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Structure and function of prostanoid receptors

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Summary

We have recently cloned cDNAs for the human and mouse TXA_2/PGH_2 receptors and a cDNA for the mouse PGE receptor. Sequencing, homology and hydrophobicity analyses revealed that they are proteins of 343, 341 and 365 amino acid residues, respectively, and all are rhodopsin-type receptors with putative seven transmembrane domains. Homology between the human and mouse TXA_2 receptors is 76% in total and that between the human TXA_2 and mouse PGE receptors is 38%. The homology increases in the putative transmembrane regions to 85 and 45%, respectively, and there observed several features common to the three receptors. These results indicate that the prostanoid receptors constitute a family of receptors of similar structure. The cloned PGE receptor showed binding activity specific to so-called EP3 agonists, which verified for the first time that pharmacologically defined PGE receptor subtypes consist of different molecules. This receptor also displayed different efficiency in signal transduction to an EP3 agonist, M & B-28767, and PGE_2, providing an interesting model for the analysis of receptor-G protein coupling. In addition to the above biochemical results, these studies have revealed the characteristic tissue distribution of these receptors, which will open up a new biology of these prostanoids.

Key words: Prostaglandin E receptor EP3 subtype; Thromboxane A_2 receptor; G-protein; Rhodopsin-type receptor; Northern blot analysis

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1. Introduction

Prostanoids including PGs and TXs elicit a wide variety of actions in different cells and tissues. Characterization of the prostanoid receptors has been carried out pharmacologically by comparing the actions and potencies of various types of PGs in different bioassay systems and then by using PG analogues more specific to each action. Through these studies it is suggested that each prostanoid has a specific receptor and there may be several subtypes for some receptors (see Coleman et al. (1990) for review). PG receptors have also been characterized biochemically as ligand-binding activities for radiolabelled PGs and their analogues, and by analyzing second messengers generated in cells after PG addition. Such analyses suggested that the PG receptor is a G protein-coupled receptor, mediating via a G protein the ligand binding stimulus to the effectors such as adenylate cyclase and phospholipase C. However, none of the receptors was purified or cloned until recently. In 1989, we purified the TXA_2/PGH_2 receptor to apparent homogeneity from membranes of human blood platelets (Ushikubi et al., 1989). Based on the partial amino acid sequences of the purified protein we then cloned cDNA for the TXA_2 receptor from cDNA libraries of cultured human megakaryocytic leukemia cells and human placenta (Hirata et al., 1991). These studies clearly demonstrated that the TXA_2 receptor is indeed a G protein-coupled rhodopsin-type receptor. We next cloned a cDNA for the mouse TXA_2 receptor (Namba et al., 1992) and, using this as a hybridization probe, cloned a cDNA for the mouse PGE receptor EP3 subtype by homology screening (Sugimoto et al., 1992). Here we review the results obtained by these studies and discuss the implciations of our findings.

2. TXA_2/PGH_2 Receptor

Fig. 1 shows a membrane topology model of the TXA_2 receptor. The human and mouse TXA_2 receptors consist of 343 and 341 amino acids, respectively, and are 76% identical in overall sequence. The receptors have an N-terminal extracellular portion of less than 30 amino acids. The fourth and sixteenth Asn are glycosylated as demonstrated by the peptide sequence of the purified protein. The transmembrane segments consist mainly of hydrophobic amino acids, the features of which are discussed below in relation to ligand binding and those of the EP3 receptor. The cytoplasmic loops are short, and there are two conserved potential c-kinase phosphorylation sites in the second cytoplasmic loop, which together with several serine and threonine residues in the C-terminal, may be involved in phosphorylation-mediated receptor desensitization. The carboxy terminal cytoplasmic tail is also short and there is no cysteine residue in this region, suggesting that the C-terminal of these receptors is not membrane-attached by myristoylation but is present free in the cytoplasm.

