor rhythms and altered molecular rhythms
27 of core clockwork, particularly the amplitude of *Bmal1*. It is likely that PROK2 could
28 exert a signalling role from the intrinsically photosensitive retinal ganglion cells that
29 project to SCN neurons [54].

30 Interestingly, in zebrafish, a diurnal vertebrate, both overexpression and mutation of
31 the *Prok2* affect sleep/wake behaviors in a light-dependent but circadian-independent
32 manner. Indeed, in light, *Prok2* overexpression increases sleep and induces
33 expression of *galanin* (*galn*), a hypothalamic sleep-inducing peptide [55].

3.2 Role in feeding behavior and glucose homeostasis

In rats and mice, acute peripheral administration of PROK2 decreases food intake while it reduces both food intake and body weight when administered chronically, in lean and diet-induced obese (DIO) mice [19]. Interestingly, DIO mice pair fed to the PROK2-treated group lost a similar amount of weight as the mice given PROK2, suggesting that the effect of PROK2 on body weight was mediated predominantly via a reduction in food intake. Mice models lacking *Prok2* have not been reported to show an increased body weight compared with their wild-type littermates, that could be explained by a compensation in embryonic knockout models [19]. Peripheral injection of PROK2 does not alter food intake in *Prokr1* deficient mice or in wild type mice with co-administration of a PROKR1 antagonist, suggesting that the anorectic effect induced by peripheral PROK2 is PROKR1-dependent. It also increases c-fos-like immunoreactivity in the DMV of the brainstem, suggesting that peripherally administered PROK2 may directly act through the DMV to inhibit food intake. Acute or chronic PROK2 administration did not have any adverse effect on glucose tolerance or insulin sensitivity in wild type mice [56]. However, we may assume that *Prokr1* deficiency would alter glucose homeostasis since endothelial PROKR1 has been characterized as a positive regulator of insulin sensitivity [57]. Moreover, in palmitate-induced insulin-resistant myotubes, PROKR1 enhanced insulin-stimulated AKT phosphorylation, GLUT4 translocation, and glucose uptake. mRNA and protein levels of PROKR1 were significantly decreased in skeletal muscle and white adipose tissue of DIO mice, and the amount of PROKR1 was significantly decreased in human skeletal muscle cell-derived myotubes under insulin resistance conditions [39].

In the hypothalamus which plays a key role in the regulation of food intake both in humans and rodents, *Prok2* expression is reduced by fasting [19], as soon as the early neonatal period [58]. This is in contrast to what was evidenced in the PVN and may be linked to the study of the entire hypothalamus compared to a single nucleus. ICV administration of Bv8/PROK2 reduces food intake in rodents and significantly decreases the number of feeding episodes without impacting energy expenditure or locomotor activity [19,59]. It also significantly increases the number of arcuate proopiomelanocortin (POMC)-expressing neurons exhibiting c-fos immunoreactivity, while peripheral administration of PROK2 does not [19,56]. This suggests that the anorectic effect of central PROK2 is dependent on hypothalamic activation. In the

1 same way, PROK2 significantly stimulates the release by hypothalamic explants of
2 anorexigenic POMC-derived peptide alpha-melanocyte-stimulating hormone (α -
3 MSH), while coadministration of the orexigenic neuropeptide Agouti-related protein
4 (AgRP) attenuated the PROK2 effect. However ICV injection of PROK2 significantly
5 decreases food intake both in wild type and *Mc4r* (melanocortin-4 receptor) -deficient
6 fasted mice [40]. This implies that PROK2 can promote an anorectic effect through a
7 mechanism that is distinct and independent from the activation of MC4R. MRAP2 is a
8 potentiator of MC4R signaling and loss of function mutations in *Mrap2* have been
9 recently associated to hyperphagic obesity complicated with hyperglycemia and
10 hypertension [60]. MRAP2 likely regulates energy homeostasis through both MC4R-
11 mediated and MC4R-independent pathways [61]. Interestingly, in contrast to its
12 activity on MC4R, MRAP2 inhibits PROKR1 signaling both *in vitro* and *in vivo*.
13 Indeed, the anorexigenic effect induced by ICV injection of PROK2 was more
14 pronounced in *Mrap2*-deficient mice than in wild type mice. This suggests that
15 MRAP2-mediated inhibition of PROKR1 promotes hunger and that PROK2-
16 dependent activation of PROKR1 may counteract this effect [40,61].

