THROMBIN AND TRYPSIN RECEPTORS: THE SAME MECHANISM OF SIGNALLING ON CELLULAR SURFACES

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RECEPTORY PRO TROMBIN A TRYPSIN, STEJNÝ MECHANISMUS SIGNALIZACE NA BUNĚČNÝCH POVRŠÍCH

Abstract

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Proteinase-activated receptors (PARs), ubiquitous surface molecules participating on many biological processes have been recently discovered. Specific receptors for thrombin (PAR-1 and PAR-3) and trypsin (PAR-2) are described in this review. They belong to a family of G protein-coupled receptors activated by amino acid sequence of N-terminal part of bound ligand revealed by site-specific proteolysis. PARs participate in tissue growth and differentiation, regeneration and reparation, inflammatory response regulation, malignant transformation, but even in vascular tonus and blood pressure regulation. ***(Fig. 5, Ref. 35.)***
Key words: PARs, trypsin, thrombin, tethered ligand.

Abstrakt

Olejár T., Nouza K.:
Receptory pro trombin a trypsin, stejný mechanismus signalizace na buněčných površích
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V nedávné době byly popsány tzv. receptory aktivovatelné proteinázami (PAR; Proteinase-activated receptors). Jde o ubiquitní povrchové molekuly, které se podílejí na regulaci celé řady biologických pochodů. V tomto přehledném referátu jsou popsány specifické receptory pro trombin (PAR-1 a PAR-3) a trypsin (PAR-2). Patří do rodiny receptorů spřažených s G-proteinem, aktivovatelných aminokyselinovou sekvencí vázaného ligandu z vlastního N-konce, který je odkryt specifickou proteolýzou. Uplatňují se při růstu a diferenciaci, regeneračních a reparačních procesech, regulaci zánětlivé odpovědi, maligní transformaci, ale i při regulaci cévního tonu a krevního tlaku. *(Obr. 5, lit. 35.)*
Klíčová slova: PAR, trypsin, trombin, vázaný ligand.

The currently known signal mechanisms of the cell communication rely on the production of cytokines, adhesive molecules, relevant receptors and in their interactions. Tens of cytokines, adhesive molecules and their natural ligands are known, which are interconnected to form very complex networks. The newest knowledge, however, demonstrates that there is a further mechanism of cell communication provided by cell receptors activable by the action of proteinases (proteinase-activated receptors, PAR). The receptors for thrombin and trypsin are expressed on the surface of various cells. The activation of these receptors results in a number of biochemical changes — mobilization of calcium ions, synthesis of prostaglandins, etc. — in the cell interior.

The sites of proteinase-activated receptors occurrence

Proteinase-activated receptors are essentially ubiquitous. They are particularly present on normal as well as tumorously transformed immunocompetent cells, on endothelial and muscle cells of major as well as minor vessels. Their presence was also immunohistochemically demonstrated on intestinal epithelial cells, epithelial cells of endocrine as well as exocrine organs, keratinocytes, fibroblasts and further cells (Baffy et al., 1994; Aj-Ani et al., 1995; Santulli et al., 1995; Bohm et al., 1996 b; Mari et al., 1996; Mirza et al., 1996; Saifeddine et al., 1996; Corvera et al., 1997; Howells et al., 1997; Kong et al., 1997; Even-Ram et al., 1998).

Function of proteinase-activated receptors

PAR are bound to transmembrane G-protein. There are receptors specific for α-thrombin (PAR-1 and PAR-3) and for trypsin and tryptase (PAR-2). These proteinases activate PAR through the mediation of a unique process characterising by the recognition of the receptor by an enzyme, subsequent cleavage at a specific site of the NH_2 terminal and presentation of a new NH_2 terminal,

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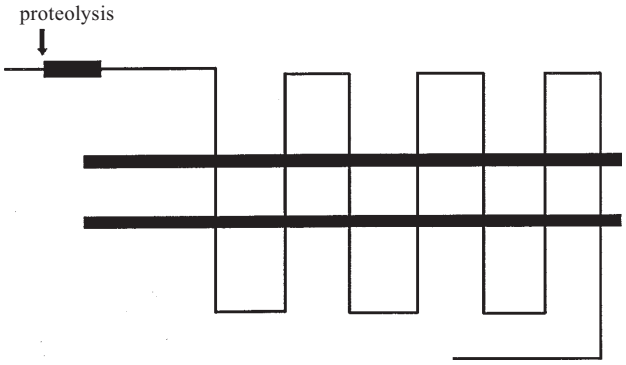

Fig. 1 a. Mechanism of PAR activation: in the first phase an oligopeptide is cleaved from the N-terminal part and tethered ligand is expressed. Adapted according to Deri et al. (1998).
Obr. 1 a. Mechanismus aktivace PAR: v první fázi dochází k odštěpení oligopeptidu z N-konce a expresi vázaného ligandu. Upraveno podle Deriho a spol. (1998).

