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Potentiation of bradykinin actions by analogues of the bradykinin potentiating nonapeptide $BPP_{9\alpha}$

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Abstract

Synthetic analogues of the bradykinin potentiating nonapeptide $BPP_{9\alpha}$ indicate significantly different structural requirements for potentiation of the bradykinin (BK)-induced smooth muscle contraction (GPI) and the inhibition of isolated somatic angiotensin I-converting enzyme (ACE). The results disprove the ACE inhibition as the only single mechanism and also the direct interaction of potentiating peptides with the bradykinin receptors in transfected COS-7 cells as molecular mechanism of potentiation. Our results indicate a stimulation of inositol phosphates (IP_n) formation independently from the B_2 receptor. Furthermore, the results with La^{3+} support the role of extracellular Ca^{2+} and its influx through corresponding channels. The missing effect of calyculin on the GPI disproves the role of phosphatases in the potentiating action. These experimental studies should not only contribute to a better understanding of the potentiating mechanisms but also incorporate a shift in the research towards the immune system, in particular towards the immunocompetent polymorphonuclear leukocytes. The chemotaxis of these cells can be potentiated most likely by exclusive inhibition of the enzymatic degradation of bradykinin. Thus the obtained results give evidence that the potentiation of the bradykinin action can occur by different mechanisms, depending on the system and on the applied potentiating factor.

Keywords: Potentiation; Bradykinin; Bradykinin potentiating peptide; Angiotensin I-converting enzyme; Inositol phosphate; Arachidonic acid; Ca^{2+}-influx; Protein phosphatases; Polymorphonuclear leukocytes; Chemotaxis; Smooth muscle contraction; Radioligand binding

Abbreviations: AA, arachidonic acid; ABA, 4-azidobenzoic acid; ACE, angiotensin I-converting enzyme; Aloc, allyl oxycarbonyl; ASA, 4-azidosalicylic acid; BK, bradykinin; BKR, bradykinin receptor; $BKR\text{-}B_1$, bradykinin B1 receptor; $BKR\text{-}B_2$, bradykinin B2 receptor; Boc, *tert*-butyloxycarbonyl; BPA, *p*-benzoylphenylalanine; BPP, bradykinin potentiating peptide; $BPP_{9\alpha}$, bradykinin potentiating peptide 9α (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro); BOP, benzotriazole-1-yl-oxy-tris (dimethylamino) phosphonium hexafluorophosphate; DCM, dichloromethane; Dde, *N*-(1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl; Ddz, α,α-dimethyl-3,5-dimethoxy-benzyloxycarbonyl; DEAE, diethylaminoethyl; DIEA, diisopropylethylamine; DIC, diisopropylcarbodiimide; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; DTE, dithioerithritol; ED, effective dose; Fmoc, 9-fluorenylmethyl oxycarbonyl; εAbu(ßPhe), erythro-α-amino-ß-phenyl-butyric acid; FR190997, 8-[2,6-dichloro-3-[*N*-(*E*)-4-(*N*-methylcarbamoyl)cinnamidoacetyl]-*N*-methylamino]benzyloxy]-2-methyl-4-(2-pyridyl-methoxy)quinoline; GPI, guinea pig ileum; HOAt, 1-hydroxy-7-azabenzotriazole; HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethylguanidinium hexafluorophosphate; HOBt, 1-hydroxybenzotriazole; HOCr, hydroxycrotonic acid; HYCRAM, hydroxycrotonyl amidomethyl linker; IP_3, inositol 1,4,5-trisphosphate; IP_n, inositol phosphates; J526, Pyr-Trp-Pro-Lys(ASA)-Pro-Gln-Ile-Pro-Pro; J527, Pro-Trp-Pro-Lys-Pro-Gln-Ile-Pro-Pro; J725, DArg-Arg-Pro-Hyp-Gly-Thi-Ser-Pro-εAbu(ßPh)-Arg; MEM, Eagle's minimal essential medium; Mtr, methoxytrimethylbenzene sulphonyl; Pd^0, palladium *tetrakis* triphenylphosphine; PMN, polymorphonuclear leukocytes (neutrophils); Ram, ramiprilat; TBTU, 2-(1H-benzotriazol-1-yl)1,1,3,3-tetramethylguanidinium tetrafluoroborate; TFA, trifluoroacetic acid; Trt, triphenylmethyl

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1. Introduction

Hormone actions can be potentiated by different factors interacting with the receptor, by enzymatic degradation or by signal pathways. However the entire overall process has been studied in detail for only very few hormones [30,69,45].

At least 40 years ago a potentiating action was observed for the nonapeptide hormone bradykinin (BK). Indeed the history of BK isolation and characterization has long been closely related to the use of potentiating factors. Werle and coworkers [74] used snake venoms to trigger the formation of BK from plasma and to describe this tissue hormone functionally. Immediately after the isolation, chemical characterization, synthesis and functional characterization, certain snake venoms were described as bradykinin potentiating compounds. Kato and Suzuki [39,40], Ferreira et al. [26,27], and Ondetti et al. [13,59] isolated different oligopeptides with bradykinin potentiating activity from the venoms of the two snakes *Agkistrodon halys blomhoffii* and *Bothrops jararaca*, including the bradykinin potentiating nonapeptide $BPP_{9\alpha}$ (trade name TEPROTIDE).

