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PERSPECTIVE

Nitric Oxide Signaling in Brain: Potentiating the Gain with YC-1

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The remarkable discovery of endothelial derived relaxing factor by Furchgott and Zawadzki (1980) initiated research that ultimately led to the discovery of nitric oxide (NO) as an endogenous mediator (Ignarro, 1993; Moncada and Higgs, 1993; McDonald and Murad, 1995). In the vascular system, NO diffuses from its site of synthesis in endothelial cells and enters the surrounding smooth muscle, where it binds to the soluble isoform of guanylyl cyclase (sGC). NO binding dramatically increases sGC activity to produce cGMP, and this leads to smooth muscle relaxation.

Although first discovered as a signaling molecule in the vasculature, the highest levels of NO occur in neurons, where NO functions as a unique neurotransmitter (Garthwaite, 1991; Snyder and Bredt, 1991). The high levels of NOS in neurons facilitated the initial identification of a nitric-oxide synthase from neurons (Bredt et al., 1991). Neuron-derived NO serves as a major neurotransmitter whose functions are best characterized in the peripheral nervous system. Release of NO from enteric neurons dilates gastric and intestinal smooth muscle (Desai et al., 1991), and release of NO from neurons in the corpora cavernosa dilates vessels that mediate penile erection (Burnett et al., 1992; Rajfer et al., 1992). In fact, sildenafil (Viagra) treats erectile dysfunction by functioning as a cGMP phosphodiesterase inhibitor that prolongs the actions of NO to increase penile blood flow.

Whereas actions of neuron-derived NO outside the brain are well established, the roles for NO in the central nervous system have been more difficult to determine. As a free radical gas, NO is a unique messenger molecule that can readily penetrate cells and tissues. This property of NO suggested that it would be ideally suited to mediate actions at brain synapses, the sites at which neurons communicate with each other. Indeed, a number of studies showed that NO is critically involved in long-term potentiation (LTP), a form of synaptic plasticity in the hippocampus (Bohme et al., 1991; Schuman and Madison, 1991), a brain region essential for memory formation. LTP can be elicited by providing a strong stimulus (or tetanus) to Schaffer collateral fibers that innervate pyramidal cells in the CA1 region of hippocampus. After this tetanus, the synapses that were stimulated in the CA1 region are specifically strengthened, and this plasticity may participate in learning and memory functions by the hippocampus. A role for NO in LTP was first suggested by pharmacological experiments showing that NOS inhibitors do not alter baseline synaptic transmission but can completely prevent the LTP (Bohme et al., 1991; Schuman and Madison, 1991).

However, the role of NO in LTP has been mired in controversy; numerous labs have found that LTP can occur in the absence of NO signaling (Selig et al., 1996). Furthermore, mutant mice that lack NOS in brain show largely intact LTP in hippocampus (Son et al., 1996). In a report of a new study beginning on page 1322 of this issue of *Molecular Pharmacology*, Chien et al. (2003) provide the latest argument to this controversy. The authors show that a recently discovered drug, YC-1, which sensitizes the sGC toward activation by NO, dramatically augments induction of hippocampal LTP. In addition to suggesting that NO may participate in LTP, this study demonstrates a potential role for YC-1 as a novel modulator of the NO-sGC pathway in brain.

Soluble guanylyl cyclase is a heterodimeric enzyme comprising an α and a β subunit (Garbers, 1979). Nitric oxide regulates sGC by binding to a heme prosthetic group (Ignarro et al., 1982; Martin et al., 2000). Under resting conditions, the iron in the heme of sGC is five-coordinated. Specifically, the iron binds to the four nitrogens in the center of the heme ring and also has a histidine group from the β subunit of sGC as the axial ligand (Fig. 1). Binding of NO at the opposite pole of the heme iron ruptures the histidine-to-iron bond (Fig. 1). This leads to an allosteric change in the sGC that increases the enzyme's specific activity to produce cGMP by more than 100-fold (Ignarro et al., 1984). Many vasodilator drugs activate sGC through a similar mechanism. The dramatic coronary artery relaxing actions of nitroglycerin and nitroprusside that relieve angina are explained by the metabolism of these "nitrovasodilator" drugs to NO.

