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Pleiotropic effects of prokineticin 2 in the control of energy metabolism

Christophe Magnan, Stephanie Migrenne-Li

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1 **Abstract**

2 Prokineticins are family of small proteins involved in many important biological
3 processes including food intake and control of energy balance. The prokineticin 2
4 (PROK2) is expressed in several peripheral tissues and areas in the central nervous
5 system. PROK2 activates G protein-coupled receptors, namely, prokineticin receptor
6 1 (PROKR1) and prokineticin receptor 2 (PROKR2). Preclinical models exhibiting
7 disturbances of the PROK2 pathway (at the level of PROK2 or its receptors) are
8 characterized by changes in food intake, feeding behavior and insulin sensitivity
9 related to a dysfunction of the energy balance control. In Humans, mutations of
10 *PROK2* and *PROKR2* genes are associated to the Kallmann syndrome (KS) that
11 affects both the hormonal reproductive axis and the sense of smell and may also lead
12 to obesity. Moreover, plasma PROK2 concentration has been correlated with various
13 cardiometabolic risk factors and type 2 diabetes (T2D). The present review
14 summarizes knowledge on PROK2 structure, signaling and function focusing on its
15 role in control of food intake and energy homeostasis.

16

1 **1. Introduction**

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

20

21 **2. PROK2 and PROK2 receptors**

22 ***2.1. PROK2***

23 ***2.1.1. The prokineticin family***

24 The first member of the prokineticin family to be identified was Mamba Intestinal
25 Toxin 1 (MIT1) isolated from the venom of the black mamba snake [1] that showed
26 the ability to stimulate contraction of the guinea pig ileum [2,3]. The Bombina
27 variegata (Bv8) was then isolated from the skin secretion of a frog and was found to
28 stimulate the contraction of gastrointestinal (GI) smooth muscle with high potency.
29 Bv8 has 58% identity with MIT1 and was reported to induce hyperalgesia and colon
30 relaxation in rats in addition to its contracting effects on guinea pig, rat, and mouse
31 ileum. Homologues of Bv8 were identified in other amphibians, lizards, fishes, and
32 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

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

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

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

14

15 **2.1.3. Gene regulation**

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

30 **2.2. Prokineticin receptors**

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

1 20p13 in humans and chromosome 2 in mouse). They have about 85% sequence
2 identity [29] and show ~80% identity to the previously described mouse orphan
3 receptor GPR73 [30].

4

5 **2.2.1. Tissue expression**

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

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

33

34

1 **2.2.2. Signaling pathways**

2 PROKRs have been reported to couple to i) Gq proteins [11,29,33,34,38–43] to
3 mediate the intracellular calcium mobilization or mitogen-activated protein kinase
4 (MAPK)/Akt pathways activation, ii) Gi proteins [4,29,44], leading to phosphorylation
5 of p44/p42 MAPK, or iii) Gs proteins [8,40], leading to adenylate cyclase activation
6 and cAMP accumulation (Table 2). However it seems that Gq-signaling pathways
7 are the most common ones associated with PROKRs stimulation. For instance,
8 addition of PROK2 to mouse cultured astrocytes causes increases in astrocytic
9 cytosolic Ca²⁺ levels through PROKR1 activation and astrocytic proliferation [38].
10 PROK2 also induced calcium mobilization in a dose-dependent manner in PROKR1-
11 overexpressed HEK293T cells. In skeletal muscle cells, PROKR1 signaling activated
12 Gq-mediated PI3K/AKT and MAPK/ERK signaling pathways and also promoted the
13 translocation of glucose transporter 4 (GLUT4) into the plasma membrane that
14 influences insulin sensitivity [39]. Interestingly, PROKR1 signaling is significantly and
15 specifically inhibited by the Melanocortin Receptor Accessory Protein 2 (MRAP2) an
16 important regulator of energy homeostasis [40].