17 *Prok2* expression and function have been also studied in the OB which is another
18 main CNS structure involved in the regulation of food intake and energy balance [62]
19 and whose neuro- and morphogenesis are dependent on PROK2 signaling pathway
20 [24, 25, 27]. Our group showed that 24 h fasting had no effect on *Prok2* mRNA
21 expression in the OB compared to the fed state, whereas it decreased that of *Prok2L*
22 [23]. This was concomitant with a lower *Prokr1* and a higher *Prokr2* mRNAs
23 expression. Because *Prok2L* encodes the PROK2beta isoform that was shown to
24 preferentially bind PROKR1, these results suggest that, in the OB, the
25 PROK2beta/PROKR1 could be the main couple regulator of feeding behavior.
26 Interestingly, we observed that PROK2 injection in the OB strongly decreased food
27 intake and induced an increase in the buried food discovery time of fasted mice [23].
28 Moreover mice injected with *Prok2* shRNA in the OB showed altered pattern of
29 feeding behavior, as increased feeding frequency and meal duration associated with
30 decreased meal size. Concerning glucose homeostasis, *Prok2* shRNA-injected mice
31 developed insulin resistance and showed decreased glucose uptake in the
32 subcutaneous adipose tissue. These data highlight PROK2 as a new target in the OB
33 that links olfaction with eating behavior and energy balance [23].

3.3 Role in adipose tissue expansion

In addition to its anorectic role, PROK2 also influences body weight homeostasis through a suppressive effect of adipose tissue expansion mediated by PROKR1. Indeed, *Prokr1*-null mice (*Prokr1*^{-/-}) have excessive abdominal adipose tissue and develop severe obesity and impaired adipocyte insulin signaling, that are exacerbated by a high fat diet. The loss of PROKR1 in endothelial cells also led to obstruction of new capillaries development, what created a hypoxic environment. Interestingly, adipocyte-specific mutant mice (*Prokr1*^{ad/-}) do not show such a severe phenotype, despite increased proliferating preadipocytes and newly formed adipocyte levels, leading to expansion of adipose tissue. They also display substantial infiltration of macrophages in the adipose tissue, which is one other component of obesity. How does PROKR1 do to preserve adequate adipocyte function in obesity is unknown but it strongly suggests that PROKR1 agonists are potent therapeutic targets against obesity [31,63].

The origin of PROK2 acting in the adipose tissue is not clear but it is suggested that PROK2 is an adipokine which exhibits an angiogenic activity [31]. As PROK2 is released by inflammatory cells [13] and expressed in lymphocytic tissues, cells, and cell lines [4], we can not exclude that endothelial cells, pericytes, T cells, and M2 macrophages contained in the stroma vascular fraction could secrete PROK2.

3.4. Role in angiogenesis and heart protection

In mouse and human myocardial tissue, PROK2 promotes cardiomyocyte survival and angiogenesis, mainly through PROKR1 signaling. PROK2 or PROKR1 overexpression induce vessel-like formation in cultured cardiac endothelial cells and activate Akt in cardiomyocytes to protect cardiomyocytes against oxidative stress [11]. Exposure of cardiomyocytes to high glucose/high palmitic acid induces oxydative stress that is accompagnied by suppression of PROK2, PROKR1, and PROKR2 expression. These effects are robustly prevented by PROK2, through an Akt-related signalling pathway [64]. Interestingly, the antidiabetic metformin (Met) that exerts cardioprotective effects, attenuated the decreases of PROK2, PROKR1, and PROKR2 expression observed in cardiomyocytes of streptozotocin-treated mice [65]. In mouse heart, as well as in kidney, PROKR1 up regulates PROK2 to promote progenitor cells differentiation into a vasculogenic cell type or into endothelial and smooth muscle cells respectively [66]. The crucial transcription factor for embryonic

