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1 NEUROBIOLOGY OF HEADACHE

2 The prokineticin system: an interface between neural
3 inflammation and pain

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7 **Abstract** Prokineticins (PK) 1 and 2 belong to a new
8 family of chemokines capable to interact with two different
9 G coupled receptors: Prokineticin receptor (PKR)1 and 2.
10 Both prokineticins and their receptors are widely dis-
11 tributed in different tissues and regulate several biological
12 functions. In particular, a role of the PK system in
13 inflammation and nociception has been established. PKRs
14 are expressed in regions of the nervous system associated
15 with pain and in primary sensitive neurons they colocalize
16 with transient potential receptor vanilloid–TRPV1 provid-
17 ing an anatomical interaction in nociceptor sensitization.
18 Moreover, PKs are strongly upregulated in immune and
19 glial cells and sustain a proinflammatory loop in inflamed
20 tissues. Recent evidences indicate that the block of the PK
21 system represents a promising strategy to contrast inflam-
23 mation and pain.

24 **Keywords** Prokineticins · Neuroinflammation · Pain

25 Prokineticins

26 What are they? and why are they becoming
27 popular?

28 Prokinetincins belong to a family of small peptide dis-
29 covered about twenty years ago in the skin secretions of
30 *Bombina Variegata* frog, from which the alternative
31 denomination Bv8 derives [1], and in the venum of *Black*
Mamba snake and were initially described for their ability 32
to induce gastrointestinal motility in rodents. Soon the 33
mammal homologs of Bv8: the prokineticin 1 (PK1 or 34
endocrine gland derived vascular endothelial growth factor, 35
EG-VEGF) and PK2 (mammalian Bv8) were also descri- 36
bed. All members of the PK family weigh approximately 37
8 kDa and have a structural conserved motif characterized 38
by an N-terminal AVITGA sequence, a Trp residue in 39
position 24 and the presence of five disulfide bridges. 40
These peptides activate two closely related G-protein 41
coupled receptors: prokineticin receptor 1 and 2 (PKR1 and 42
PKR2) that belong to the family of neuropeptide Y receptor 43
and have an amino acid identity of 85%. 44

PKs and their receptors are widely distributed in many 45
human tissues such as ovary, testis, adrenal gland, placenta, 46
uterus, brain, intestinal tract, heart, bone marrow and 47
peripheral blood. This wide and strategic presence in the 48
body tissues allows them to be involved in many biological 49
activities and to coordinate complex behaviors like feed- 50
ing, drinking, circadian rhythm, neurogenesis, angiogene- 51
sis, haematopoiesis [2–4] activating multiple intracellular 52
signals such as mitogen activated protein kinase (MAPK), 53
AKT and STAT3. Moreover, the presence of PK members 54
(PK and PKRs) in immune cells and in the main stations 55
involved in pain transmission makes PK important players 56
in inflammation and pain pathophysiology. 57

Prokineticins as regulators of inflammation 58

A parallelism between the structure, size, signaling and 59
biological activities of prokineticins and chemokine 60
superfamily was soon suggested [5] and PKs are now 61
recognized in all respects as chemokines. Lymphoid 62
organs, circulating leukocytes and hematopoietic cells, 63
synoviocytes and dendritic cells constitutively express 64

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65 moderate levels of prokineticins [6, 7] and their levels are
66 increased in inflamed tissues [7].

67 We demonstrated that the administration of Bv8 (a
68 valuable research tool to study PK system) in mice induced
69 a proinflammatory phenotype stimulating macrophage
70 chemotaxis and proinflammatory cytokine release [8] and
71 skewing a Th1/Th2 balance towards a Th1 response [9]. A
72 similar effect was also described for human monocytes.
73 Our studies demonstrated that these effects are mediated by
74 PKR1 receptor with the involvement of a Gq protein [8].
75 Moreover, the literature suggests that multiple pathways
76 are involved in PK signaling and PKRs can also couple to
77 Gi and Gs proteins [6, 10].

