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Biological Function of Prokineticins

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Abstract Secreted peptides have been implicated in diverse physiological functions. Prokineticins are a pair of regulatory peptides that signal through two highly homologous G protein-coupled receptors. Prokineticins possess a unique structural motif of five disulfide bonds and conserved N-terminal stretches. Diverse biological functions, ranging from development to adult physiology, have been attributed to prokineticins. Herein we provide an overview of current knowledge of this interesting pair of regulatory peptides.

Keywords Angiogenesis · Basic helix-loop-helix transcriptional factors · Circadian rhythm · G protein-coupled receptor · Neurogenesis · Neuropeptides · Prokineticin

1 Introduction

Secreted peptides play critical roles in the integration of body physiology and brain functions. Snake venom and skin secretion from frogs have been rich sources for identification of biologically active secreted peptides. Before the molecular identification of mammalian prokineticins, peptides with a unique structural motif of five disulfide bonds had been isolated from snake venom and frog skin secretions (Joubert and Strydom 1980; Schweitz et al. 1990, 1999; Mollay et al. 1999). Mammalian prokineticins (prokineticin 1, PK1 and prokineticin 2, PK2) consist of two secreted proteins of about 80 residues (Wechselburger et al. 1999; Li et al. 2001; LeCouter et al. 2001; Chen et al. 2005). PK1 and PK2 have about 45% amino acid identity between them (Li et al. 2001). In addition to the five disulfide bond motif (Boisbouvier et al. 1998; Li et al. 2001), sequence alignment clearly shows that PK1 or PK2 and their vertebrate homologs exhibit a complete conservation of the first six amino acids (AVITGA) (Bullock et al. 2004). Mutagenesis and protease digestion experiments have revealed that the disulfide bonds and the conserved N-terminal stretch are critical for the bioactivities of prokineticins (Bullock et al. 2004; Negri et al. 2005). Bioassays indicate that the receptors that mediate the contracticle effect of prokineticins on smooth muscle cells belong to the G protein-coupled receptors (Li et al. 2001), and this was subsequently confirmed by three independent groups (Lin et al. 2002a; Masuda et al. 2002; Soga et al. 2002). The current knowledge of biological functions of proki-

neticins has been the subject of a number of recent reviews (Kaser et al. 2003; Ferrara et al. 2004; Zhou and Cheng 2005; Zhou 2006; Maldonado-Perez et al. 2007; Negri et al. 2007)

2
Distribution of Prokineticin Receptors

Two G-protein-coupled receptors, prokineticin receptor 1 (PKR1) and prokineticin receptor 2, with an unusually high degree of sequence identity (>85%) have been identified as receptors for prokineticins (Lin et al. 2002a; Masuda et al. 2002; Soga et al. 2002). This high degree of receptor sequence conservation suggests the critical functions of prokineticin receptors for essential biological processes. Intriguingly, even with the high degree of conservation, PKR1 and PKR2 reside in different human and mouse chromosomes (Lin et al. 2002a). In essentially all functional assays, PK1 or PK2 exhibit little selectivity over PKR1 or PKR2 (Lin et al. 2002a). This apparent non-selectivity of ligand/receptor activation implies that the availability of ligands (PK1 or PK2) and receptors (PKR1 or PKR2), i.e., the gene expression regulation, likely determines which possible signaling pair is involved for a particular biological process. Distribution studies have revealed that PKR1 is widely distributed in the peripheral organs, including the gastrointestinal system, lungs, blood system, and various endocrine organs (Lin et al. 2002a; Masuda et al. 2002; Soga et al. 2002). PKR2 may also be expressed in various endocrine tissues, including thyroid, pituitary, adrenal gland, testis and ovary (Lin et al. 2002a; Masuda et al. 2002; Soga et al. 2002). In situ analysis indicates that PKR2 is the dominant receptor in the adult brain, with particularly high expression of PKR2 in the hypothalamus, the olfactory ventricular regions and the limbic system (Cheng et al. 2002, 2006; Ng et al. 2005), which has been confirmed by a series of functional studies.