Ligand binding properties of the cloned receptors were examined by expression in cultured COS cells. The human and mouse receptors showed specific binding of the selective TXA_2 receptor ligand, [^{3}H]S-145, with a K_d of 1.2 and 3.3 nM,

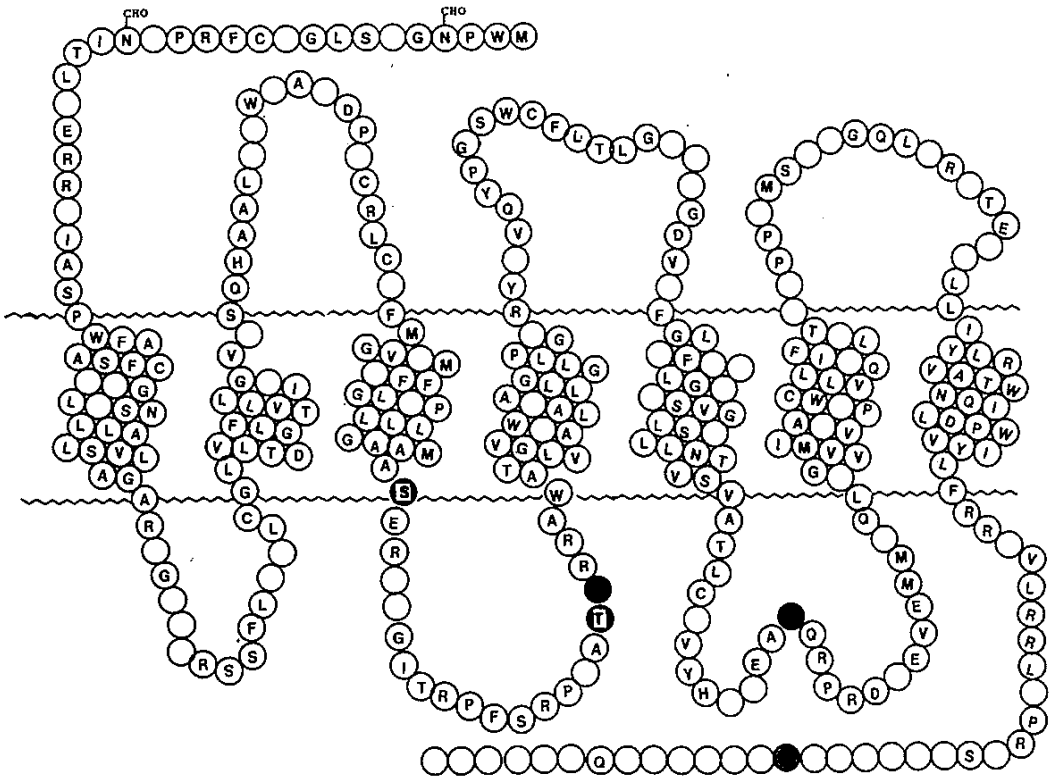

Fig. 1. Membrane topology model of the TXA_2 receptor. The model was constructed on the sequence of the mouse TXA_2 receptor and residues identical in the mouse and human receptors are shown in single-letter code.

respectively, and this binding was displaced selectively by various TXA_2/PGH_2 analogues in the order of S-145 > ONO-3708 > STA2 > U-46619. Other PGs such as PGD_2 also displaced the binding, but their potencies were at least two orders of magnitudes less than those of these compounds. Signal transduction following ligand binding was examined by expression of the cloned receptors in *Xenopus* oocytes. In these oocytes application of TXA_2 agonists evoked an inward current of an amplitude of several hundred nanoamperes. This response was blocked by prior treatment with the TXA_2 receptor antagonists and became much weaker upon repetitive application of the agonists, suggesting that they underwent homologous desensitization. There were no responses found with other PGs and TXB_2 up to 1 μM concentration, confirming the specificity of the cloned receptor. Because the observed inward current was induced by the rise in free Ca^{2+} in oocytes, these results suggested that the cloned receptor evoked PI turnover and subsequent calcium release upon agonist stimulation as suggested in cells and membranes containing the native TXA_2 receptor.

