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International Union of Pharmacology. LXIX. Status of the Calcitonin Gene-Related Peptide Subtype 2 Receptor

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***Abstract*——Historically, calcitonin gene-related peptide (CGRP) receptors have been divided into two classes, $CGRP_1$ and $CGRP_2$. After the cloning of calcitonin receptor-like receptor (CLR) and receptor activity-modifying proteins (RAMPs), it became clear that the $CGRP_1$ receptor was a complex between CLR and RAMP1. It is now apparent that the $CGRP_2$ receptor phenotype is the result of CGRP acting at receptors for amylin and adrenomedullin. Accordingly, the term "$CGRP_2$" receptor should no longer be used, and the "$CGRP_1$" receptor should be known as the "CGRP" receptor.**

I. Introduction

Heterogeneity among calcitonin gene-related peptide (CGRP[2]) receptors was first detected in 1989, when it was shown that the truncated CGRP antagonist, $CGRP_{12–37}$, preferentially antagonized the chronotropic and ionotropic actions of CGRP on the guinea pig atrium but not its ability to inhibit contraction of the electrically stimulated rat vas deferens. In contrast, the linear CGRP agonist, $Cys(ACM)^{2,7}$-hαCGRP, selectively activated CGRP receptors on the vas deferens (Dennis et al., 1989). Based on these data, it was suggested that there were two subtypes of CGRP receptor, $CGRP_1$ and $CGRP_2$. Subsequent work with the antagonist $CGRP_{8–37}$ confirmed these observations (Dennis et al., 1990). $CGRP_1$ receptors were classified as being antagonized with high potency by $CGRP_{8–37}$, whereas $CGRP_2$ receptors were less sensitive to the effects of this antagonist. The interpretation of data with $Cys(ACM)^{2,7}$-hαCGRP is complicated by the fact that it is a partial agonist (Waugh et al., 1999), but observations with $CGRP_{8–37}$ have demonstrated that a broad range of $CGRP_{8–37}$ affinities can be observed within a single species (Fig. 1a). A more limited range of studies with the nonpeptide antagonist BIBN4096BS also suggests heterogeneity in receptors responding to CGRP (Fig. 1b). Thus, there is good evidence that CGRP can act via more than one receptor when applied pharmacologically. When the International Union of Pharmacology nomenclature subcommittee previously considered CGRP receptors, that status of the $CGRP_2$ receptor was unclear (Poyner et al., 2002); this situation has now been clarified.

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[2] Abbreviations: CGRP, calcitonin gene-related peptide; $Cys(ACM)^{2,7}$-hαCGRP, [acetamidomethyl-cysteine^{2,7}]-human α-CGRP; BIBN4096BS, *N*-[2-[[5-amino-*l*-[[4-(4-pyridinyl)-*l*-piperazinyl]carbonyl]pentyl]amino]-1-[(3,5-dibromo-4-hydroxyphenyl)methyl]-2-oxoethyl]-4-(1,4-dihydro-2-oxo-3(2*H*)-quinazolinyl); AM, adrenomedullin; CLR, calcitonin receptor-like receptor; RAMP, receptor activity-modifying protein; CT, calcitonin; AMY, amylin.

II. Studies on Cloned Receptors

A. Calcitonin Gene-Related Peptide and Adrenomedullin Receptors

The best characterized CGRP receptor has two transmembrane-spanning components; a G protein-coupled receptor-like protein, CLR, and also an accessory protein, RAMP1. This receptor has the pharmacological