3
Regulatory Function of PK2 in Circadian Rhythms

The suprachiasmatic nucleus (SCN) in the anterior hypothalamus is the primary mammalian circadian clock that drives daily rhythms of diverse physiology and behavior (Reppert and Weaver 2002). While an understanding of the molecular mechanism for the circadian clockwork has emerged (Reppert and Weaver 2002; Lowrey and Takahashi 2004), much less is known about how the timing information from the SCN clock is transmitted to control physiology and behavior. The initial observation that PK2 mRNA in the SCN displays dramatic daily oscillation has implicated the potential regulatory function of PK2 in the circadian rhythm (Cheng et al. 2002). Multiple lines of evidence

have emerged to support that PK2 function as a prominent communicating signal from the SCN to its targets in the generation of circadian rhythms. In vitro studies have revealed that PK2 is a first-order clock-controlled gene with its expression controlled by CLOCK and BMAL1 acting on the E-box elements in its promoter (Cheng et al. 2002). CLOCK and BMAL1 are critical positive components of the circadian clock (Reppert and Weaver 2002). Correlative in vivo experiments have confirmed that PK2 is a clock-controlled gene that lies downstream of CLOCK and BMAL1. In mutant mice lacking functional clockwork, including Clock mutant and $Cry1^{-/-}Cry2^{-/-}$ mice, the PK2 mRNA oscillation in the SCN is abolished (Cheng et al. 2002). Moreover, the molecular rhythm of PK2 in the SCN was shown to adapt faster to the delay of light cycles than to the advance of light cycles (Cheng et al. 2005). It is well known that the circadian clock is asymmetrically built. The differential rates of adaptation of the PK2 rhythm to the delay and advance of light cycles were also consistent with the respective rates of behavioral and physiological adaptation observed in animals and humans (Yamazaki et al. 2000). Furthermore, intracerebroventricular delivery of recombinant PK2 suppressed the nocturnal wheel-running activity (Cheng et al. 2002) and feeding behavior (Negri et al. 2004), when endogenous PK2 level is minimal. Receptor distribution studies also revealed the capability of SCN targets to respond to an oscillatory PK2 signal from the SCN. The receptor for PK2 (PKR2) is expressed in virtually all known primary SCN targets, the paraventricular nucleus of the hypothalamus (PVN), the dorsal medial nucleus of the hypothalamus (DMH), paraventricular and paratenial nuclei of the thalamus (PVT/PT), and lateral septal nucleus (LS) (Cheng et al. 2002)

Recently, direct genetic evidence for the role of PK2 in the control of the circadian rhythm has been reported. The generation and characterization of mice lacking the PK2 or PKR2 gene has confirmed the critical role of PK2 signaling for the maintenance of robust circadian rhythms (Li et al. 2006; Prosser et al. 2007). Under both normal light/dark and constant dark housing conditions, the reduction of circadian locomotor rhythmicity in PK2-null mice was apparent (Li et al. 2006). PK2-null mice also displayed significantly reduced rhythmicity for a variety of other circadian indices, including sleep-wake cycle, body temperature, circulating glucocorticoid and glucose levels as well as the expression of peripheral clock genes. The fact that PK2-null mice have essentially normal clockwork genes oscillation in the SCN is consistent with the postulated role of PK2 as an output molecule. The circadian phenotypes of PKR2-mutant mice are almost identical with that of PK2-null mice (Prosser et al. 2007). The targeted null mutation of PKR2 disrupts circadian coordination of the activity cycle and thermoregulation. Specifically, mice lacking PKR2 lost precision in timing the onset of nocturnal locomotor activity; and under both a light/dark cycle and continuous darkness, there was a pronounced temporal redistribution of activity away from early to late circadian night. Moreover, the coherence of circadian locomotor behavior

was significantly reduced, and nocturnal body temperature was depressed. As with PK2-null mutants, entrainment by light is not dependent on PKR2, and bioluminescence real-time imaging of organotypical SCN slices showed that the mutant SCN is fully competent as a circadian oscillator, consistent with the notion that PKR2 only functions in the output pathway. In a transgenic model of Huntington's disease, a correlation between increased daytime locomotor activity and reduced SCN expression of PK2 molecular rhythm was also observed (Morton et al. 2005). Studies from these mutant mice strongly supported the notion that PK2 signaling is an essential link for coordination of circadian behavior and physiology controlled by the SCN.