Bradykinin potentiating peptides have also been isolated from other snakes [8,9,23–25,35] or other toxins [3,21,22,44,52,73] as well. Very recently new potentiating peptides have been isolated from the venom of *B. jararaca* [36]. Surprisingly peptides with potentiating activity have also been formed by the partial hydrolysis of proteins taken from serum [80], hemoglobin [38,66,81], milk [34,46], or wheat germ [51]. Also degradation fragments of angiotensin such as the heptapeptide 1–7 were found to potentiate the BK action [64]. In addition linear BK analogues, partial sequences [7], certain active and inactive side chain and backbone cyclic agonists are able to potentiate the BK action on GPI [68].

The angiotensin I-converting enzyme (ACE) cleaves dipeptides from the C-terminus of angiotensin I and bradykinin resulting on the one hand in the formation of the highly hypertensive hormone angiotensin II and on the other hand in the inactivation of the hypotensive BK. This enzyme has been extensively studied because of these important functions in the blood pressure regulation. ACE contains a N-terminal as well as a C-terminal catalytic domain, described in most publications as having only slight differences in their structural requirements [14,79]. More recently Cotton et al. demonstrated in an excellent investigation using domain-specific substrates and inhibitors, affinity differences between N- and C-terminal catalytic domains of about three orders of magnitude [11].

The membrane bound form of this enzyme seems to play an important role in the potentiation of the BK action. Inhibitors of this enzyme are used as drugs for treatment of different forms of hypertension and heart failure. For therapeutically used hormones this knowledge about potentiating compounds and their action mechanisms is very important. This knowledge can help to improve the therapeutic effect, to prevent not only an excessive dose, but also interaction with other drugs and side effects. On the other hand the therapeutic use of potentiating compounds requires the knowledge about the interaction with the hormone action on the molecular basis as well. As shown by Li et al. ACE may also act as a receptor for SARS coronavirus [47].

With extensive therapeutical application of bradykinin potentiating compounds such as captopril [12], enalaprilat [64], ramiprilat [75], quinaprilat [41] and lisinopril [6], studies of the molecular action mechanism have become more and more important. Many other proteases are also able to inactivate BK including the neutral endopeptidase (NEP, Neprolysin; E.C. 3.4.24.11) [42,71], metalloendopeptidase (E.C. 3.4.25.15/16) [55,58], aminopeptidase P (E.C. 3.4.11.9) [67], aminopeptidase N (E.C. 3.4.11.2) [63] and carboxypeptidase M (E.C. 3.4.17.12) [72].

Potentiation of BK action has also been studied in in vivo models by pharmacological tests on isolated organs and on the cellular level by biochemical methods. In the in vivo models, potentiation of BK action has been measured on the pressor response to intravenous BK in conscious rabbits [68] or on the hypotensive effect in freely moving Wistar rats [65]. Isolated organs such as the guinea pig ileum (GPI) [54], rat heart [34], rabbit jugular vein [17,33], cerebral microvasculature (estimation of permeability) [58] and porcine coronary arteries [76] have been used for in vitro tests.

Yet even at the onset of the search for the molecular mechanism of BK potentiation, certain contradictory findings have been observed. The inhibition of ACE by various peptide and nonpeptide compounds did not correlate well with the potentiating activity [5,10]. Furthermore, the maximum of the BK-induced contraction of guinea pig ileum can be enhanced by potentiating compounds [77]. Also the action evoked by enzymatically stable BK agonists can be potentiated in some test systems [78]. Repeated exposure of porcine coronary arteries to BK has led to receptor desensitization. The addition of the potentiating compounds quinaprilat or angiotensin 1–7 fully restored the relaxant effect at a point when BK alone was no longer able to induce relaxation [76]. At the molecular level the co-immunoprecipitation of ACE and the B_2 receptor with an anti receptor antibody clearly indicates an interaction of both partners on the cell membrane [50]. Despite all these contradictory and to some degree confusing findings Regoli and coworkers [29] and Dendorfer et al. [17] have demonstrated that in their test systems (rabbit isolated aorta and venoconstriction) the potentiation by therapeutically used ACE inhibitors results exclusively from the inhibition of enzymatic BK degradation. Nevertheless, the group of Regoli found under influence of ACE inhibitors, a resensitization of the rabbit jugular vein [31] and a change in the density of B_2 receptors in rat spinal cord [60].

To understand the molecular basis for these discrepancies many approaches have been undertaken to elucidate the influence of potentiating compounds on the different bradykinin destroying enzymes, on the bradykinin receptors (BKR) and on signal pathways.