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COMMENTARY

Soluble guanylate cyclase: an old therapeutic target re-visited

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The heterodimeric haemoprotein soluble guanylate cyclase (sGC) acts as the principal intracellular receptor for nitric oxide (NO) and facilitates the formation of the second messenger cyclic guanosine-3′,5′-monophosphate (cGMP), which in turn governs many aspects of cellular function *via* interaction with specific kinases, ion channels and phosphodiesterases (PDEs; Hobbs & Ignarro, 1996; Hobbs, 1997). This signal transduction pathway underlies the majority of physiological actions attributed to NO and is important in the regulation of the cardiovascular, gastrointestinal, urogenital, nervous and immune systems. As a consequence, aberrant sGC-dependent signalling may be fundamental to the aetiology of a wide variety of pathologies; agents that can modulate enzyme activity in a selective manner should therefore possess considerable therapeutic potential.

Yet sGC can hardly be described as a novel therapeutic target! The use of organic nitrates (e.g. glyceryl trinitrate, GTN; isosorbide dinitrate) for the treatment of conditions such as angina and heart failure has been advocated for over a century (Brunton, 1867), although the mechanism of action of such compounds was not elucidated until the late 1970s and found to involve metabolic conversion to NO and subsequent activation of sGC (Ignarro *et al*., 1981). In contrast, recent attempts to manipulate sGC signalling for medical benefit have focused almost exclusively toward inhibition of NO synthesis; in terms of novel therapeutics, however, this has proven to be somewhat of a fruitless exercise. Surprisingly perhaps, little attention has focused on the identification of selective sGC-modulating compounds (indeed, they have been notoriously hard to come by!), particularly enzyme activators that are probably of greater interest therapeutically. This is despite the fact that sGC dysfunction is likely to have an equivalent impact on pathogenesis as inappropriate NO production and tissue-specific distribution of sGC isoforms (Budworth *et al*., 1999) may provide a means of targeting drug therapy.

Although clinicians have at their disposal organic nitrates (and other NO-donor or 'nitrovasodilator' drugs), which release the endogenous ligand NO to activate sGC, the use of such compounds is problematic. First, NO-donor compounds, particularly organic nitrates, suffer from the development of tolerance following prolonged administration. The mechanism(s) underlying this tachyphylaxis remain unclear but may be linked to decreased metabolic activation of the compounds (Needleman & Johnson, 1973), excessive superoxide, endothelin or angiotensin II levels (Buchmuller-Rouiller & Mauel, 1991; Munzel *et al*., 1996), or a reduction in the sensitivity/activity of the NO receptor, sGC (Hussain *et al*., 1999). Second, the use of NO-donors *in vivo* is potentially troublesome due to non-specific interaction of NO with other biological molecules; reactions that are difficult to control due to the spontaneous release of NO from nitrovasodilators and its free diffusion in biological systems. Current dogma suggests that the beneficial (physiological) actions of NO are mediated predominantly *via* activation of sGC (i.e. cGMP-dependent) and the detrimental (pathological) actions of NO are exerted primarily *via* direct (i.e. cGMP-independent) modifications of proteins (e.g. nitrosation, nitration), lipids (e.g. peroxidation) and nucleic acids (e.g. DNA strand breaks). Thus, the use of NO-based therapeutics will always represent a double-edged sword. Even if doses are titred to minimize these side effects, the majority are not readily reversible and will accumulate over time, potentially manifesting as long-term problems. Moreover, persistent inhibition of oxidative phosphorylation by NO may trigger apoptosis and cell death (Beltran *et al*., 2000). In light of these shortcomings, compounds which can activate sGC in an NO-independent manner, and not suffer from tachyphylaxis, will therefore offer a considerable advance on current therapy.

Stasch *et al*. (2002a, b) have reported just such a series of compounds in a succession of papers in this journal and others (Stasch *et al*., 2001; Becker *et al*., 2001; Straub *et al*., 2001) in which they have identified and characterized novel non NO-based sGC activators. This family of reagents is based loosely on YC-1 (Figure 1), a recently described sGC activator with hypotensive and anti-platelet properties (Ko *et al*., 1994; Mulsch *et al*., 1997; Rothermund *et al*., 2000). However, the BAY series of compounds are considerably more potent and do not appear to inhibit PDE activity (at least at therapeutic doses) thus offering significantly greater selectivity. The authors have conducted a thorough pharmacological evaluation of these compounds both *in vitro* and *in vivo* and they appear to fall into two distinct classes: haem-dependent and haem-independent activators.

The haem-dependent sGC activators, exemplified by BAY 41-2272 and BAY 41-8543, activate purified enzyme in a synergistic fashion with NO and require the presence of haem (sensitive to blockade by the sGC inhibitor, ODQ). These

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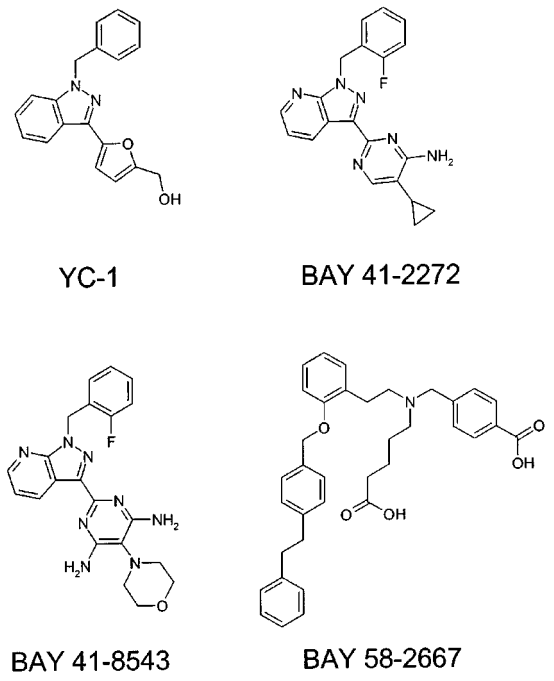