The daily cycle of sleep/wakefulness is probably the most important physiological process regulated by the SCN circadian clock. The sleep characterization of PK2-null mice has recently been reported (Hu et al. 2007). Sleep regulation has been postulated to consist of homeostatic and circadian processes (Borbely 1982). The homeostatic process determines the duration and intensity of sleep, which builds up in the absence of sleep and dissipates during sleep. The circadian process, controlled by SCN, determines the timing of sleep (Klein et al. 1991). However, the relationship of these two processes is still controversial. Earlier results implied that the homeostatic process is independent of the circadian process, since sleep amount and recovery sleep after sleep deprivation are unchanged in SCN-lesioned rats that lack normal circadian expression of sleep (Mouret et al. 1978; Mistlberger et al. 1983; Tobler et al. 1983; Eastman et al. 1984; Borbely et al. 1989; Trachsel et al. 1992). However, in more recent studies on SCN-lesioned rats it has been interpreted that SCN is also involved in the homeostatic regulation of sleep (Wurts and Edgar 2000). Furthermore, SCN lesion in the monkeys indicated that the circadian process is an intimate component of the homeostatic process. Complete SCN lesion of the squirrel monkey led to a loss of circadian timing as well as a 4 h increase in daily sleep time (Edgar et al. 1993). Studies with circadian gene mutant mice have revealed that these circadian genes affect not only circadian sleep distribution, but the homeostatic regulation of sleep as well. Clock gene mutant mice sleep 2 h less than wild-type mice daily (Naylor et al. 2000), whereas the Bmall/Mop3 mutant mice (Laposky et al. 2005), and double Cry1 and Cry2 mutant mice (Wisor et al. 2002) displayed 1.5 h and 1.8 h increases in total sleep amount under baseline conditions, respectively. In wild-type mice, sleep deprivation (SD) is followed by a compensatory sleep increase. Mice lacking both Cry1 and Cry2 (Wisor et al. 2002) had a reduced non-rapid eye movement (NREM) sleep rebound, while mice lacking Clock (Naylor et al. 2000), the Bmall/Mop3 (Laposky et al. 2005) had a reduced rapid eye movement (REM) sleep rebound in response to SD. In PK2-null mice, the total sleep time under entrained light-dark and constant darkness conditions was reduced (Hu et al. 2007). Furthermore, the reduced sleep time occurred predominantly during the light period, consistent with the expression of PK2 during this period. Intriguingly, PK2-null mutant mice displayed

an impaired response to sleep deprivation (Hu et al. 2007). These studies indicate that PK2 plays roles in both circadian and homeostatic regulation of sleep.

Daytime restricted feeding (RF), is known to compete with SCN, to control locomotor rhythm (Damiola et al. 2000; Stokkan et al. 2001). In response to a daytime RF, rodents will feed at unusual periods for the sake of survival and gradually become active before the food is made available, a phenomenon called food anticipatory activity (FAA). FAA is a rhythmic event and has been associated with the food entrained oscillators (FEO) in the brain (Gooley et al. 2006). Thus, during RF, the FEO competes with the light-entrained oscillator (LEO, i.e. SCN) for the control of activity and physiological events. The increased FAA has been interpreted as a relatively stronger FEO control (Dudley et al. 2003; Pitts et al. 2003). When the FAAs of PK2-null and control mice were monitored in response to RF, PK2-null mice displayed significantly higher FAA (Li et al. 2006). Thus, in the absence of the PK2 signal, the control of locomotor rhythm was weakened, and their response to RF was enhanced. This study supports the notion that PK2 functions as a signal from the SCN that suppresses inappropriate feeding activity.

The molecular rhythm of PK2 in the SCN of a diurnal rodent has also been investigated (Lambert et al. 2005). Similar to the oscillation pattern observed in nocturnal mouse and rat (Cheng et al. 2002; Masumoto et al. 2006), PK2 mRNA in the SCN of diurnal *Arvicanthis niloticus* was rhythmically expressed, with peak levels in the morning hours and essentially absent during the night phase (Lambert et al. 2005). Thus, the phase of PK2 expression in the SCN of diurnal rodents is the same as that of nocturnal rodents, consistent with a growing body of evidence suggesting that the key to diurnality lies downstream of the SCN circadian clock. It will be very interesting to demonstrate whether nocturnal and diurnal animals respond differentially to the same-phase PK2 signaling from the SCN clock.

Recent studies have identified a possible neurophysiological mechanism of PK2 in mediating SCN output in controlling diverse circadian rhythms. PK2 was shown to excite neurons that express the PK2 receptor (Cottrell et al. 2004). This first physiological study of PK2 revealed a possible link between this oscillating PK2 messenger and the neuronal firing rates. The neurophysiological effect of SCN-derived PK2 on the PVN, one critical SCN target, has recently been reported (Yuill et al. 2007). PVN, a primary SCN efferent nucleus, is involved in the regulation of endocrine rhythms or oscillation of the autonomic nervous system. PK2 was able to excite PVN neurons of both parvocellular and magnocellular branches (Yuill et al. 2007). Importantly, a peptide-based PK2 receptor antagonist was able to decrease the basal activity of parvocellular neurons in the hypothalamus slice (including SCN and PVN) only during the light phase, when PK2 is highly expressed in the SCN (Yuill et al. 2007). Thus, endogenous SCN-derived PK2 excites the parvocellular branch of PVN neurons in a phase-dependent manner.