development, T-Box Transcription Factor 20 (TBX20), regulates angiogenesis through the PROK2-PROKR1 pathway in both development and disease [67]. PROKR1 signaling is also involved in regulation of cardiac, renal, and metabolic functions by regulating transendothelial insulin uptake and endothelial cell proliferation. Indeed, a genetically -induced PROKR1 loss in the endothelial cells (ec-PROKR1^{-/-}) causes the impaired capillary formation and transendothelial insulin delivery, leading to insulin resistance and cardiovascular and renal disorders. Despite having a lean body phenotype, ec-PROKR1^{-/-} mice exhibited polyphagia, polydipsia, polyurinemias, and hyperinsulinemia, which are reminiscent of human lipodystrophy [57].

4. Humans data

In Humans, *PROK2* or *PROKR2* gene deficiency cause the development of the Kallmann syndrome (KS) [68–71]. KS is genetically heterogeneous and its clinical features are congenital hypogonadotropic hypogonadism (HH) with anosmia or hyposmia that is sometimes accompanied by a deregulation of the energy balance. Patients carrying biallelic mutations in *PROK2* or *PROKR2* have consistently a severe, complete KS phenotype. Such mutations in homozygous state were found in a few patients, the main part of the KS patients carrying only heterozygous mutations. In that case of monoallelic mutations, the phenotype of patients is more variable and likely depends on the additional genetic hits [72].

Dodé et al. studied a cohort of 192 KS patients using a candidate gene strategy and identified ten and four different point mutations in the genes encoding *PROKR2* and *PROK2* respectively. Interestingly, one patient carrying the mutation p.R73C in *PROK2* gene suffered from a severe sleep disorder and marked obesity, which might be related to the known circadian function of PROK2 and its potential roles in sleep-wake regulation and ingestive behavior [71]. Abreu et al. reported the case of three KS patients who presented mutations in the *PROKR2* gene (p.L173R or p.R268C mutations in the heterozygous state), all were obese and one developed metabolic syndrome [70].

Two other human studies addressed the relationship between PROK2 and metabolic syndrom (MetS), since PROK2 is involved in inflammation which plays an important role in the pathogenesis of MetS. Although its origin has not been clearly

demonstrated, it is thus likely that the main source of plasma PROK2 are lymphoid organs, circulating leukocytes and hematopoietic cells in humans, as it is the case in mice. In the first study, serum PROK2 levels were measured in 162 middle-aged and elderly Chinese patients with cardiovascular risk factors [73]. They were found to be positively correlated with various cardiometabolic risk factors including blood lipid, fasting plasma glucose, HbA1c, blood pressure, body mass index (BMI), and uric acid. One limitation of this study was the lack of healthy control group [73]. In the second study, our group assumed that circulating PROK2 could be used as a biomarker of MetS or T2D in subjects from the DESIR cohort (Data from an Epidemiological Study on the Insulin-Resistance syndrome) [74]. We found that plasma PROK2 was lower in T2D patients compared to the nondiabetic ones, but this association between plasma PROK2 and T2D disappeared after adjustment for BMI and/or caloric intake [23]. In univariate regression studies, PROK2 was significantly inversely associated with BMI, waist girth, fasting glycemia, HbA1c, total cholesterol, and LDL cholesterol [23]. Associations with incident hyperglycemia and incident MetS were observed even after adjustment for BMI and with obesity incidence. They were compatible with the additive model, showing an allele dose effect, but also most of the times with the dominant model meaning that carrying one or two alleles makes no difference. Actually, these results indicate associations between phenotypes and genetic variation but cannot allow to conclude firmly on the genetic model.