Fig. 2 shows Northern blot analysis. The receptor mRNA was most abundantly expressed in thymus, followed by spleen, lung, kidney, heart and uterus in that order. Slight but significant expression was also observed in brain. Similar analysis

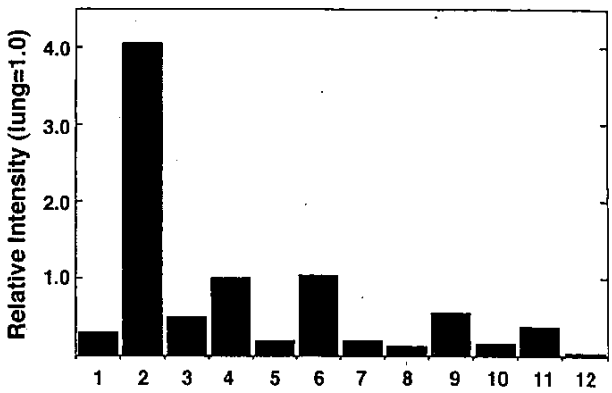

Fig. 2. Tissue distribution of the TXA_2 receptor mRNA expression in mouse tissues. Results of the Northern blot were quantified by an image analyzer: (1) brain; (2) thymus; (3) heart; (4) lung; (5) liver; (6) spleen; (7) stomach; (8) ileum; (9) kidney; (10) testis; (11) uterus; (12) background.

in human tissues demonstrated greater expression in placenta and cultured megakaryocytic leukemia cells than lung. Thus, thymus is one of the richest tissues as are the placenta and platelets. The role of TXA_2 in the immune system was analyzed thus far only in peripheral blood lymphocytes and monocytes with mostly negative results. The present results together with the finding that TX synthase is rich in dendritic cells and macrophages in these tissues (Nüsing et al., 1990) have motivated us to re-evaluate its role in thymus and spleen.

3. PGE Receptor EP3 Subtype

This is a polypeptide consisting of 365 amino acid residues with an estimated molecular weight of 40077. This receptor also possesses seven hydrophobic segments and shares a significant sequence homology with the human and mouse TXA_2 receptor (38 and 33% in total, respectively). It has two potential glycosylation sites, one in the N-terminal region and the other in the second extracellular loop. At the C-terminal tail nine serine and threonine residues are seen as possible phosphorylation sites.

The receptor expressed in cultured COS cells specifically bound [^{3}H]PGE_2 with a K_d of 2.9 nM. This [^{3}H]PGE_2 binding was displaced by unlabelled PGs in the order PGE_2=PGE_1 > iloprost > $PGF_{2\alpha}$ > PGD_2. In addition, the binding was effectively displaced by ligands specific to the EP3 subtype of PGE receptor such as M&B-28767 and GR-63799 but not by EP1 and EP2 ligands such as AH-6809, SC-19220 and butaprost. In CHO cells stably expressing the receptor, PGE_2 and EP3 agonists concentration-dependently inhibited forskolin-induced cAMP synthesis, suggesting that the receptor couples to adenylate cyclase in an inhibitory manner. These results clearly established that the cloned receptor represents the PGE receptor EP3 subtype. One interesting finding in this study is that M & B 28767 exerts inhibition of adenylate cyclase at much lower concentrations than

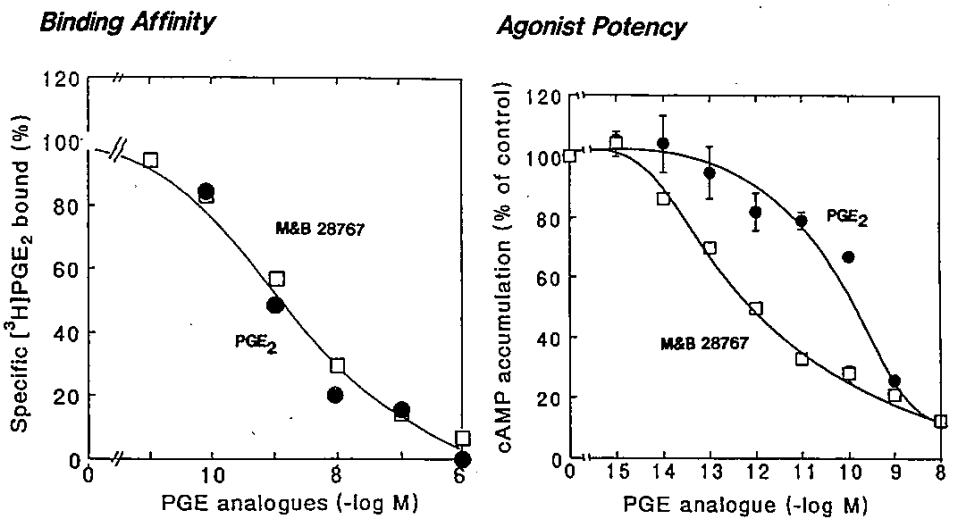

Fig. 3. Comparison of binding affinities and agonist potencies of PGE_2 and M & B-28767. Various concentrations of PGE_2 and M & B-28767 were added to the membrane of COS cells (left) and to CHO cells (right), both expressing the cloned EP3 receptor, and displacement of $[^3H]PGE_2$ and inhibition of forskolin-induced cAMP synthesis were examined, respectively.