4 Function of PK2 in Neurogenesis

In mammals, neurogenesis occurs mainly during embryonic to early postnatal stages. However, neurogenesis persists in certain regions of adult mammalian brains, including the olfactory bulb (OB) and the dentate gyrus (DG) of the hippocampus (Altman 1969; Gage 2000). New neurons are continuously generated in the OB from progenitor cells of the subventricular zone (SVZ) of the lateral ventricle. These progenitor cells proliferate and migrate through the rostral migratory stream (RMS) to the OB to form mature granular and periglomerular cells through the adult life of mammals (Kaplan and Hinds 1977; Luskin 1993; Lois and Alvarez-Buylla 1994). Recently, it was demonstrated that PK2 functions as a chemoattractant for SVZ-derived neuronal progenitors, and this PK2 signal is essential for the normal development of OB architecture (Ng et al. 2005). The expression of PK2 and its receptor was complementary in the OB, with PK2 expressed in the mature granular and periglomerular layers of the OB whereas its receptors (PKR1 and PKR2) are expressed in the immature ependyma and subependymal layers of the olfactory ventricle (Ng et al. 2005; Cheng et al. 2006). In vitro migration assays indicated that PK2 stimulates migration of neuronal progenitors from the SVZ in both adult and postnatal rats, and this migration is directional and could be inhibited by the PK antagonist (Ng et al. 2005). The critical role of PK2 in OB development was confirmed by studies with PK2-null mice. PK2-deficient mice have abnormal development of OB, including the dramatic reduction in OB volume, and the loss of normal OB layer architecture (Ng et al. 2005). Although both PKR1 and PKR2 are expressed in the immature ependyma and subependymal layers of the olfactory ventricle, genetic analysis indicated that PKR2, but not PKR1, is a critical receptor for OB development (Matsumoto et al. 2006). Whereas the OB development in PKR1-deficient mice is essentially normal, PKR2-deficient mutant mice exhibited similar abnormal development of the OB, as that of PK2-null mice. These genetic studies reveal that PK2-PKR2 signaling is essential for normal neurogenesis in the OB.

It has been demonstrated that CLOCK and BMAL1 are the positive upstream regulators of PK2 in mediating circadian rhythms (Cheng et al. 2002). The normal OB development in Clock and Bmal1 mutant mice (Vitaterna et al. 1994; Bunger et al. 2000) indicates that PK2 is likely under the control of different transcriptional factors in regulating neurogenesis of the OB. Recent studies have elucidated the identities of these upstream regulators of the PK2 gene for OB neurogenesis (Zhang et al. 2007). In the OB, the PK2 gene is found to be a functional target of proneural basic Helix-Loop-Helix (bHLH) factors NGN1 and MASH1. bHLH transcription factors have been shown to be crucial regulators of neurogenesis (Bertrand et al. 2002). During development, NGN1 and MASH1 regulate several important steps of neurogenesis,

including the commitment of stem cells to neuronal and glial lineages, the specification of neuronal subtype identities, and neuronal migration. The evidence that NGN1 and MASH1 are the upstream regulators of the PK2 gene in OB neurogenesis includes: (1) NGN1 and MASH1 activate PK2 transcription by binding to E-boxes on the PK2 promoter; (2) NGN1 and MASH1 are co-expressed with PK2 in OB neurons; (3) Chromatin immunoprecipitation has demonstrated the association of NGN1 and MASH1 with the PK2 promoter in vivo; (4) Similar defects in OB neurogenesis have been identified in the mutant mice that lack Ngn1, Mash1 and PK2. These results indicate that PK2 is a critical downstream target gene of NGN1 and MASH1 in regulating OB neurogenesis.

Thus, it is interesting to learn that the same PK2/PKR2 signaling pair plays a critical role in apparently completely different biological processes: OB neurogenesis during development and circadian clock output of adult physiology. For both processes, the PK2 gene is the common functional target of different families of bHLH transcriptional factor: Ngn1 and MASH1 for OB neurogenesis and Clock and Bmal1 for circadian clock output. It is truly intriguing to learn that the same set of E-boxes in the promoter of the PK2 gene is utilized by Ngn1/MASH1 and CLOCK/BMAL1 (Zhang et al. 2007). Thus, PK2 has been established as a common functional target gene for different bHLH transcriptional factors in regulating their respective functions (Fig. 1). It is clear that NGN1/MASH1 and ClOCK/BMAL1 also have other target genes. This gene network of transcriptional control of PK2 and its upstream regulators has provided an elegant example showing that the complexity of the mammalian genome is probably not due to a sheer increase in the quantity of genes, but more likely to complicated gene transcriptional control.