5. Conclusion

PROK2 is involved in many physiological processes both in rodents and mammals including Humans. In the metabolic field, it is involved in the regulation of physiological circadian rhythms as body temperature, sleep behavior and glucose homeostasis, bearing in mind that the extrapolation from rodents data to humans can not be automatic. PROK2 reduces food intake when administered in an acute peripheral or central way to mice [19, 23, 56]. Activation of the PROKR1 pathway restrains adipose tissue expansion and positively regulates insulin sensitivity in adipocytes, skeletal muscle and endothelial cells [31,39,57]. Finally, PROK2 attenuates high glucose/high palmitic acid-induced cardiomyocyte apoptosis [64] and it has been suggested that the known cardioprotective effects of metformin could be mediated, at least in part, through an increase in PROK2 signalling [65]. All of these studies suggest that PROKR agonists could be used as potent therapeutic targets for

the treatment of obesity and associated disorders, such as cardiovascular diseases [75]. A PROKR1 non-peptide agonist has already been evidenced to contribute to myocardial repair and collateral vessel growth. However, it should not be forgotten that activation of PROKR signalling pathway may also have adverse effects, as exacerbation of pain, inflammation or CNS neurotoxicity. Thus, much work is still needed to precisely characterize the multiple physiological effects of this promising family of proteins in the hope of developing new therapeutic molecules targeting this signalling pathway.

Declarations of interest

None.

Ethical approval

This is a review article that cites the peer reviewed papers from PubMed. The study designs and ethical statements are detailed in the primary papers, and therefore this review article does not include a study design with direct ethical statements.

Statement of original publication

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Tables

Table 1. Expression pattern of prokineticin 2 in adult mouse peripheral tissues and brain regions.

Table 2. G protein-dependent signaling pathways induced by activation of prokineticin receptors.

Figure legend

Figure 1. Main effects of prokineticin 2 in the control of metabolism and energy balance. GLUT4: glucose transporter 4.

