British Journal of Pharmacology (2006) 147, 835–837
www.nature.com/bjp

COMMENTARY

Common ligands of G-protein-coupled receptors and arginine-utilizing enzymes

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A new family of G-protein-coupled receptors (GPRC6A) has recently been described and characterized with unknown physiological role. Three isoforms of GPRC6A are expressed from the same gene by alternative splicing. Agonists for GPRC6A are basic amino acids, particularly the analogues and derivatives of L-arginine and L-ornithine. These compounds are known ligands of nitric oxide synthase and arginase isoenzymes, but amino-acid sequences of these enzymes have not shown significant homologies. Various hypotheses for physiological roles of these receptors have been proposed but not yet substantiated. In order to define the most potent agonists, and elucidate further the biological significance of the receptor family, a detailed ligand-based quantitative structure–activity relationship study may be helpful.
British Journal of Pharmacology (2006) **147,** 835–837. doi:10.1038/sj.bjp.0706683;
published online 20 February 2006

Keywords: GPRC6A; inhibitors of NOS/arginase; sequence comparison; QSAR

Abbreviations: GPRC6A, G-protein-coupled receptor 6A; NOS, nitric oxide synthase; QSAR, quantitative structure–activity relationship

A new member of the C family of G-protein-binding receptors (GPRC6A) has recently been discovered and characterized in human and mouse tissues. This receptor family consists of a long amino-terminal domain including the ligand-binding region and a seven-transmembrane domain involved in the G-protein coupling (Pin *et al*., 2003). The C family includes glutamate and GABA-B receptors, taste and calcium sensing receptors and also orphan receptors in several vertebrates (Wellendorph & Bräuner-Osborne, 2004). Cloning, expression and sequence analysis of three isoforms of GPRC6A were carried out by Wellendorph & Bräuner-Osborne (2004). The isoforms are coded by the same gene, and alternative splicing leads to three proteins of different lengths and abundances. Although their biological function is not yet known, a considerable amino-acid sequence homology with various vertebrate sensor proteins has been proved (Brown *et al*., 1993; Speca *et al*., 1999; Kuang *et al*., 2003), suggesting some similar functions in the human organism, for example a sensor function to detect free amino-acid concentrations in the blood. Alternative functions such as receptor role in the nervous system (Wellendorph *et al*., 2005), cell-to-cell communication (Kuang *et al*., 2005) or sensor for cell death (Civelli, 2005) were also proposed. It has also been found that basic amino acids and their analogues and derivatives are agonists or antagonists of this receptor and, for this reason, a regulatory function concerning the urea cycle has been suggested. As it is known, L-arginine and related compounds are substrates or inhibitors of nitric oxide synthases (NOSs) and arginases. For this reason, a systematic investigation of the effects of these compounds on GPRC6A has recently been performed by Christiansen *et al*. (2006) and the results are published in this issue of *British Journal of Pharmacology*.

The NOS and arginase isoforms utilize the same physiological substrate, L-arginine. However, mechanism of the catalysed reactions is different: while NOS is an oxygenase with a relatively complicated structure and regulation, arginase is a hydrolase without tight regulation. Nevertheless, in cells (e.g. macrophages) where both enzymes are present, a reciprocal regulation between the two enzymes has been found. N^G-hydroxy-L-arginine, the intermediate of the reaction catalysed by NOS, or higher nitrite (end product of NO conversion) concentrations are inhibitors of arginase (Daghigh *et al*., 1994; Hrabák *et al*., 1996a); whereas putrescine, a derivative of ornithine inhibits NOS activity (Hrabák *et al*., 1996b). The expression of inducible NOS and arginase is also regulated reciprocally by different cytokines (Munder *et al*., 1998). In these studies, the functional groups most important in the binding of substrate and inhibitors were defined: (i) both enzymes are strictly specific for the L-configuration of the α-amino and α-carboxyl groups; (ii) there is an optimal carbon chain length and; (iii) in optimal case, a guanidino group or a nitrogen atom in proper position. According to studies on inhibitor specificity, when compared to arginase, a less tightly fitting binding site on NOS isoforms could be determined: L-homoarginine is substrate for NOS only, most of N^G-substituted arginines are inhibitors for NOS but not for arginase (Hrabák *et al*., 1994; 1996b). Interestingly, arginase is also inhibited by amino acids lacking the guanidino group, provided that they have the optimal length of carbon chain. In addition, new, selective arginase inhibitors containing hydroxyl groups coupled to terminal nitrogen atoms were designed (Custot *et al*., 1996). Their inhibitory effect may be due to their binding to the essential manganese cluster of arginase by the hydroxyl groups, while the aliphatic carbon chain secures good