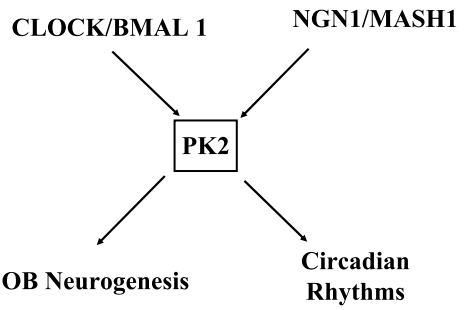

Fig. 1 Transcriptional regulation of prokineticin 2 (PK2) by basic helix-loop-helix (bHLH) transcription factors. The promoter of the PK2 gene contains multiple E-box sequences (CACGTG) that are targeted by bHLH transcription factors CLOCK/BMAL1 and NGN1/MASH1. PK2 is a common functional target gene for both sets of transcriptional factors in regulating their respective biological processes: circadian rhythm and olfactory bulb (OB) neurogenesis

5 Functions in Angiogenesis

Angiogenesis is crucial for diverse biological processes including development, tumorigenesis, reproduction, and wound healing. When screening a library of secreted molecules, LeCouter et al. identified PK1 as a molecule that was capable of inducing proliferation of primary bovine adrenal-cortex-derived capillary endothelial (ACE) cells (LeCouter et al. 2001). It was later found that, similarly to adrenal gland-derived endothelial cells (EC), PKs enhanced the proliferation and inhibited apoptosis in EC derived from another endocrine gland, the corpus luteum (Kisliouk et al. 2005; Podlovni et al. 2006). In fact, PKs also induced proliferation of bovine aortic EC (BAEC), a well-characterized model for a macrovessel, indicating that PKs are mitogens and serve as survival factors for microvascular (AEC and luteal EC) and macrovascular (BAEC) cells (Kisliouk et al. 2005; Podlovni et al. 2006). However, these different EC types each have a distinct pattern of PKR expression (Fig. 2). In contrast to ACE or luteal EC, which expressed both PKR1 and PKR2, BAEC mainly expressed PKR1. BAEC and luteal EC differed not only in their receptor repertoire, but also in their regulation in response to apoptotic cues such as TNFa and serum starvation (Fig. 2) (Podlovni et al. 2006), suggesting cell-specific biological effects. Under conditions of serum starvation, PK1 maintains its antiapoptotic effects only in luteal EC (and not in BAEC) (Podlovni et al. 2006). These findings highlight the importance of PK1 action on stressed EC, suggesting that the presence of PK-R2 provides luteal EC with

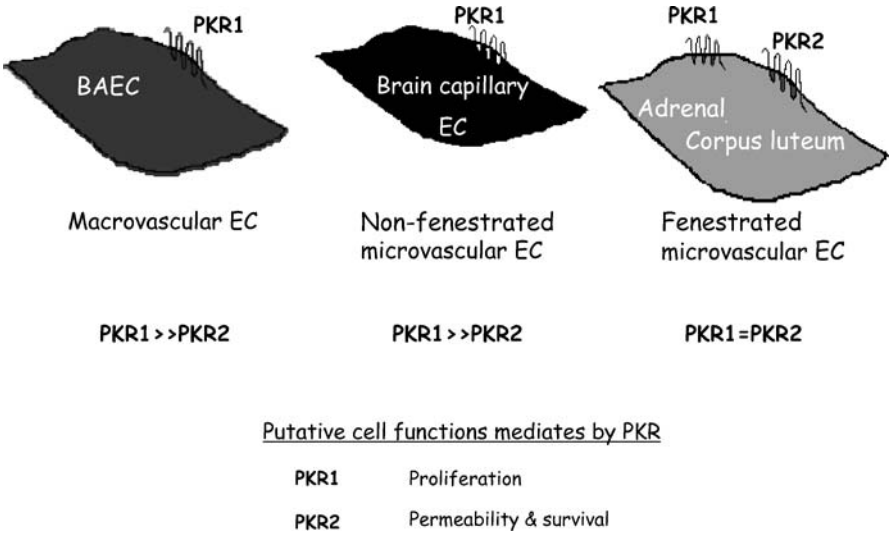

Fig. 2 Prokineticins receptor distribution in different endothelial cell types

an additional tool to resist stress-induced apoptosis. Indeed, due to their tissue microenvironment, luteal EC are more prone than BAEC to experience extreme conditions such as low oxygen tension and a shortage of nutrients.