78 In the last years, several papers described in animals and
79 humans a direct correlation between alterations in PK
80 system and the development of different inflammatory and
81 autoimmune diseases suggesting that the PK antagonism
82 could ameliorate the pathological condition. The involve-
83 ment of PK system was described in a mice model of
84 arthritis (collagen-induced arthritis, CIA) where the
85 expression levels of PK2 and PKR2 were found to be
86 elevated in the CIA joints due to the presence of macro-
87 phage cells in the synovial membrane and these levels
88 correlated with the arthritis severity. The administration of
89 a prokineticin receptor antagonist decreased or suppressed
90 the severity of arthritis by inhibiting PK2-PKR2 signaling
91 in macrophages and reducing the levels of proinflammatory
92 cytokines [11].

93 Pedotti' group recently demonstrated a role of the PK
94 system in multiple sclerosis, in an animal model (experi-
95 mental autoimmune encephalomyelitis, EAE) and patients
96 and showed how the use of a PKR antagonist could inhibit
97 proinflammatory T cell responses and reduce neurologic
98 signs and central nervous system damage in mice [12]. The
99 role of PK was also suggested in the development of
100 Psoriasis, a chronic systemic inflammatory and autoim-
101 mune skin disease [13]. The author demonstrated a positive
102 PK2/IL-1 proinflammatory loop which sustained chronic
103 inflammation and keratinocyte hyperproliferation. The
104 knock-down of PK2 improved the inflammatory condition
105 while PK2 overexpression aggravated psoriasis [13].

106 These results suggest that prokineticins appear strongly
107 upregulated in inflammatory cells and sustain a positive
108 inflammatory loop at the basis of the development of
109 pathological condition.

Prokineticins in nociceptive pain

111 The first evidences of a pronociceptive role of PK were
112 derived from the observation that systemic injection of Bv8
113 and PK2 in rodents induces hyperalgesia to mechanical and
114 thermal stimuli [1, 14] by activating PKRs receptors
115 localized in the main stations of pain pathway. Both PKR1

116 and PKR2 are expressed in the superficial layers of the
117 spinal cord, dorsal root ganglia (DRG) and peripheral ter-
118 minal of nociceptors. The release of the neuropeptides like
119 calcitonine gene related peptide (CGRP) and substance P in
120 the spinal cord [1, 15] together with TRPV1 sensitization in
121 primary dorsal root ganglia (DRG) neurons [1] was pri-
122 marily involved in the development of hyperalgesia.
123 Experiments of co-localization indicated that the majority
124 of PKR1-positive DRG neurons also express TRPV1 and a
125 reduced response to Bv8 is observed in TRPV1 deficient
126 mice. Moreover, 50% of the Bv8-responding DRG neurons
127 also express neuromediators involved in pain processing
128 such as substance P and CGRP and release them after Bv8/
129 PK exposure.

130 It was suggested that PKs are also able to modulate
131 central pain mechanism. Maione and collaborators
132 demonstrated that the microinjection of Bv8/PK into the
133 periaqueductal grey (PAG) exerted a pronociceptive effect
134 increasing the intrinsic GABAergic tone which, in turn,
135 was responsible for the inhibition of PAG antinociceptive
136 output neurons impinging on rostro ventromedial medulla
137 (RVM) neurons [17].

138 Further studies conducted in mice lacking pkr1 or pkr2
139 or pk2 genes demonstrated a direct role of the prokineticin
140 system in pain perception in fact all these genotypes were
141 characterized by higher thermal, mechanical and tactile
142 pain threshold in comparison to normal wild type mice
143 [18, 19].