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fitting to the arginine-binding site. The inhibitory effect of sulphur-containing guanidines on NOS activity can rather be explained by the interaction of the sulphur atom with the heme iron of the NOS active site (Southan *et al*., 1995). Later, the essential amino-acid residues involved in the substrate binding both in NOS (Boucher *et al*., 1999) and arginase (Kanyo *et al*., 1996) were identified. In NOS isoforms, a glutamate side chain has been proposed for a role in substrate binding and oxygen activation, together with a tryptophane, which stabilizes the binding by H-bonds (Crane *et al*., 1998). In arginase, also a glutamate side chain binds the guanidino group of the substrate, an aspartate side chain is responsible for the proper position of manganese cluster and a histidine residue helps the proton transfer in the reaction (Kanyo *et al*., 1996).

In their studies, Christiansen *et al*. (2006) have found similar requirements for the strong agonist effect on GPRC6A and for the inhibitor efficiency of NOS and arginase with the same compounds. The presence and proper L-configuration of the α-amino and α-carboxyl groups, and the presence of a basic guanidino group or distal amino groups (L-ornithine, L-lysine) were the most essential prerequisites. Nevertheless, when a comparison of the primary sequence of GPRC6A with that of NOS or arginase isoforms has been carried out, only poor sequence identity (12–14%) was observed, suggesting that the structure of their arginine-binding sites are not related. Interestingly, the amino-acid sequence homology between various NOS isoenzymes and arginase isoforms was also poor (11–12%, using http://www2.igh.cnrs.fr/bin/align-guess.cgi website). Although a similar folding pattern of arginine-binding domains of these proteins cannot be excluded, there is no evidence for homology in binding pockets. In addition, the approximation of the ligand binding of GPRC6A, based on a structure-based quantitative structure–activity relationship (QSAR) does not seem promising. First of all, NOS and arginase use L-arginine and its analogues and derivatives as substrate and inhibitors, respectively. Therefore, the related inhibitory molecules must act at the active site. In addition, the inhibition of enzyme activity may be due not only to the decreased binding, but also to the block of the reaction mechanism involving a distinct catalytic site. Moreover, the function of a protein is also influenced by interactions with its environment, which cannot be identified from a sequence homology alone. Both NOSs and arginases contain metals (heme iron and manganese, respectively) essential for their activities and several inhibitors may inhibit their activity by binding to these metal ions (Southan *et al*., 1995; Kanyo *et al*., 1996). On the contrary, for GPRC6A, these basic amino acids are only ligands, without a substrate function, and the involvement of metal ions or atoms in their binding is not known.

If the authors would like to define the structure of the most potent agonists or antagonists for the receptor, a ligand-based QSAR study seems adequate and simple for this purpose. On the basis of their systematic study on the effect of basic amino acids and their analogues and derivatives on calcium sensor function, an abundant database of biological activities is available for this analysis. As mentioned above, structure-based QSAR is less advantageous for this purpose, because: (1) sequence identities are not evidences for a binding site, (2) sequence or even 3D data cannot give information about the functional interactions of the receptor with its environment. Testing of nonarginine hydroxy-substituted derivatives (e.g. N^{ε}-OH-lysine or the corresponding ornithine; strong arginase inhibitors) is also recommended in order to compare their effects with that of hydroxyarginines. This may give information on whether the hydroxyl group must be positioned on a guanidino group or any terminal basic group can be substituted by that. It is likely that a QSAR study mentioned above may lead to the definition of the structural features of the most efficient agonists/antagonists. On the basis of the results, it would be possible to define, design and synthesize novel arginine/ornithine derivatives with an increased potency at GPRC6A. In addition, the studies on the ligand specificity may contribute not only to reveal the biological function(s) of GPRC6A but also to the elucidation of the possible signalling pathways involved in the transmission of the ligand binding to the biological response.

The work of the author is supported by the Grants T-043075 of National Science Research Foundation (OTKA), and 213/2003 of Hungarian Ministry of Health (ETT). The critical review of this commentary by Professor Miklós Tóth is highly appreciated.

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(Received December 15, 2005
Revised January 5, 2006
Accepted January 10, 2006
Published online 20 February 2006)