Besides mitogenesis, endothelial permeability plays an important role in angiogenesis. In addition to the functions mentioned above, the presence of PKR2 appears to be indispensable for PK1-induced endothelial cell permeability. These effects were only observed in PKR2 expressing LEC and not in BAEC or another cell examined: brain capillary EC (BCEC; Fig. 2) (Podlovni et al. 2006). The case of BCEC is particularly interesting: in contrast to other microvascular EC, the brain capillary endothelium serves as a protective shield or barrier between blood and the underlying tissue (the central nervous system) (Abbott 2000; Kniesel and Wolburg 2000). To date, PKR2 has only been identified in fenestrated EC such as those found in the adrenal cortex, CL, kidney, and liver, in agreement with the notion that PKR2 plays a role in EC permeability (Kisliouk et al. 2005; LeCouter et al. 2003c; Lin et al. 2002) (Fig. 2). Notably, in aorta and corpus luteum, PK1 was immunolocalized to the smooth muscle layer in blood vessel walls, and could not be detected in EC (Podlovni et al. 2006). Hence, PK1, which is necessary for activating EC types via PK-Rs, is produced in neighboring cells, suggesting that PK1 has a paracrine mode of action.

The promoter of PK1 possesses putative HIF-1 (hypoxia-inducing factor) binding sites, and the expression of PK1 is induced by hypoxia. Delivery of recombinant virus, which expresses PK1 in ovaries, elicited potent angiogenesis, but the angiogenic effect was absent when delivered to cornea or skeletal muscles. PK1 was hypothesized as the first example of a tissue-specific angiogenic factor (LeCouter et al. 2003a), although its angiogenic effect is most likely broader than originally claimed (Tanaka et al. 2006; Podlovni et al. 2006). Interestingly, PK1 is up-regulated under certain diseased conditions, including malignancy (Ferrara et al. 2003; Zhang et al. 2003; Pasquali et al. 2006; Ngan et al. 2007). In essentially all in vitro angiogenic assays, including the proliferation, survival, and migration of different EC types (BAEC, luteal EC, and ACE), PK2 behaved similarly to PK1 (LeCouter et al. 2003b; Kisliouk et al. 2005; Podlovni et al. 2006). The expression of PK2 is also hypoxia-inducible (LeCouter et al. 2003b), and the PK2 promoter possesses more probable consensus HIF1 binding sites than that of PK1. To date, however, the expression studies of PK2 in tumors are rather limited.

6 Functions in the Reproductive System

Since angiogenesis is crucial for normal development and function of the corpus luteum and the placenta, several groups have examined the possible roles of PKs in reproductive organs. Subsequently, it was reported that PK1

and its receptors are expressed in the ovary, uterus, placenta, testis, and prostate, with functions extending beyond the vascular network. A possible paracrine role for PKs in the function of the endometrium and placenta has been suggested by functional studies and expression analyses. Battersby et al. demonstrated that PK1 expression, but not PK2, was elevated in the endometrium during the secretary phase of the menstrual cycle, and this PK1 elevation was induced by treatment with progesterone (Battersby et al. 2004). The presence of PKR1 and PKR2 in the glandular epithelial cells and smooth muscle cells, along with endothelial cells, suggests that PKs have angiogenic as well as nonangiogenic functions such as myometrial contraction.

Hoffmann and colleagues (Hoffmann et al. 2006) have shown that in the human placenta, expression of PK1 and PKR1 peaks during the first trimester of pregnancy, corresponding to the hypoxic period of placental development. Moreover, in cultured trophoblast cells, PK1 and its type 1 receptors were up-regulated by hypoxia. Interestingly, although both PK1 and VEGF were induced by hypoxia, these two peptides exhibited distinct spatiotemporal patterns of expression, with PK1 mainly localized to the syncytiotrophoblast layer and VEGF to the cytotrophoblast and extravillous trophoblast cells (Hoffmann et al. 2006). Similar findings were reported for the mouse placenta, where PK1 was elevated during early gestation (Hoffmann et al. 2007).

It has recently been shown that PK1 is also localized to the ovary (Frazer et al. 2005; Kisliouk et al. 2005b). In the corpus luteum its expression increased throughout the luteal phase, with the highest levels found during the late luteal phase including in regressed glands undergoing apoptosis (Frazer et al. 2005; Kisliouk et al. 2005b, 2007). As in the placenta, the relative abundance of PK2 mRNA in human corpus luteum was low (Kisliouk et al. 2003; Frazer et al. 2005). Elevated PK1 mRNA levels were also detected in another ovarian apoptotic tissue: the granulose cell layer of atretic follicles. Immunostaining and FACS analysis eventually showed that PK1 is expressed by macrophages infiltrating the regressed corpus luteum (Kisliouk et al. 2007). Functional studies demonstrated the involvement of PK1 in enhancing the recruitment and subsequent activation of leukocytes in atretic follicles and regressing CL (Kisliouk et al. 2007). Therefore during different reproductive stages in the ovary as in placenta and uterus, PK1 undertakes different roles, vascular as well as non-vascular functions.