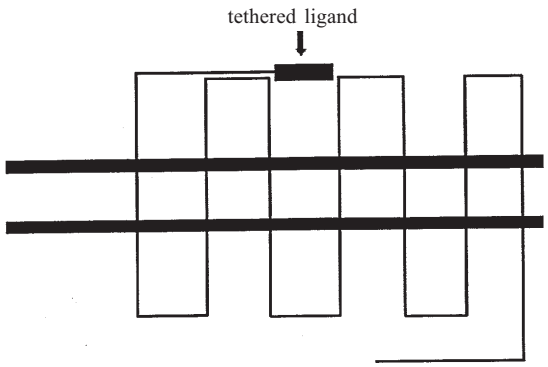

Fig. 1 b. Mechanism of PAR activation: the tethered ligand activates the receptor in the reaction with its 1. and 2. extracellular domain. Adapted according to Deri et al. (1998).
Obr. 1 b. Mechanismus aktivace PAR: vázaný ligand aktivuje receptor reakcí s jeho 1. a 2. extracelulární doménou. Upraveno podle Deriho a spol. (1998).

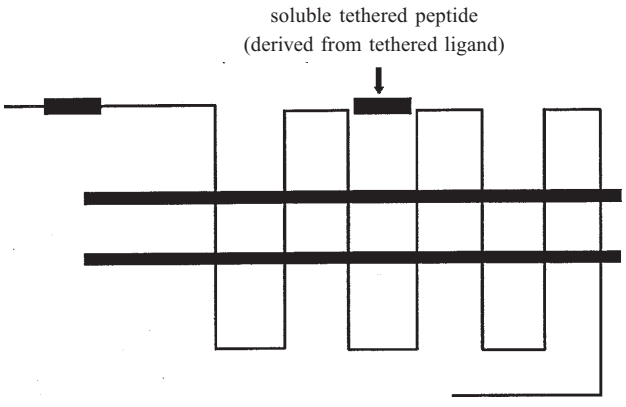

Fig. 1 c. Mechanism of PAR activation: receptor can be activated also by free oligopeptide ligand without proteolysis. Adapted according to Deri et al. (1998).
Obr. 1 c. Mechanismus aktivace PAR: receptor je aktivován i volným oligopeptidovým ligandem bez proteolýzy. Upraveno podle Deriho a spol. (1998).

which behaves as "a tethered ligand" and which is bound to the extracellular domain of the molecules split (Figs. 1 a, b). Thus, PAR are receptors, whose ligand is a physical part of the receptor molecule (Deri et al., 1998).

PAR-1 activation

The extracellular NH_2 terminal of human PAR-1 contains a cleavage site for α-thrombin (LDPR^{41} ! S^{41} FLLRN), after which a sequence of residues follows with a negative electric charge (D^{51}.KYEPF^{56}) (Vu et al., 1991 a, b). This is very similar to the COOH part of the leech anticoagulant hirudin. Hirudin inhibits thrombin through an analogous bond at the same site. The domain D^{51}.KYEPF^{56} reacts (in the same way as hirudin) with the area binding anions on an α-thrombin molecule and it probably induces conformational changes of the receptor molecule, which adjusts its cleavage site for catalytic domains of α-thrombin (Fig. 2). The PAR-1 activation with γ-thrombin, which contains no area binding anions, is weaker by a factor of 100. The deletion of this domain (D^{51}.KYEPF^{56}) results in losing the activability with thrombin (Vu et al., 1991 b).

Thus, thrombin splits PAR at the NH2 terminal. Synthetic, free peptide ligands can, however, activate PAR without splitting the receptor (Vu et al., 1991 a; Scarboroough et al., 1992); they are, however, very weak agonists in comparison with proteinases (Fig. 1 c). The reason for this is obviously an imperfect presentation of the soluble peptide to the binding domain in comparison with "the tethered ligand". In addition, soluble peptides are immediately degraded by proteolysis (Godin et al., 1994).