Figure 1 Novel non NO-based sGC activators.

compounds are potent relaxants of vascular smooth muscle in both arteries and veins, including the coronary circulation, and in the rat Langendorff preparation they reduce coronary perfusion pressure without affecting left ventricular pressure or heart rate. Remarkably, the EC_{50} values for BAY 41-8543 are between 1 and 2 orders of magnitude lower than 'classical' nitrovasodilators, including clinically used nitrate esters (Figure 2). Perhaps of even greater interest is that these 'beneficial' cardiovascular actions of the BAY compounds remain intact in tissues tolerant to GTN. Not only is this valuable for therapeutic use, but also suggests that the tachyphylaxis to organic nitrates is specific to NO-mediated activation of the enzyme. These novel sGC activators also have a pronounced inhibitory effect on aggregation in washed platelets and platelet rich plasma (although in the latter the potency is somewhat diminished) which is mediated, at least in part, *via* phosphorylation of the vasodilator-stimulated phosphoprotein (VASP). The vasorelaxant and anti-platelet actions of BAY 41-8543 *in vitro* are mirrored *in vivo*. In anaesthetized dogs and rats the compound causes a dose-dependent decrease in blood pressure (lasting up to 24 h) whilst increasing coronary blood flow. Moreover, in a high-renin model of hypertension, BAY 41-8543 prevents the blood pressure increase in response to L-NAME and affords renal protection. As intimated by *in vitro* experimentation, no tolerance is observed to BAY 41-8543 following repeated administration; furthermore, the sGC-activators are orally active, a much sought-after therapeutic trait. Also in accord with *in vitro* observations, BAY 41-8543 prolongs rat tail bleeding time and inhibits $FeCl_3$-induced thrombus formation.

Not only do these compounds offer novel therapeutic approaches to treating cardiovascular disease, but they have also highlighted a unique, allosteric regulatory site on sGC. Photoaffinity labelling studies revealed that the sGC-activators bind to the N-terminal region of the α subunit in

Compound	Rabbit saphenous vein EC_{50} (μM)	Relative potency (GTN = 1)
GTN	23	1
SIN-1	1.1	21
SNP	0.6	38
BAY 41-8543	0.1	230
BAY 41-2272	0.06	383
BAY 58-2667	0.0004	57,500

Figure 2 Example of the relative potency of nitrovasodilator drugs and novel non NO-based sGC activators on vascular tissue.

close proximity to two cysteine residues (αCys^{238} and αCys^{243}) to mediate activation of the enzyme (Stasch *et al.*, 2001), although the precise binding site will only be revealed following crystallization studies. It is likely that this stretch of amino acids is important in regulating enzyme activity physiologically, perhaps in response to an endogenous allosteric activator which sensitizes the enzyme to NO. Alternatively, modification (e.g. nitrosation) of the enzyme at these cysteines by NO (or related species) might represent a feedback regulatory loop governing NO signalling.

In this issue of the *British Journal of Pharmacology*, Stasch *et al.* report the first pharmacological characterization of the haem-independent sGC activators, represented by BAY 58-2667. This compound also elicits increases in cGMP *via* activation of sGC, but intriguingly the activity is maintained, indeed enhanced, in haem-deficient enzyme or in the presence of ODQ. BAY 58-2667 relaxes rabbit saphenous artery and vein with a potency some two orders of magnitude greater than BAY 41-2272 and 1000 fold greater than SNP and SIN-1 (Figure 2). The compound also reduces coronary perfusion pressure in a rat Langendorff preparation. Akin to its predecessors, BAY 58-2667 remains active in tissues made tolerant to GTN. *In vivo*, BAY 58-2667 causes a prolonged fall in blood pressure in anaesthetized dogs and has a similar effect to GTN on the arterial and venous circulation; the compound also reverses the rise in blood pressure in spontaneously hypertensive rats. BAY 58-2667 also has potent anti-platelet activity both *in vitro* (washed platelets and platelet rich plasma) and *in vivo* (rat tail bleeding time and $FeCl_3$-induced thrombosis). The differential activity of BAY 58-2667 compared to BAY 41-2272 and BAY 41-8543 may be the result of the former interacting with a second, distinct allosteric site on the enzyme. Evidence is presented indicating that BAY 58-2667 binds to the protein on the α subunit at position 371 and the β subunit between positions 231–310, identifying yet another potential regulatory site governing cGMP-dependent signalling.

The identification of these novel non NO-based sGC activators is important both as pharmacological tools and in the development of new therapeutics. These compounds have revealed previously unknown regulatory sites on the enzyme which may be important physiologically, representing target sites for endogenous molecules modulating sGC activity. The therapeutic benefits of these compounds are clear in the cardiovascular system, having a profile of activity similar to organic nitrates, but devoid of the problem of tolerance and the potential cytotoxic actions of NO; these reagents should therefore offer significant advantage over current therapy. Moreover, these compounds have significant anti-platelet activity, which organic nitrates do not, which is theoretically beneficial for diseases such as angina and heart failure. The

value of sGC activators should also be realized in other organ systems, particularly in the treatment of erectile dysfunction. Current therapy, PDE V inhibitors, rely upon residual endogenous NO production to be effective. Nearly a third of cases do not respond to PDE V inhibitors (i.e. Viagra), perhaps indicating that endogeous NO production is impaired to such an extent that inhibition of cGMP breakdown has no significant beneficial effect. However, the novel non NO-based sGC activators might still be functional under such circumstances.

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