The PK system has also been indirectly implicated in reproductive organ development (Matsumoto et al. 2006; Pettiloud et al. 2007). For example, PK2-deficient mice exhibit hypogonadotropic hypogonadism, and are essentially infertile (Pettiloud et al. 2007). The hypogonadotropic hypogonadism in PK2-deficient mice is due to a dramatic decrease in the GnRH neuron population in the hypothalamus. The fact that PK2-deficient mice responded normally to exogenous human chorionic gonadotropin indicates that the reproductive phenotype is most likely due to a migration defect in GnRH neurons in

these mice (Pettiloud et al. 2007). Earlier studies have revealed that OB is required for the proper migration of GnRH neurons from the nasal cavity to the hypothalamus during development (Wierman et al. 2004). A similar reproductive defect was also observed in PKR2-deficient mice (Matsumoto et al. 2006). Thus, the PK2/PKR2 signaling pathway is essential for OB ontogenesis and therefore critical for the normal migration of GnRH neurons (Ng et al. 2005; Matsumoto et al. 2006; Pettiloud et al. 2007). Recently, homozygous deletion mutations in the PK2 gene have been reported to account for two patients with Kallman Syndrome and one patient with normosmic idiopathic hypogonadotropic hypogonadism (Pettiloud et al. 2007). The identified deletion results in a truncated PK2 protein of only 27 amino acids (rather than 81 in its mature form), which completely lacks bioactivity. Thus, homozygous loss-of-function PK2 mutations cause both Kallman Syndrome and normosmic idiopathic hypogonadotropic hypogonadism. Heterozygous mutations in PK2 or PKR2, including compound heterozygotes, have also been associated with Kallman syndrome (Dode et al. 2006). This indicates that insufficient PK2 signaling through PKR2 leads to abnormal development of the olfactory system and reproductive axis in humans. It will be very interesting to examine the OB and reproductive phenotype of PK2 and PKR2 compound heterozygous mice.

7
Prokineticins as Regulators of Gastrointestinal Motility

The contractile activity of prokineticins on gastrointestinal smooth muscle was the first demonstrated biological activity for this family of regulatory peptides (Mollay et al. 1999; Schweitz et al. 1999; Li et al. 2001). The ligand-binding studies and functional studies with gastrointestinal preparations had revealed the presence of high-affinity prokineticin receptors in the smooth muscle cells of small intestines (Li et al. 2001), and these observations have helped the initial characterization of prokineticin receptors and eventual molecular identification (Lin et al. 2002a). Whereas it is still unclear whether the effects of prokineticins on gastrointestinal smooth muscle cells are pharmacological or physiological, in vivo studies have indicated that exogenous PK1 and PK2 stimulate the gastrointestinal transit (US patent filing US2004/0162238A1; Owyang C, personal communication), and thus the contractile effects of prokineticins on gastrointestinal smooth muscle might be propulsive. The role of prokineticins in gastric and colonic contractility has also been investigated (Bassil et al. 2005; Hoogerwerf 2006). PK2 was found to increase the emptying of a liquid meal from rat stomach (Lewis 2004), although this observation was not confirmed by a different study (Bassil et al. 2005). In colons, PK1 was found to suppress giant contractions of the circular muscle via the release of nitric oxide indicating an indirect effect of proki-

neticins on gastrointestinal motility is also likely (Hoogerwerf 2006). This study further revealed that PKR1 is expressed on myenteric plexus neurons and it co-localizes with a small subset of NOS-expressing neurons. Thus, PK1 or PK2 may regulate gastrointestinal motility directly via activating smooth muscle cells, and indirectly via modulating the activities of enteric neurons. Additional studies, including those with mutant mice as well as small molecule receptor antagonists, need to be carried out to increase our understanding of the roles of prokineticins in regulating gastrointestinal motility.

8 Prokineticins and Pain Perception

A series of studies have indicated the role of prokineticins or their non-mammalian homologues in sensitivity of pain perception. Intraplantar injection of PK2 and Bv8 causes a strong and localized hyperalgesia by reducing the nociceptive thresholds to thermal and mechanical stimuli (Mollay et al. 1999; Negri et al. 2002; Hu et al. 2006). Systemic injection of Bv8 into rats also induces hyperalgesia to tactile and thermal stimuli (Negri et al. 2002). The hyperalgesia caused by PK2 or Bv8 is likely due to activation of dorsal root ganglia (DRG) neurons, which express both PKR1 and PKR2 mRNAs (Negri et al. 2002; Vellani et al. 2006). Functional assays indicate that PK2 and Bv8 were able to mobilize calcium in cultured rat DRG neurons (Negri et al. 2002; Hu et al. 2006). Transient receptor potential vanilloid 1 (TRPV1) has recently been identified as a possible molecular link between PKR1/R2 and DRG neuron activation. TRPV1 is an excitatory ion channel that is critical for the detection and integration of pain-producing chemical and thermal stimuli by DRG neurons (Caterina and Julius 2001). Colocalization experiments have revealed that the majority of PKR-positive DRG neurons also express TRPV1 (Vellani et al. 2006), and calcium image studies have revealed that the majority of DRG neurons that respond to PK2 were also activated by capsaicin (Hu et al. 2006). Mice lacking the PKR1 gene exhibited impaired pain perception to various stimuli, including noxious heat, mechanical, capsaicin, and protons (Negri et al. 2006), indicating that PKR1 is probably the dominant receptor that exerts a tonic activation of TRPV1 in DRG neurons. The reduced response of TRPV1-null mice to Bv8 (Negri et al. 2006) further indicates that TRPV1 is a critical downstream signaling component of PKR1 in pain perception.