PAR-2 activation

The second member of the family of proteinase-activated receptors, PAR-2 was identified in the course of a PCR screening of the mouse genome with the use of primers for the second and sixth transmembrane domain of the receptor for neurokinin 2 (Nysted et al., 1994, 1995). It was shown that in this case, the protein is encoded with typical characteristics of a receptor coupled with G-protein and with a 30 % degree of identity with human PAR-1. The mouse PAR-2 contains a cleavage site for trypsin (SKGR^{34} ! S^{35} LIGR). PAR-2 was later identified in humans and rats (Nysted et al., 1995; Bohm et al., 1996; Saifeddine et al., 1996). Trypsin, similarly as thrombin in the case of PAR-1, splits PAR-2 at a certain site (Fig. 2) and it makes an exposure of a bound ligand to its own extracellular domain possible (Nysted et al., 1994; Bohm et al., 1996). Synthetic peptides (SLIGRL in mice, SLIGKV in humans) also activate PAR-2 without necessary cleavage of their own receptor (Nysted et al., 1994, 1995 a, b; Bohm et al., 1996).

Thus, PAR-1 and PAR-2 are activated by the same mechanism — cleavage of the receptor and exposure of the bound ligand. One difference was, however demonstrated: there is no evidence for the interaction of trypsin with other parts of the receptor than with the split one.

PAR-3 activation

The demonstration of PAR-2 presented a possibility of searching for the existence of further receptors from this family. The

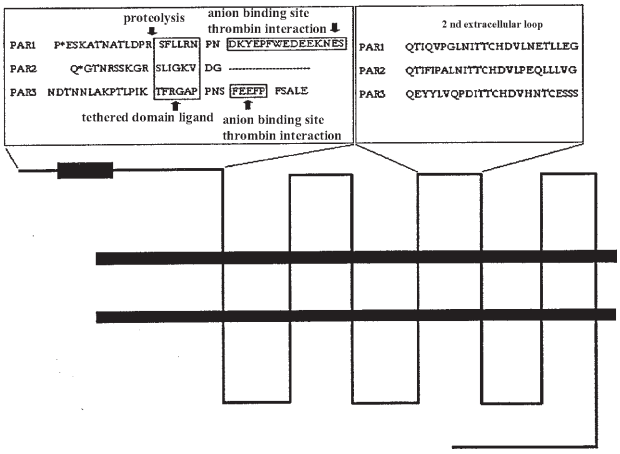

Fig. 2. PAR structure: aminoacid sequence of N-terminal part and second extracellular domain are principal prerequisities for the receptor activation.Frames denote domains of tethered ligands and negatively charged parts reacting with thrombin (PAR1 and PAR3). Adapted according to Deri et al. (1998).
Obr. 2. Struktura PAR: sekvence aminokyselin N-konce a druhé extracelulární domény jsou pro aktivaci receptoru principiální. Rámečky označují domény vázaných ligandů a záporně nabité oblasti reagující s trombinem (PAR1 a PAR3). Upraveno podle Deriho a spol. (1998).

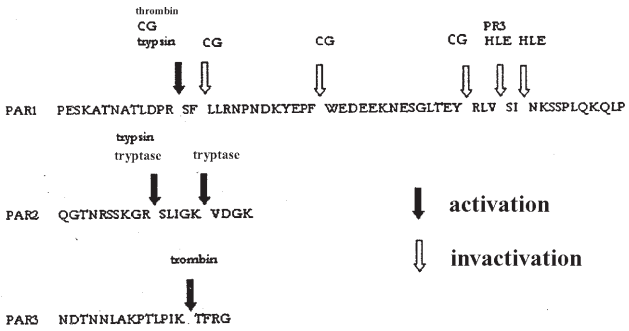

Fig. 3. Proteolytical cleavage of PAR by various enzymes. Only the influence of thrombin, trypsin and tryptase leads to activation of the process. (CG — catepsin, PR3 — proteinase 3, HLE — elastase of human leukocytes). Adapted according to Deri et al. (1998).
Obr. 3. Proteolytické štěpení PAR různými enzymy. K aktivaci dochází pouze vlivem trombinu, trypsinu a tryptázy (CG — katepsin, PR3 — proteináza 3, HLE — elastáza lidských leukocytů). Upraveno podle Deriho a spol. (1998).

existence of a further receptor for thrombin, PAR-3, was demonstrated with the help of primers for different domains of PAR-1 and PAR-2. RNA of rat thrombocytes was used (Ishihara et al., 1997). Thrombin splits the LPIK[38] ! T[39] FRG PAR-3 sequence. Similarly as in the case of PAR-1, PAR-3 contains a site similar to hirudin (FEEFP), which reacts with α-thrombin. In contrast to PAR-1, peptides analogous to the bound ligand, however, do not activate the receptor. Here, a stereochemical change of the molecule due to the thrombin bond is necessary (Deri et al., 1998).