PGE_2, while the two compounds show almost identical affinities to the expressed receptor (Fig. 3). We also found such differences in their potencies for stimulating GTP hydrolysis (Sugimoto et al., submitted). These results strongly suggest that the receptor · M & B complex has much higher affinity to the coupling G_i protein than the receptor · PGE_2 complex, so that only a small amount of the former complex is sufficient to stimulate the G protein to transduce signal to the effector.

Distribution of the EP3 receptor was examined by Northern blot analysis of the cloned receptor. The tissue most highly expressing the EP3 receptor is kidney, followed by uterus and stomach. This distribution is consistent with known functions of the EP3 receptor such as antagonism to vasopressin in water and sodium reabsorption in kidney, contraction of uterine smooth muscle, and antagonism to histamine in gastric acid secretion in stomach. Significant expression was also found in brain, thymus, heart, lung and spleen. Actions of the EP3 receptor in these tissues are less clear, and identification of cells expressing the EP3 receptor by in situ hybridization will help to unravel the function of this receptor therein

4. Implications for Putative Ligand Binding Domains in the Prostanoid Receptors

Ligand binding and recognition in the rhodopsin-type receptors occur in the outside half of the seven transmembrane segments (see Savarese and Fraser (1992) for review). Fig. 4 compares the transmembrane segments of the TXA_2, PGE and β-adrenergic receptors. The transmembrane segments in the two prostanoid recep-

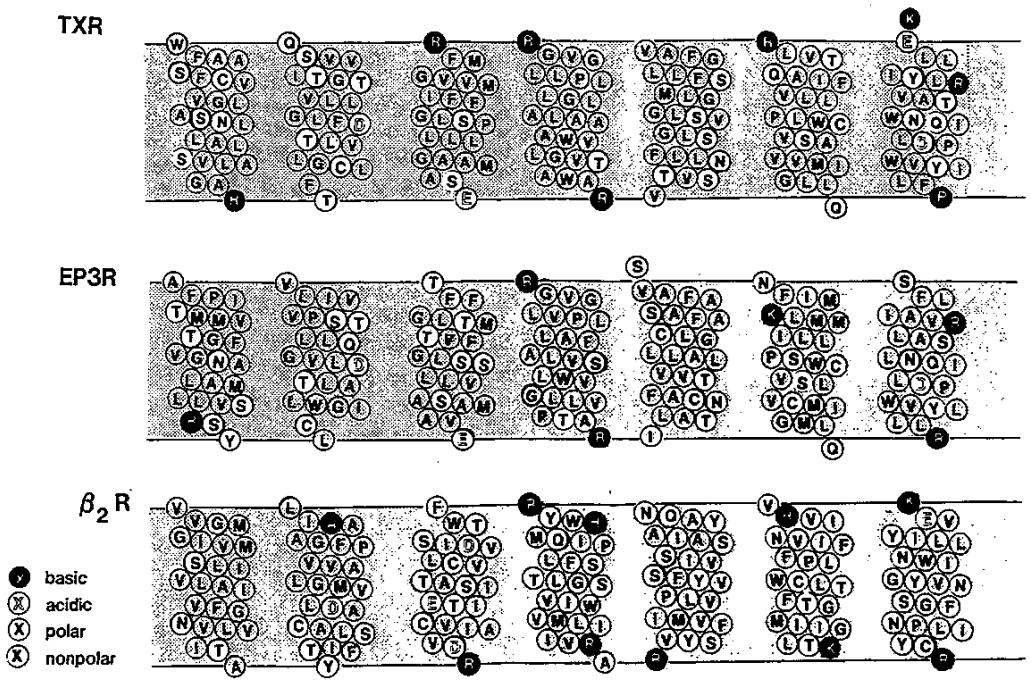

Fig. 4. Comparison of transmembrane segments of the TXA_2, EP3 and β-adrenergic receptors.