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

19 Prokineticins exhibit different affinities for their receptors : PROK2 binds PROKRs
20 with higher affinity than does PROK1 [8] and PROK2beta displays clear selectivity for
21 PROKR1 [9]. However, all the natural prokineticins bind and activate both receptors
22 in nanomolar range. Functional studies showed that PROK2 and PROK2beta
23 stimulate intracellular Ca²⁺ responses in PROKR1-expressing cells with similar
24 potencies. However, the PROK2beta stimulus of Ca²⁺ responses in PROKR2-
25 expressing cells is at least 10-fold less potent than that of PROK2.

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

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

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

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

30

31 **3.1 Role in the circadian clock of physiologic functions**

32 In mammals, the pacemaker that drives circadian rhythms of physiology and behavior
33 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].

1 **3.2 Role in feeding behavior and glucose homeostasis**

2 In rats and mice, acute peripheral administration of PROK2 decreases food intake
3 while it reduces both food intake and body weight when administered chronically, in
4 lean and diet-induced obese (DIO) mice [19]. Interestingly, DIO mice pair fed to the
5 PROK2-treated group lost a similar amount of weight as the mice given PROK2,
6 suggesting that the effect of PROK2 on body weight was mediated predominantly via
7 a reduction in food intake. Mice models lacking *Prok2* have not been reported to
8 show an increased body weight compared with their wild-type littermates, that could
9 be explained by a compensation in embryonic knockout models [19]. Peripheral
10 injection of PROK2 does not alter food intake in *Prokr1* deficient mice or in wild type
11 mice with co-administration of a PROKR1 antagonist, suggesting that the anorectic
12 effect induced by peripheral PROK2 is PROKR1-dependent. It also increases c-fos-
13 like immunoreactivity in the DMV of the brainstem, suggesting that peripherally
14 administered PROK2 may directly act through the DMV to inhibit food intake. Acute
15 or chronic PROK2 administration did not have any adverse effect on glucose
16 tolerance or insulin sensitivity in wild type mice [56]. However, we may assume that
17 *Prokr1* deficiency would alter glucose homeostasis since endothelial PROKR1 has
18 been characterized as a positive regulator of insulin sensitivity [57]. Moreover, in
19 palmitate-induced insulin-resistant myotubes, PROKR1 enhanced insulin-stimulated
20 AKT phosphorylation, GLUT4 translocation, and glucose uptake. mRNA and protein
21 levels of PROKR1 were significantly decreased in skeletal muscle and white adipose
22 tissue of DIO mice, and the amount of PROKR1 was significantly decreased in
23 human skeletal muscle cell-derived myotubes under insulin resistance conditions
24 [39].

25 In the hypothalamus which plays a key role in the regulation of food intake both in
26 humans and rodents, *Prok2* expression is reduced by fasting [19], as soon as the
27 early neonatal period [58]. This is in contrast to what was evidenced in the PVN and
28 may be linked to the study of the entire hypothalamus compared to a single nucleus.
29 ICV administration of Bv8/PROK2 reduces food intake in rodents and significantly
30 decreases the number of feeding episodes without impacting energy expenditure or
31 locomotor activity [19,59]. It also significantly increases the number of arcuate
32 proopiomelanocortin (POMC)-expressing neurons exhibiting c-fos immunoreactivity,
33 while peripheral administration of PROK2 does not [19,56]. This suggests that the
34 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].

1 **3.3 Role in adipose tissue expansion**

2 In addition to its anorectic role, PROK2 also influences body weight homeostasis
3 through a suppressive effect of adipose tissue expansion mediated by PROKR1.
4 Indeed, *ProkR1*-null mice (*ProkR1*^{-/-}) have excessive abdominal adipose tissue and
5 develop severe obesity and impaired adipocyte insulin signaling, that are
6 exacerbated by a high fat diet. The loss of PROKR1 in endothelial cells also led to
7 obstruction of new capillaries development, what created a hypoxic environment.
8 Interestingly, adipocyte-specific mutant mice (*ProkR1*^{ad-/-}) do not show such a severe
9 phenotype, despite increased proliferating preadipocytes and newly formed
10 adipocyte levels, leading to expansion of adipose tissue. They also display
11 substantial infiltration of macrophages in the adipose tissue, which is one other
12 component of obesity. How does PROKR1 do to preserve adequate adipocyte
13 function in obesity is unknown but it strongly suggests that PROKR1 agonists are
14 potent therapeutic targets against obesity [31,63].