PAR interactions with other proteinases

The other proteinases (cathepsin, proteinase 3, human leukocyte elastase) split PAR at a site different from the activation site (Fig. 3) and thus, the receptor is not activated (Molino et al., 1995, 1997). Thus, on thrombocytes and endothelial cells, these enzymes can block the biological effect of thrombin (Renesto et al., 1997). In contrast to this, the heparinocyte tryptase, which splits the ligand from the C- as well as N-terminal is able to activate the cell (Molino et al., 1995).

Signal transfer

The PAR activation results in a conformational change of heterodimeric G protein, which catalyzes replacement of GDP with GTP at the α-subunit of G-protein. The α-subunit and ßγ-heterodimer activate numerous effector enzymes or ion channels leading to GTP hydrolysis. G protein is then returned to the inactive state. The transfer of the signal through the PAR-1 activation has been most thoroughly studied till now (Grand et al., 1996).

Substrate specificity and role of PAR

In spite of a relatively good description of the mechanism of the action mediated through the mentioned three types of receptors, a further potential substrate specificity of these receptors for the other enzymes is not yet quite clear. The effect of α-thrombin (PAR-1 and PAR-3), trypsin, tryptase and acrosin (PAR-2) (Deri et al., 1998) has been already unambiguously documented. In the other enzymes (chymotrypsin, cathepsin G, thermolysin, neutral endopeptidase) the ability of the PAR activation was not observed and, in contrast to this, some of these enzymes block the PAR activation (Molino et al., 1995; Renesto et al., 1997; Deri et al., 1998). In experiments it is, however, possible to induce the apoptosis of neutrophils with elastase, trypsin and chymotrypsin (Trevani et al., 1996), i.e. with enzymes exerting either antagonistic or zero effects on the known PAR. It is assumed that the induction of the neutrophil apoptosis through the mediation of PAR could be an important mechanism regulating (restricting) the inflammatory reaction. Experimental works on SD/Ipcv rats with the spontaneous T-lymphoblastic leukemia demonstrated that rectally administered proteinases (trypsin, chymotrypsin and papain) reduce absolute counts of blasts in the peripheral blood and that they are able to induce a transient laboratory remission in experiments (Wald et al., 1998). PAR can also perhaps be a target structure for endopeptidases (as e.g. CD 10, ECE, KELL, PEX), which are common parts of cellular membranes (Mari et al., 1997; Turner and Tanzawa, 1997). The biological importance of PAR is not yet quite clear. Based on the results of experimental works, a possibility is suggested of a feedback control of different processes. The PAR-2 activation on epithelial cells of the large intestine with trypsin resulted in the inhibition of spontaneous contractions under experimental conditions (Corvera et al., 1997). Through the mediation of activated PAR-2, the phosphatidyl-inositol system is also activated on intestinal epithelial cells, which results in the intracellular utilization of arachidonic acid and secretion of prostaglandins (Kong et al., 1997). Thus, trypsin obviously directly participates in re-

sorption processes in the intestine The other studies demonstrate that the cellular response of immunocompetent cells to the PAR-1 stimulation with α-thrombin or SLIGRL depend on the degree of its activation (Joyce et al., 1997). In this way, thrombin mediates a direct functional binding between the hemostatic and immune systems. In the regulation of the systemic inflammatory response, the proteolytic activity of plasma seems to be of importance, particularly the specific plasmatic activity of trypsin. The biphasic, alimentarily independent secretion of trypsin through the basolateral membrane of acinar cells of the pancreas is thus obviously not useless. The knowledge concerning the effect of PAR on the blood circulation is also of interest. For example, in rats, the intravenous administration of the synthetic peptide SLIGRLETQPPI in a dose of 150 nmol/kg leads to a considerable drop of blood pressure (from 110 to 60 mmHg) (Emilsson et al., 1997). Under in vivo conditions, by the action of trypsin or SLIGRL, through the PAR-2 activation, dilatation of the basilar artery occurs in rats (Sobey and Cocks, 1998). Thrombin and trypsin cause a dilatation of the contracted segment of the human coronary artery ring ex vivo (Hamilton et al., 1998). Certain works suggesting the expression of PAR-1 on atheromatous plates assume a contribution of PAR to sclerotic and inflammatory processes in vessel walls (Nelken et al., 1992). A connection was demonstrated between the PAR-1 expression and histological grading with a high degree of the invasion of human breast carcinoma. Thus, it seems that PAR play a crucial role in processes of the physiological as well as pathological invasion, particularly in tumor processes connected with the metastatic dissemination (Even-Ram et al., 1998). In a model of the cells of pancreas carcinoma in vitro (PaCa-2), trypsin or its agonistic peptide caused a mobilization of Ca^{2+} ions, transient enhancement of the I-P_3 level and PKC translocation. In addition, there was a decrease of the DNA synthesis in these cells. The authors assume that PAR-2 can play a role of a negative regulator of the tumor growth (Kaufmann et al., 1998). This fact is also indirectly supported by experimental experiences with a certain antiproliferative action of a mixture of proteinases (trypsin, chymotrypsin and papain) on the growth of human pancreas carcinoma and carcinoma of tonsils in nude mice (Wald et al., personal communication).