The expression pattern indicates that PK2 might be the ligand for PKR1 in regulation of pain perception, especially in inflammatory pain. PK2 is highly expressed in peripheral blood cells, notably in monocytes, neutrophils, and dendritic cells (LeCouter et al. 2004; Dorsch et al. 2005). Thus, PK2 could be released into the sites of inflammation by neutrophils, activate macrophages and subsequently induce the release of other proinflammatory cytokines such

as interleukin 1 and interleukin 12 (Martucci et al. 2006). Genetic studies with PK2-deficient mice confirm the critical involvement of PK2 in acute and inflammatory pain (Hu et al. 2006). PK2-deficient mice displayed significant reduction in nociception induced by thermal and chemical stimuli. Thus, PK2-PKR1 appears to be the dominant ligand/receptor pair in the regulation of pain sensation.

9 Role of Prokineticins in the Development and Function of Blood Cells

The detection of the mRNAs of prokineticins and their receptors in bone marrows and other hematopoietic organs implicates a possible role in hematopoiesis (Li et al. 2001). Detailed expression analyses indicate that both PKR1 and PKR2 are expressed in the hematopoietic stem cells (LeCouter et al. 2004). Dorsch et al. (2005) first investigated the differentiative effect of PK1 on bone marrow cells and demonstrated that PK1 drastically promoted the differentiation of mouse and human bone marrow cells into the monocyte/macrophage lineage. LeCouter et al. (2004) showed the similar effect of PK2 on monocyte lineage, and further demonstrated that PK2 promoted the survival and differentiation of granulocytic lineages in cultures of human or mouse hematopoietic stem cells. Taken together, these studies imply the possible role of prokineticins, particularly PK2, in hematopoiesis, which may be conserved during evolution (Soderhall et al. 2005).

As both PKR1 and PKR2 are expressed in mature blood cells, prokineticins may also regulate the functionality of specific mature blood cells by altering their behaviors. PK2 and Bv8 induced chemotaxis of macrophages with very high potency (LeCouter et al. 2004; Martucci et al. 2006). PK1 treatment altered the morphology of human peripheral monocytes and expression of several cytokines or cytokine receptors, such as interleukin-10, interleukin-12, and tumor necrosis factor α (Dosrch et al. 2005; Kisliouk et al. 2007). These observations are interesting in the context of the sites of PK1 and PK2 expression. PK1 is expressed constitutively in B cells, T cells, and also in inflamed tissues (LeCouter et al. 2001; Dosrch et al. 2005). PK2 is detected in dendritic cells, neutrophils, and macrophages (LeCouter et al. 2004; Martucci et al. 2006). PK2 is also highly expressed in infiltrating cells at sites of inflammation, predominantly in neutrophils, and the expression of PK2 and both PK receptors are induced in monocytes upon exposure to lipopolysaccharide (LeCouter et al. 2004). These results indicate that PK1 or PK2 may regulate an immune response by altering monocyte activation, migration of granulocytes and monocytes. Thus, it is likely that prokineticins are hematopoietic cytokines that modulate the innate and the adaptive immune systems.

10 Summary and Perspectives

Over the last few years, prokineticins have evolved as a pair of signaling peptides that regulate diverse functions ranging from embryonic development to adult physiology. These diverse functions could be generally classified into two categories: cell excitability and cell differentiation/migration. The regulatory function of prokineticins in circadian rhythm, pain perception, and gastrointestinal motility relates to the enhanced cell excitability, whereas the functions in angiogenesis, neurogenesis, and hematopoiesis are linked to the effects of prokineticins on cell differentiation and migration. Another emerging theme is that PK2 has been shown as a common functional target gene for different sets of upstream transcriptional factors in regulating separate biological processes. It is obvious that the majority of the biological functions of prokineticins are yet to be discovered.

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