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

20 **3.4. Role in angiogenesis and heart protection**

21 In mouse and human myocardial tissue, PROK2 promotes cardiomyocyte survival
22 and angiogenesis, mainly through PROKR1 signaling. PROK2 or PROKR1
23 overexpression induce vessel-like formation in cultured cardiac endothelial cells and
24 activate Akt in cardiomyocytes to protect cardiomyocytes against oxidative stress
25 [11]. Exposure of cardiomyocytes to high glucose/high palmitic acid induces
26 oxydative stress that is accompanied by suppression of PROK2, PROKR1, and
27 PROKR2 expression. These effects are robustly prevented by PROK2, through an
28 Akt-related signalling pathway [64]. Interestingly, the antidiabetic metformin (Met) that
29 exerts cardioprotective effects, attenuated the decreases of PROK2, PROKR1, and
30 PROKR2 expression observed in cardiomyocytes of streptozotocin-treated mice [65].
31 In mouse heart, as well as in kidney, PROKR1 up regulates PROK2 to promote
32 progenitor cells differentiation into a vasculogenic cell type or into endothelial and
33 smooth muscle cells respectively [66]. The crucial transcription factor for embryonic

1 development, T-Box Transcription Factor 20 (TBX20), regulates angiogenesis
2 through the PROK2-PROKR1 pathway in both development and disease [67].
3 PROKR1 signaling is also involved in regulation of cardiac, renal, and metabolic
4 functions by regulating transendothelial insulin uptake and endothelial cell
5 proliferation. Indeed, a genetically -induced PROKR1 loss in the endothelial cells (ec-
6 PROKR1^{-/-}) causes the impaired capillary formation and transendothelial insulin
7 delivery, leading to insulin resistance and cardiovascular and renal disorders. Despite
8 having a lean body phenotype, ec-PROKR1^{-/-} mice exhibited polyphagia, polydipsia,
9 polyurinemias, and hyperinsulinemia, which are reminiscent of human lipodystrophy
10 [57].

11

12 **4. Humans data**

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

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

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

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

21 **5. Conclusion**

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

1 the treatment of obesity and associated disorders, such as cardiovascular diseases
2 [75]. A PROKR1 non-peptide agonist has already been evidenced to contribute to
3 myocardial repair and collateral vessel growth. However, it should not be forgotten
4 that activation of PROKR signalling pathway may also have adverse effects, as
5 exacerbation of pain, inflammation or CNS neurotoxicity. Thus, much work is still
6 needed to precisely characterize the multiple physiological effects of this promising
7 family of proteins in the hope of developing new therapeutic molecules targeting this
8 signalling pathway.