Prokineticines in chronic pain (inflammatory and neuropathic pain)

146 It has recently emerged that pathological pain development
147 and maintenance are not confined only to changes in the
148 activity of neuronal systems, but involve interactions
149 between neurons, inflammatory immune cells, glial cells,
150 as well as a wide cascade of pro- and anti-inflammatory
151 cytokines [20]. In this view, PKs can be considered
152 important modulators capable to interfere both with
153 peripheral and central pain mechanism. Consistently in the
154 last years, a role of the PK system was suggested in the
155 development and maintenance of inflammatory and neu-
156 ropathic pain. Negri [21] demonstrated in an animal model
157 of chronic inflammation, Complete Freund Adjuvant
158 (CFA), that inflammation was highly correlated with an
159 overexpression of PK2 in the granulocytes that infiltrate the
160 inflamed tissue and the up-regulation was responsible for
161 inflammation-associated hyperalgesia [21]. The authors
162 showed how the hyperalgesia induced by CFA was abol-
163 ished by PC1, a non-peptidic PKR1 preferred antagonist.

164 Regarding neuropathic pain (NP), the involvement of
165 prokineticins was investigated in different experimental
166 animal models: NP derived from an injury of sciatic nerve:

167 chronic constriction injury model (CCI) [22] and spared
168 nerve injury [23], diabetes [24] and cancer-induced NP
169 [25]. In all these models, the presence of aberrant pain well
170 correlates with an increase of the levels of PK2 in the
171 spinal cord, especially in activated astrocytes [22–24]. The
172 treatment with a PK system antagonist like PC1 or with PK
173 neutralizing antibody [25] was able to counteract thermal
174 hyperalgesia and allodynia, reduce the injury-induced
175 overexpression of PK2 and restore the physiological levels
176 of proinflammatory and anti-inflammatory cytokines both
177 in periphery and spinal cord.

Conclusions

179 The above presented evidences suggest that PK system
180 plays a central role in the development and maintenance of
181 inflammation and acute and chronic pain and indicate that
182 the antagonism of the PKRs could represent a new phar-
183 macological strategy to control pathological pain, acting at
184 different levels. The PKR block may contrast the prono-
185 ciceptive role of endogenous PK, reduce immune cells
186 infiltration and neuroinflammation in the main pain sta-
187 tions, and prevent the release of CGRP and TRPV1
188 sensitization.

Perspective: what about a possible role
of prokineticins in migraine?

191 Abundant evidences accumulated from animal and human
192 have demonstrated that the activation of meningeal affer-
193 ents, neuropeptide release, and neurogenic inflammation
194 plays a pivotal role in the generation of pain in migraine
195 headache. CGRP is no doubt a crucial player and upon
196 stimulation, it is released not only at the nerve endings,
197 where it mediates vasodilation via smooth muscle cell
198 receptors and plasma extravasation [26] but also from the
199 neuronal cell bodies in the trigeminal ganglia. It is now
200 recognized that CGRP represents a regulator of the intra-
201 ganglionic crosstalk between neurons and glial cells
202 prompting an inflammatory cascade that could lead to
203 sensitization throughout the release of proinflammatory
204 cytokines and chemokines which in turn activate TRPV1 or
205 purinergic P2X receptors. Moreover, recent evidences
206 suggested that also in migraine a significant neuronal, glia,
207 immune interaction exists and an involvement of proin-
208 flammatory cytokines in the pathophysiology of primary
209 headaches is probable. As previously described, proki-
210 neticins induce CGRP release in the spinal cord and DRG
211 and recent findings demonstrated that also the trigeminal
212 ganglia can be a target for prokineticins. In fact the
213 application of Bv8/PK to trigeminal ganglia significantly
214 elevated their heat-induced CGRP release and

215 immunohistochemistry experiments revealed a co-local-
216 ization of PK2 and CGRP in the same trigeminal neurons
217 [27]. It was also suggested that PK2 suppressed GABA
218 activated current in trigeminal ganglion neurons [28]. All
219 these evidences support the idea of the presence of proki-
220 neticin receptors in the vascular trigeminal system. In the
221 same way, we cannot exclude the possibility that PKs may
222 also directly sensitize meningeal nociceptors also by
223 inducing proinflammatory cytokines release. Finally, we
224 can speculate that PK may also sustain a central nervous
225 system origin of migraine acting on PAG-RVM circuit
226 [29].

227 Future studies aimed at identifying novel targets, such as
228 PK system, in migraine will be of great importance, also
229 considering that the treatment of this condition is still far
230 from being satisfactory.

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