Commentary

Toward a new anti-inflammatory and analgesic agent

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A comparative study on normal and B1-receptor deficient (B1-KO) mice, Pesquero et al. (1) provide evidence in this issue of PNAS in favor of crucial roles played by the kinin B1 receptor in toxic shock, inflammation, and nociception. B1 receptors are generally silent or absent in healthy states and are induced or activated by pathological challenges. When they are absent, as in B1-KO mice, tissue reactions to microbial toxins, local inflammatory agents, and painful stimulations are reduced without any apparent drawback. The B1 receptor appears therefore to favor the appearance and worsening of pathological lesions and symptoms. From this paper, the B1 receptor emerges as a new target of great potential for the development of agents directed to reduce the generally excessive responses that are used by the body to counteract noxious stimuli. These agents should be antagonists, possibly specific and selective for the B1 receptor.

In the late '70s, two different types of receptors for bradykinin and related kinins were identified, using pharmacological in vitro assays on rabbit isolated vessels, the aorta for the B1 and the jugular vein for the B2 receptor (2). The B1 receptor was characterized earlier than the B2, thanks to the discovery of selective and specific antagonists. It soon became evident that kinins [bradykinin (BK) and kallidin (LysBK)] are the natural ligands for the B2, and their desArg⁹ metabolites (desArg⁹ BK and Lys desArg⁹ BK) are the ligands of the B1 receptor (Fig. 1). Indeed, treatment of rabbit aorta with Mergetpa (a carboxypeptidase M inhibitor) prevented the myotrop effect of BK, while leaving intact the activity of desArg⁹ BK, which indicated that activation of the B1 receptor by BK requires the conversion of the nonapeptide to desArg⁹ BK by intramural carboxypeptidases (3). This appears today to be the general pathway by which B1 receptor ligands are generated in tissues in pathological conditions, when the B1 receptor is made to express itself and needs the ligands for its activation (4). In fact, BK and LysBK are the only species that are released when plasma kallikrein acts on high molecular weight kininogen (to release BK) and tissue kallikreins hydrolyze the low molecular weight kinino-

![Fig. 1. Kinins and their receptors.](Image)

To release LysBK (5); the B1 selective agonists can derive only from the kinins, which are therefore to be considered as biologically active agents as well as precursors of other species (Fig. 1).

In the early '90s, B1 and B2 receptors were cloned in animals and humans (4, 6) and found to lie close to each other in human chromosome no. 14. No genetic or pharmacological evidence has been reported until now in favor of the existence of other types of kinin receptors (4, 6, 7) in mammals. B1 and B2 receptors differ, however, in their sequence, expression, and function: they are different pharmacological entities and their physiopathological roles are certainly distinct. In fact, sequence homology between B1 and B2 receptors is only 36%, approximately the same as between B1 and AT₁, the functional receptor for angiotensin II. B2 receptors are constitutive structures that are expressed by a variety of cells under physiological conditions, whereas B1 are generally absent from healthy tissues, but rapidly appear after injuries of various nature (e.g., injection of lipopolysaccharide or IL-1B), eventually in parallel with cyclooxygenase 2 and carboxypeptidases or other functional tissue components. Interestingly, a reduction of NO synthase activity is associated to the blunted expression of the B1 receptor in B1-KO mice. Thus, the B1 receptor up-regulation appears to be part of a more generalized response that includes the local coexpression (eventually up-regulation) of enzymes, receptors, and autacoids that notoriously play key roles in the early and late responses of tissues to various types of injury. Worthy of mention is that studies on B1 receptors have been important for demonstrating differences between responses of normal or pathological tissues to the same agents, opening the way to modern pathopharmacology.

An eloquent demonstration of these facts is provided by the results presented by Pesquero et al. (1), who first cloned the mouse B1 receptor (7) and then disrupted its gene by targeting technology to obtain B1-KO mice. When lipopolysaccharide is injected to induced toxic shock, the control mice respond with a marked fall of blood pressure, whereas little, if any, change is observed in the B1-KO. The blood pressure fall is not immediate and progresses over 30–40 min, as expected by the timing needed for lipopolysaccharide to release interleukins (IL-1B, according to ref. 4) and the subsequent induction of the B1 receptor, which is known to require new protein synthesis (4). The acute blood pressure fall is attributed in large part to the release of NO (from the endothelium). A reduced level of NO synthase activity may contribute to the blunted blood pressure response to lipopolysaccharide, observed in B1-KO mice, because it may reduce the massive and rapid production of NO that worsen toxic shock. In normal conditions, reduction of NO synthase activity could interfere, however, with the protective role of NO on endothelia, a role that may account for part of the beneficial effects of angiotensin-converting enzyme (ACE) inhibitors (8) in hypertension, heart failures, and diabetes. Induction of these diseases in the B1-KO mice and the subsequent treatment with ACE inhibitors could help to assess the possible implication of the B1 receptor, both in some cardiovascular disorders and in the therapeutic efficacy of ACE inhibitors. Much remains to be done with B1-KO mice.