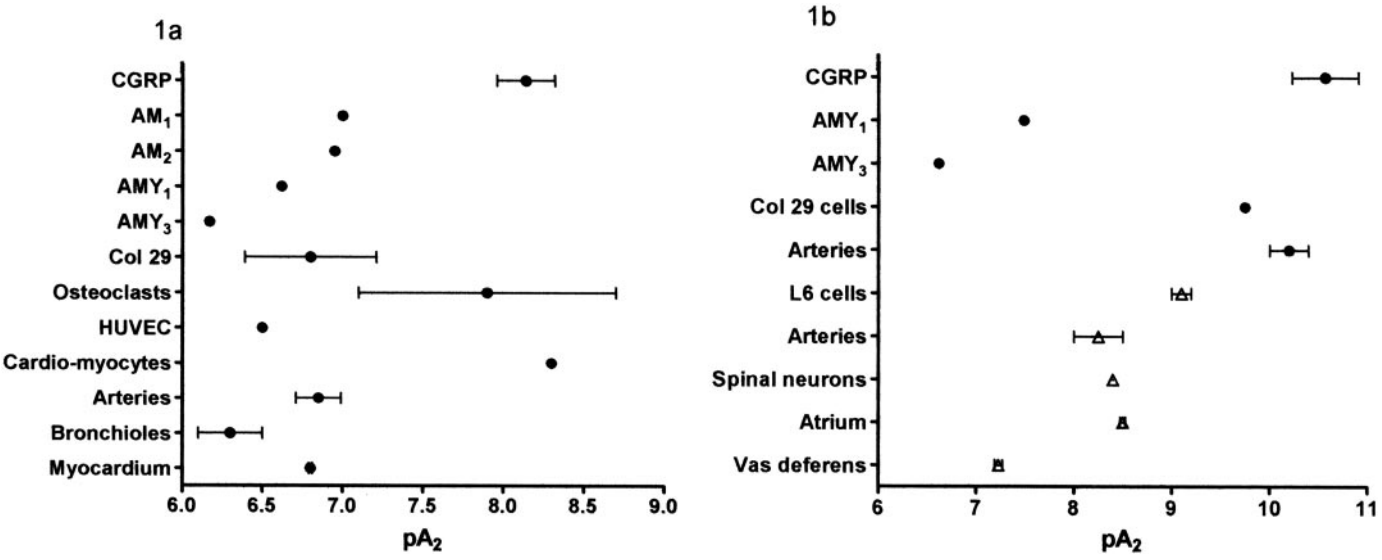

FIG. 1. Antagonist affinities on CGRP receptors. a, apparent pA_2 values for $CGRP_{8-37}$ on human cells and tissues. For CGRP receptors (CLR/RAMP1), data have been combined from studies on recombinant receptors and also SK-N-MC cells. b, apparent pA_2 values for BIBN4096BS on rat (△) and human (●) cells and tissues. It should be noted that because BIBN4096BS is probably an allosteric antagonist (Hay et al., 2006), the apparent pA_2 values are simply to give a guide as to its affinity at the preparations illustrated here. Data for both figures from Hay et al. (2004), updated to 2008 (Sheykhzade et al., 2004; Springer et al., 2004; Kawase et al., 2005; Nodin et al., 2005; Verheggen et al., 2005; Bailey and Hay, 2006; Gupta et al., 2006a,b; Takhshid et al., 2006; Edvinsson et al., 2007; De Mey et al., 2008; Wunder et al., 2008). In addition, an apparent pA_2 for BIBN4096BS of 14 on human pial arteries has been reported (Moreno et al., 2002). Where no error bar is shown, the result is from a single study; otherwise $n = 2$ to 12. HUVEC, human umbilical vein endothelial cells.

profile of a $CGRP_1$ receptor (McLatchie et al., 1998). Coexpression of CLR with RAMP2 and RAMP3 gives receptors that preferentially bind AM (McLatchie et al., 1998). These are the AM_1 and AM_2 receptors (Poyner et al., 2002). The AM_2 receptor, in particular, can have significant affinity for CGRP and therefore might be activated by this peptide at pharmacological concentrations (Hay et al., 2003). $CGRP_{8-37}$ can antagonize AM_1 and AM_2 receptors with estimated pA_2 values in the range of 6.0 to 7.0 (Fig. 1a); BIBN4096BS has no appreciable affinity at either of these receptors (Hay et al., 2003). Therefore, under conditions of high receptor expression, the AM_2 receptor could be activated pharmacologically by CGRP and antagonized by $CGRP_{8-37}$ with low potency; the characteristics of a $CGRP_2$ receptor.

B. Calcitonin and Amylin Receptors

CT receptors are activated only very weakly by CGRP so need not be considered here. On the other hand, AMY receptors can show significant affinity for CGRP. In particular, the $AMY_{1(a)}$ receptor (insert negative CT receptor [$CT_{(a)}$] plus RAMP1), at least in transfected cells, may potentially be activated by CGRP (Kuwasako et al., 2004; Hay et al., 2005). The $AMY_{3(a)}$ [$CT_{(a)}$/RAMP3] receptor shows activation by CGRP similar to that of the AM_2 receptor (Hay et al., 2005). Furthermore, both of these AMY receptor subtypes were shown to be weakly antagonized by $CGRP_{8-37}$. Similar observations have also been made for rat $AMY_{1(a)}$ receptors (D. L. Hay and A. Ferner, unpublished observations). Therefore, AMY_1 and AMY_3 receptors also have the characteristics of a $CGRP_2$ receptor. The $AMY_{1(a)}$ receptors [but not the $AMY_{3(a)}$ receptors] show significant affinity for BIBN4096BS (Hay et al., 2006) but at approximately 150-fold lower than that seen at $CGRP_1$ receptors (Fig. 1b).

III. Conclusions

There are now clear molecular correlates for CGRP receptors identified pharmacologically. The $CGRP_1$ receptor corresponds to the CLR/RAMP1 complex. The pharmacological profile of the $CGRP_2$ receptor can be generated by the AMY_1 receptor and, to a lesser extent, by the AMY_3 and AM_2 receptors. Accordingly, it is recommended that the "$CGRP_1$" receptor should now be called the "CGRP" receptor and the term "$CGRP_2$" receptor should not be used. There remain significant differences between antagonist affinities found on cell lines and tissues for the same receptor subtype (Fig. 1). These complicate the pharmacological identification of receptors and may relate to accessibility or stability issues of the currently available antagonists.

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