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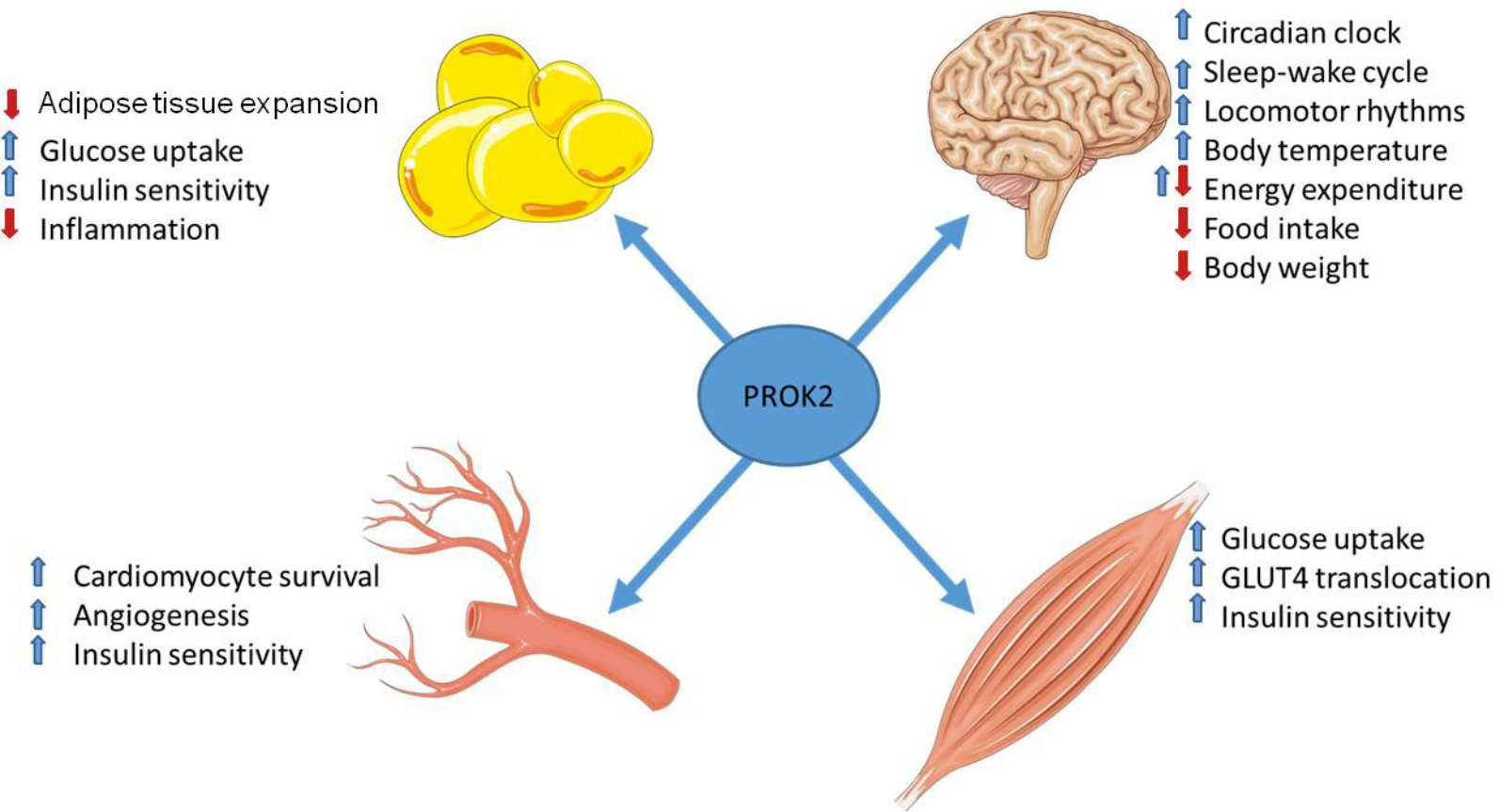
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	Tissue	Expression level	References
Peripheral tissues	Blood cells and bone marrow /inflammatory blood cells	++/+++	[4,10,13]
	Urogenital tract (Testis, Uterus)	++	[10,12]
	Myocardial tissue	++	[8,10,11]
	GI tract, Enteric plexus	++	[5,8,10]
	Kupfer cells in the liver	++	[8,10]
	Some dorsal root ganglion (DRG) neurons	++	[10,20,21]
	Adrenal glands, Spleen	++	[8]
Brain regions	Suprachiasmatic nucleus of the hypothalamus	++++	[14-17]
	Olfactory bulb	++++	[10,17,18,23,24]
	Medial preoptic area of the hypothalamus	+++	[10,17]
	Hippocampus	+++	[10,17,18]
	Islands of Calleja	++	[17]
	Cerebral cortex (high levels in layer II)	++	[10,18]
	Medial amygdala	++	[10,17,18]
	Cerebellar cortex	++	[10,18]
	Spinal Cord (dorsal and ventral horns)	++	[10,18]
	Nucleus of solitary tract	++	[18]
	Arcuate nucleus of the hypothalamus	++/+	[10,17]
	Nucleus accumbens shell	++/+	[17]

Table 1 : Expression pattern of Prokineticin 2 in Adult Mouse Peripheral Tissues and Brain regions.

G protein	Signaling pathway	Tissue /cell type	References
Gq	<p>Ca²⁺ mobilization via activation of phospholipase C (PLC)-β and formation of IP₃</p> <p>Gα_q MAPK and Akt kinases</p> <p>PI3/Akt pathway</p>	<p>Rat DRG neurons,</p> <p>Mouse cultured astrocytes,</p> <p>Mouse macrophages,</p> <p>COS-7 cells,</p> <p>CHO cells</p> <p>HEK 293 cells,</p> <p>Skeletal muscle cells,</p> <p>Cultured capillary endothelial cells derived from heart,</p> <p>Cardiomyocytes,</p> <p>H9c2 cardioblast cell line</p>	<p>[29,34,38,41]</p> <p>[10,11,29,39,42,43]</p>
Gs	<p>Activation of adenylate cyclase and cAMP accumulation</p>	<p>HEK 293 cells,</p> <p>CHO cells</p>	[8,40]
Gi	<p>P hosphorylati on of p44/p42 MAPK</p>	Human monocytes	[4,29,44]

Table 2 : G protein-dependent signaling pathways induced by stimulation of Prokineticin receptors.