Other findings by Pesquero et al. (1) concern inflammation, namely pleurisy...
and peritonitis induced by carragein, a chemical stimulus that activates kinin release and, according to Campos et al. (9), also induces the B₁ receptor. Accumulation of polymorphonuclear leukocytes (PMN) in the pleurisy exudate occurs in control but not in B₁-KO animals and strongly suggests that B₁ receptors are involved in one determinant step of the PMN activation. These steps (homing, rolling, adhesion, diapedesis) result from the cooperative interactions of adhesion molecules (of which some are induced by interleukins or tumor necrosis factor, for instance E selectine) and factors (eventually receptors) that are expressed on the surface of endothelial and/or white blood cells. As a matter of fact, the B₁ receptor has been shown to be present in both the endothelia and the PMN (see ref. 4, for a review). The step of the PMN migration that is facilitated and the mechanism by which the B₁ activation may intervene in the process remain to be elucidated. The great merit of Pesquero et al. has been to provide a first consistent evidence that the B₁ receptor is involved specifically in the migration of PMN while other similar processes (e.g., migration of monocytes or symptoms of the initial inflammatory reaction (e.g., plasma extravasation) occur normally in B₁-KO mice. The consequences of reduced or abolished PMN migration in the evolution of inflammation and other tissue injuries processes remains to be classified; in this respect, it would be interesting to see whether reactions of B₁-KO mice to ischemia (e.g., by coronary artery ligation) differ from those of controls, because accumulation of PMN outside the blood stream has been shown to occur in ischemia. Last but not least, results emerging from the Pesquero et al. study should be consolidated by the use of B₁ receptor antagonists, using the available peptides, suitable for the mouse (10) and the emerging nonpeptide compounds.

A role for the B₁ receptor in the generation and the perception of pain in various forms of inflammation, especially in the second phase of hyperalgesia, has been proposed, based on results obtained with selective antagonists (4). While supporting this proposal with findings in B₁-deficient mice, the role of B₁ receptors in nociception has been proven by Pesquero et al. (1) by showing that only responses involving complex (spinal and supraspinal) neuronal circuits (hot plate test) are modulated by the B₁ receptor, whereas acute nociceptive responses (tail flick) and other defensive mechanisms (heat) are not. This finding speaks in favor of B₁ being expressed not only in the sensory but also in motor neurons and interneurons, particularly in connection with inflammatory lesions induced by chemical or physical challenge (e.g., carragein), that are known to induce release of IL-1B and the B₁ receptor. This is the basic mechanism already discussed for toxic shock and inflammation. However, the most original aspect of the present work is that B₁ receptors may be implicated in the acute phase of hyperalgesia, with a new mechanism of central sensitization. For this to occur, B₁ receptors must be there from the beginning, because induction requires timing that is incompatible with acute responses to formalin, capsaicin, and the central sensitization (wind up) that have been shown to be reduced or abolished in B₁-deficient mice. As a matter of fact, mRNA for B₁ receptors has been shown to be present in sympathetic and sensory ganglia in the mouse (11), possibly to allow for a rapid setting of functional B₁ receptor sites (Table 1). This process could be independent of IL-1B, the hyperexpression of cyclooxygenase 2, the increased production of prostaglandins (that makes neurons more sensitive to tissue kinins), and the induction of the tachykinin NK-1 receptor. The merit of Pesquero et al.’s paper (1) is to propose a second mechanism by which the B₁ receptor can produce hyperalgesia. This new mechanism is illustrated (and compared with the classical IL-1B induction of B₁) in Table 1. It appears to concern the synaptic connectivity between nociceptive sensory neurons and the spinal reflex circuits that are altered in the B₁-deficient mice and therefore to involve primarily the central neurons. It does not require new synthesis (up-regulation) of B₁ receptors; the already existing functional unit needs to be accessible or ready to work in a very short time. It should act not through prostanoids but by lowering the threshold of the nociceptive circuit, specifically to painful stimuli perhaps through the action of another interleukin, IL-8, as suggested by the results of Cunha et al. (12).

In conclusion, the paper by Pesquero et al. (1) confirms the knowledge that B₁ receptors are generally inductible in pathological states and their induction is controlled by interleukins (IL-1B) together with the induction of other factors (cyclooxygenase 2) and receptors (NK-1) in the frame of a generalized tissue response to injury. Pesquero et al. have provided evidence for the existence of a new way by which, apparently silent B₁ receptors can be rapidly made to work by chemical (capsaicin, formalin) and electrical stimuli which selectively activate B₁ receptors, whose major function is to sensitize the sensory system to noxious stimuli. Overall, the paper is an elegant demonstration of important pathological roles sustained by the B₁ receptor and provides strong evidence for the use of B₁ receptor antagonists as new anti-inflammatory and analgesic agents.

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Table 1. B₁ receptors in pathologies

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Lesion</th>
<th>Cell</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxic shock</td>
<td>Massive release of NO</td>
<td>Endothelium</td>
<td>B₁ up-regulation by IL-1B</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Migration of leucocytes</td>
<td>PMN</td>
<td>B₁ up-regulation by IL-1B and IL-8</td>
</tr>
<tr>
<td>Chronic carragecin pain</td>
<td>Increased nociception</td>
<td>Sensory neuron</td>
<td>B₁ up-regulation by IL-1B</td>
</tr>
<tr>
<td>Acute formalin pain, capsaicin, wind up</td>
<td>Increased nociception</td>
<td>Spinal neurons</td>
<td>B₁ up-regulation by IL-1B</td>
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