Conclusion

PAR are a relatively newly described unit in concepts of activation and regulatory processes of the cellular differentiation, regulation or possibly decay. In spite of the fact that their exact function in the organism has no yet been perfectly elucidated, the ubiquity of PAR in tissues suggests their considerable importance in physiological as well as pathophysiological processes and indirectly also irreplaceable role of proteinases as a triggering or regulatory factor in a number of biological processes on cellular membranes and inside of cells as well as in the course of subsequent cellular responses.

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RECENZIA KNIHY

Jíra J.: Lékařská helmintologie. Praha, Galén 1998, 120 obrázkov, 9 tabuliek, 491 strán.

Popredný český parazitológ prof. MUDr. RNDr. Jindřich Jíra, DrSc., napísal aj v európskom meradle ojedinelú monografiu o helmintózach, častých parazitírnych nákazách človeka. Rozsah publikácie vzhľadom na významnosť odboru sa môže zdať neúmerný. Na druhej strane nám chýbala publikácia s komplexným pohľadom na helmintológiu ako vednú disciplínu.

Monografia hovorí o nákazách, ktoré postihujú státisíce obyvateľov najmä v tropických a subtropických oblastiach a ktoré ohrozujú podstatne širší okruh ľudí mierneho pásma, ktorí stále častejšie tieto oblasti navštevujú. Nehovoriac o helmintózach, ktoré sa kozmopolitne vyskytujú na všetkých kontinentoch.

Kniha má 491 strán, je obohatená 47 väčšinou farebnými obrázkami a mikrofotografiami, 73 perokresbami a 9 tabuľkami. Pri jednotlivých kapitolách je zoznam použitej literatúry a v závereč-nej časti bibliografia učebníc, monografií a prehľadových článkov. V závere autor uvádza zoznam periodík, špecializovaných na helmintológiu a parazitológiu.

Monografia je rozdelená na 6 väčších kapitol, z nich prvú tvorí všeobecná helmintológia, ktorá hovorí o prabidlách nomenklatúry v helmintológii, o vzťahoch hostiteľa a parazita, o molekulovej biológii a genetike helmintov, patobiológii helmintóz a ich epidemiológii. Cenný je prehľad laboratórnych vyšetrovacích metód v lekárskej helmintológii, ako aj rozsiahla kapitola o súčasných možnostiach farmakoterapie helmintóz.

Ďalšie kapitoly sa venujú predstaviteľom trematodóz, cestodóz a nematodóz, každá so všeobecným úvodom, biologickým cyklom a základnými spoločnými morfologickými znakmi. Jednotlivé helmintózne nákazy ako klinické jednotky majú spoločnú štruktúru: pôvodca — nozogeografia a prevalencia — epidemiológia — patobiológia — klinický obraz a symptomatológia — diagnostika — terapia — zdravotnícke opatrenia (prevencia).

V *Dodatku* je prehľad histórie lekárskej helmintológie, stať o evolúcii helmintoparazitov, paleohelmintológie a základoch lekárskej malakológie.

Opätovne možno konštatovať prioritu tejto monografie z európskeho hľadiska. Je určená ako základná odborná príručka lekárom teroretických a klinických odborov, mikrobiológom, parazitológom, epidemiológom a patológom. Poslúži aj infektológom, pediatrom, gastroenterológom a lekárom zameraným na tropickú geografickú a cestovnú medicínu.

Publikácia je na vynikajúcej odbornej a vedeckej úrovni súčasných poznatkov. Je reprezentatívna obsahom aj reprografickou úrovňou a nájde určite v lekárskej komunite v česku a na Slovensku svoje uplatnenie.

G. Čatár