British Journal of Pharmacology (2002) 135, 1839–1840
www.nature.com/bjp

COMMENTARY

2-Chloro-N^6-methyl-(*N*)-methanocarba-2′-deoxyadenosine-3′,5′-bisphosphate is a selective high affinity $P2Y_1$ receptor antagonist: Commentary on Boyer *et al.*

*,[1]Brian F. King

[1]Department of Physiology and Autonomic Neuroscience Institute, University College London, Royal Free Campus, Rowland Hill Street, Hampstead, London NW3 2PF

British Journal of Pharmacology (2002) **135,** 1839–1840
Keywords: ADP; ADP analogue; antagonism (competitive); blood platelet; GPCR; nucleotide

The $P2Y_1$ receptor was the first of its kind to be cloned and identified as a metabotropic receptor for adenine nucleotides (Webb *et al*., 1993). Since its discovery, many more P2Y receptor subtypes have been identified (King *et al*., 2001) and the current series now extends to $P2Y_{13}$ (Communi *et al*., 2001). The $P2Y_1$ receptor couples to heterotrimeric G-proteins (Gq/Gα11) and, through this link, stimulates phospho-inositide turnover and release of intracellular calcium in expression systems. The human $P2Y_1$ receptor is particularly sensitive to ADP and 2-methylthioADP, whereas the agonist properties of ATP are highly controversial here. The tissue distribution for $P2Y_1$ protein, and messenger RNA, is very extensive – found in peripheral sensory nerves, CNS and ANS, most physiological systems and many cell types, including blood platelets. Thus, much has been learned about the activation of recombinant $P2Y_1$ receptors and where the native form of this receptor can be found. However, an inability to clearly distinguish $P2Y_1$ from other endogenous P2Y receptor subtypes – using the currently available pharmacological tools – has hindered the study of this receptor *in vivo*. Apart from some preliminary observations made in $P2Y_1$-deficient (knockout) mice, relatively little is known with certainty about the physiological role of the $P2Y_1$ receptor.

The present study by Boyer *et al*. (2002) '2-Chloro-N^6-methyl-(*N*)-methanocarba-2′ deoxyadenosine-3′,5′-bisphosphate is a selective high affinity $P2_1$ receptor antagonist' introduces a highly selective and competitive antagonist for the $P2Y_1$ receptor, reporting K_b values of 18 nM at the $P2Y_1$ receptor in turkey erythrocytes, 8 nM at the human $P2Y_1$ receptor expressed in astrocytoma 1321-N1 cells, and 9 nM at the $P2Y_1$ receptor in human blood platelets. This antagonist is somewhat loosely related to cyclic AMP (adenosine 3′,5′-cyclic monophosphate), although it is better viewed as a highly modified non-cyclic biphosphate analogue. Its name is 2-chloro-N^6-methyl-(*N*)-methanocarba-2′-deoxyadenosine-3′,5′-bisphosphate, but it is more easily identified and remembered by its production number MRS 2279. The acronym, MRS, stands for Molecular Recognition Section – a laboratory based at NIH (Bethesda, MD, U.S.A.) and directed by Dr Kenneth Jacobson.

*Author for correspondence; E-mail: b.king@ucl.ac.uk

The findings in this paper are remarkable for several reasons. First, MRS 2279 seems to be specific for $P2Y_1$ receptors and fails to block nucleotide signalling at most of the other known P2Y receptor subtypes: $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$ and native form of PY_{12}. Second, this antagonist is very potent, shows the classical properties of a competitive antagonist and is chemically stable. Third, this antagonist now provides the means to study the physiological role of native $P2Y_1$ receptors. In this respect, the authors touch on the ability of MRS 2279 to potently inhibit ADP-induced aggregation of human blood platelets *in vitro* – although the current utility of the drug remains a research tool, not an antithrombotic agent.

The identification of MRS 2279 as a specific $P2Y_1$ receptor antagonist represents the culmination of 6 years work by this research group. The origins of this compound can be traced back to work on the inhibitory actions of simple, and commercially available, bisphosphate nucleotides (e.g. adenosine 3′-phosphate 5′-phosphate) at $P2Y_1$ receptors – where these agents acted as competitive, yet not especially potent, antagonists (Boyer *et al*., 1996). Others drew attention to 'the limited subtype selectivity and non-P2 receptor effects' of such bisphosphate nucleotides (Bultmann *et al*., 1998), a setback that only served to drive the authors on to discover more selective analogues. The next landmark compound was MRS 2179 (N^6-methyl 2′-deoxyadenosine 3′,5′-bisphosphate), another competitive antagonist at the $P2Y_1$ receptor (Boyer *et al*., 1998). Yet again, the authors' hopes were dashed following the observed inhibitory activity of this compound at $P2X_1$ and $P2X_3$ receptors (Brown *et al*., 2000). Thereafter, focus switched to non-ribose compounds where the ribose moiety was substituted by methanocarbo pseudosugars in either Northern (N) or Southern (S) conformations – to reduce the flexibility of the sugar spacer and enhance ligand docking (Nandanan *et al*., 2000; Kim *et al*., 2002). The resultant MRS 2279 represents the most potent and selective of this series of analogues, as now reported by Boyer and colleagues in their present study.