tors are more hydrophobic than in the adrenergic receptor; hydrophobic residues accumulate especially in the fourth and fifth transmembrane segments of the two receptors, while some polar amino acids are scattered in the corresponding regions of the adrenergic receptor. This may be consistent with the hydrophobic nature of prostanoid molecules, and suggests that their alkyl chains interact with these regions. Another feature is the presence of an arginine residue at the identical position of the seventh segments of the two prostanoid receptors. In rhodopsin, a lysine residue is present at an analogous position and makes a Schiff base with its ligand, all-*trans*-retinal. Based on this analogy and on the fact that the carboxyl group is essential for the biological activities of most PGs, we presume that this arginine serves as the binding site for the carboxyl group of prostanoids. In addition to these common features, we note that there are some polar amino acids in the third segment of the EP3 receptor, while the corresponding region of the TXA_2 receptor is hydrophobic. This may reflect the structure of each ligand; PGE has one hydroxy and one carbonyl group in its ring structure, whereas the ring structure of TXA_2 is essentially hydrophobic (this can be demonstrated by the facts that the oxane ring is replaced by a pinane or bicycloheptane ring in many TXA_2 analogues and hydration of the ring to TXB_2 results in loss of biological activity). If so, the prostanoid ring is likely to interact with the third transmembrane segments of these receptors. This can be tested by making a chimera receptor of the TXA_2 and EP3 receptors, and study on this line is in progress.

5. Perspective

In this communication we have reviewed our work on cDNA cloning of three prostanoid receptors. These are the first examples of prostanoid receptor cloning and, based on our data, more PG receptors should be cloned in the coming years. Through these studies, the diversity of prostanoid receptors should be clarified. This will include more types and subtypes of prostanoid receptors than are currently being envisaged. In fact, we have already identified several isoforms for the EP3 receptors (Sugimoto et al., unpublished observation). Such studies will enable us to identify ligand binding structures more precisely. Knowledge on the diversity and ligand binding structure of prostanoid receptors will make possible the development of more specific drugs to control and mimic prostanoid actions. In addition, as seen in the distribution study of the TXA_2 receptor, these studies should provide a hint on a new action of prostanoids. Prostanoid research has been carried out mainly in smooth muscles and other systems where actions of the PGs can be evaluated readily, and it is very likely that many important effects have been overlooked. Distribution analysis of cloned receptors will be of help to elucidate these actions and open new fields in prostanoid biology.

Acknowledgements

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References

Coleman, R.A., Kennedy, I., Humphrey, P.P.A., Bunce, K. and Lumley, P. (1990) Prostanoids and their receptors. In: J.C. Emmett (Ed.), Comprehensive Medicinal Chemistry, Vol. 3, Membranes and Receptors, Pergamon, Oxford, pp. 643–714.

Hirata, M., Hayashi, Y., Ushikubi, F., Yokota, Y., Kageyama, R., Naknishi, S. and Narumiya, S. (1991) Cloning and expression of cDNA for a human thromboxane A_2 receptor. Nature 349, 617–620.

Namba, T., Sugimoto, Y., Hirata, M., Hayashi, Y., Honda, A., Watabe, A., Negishi, M., Ichikawa, A. and Narumiya, S. (1992) Mouse thromboxane A_2 receptor: cDNA cloning, expression and Northern blot analysis. Biochem. Biophys. Res. Commun. 184, 1197–1203.

Nüsing, R., Lesch, R. and Ullrich, V. (1990) Immunohistochemical localization of thromboxane synthase in human tissues. Eicosanoids 3, 53–58.

Savarese, T.M. and Fraser, C.M. (1992) In vitro mutagenesis and the search for structure-function relationships among G protein-coupled receptors. Biochem. J. 283, 1–19.

Sugimoto, Y., Namba, T., Honda, A., Hayashi, Y., Negichi, M., Ichikawa, A. and Narumiya, S. (1992) Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype. J. Biol. Chem. 267, 6463–6466.

Ushikubi, F., Nakajima, M., Hirata, M., Okuma, M., Fujiwara, M. and Narumiya, S. (1989) Purification of the thromboxane A_2/prostaglandin H_2 receptor from human blood platelets. J. Biol. Chem. 264, 16496–16501.