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COMMENTARY

Humanizing mice: catching up with elusive B1 receptors

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Bradykinin receptor activation plays an important role in pain arising following tissue inflammation, and recent studies have suggested that bradykinin B1 receptors in particular may be important in chronic pain related to arthritis and various neuropathies. The investigation of the function of the B1 receptors *in vivo* has been hampered by the lack of nonpeptide antagonists, and the development of such compounds made more difficult by the considerable species variation between human and rodent B1 receptors. In this issue, Fox and co-workers report the creation of a mouse that has had the human B1 gene inserted into the corresponding mouse locus, and they exploit this animal to study the effects of a novel, nonpeptide B1 receptor antagonist on measures of acute nociception and nociception following inflammation. By creating a platform that allows the study of human B1 receptors *in vivo*, these investigators have provided a tool to significantly advance the understanding of the kallikrein–kinin system in physiological and pathophysiological states.
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The kallikrein–kinin system is a complex system intimately involved in the regulation of blood pressure, inflammation and nociception (Marceau *et al*., 1998). The ligands for kinin B1 and B2 receptors are produced on demand by tissue proteases and while B2 receptors are constitutively expressed in many tissues, including neurons, a hallmark of B1 receptors is their induction by pathophysiological stimuli. There is considerable variation between species in the preferred natural ligands for the kinin receptors and important differences in the sequences of B1 receptors between rodents and humans. This means that while physiological studies of B1 receptor function can be performed in rats or mice, examining the *in vivo* effects of ligands with affinity for human B1 receptors has to date been problematic. The difficulties of studying an inducible receptor in the absence of good nonpeptide antagonists has previously led to the generation of B1 receptor knockout mice (Pesquero *et al*., 2000), knockin mice that constitutively express rat B1 receptors (Ni *et al*., 2003) and even a transgenic rat over-expressing human B1 receptors (Hess *et al*., 2004). While valuable, none of these models provide a platform for examining the effects of potentially therapeutic B1 ligands on a background of a physiologically regulated receptor.

This goal appears to have been achieved as a result of the impressive study reported in the current issue of this journal (Fox *et al*., 2004). The authors have 'knocked-in' the human B1 receptor into the B1 receptor gene locus in mice. By replacing only the coding sequence of the mouse gene and preserving the flanking regions containing regulatory sequences, they produced an animal in which expression of the human B1 receptor was induced by the kind of stimuli that upregulate expression of the native mouse protein, such as lipopolysaccharide treatment. Furthermore, the inflammation-induced hyperalgesia that was absent in mouse B1 knockout animals was restored after knock in of the human B1 receptor. Finally, oral administration of the novel nonpeptide B1 antagonist NVP-SAA164 reduced inflammation-induced hyperalgesia in the human B1 knockin mice.

The mouse described in this study provides an excellent example of a powerful strategy for the *in vivo* evaluation of pharmacological agents that display species variability. In addition, the study provides further support for the well-established role of B1 receptors in chronic inflammatory pain. More importantly, the current findings have implications for other chronic pain states. Recent studies have demonstrated a role for B1 receptors in neuropathic pain states, such as those caused by nerve injury and diabetes (Gabra & Sirois, 2002; Rashid *et al*., 2004). These neuropathic pain states are resistant traditional opioid analgesics, and treatment of the hyperalgesia and allodynia associated with these pain states remain elusive.

More broadly, the development of nonpeptide B1 antagonists by the present group and others (Ransom *et al*., 2004) will facilitate the investigation of the role of B1 receptors in acute nociception and other physiological processes. Although B1 receptors are dramatically induced by inflammation or injury, there is increasing evidence that B1 receptors are normally expressed to some degree in subpopulations of nociceptive sensory neurons (Vellani *et al*., 2004) and neurons in the spinal cord and brain, a situation reminiscent of how our understanding of the enzyme COX-2 developed. Studies in B1 knockout animals suggest that absence of B1 receptors leads to changes in some measures of acute nociception, and good antagonists should help resolve whether this arises from an absence of B1 receptors at some point during the development of the nervous system or if B1 receptors truly contribute to acute thermal and chemical nociception (Pesquero *et al*., 2000).

While it remains to be seen to what extent the role and regulation of B1 receptors in mice parallels that in

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humans, this humanized mouse will undoubtedly be a valuable tool, and its success will hopefully encourage other investigators to undertake the arduous task of producing knock-ins of other G-protein-coupled receptors that have substantial species divergence in their ligand binding profiles, such as substance P (Rupniak & Kramer, 1999).

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