B₂R of Bradykinin Activated by Proteases

To the Editor:

We wish to correct the misstatements of our results by Houle et al., who quoted us: “The human B₂R has been proposed to be a specific binding site for serine proteases like human and porcine tissue kallikrein and trypsin, with pharmacological activation of these receptors on binding” (p. 611); “Tissue kallikrein exerts its effect on B₂R after high-affinity binding and without the need for a catalytically active enzyme” (p. 616). Both statements are attributed to our report, but they are the opposite of what we published in abstract, on p. 831, para. 2, and on p. 834, para. 7; using cultured transfected or native cells, catalytically active enzyme is necessary to activate the bradykinin (BK) receptor and involving cleavage of a peptide bond. Just binding to B₂ R did not activate it since inactive prokallikrein and DFP-treated kallikrein bind to B₂ R (Figure 7); conversely, trypsin or cathepsin G did not bind to (Figure 6, p. 832, para. 3, and p. 836, para. 3), but activated the native receptor (p. 830, paras. 6, 7, 11, and p. 831, para. 1).

These authors also exposed HEK 293 cells to undiluted human plasma. After rinsing cells they added rabbit kallikrein, released BK, and, in Western blot, showed kininogen (Figure 5). Elsewhere they found no competition binding with [¹⁴C]BK of kallikrein added to B₂ green fluorescent protein-expressing cells. They conclude that kallikrein’s effect on B₂ was due to kinin release, but elsewhere (p. 616) to limited proteolysis of B₂ R.

They detected low levels of BK (0.01 nmol/L) in the medium of HEK 293 cells exposed only to 50 nmol/L kallikrein for 10 minutes but not to plasma. It is questionable whether this amount could be found with cells washed with zinc-free medium or containing Ca²⁺-EDTA as done by us (p. 634, para. 5) to remove traces of kininogen bound via Zn²⁺. If kallikrein released 0.01 nmol/L BK in 10 minutes, no significant amount of BK would be released in the seconds needed to elevate [Ca²⁺], by kallikrein in CHO/B₂ cells (Figure 2). Finally, the authors show that 50 nmol/L kallikrein releases arachidonate from HEK-B₂-GFP cells as does 1 nmol/L BK (Figure 3) as we reported in Figure 1, but certainly 0.01 nmol/L BK would not give such a response.

Of course kallikreins liberate kinins, but when investigating a basic mechanism in cultured cells, they are never exposed to undiluted plasma, but grown with diluted serum which presumably most kininogen was catabolized during clotting.

The authors also found that rabbit kallikrein (50 nmol/L) stimulated B₂ R, released arachidonate, and contracted the rabbit jugular vein without cross-desensitization to BK as we stated. These experiments and other ones appear to confirm our results, in contrast to misquotations.

We are puzzled how repeated statements, supported by a figure, can be cited the opposite way. Nevertheless, this does not negate the validity of our finding that B₂ R is protease-activated.

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Response: Does the Bradykinin B₂ Receptor Function as a Protease-Activated Receptor?

Our recent article was misquoted by the above correspondence of Deddish, Hecquet, and Erdös. We used human, not rabbit tissue kallikrein (TK). HEK 293 cells were washed with a zinc-free solution before TK stimulation in experiments based on enzyme immunoassays for kinins. The possibility that B₂ receptor (B₂ R) proteolysis initiates the pharmacologic effects of kallikrein was mentioned only as a nonpreferred hypothesis among others. Beyond misquotations from either side, we wish to sumarize why our experiments do not convincingly support a direct B₂ R activation by TK.

HEK 293 cells expressing B₂ R-green fluorescent protein (GFP) were exploited to assess receptor hydrolysis by extracellular proteases, as GFP-related fragments are readily detectable reaction products. The reaction was undetectable for TK (≤100 nmol/L) or for plasma kallikrein. However, the cells were pharmacologically activated by TK (50 nmol/L). A plausible explanation is that the cells have taken up or produced kininogen. Even without plasma pretreatment, a significant production of immunoreactive bradykinin was detected in TK-stimulated cells. The measured concentration (∼10 pmol/L) was low in the supernatant, but the kinin level must have been transiently higher at the cell surface and possibly sufficient for uptake by high affinity receptors, as discussed. What could be the source of kininogen for such a reaction? Kininogen production by various types of cultured cells has been documented. Kininogens also adhere to HEK 293 cells exposed to human plasma. Animal sera used in culture media are not likely source of high molecular weight kininogen, but may provide cells with low molecular weight kininogen.

Our desensitization experiments constitute the strongest evidence against a proteinase-activated receptor (PAR)-related mechanism for the action of kallikrein at B₂ Rs. The contraction of the rabbit jugular vein induced by TK (50 nmol/L) is completely desensitized after a single application. Bradykinin does not desensitize the preparation to itself or to TK if the stimulations are made at 30-minute intervals. We and others have shown that the B₂ Rs are completely recycled to the cell surface after bradykinin-induced desensitization and endocytosis. Proteolytically active kallikrein obviously consumes a substrate that is present in a limited amount to contract the vein via a mechanism sensitive to a B₂ R antagonist. This substrate is not likely to be the B₂ R, as shown by unabated responses to BK in TK-desensitized tissues. B₂ Rs hypothetically cleaved by kallikrein are not likely to remain at the cell surface to be available for subsequent bradykinin stimulation because receptors cleaved by proteases are submitted to immediate endocytosis and extensive degradation. The most reasonable explanation is that the relevant kallikrein substrate is a kininogen in the rabbit jugular vein.

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Letters to the Editor


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