A fuller understanding of the pharmacology of MRS 2279 will depend on its commercial availability – so that others might assist in testing this compound on P2 and non-P2 receptors. There are still many potential receptor targets that must be excluded – for example, the uncloned P2Y-like receptor in taenia coli, the recently cloned $P2Y_{13}$ receptor,

and the UDP-glucose receptor that, for the last 2 years, has waited patiently in the wings to join the P2Y receptor family. As yet, MRS 2279 has not been tested on recombinant P2X receptors in their multiple homomeric and heteromeric forms. Also, no mention is made of its (in)activity at adenosine receptors.

However, if their luck holds, the authors are standing at the threshold of an important era of $P2Y_1$ receptor pharmacology where the actions of MRS 2279 can help elucidate the functions of the $P2Y_1$ receptor *in vivo*. There are many examples of $P2Y_1$ receptors present in complex physiological systems – for example, in endothelial cells, vascular smooth muscle, cortical synaptosomes, pancreatic β-islet cells, osteoclasts, kidney epithelium, leucocytes and, of course, blood platelets etc – and it has never been more important that selective drugs are now made available to pharmacologically dissect the role of this signalling protein.

In the past, I have often listened to makers of medicines say the nucleotide template is wholly unsuitable for drug discovery. I have heard say the chemistry was too complex and the molecule too unstable to achieve desired levels of selectivity and potency. Here, Boyer and colleagues have confounded such critics by showing that nucleotide analogues can possess the appropriate pharmacological attributes. In this respect, MRS 2279 joins another nucleotide – 2-propylthio-D-β,γ-dichloromethylene-ATP (ARC67-085MX) – as seemingly selective competitive antagonists at P2Y receptor subtypes (for $P2Y_1$ and $P2Y_{12}$, respectively). The *British Journal of Pharmacology* looks forward to publishing more accounts of highly selective competitive antagonists for the remaining members of the P2Y (and P2X) receptor families.

Note added in proof

The commentator is a member of the editorial board of the *British Journal of Pharmacology*.

References

BOYER, J.L., ADAMS, M., RAVI, R.G., JACOBSON, K.A. & HARDEN, T.K. (2002). 2-Chloro-N^6-methyl-(*N*)-methanocarba-2′-deoxyadenosine-3′,5′-bisphosphate is a selective high affinity $P2Y_1$ receptor antagonist. *Br. J. Pharmacol.,* **135,** 2004 – 2010.

BOYER, J.L., ROMERO-AVILA, T., SCHACHTER, J.B. & HARDEN, T.K. (1996). Identification of competitive antagonists of the $P2Y_1$ receptor. *Mol. Pharmacol.,* **50,** 1323 – 1329.

BOYER, J.L., MOHANRAM, A., CAMAIONI, E., JACOBSON, K.A. & HARDEN, T.K. (1998), Competitive and selective antagonism of $P2Y_1$ receptors by N^6-methyl 2′-deoxyadenosine 3′,5′-bisphosphate. *Br. J. Pharmacol.,* **124,** 1 – 3.

BROWN, S.G., KING, B.F., KIM, Y.C., JANG, S.Y., BURNSTOCK, G. & JACOBSON. K.A. (2000), Activity of novel adenine nucleotide derivatives as agonists and antagonists at recombinant rat P2X receptors.*Drug Devel. Res.,* **49,** 253 – 259.

BULTMANN, R., TULUC, F. & STARKE, K. (1998). On the suitability of adenosine 3′-phosphate 5′-phosphosulphate as a selective P2Y receptor antagonist in intact tissues. *Eur. J. Pharmacol.,* **351,** 209 – 215.

COMMUNI, D., GONZALEZ, N.S., DETHEUX, M., BREZILLON, S., LANNOY, V., PARMENTIER, M., BOEYNAEMS, J.-M. (2001). Identification of a novel human ADP receptor coupled to G_i. *J. Biol. Chem.,* **276,** 41479 – 41485.

KIM, H.S., RAVI, R.G., MARQUEZ, V.E., MADDILETI, S., WIHLBORG, A.K., ERLINGE, D., MALMSJO, M., BOYER, J.L., HARDEN, T.K., JACOBSON, K.A. (2002). Methanocarba modification of uracil and adenine nucleotides: high potency of Northern ring conformation at $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_{11}$ but not $P2Y_6$ receptors. *J. Med. Chem.,* **45,** 208 – 218.

KING, B.F., BURNSTOCK, G., BOYER, J.L., BOEYNAEMS, J.-M., WEISMAN, G.A., KENNEDY, C., JACOBSON, K.A., HUMPHRIES, R.G., ABBRACCHIO. M.P., GACHET, C. & MIRAS-PORTUGAL, M.T. (2001). P2Y receptors. In: *IUPHAR Compendium of Receptor Characterization and Classification*, 2nd edn, pp 306 – 320.

NANDANAN, E., JANG, S.Y., MORO, S., KIM, H.O., SIDDIQUI, M.A., RUSS, P., MARQUEZ, V.E., BUSSON, R., HERDEWIJN, P., HARDEN, T.K., BOYER, J.L. & JACOBSON, K.A. (2000). Synthesis, biological activity, and molecular modeling of ribose-modified deoxyadenosine bisphosphate analogues as $P2Y_1$ receptor ligands. *J. Med. Chem.,* **43,** 829 – 842.

WEBB, T.E., SIMON, J., KRISHEK, B.J., BATESON, A.N., SMART, T.G., KING, B.F., BURNSTOCK, G. & BARNARD, E.A. (1993). Cloning and functional expression of a brain G-protein-coupled ATP receptor *FEBS Lett.,* **324,** 219 – 225.