9

10 **Declarations of interest**

11 None.

12 **Ethical approval**

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

16 **Statement of original publication**

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20 **Tables**

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

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

25 **Figure legend**

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

28

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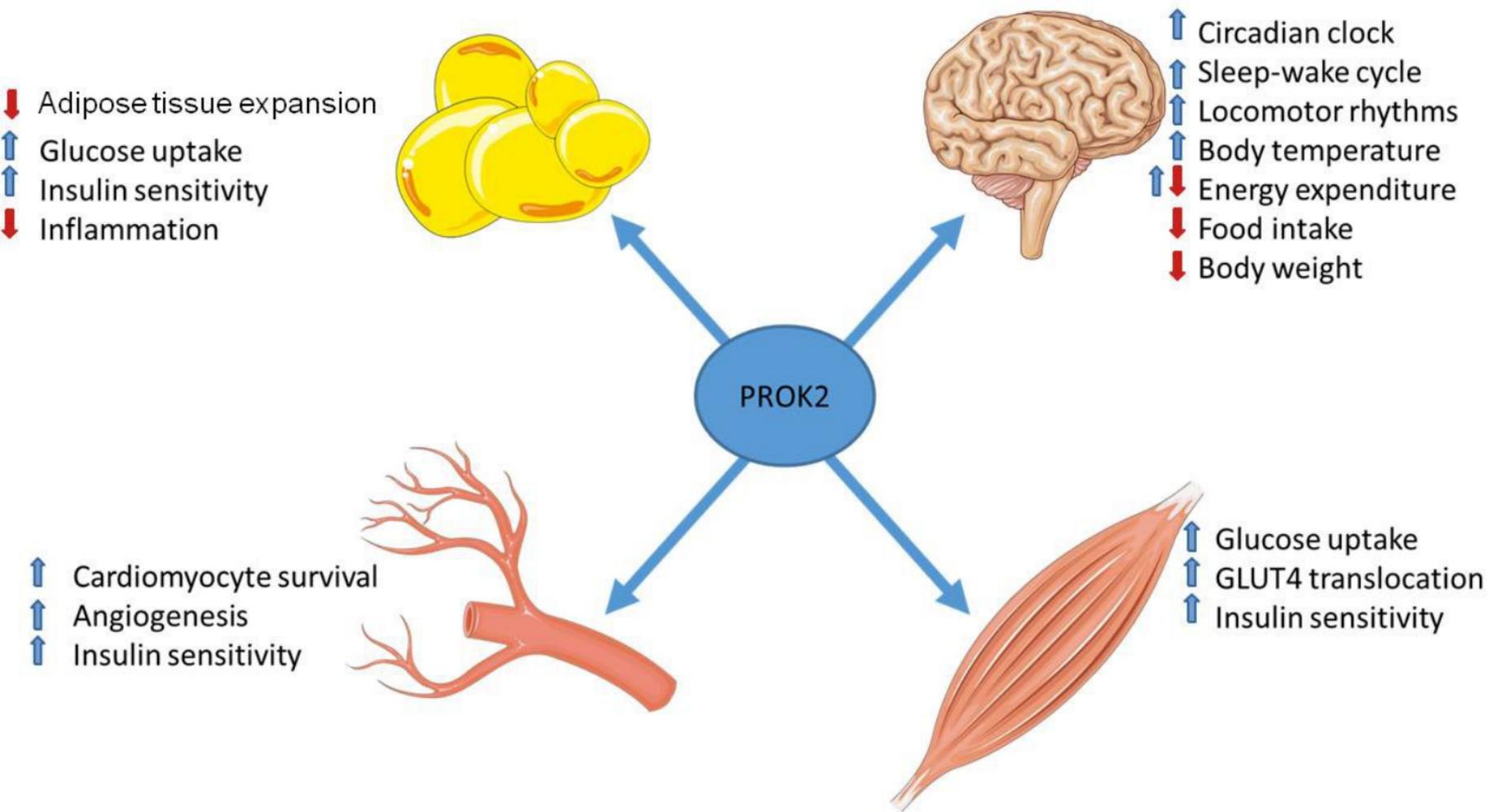
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	Tissue	Expression level	References
Peripheral tissues	Blood cells and bone marrow /inflammatory blood cells	++/+++	[4,10,13]
	Urogenital tract (Testis, Uterus)	++	[10,12]
	Myocardial tissue	++	[8,10,11]
	GI tract, Enteric plexus	++	[5,8,10]
	Kupfer 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 Callejia	++	[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	Ca ²⁺ mobilization via activation of phospholipase C (PLC)- β and formation of IP3 G α q MAPK and Akt kinases PI3/Akt pathway	Rat DRG neurons, Mouse cultured astrocytes, Mouse macrophages, COS-7 cells, CHO cells HEK 293 cells, Skeletal muscle cells, Cultured capillary endothelial cells derived from heart, Cardiomyocytes, H9c2 cardioblast cell line	[29,34,38,41] [10,11,29,39,42,43]
Gs	Activation of adenylate cyclase and cAMP accumulation	HEK 293 cells, CHO cells	[8,40]
Gi	P phosphorylation of p44/p42 MAPK	Human monocytes	[4,29,44]

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