ABBREVIATIONS: sGC, soluble guanylyl cyclase; LTP, long-term potentiation; NOS, nitric-oxide synthase; YC-1, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole; BAY 41-2272, 5-cyclopropyl-2-[1-(2-fluoro-benzyl)-1H-pyrazolo[3,4-*b*]pyridine-3-yl]pyrimidin-4-ylamine.

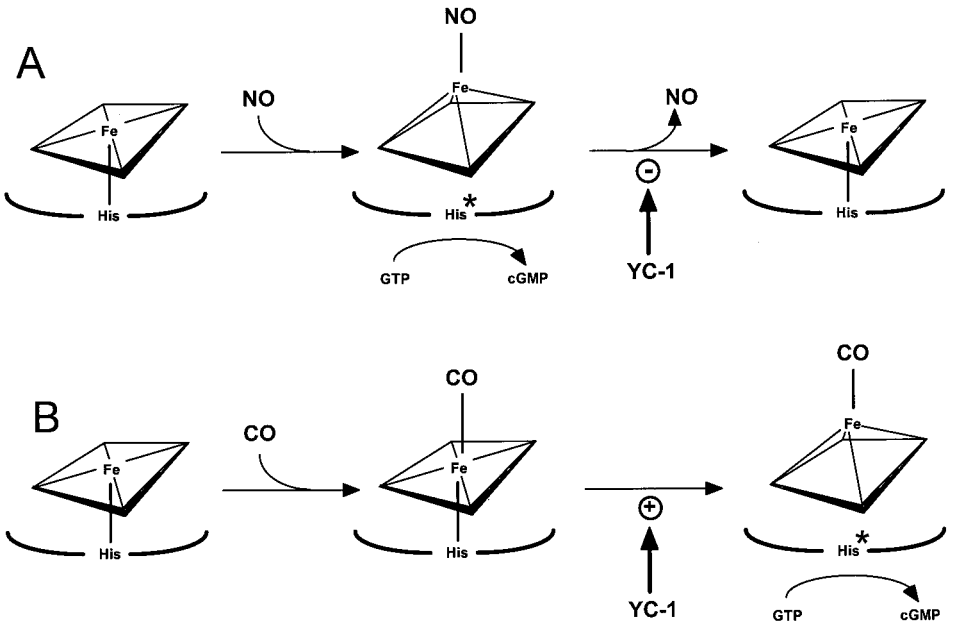

Fig. 1. YC-1 sensitizes guanylyl cyclase to activation by nitric oxide and carbon monoxide. In the resting state the heme of sGC is five-coordinated with a histidine (His) from the β subunit bound to an axial fifth coordination position. A, binding of NO to the sixth position ruptures the histidine-to-iron bond and activates the catalytic activity of sGC. YC-1 slows the dissociation of NO from sGC and thereby sensitizes the enzyme to low concentrations of NO. B, binding of CO to the sixth coordination position does not sever the histidine-to-iron bond and does not effectively increase sGC activity. In the presence of YC-1, however, CO binding does break the histidine bond and stimulates enzyme activity.

A new mechanism of sGC regulation, however, explains the actions of YC-1, a benzylindazole derivative (Ko et al., 1994). YC-1 was first identified as an inhibitor of platelet aggregation, and subsequent studies showed that activation of sGC by YC-1 explains its antiplatelet activity (Ko et al., 1994). However, unlike other sGC activators, YC-1 does not contain NO. Instead, YC-1 exerts a distinct allosteric regulation of sGC that can increase the activity of the purified enzyme by at least 10-fold. The binding site for YC-1 remains unknown but it does not directly interact with the heme iron (Friebe et al., 1996).

The actions of YC-1 in vivo are more complex, in that its effects on sGC synergize with those of NO (Hoenicka et al., 1999). The presence of YC-1 makes NO a far more potent activator of sGC, such that the enzyme can be maximally activated by the low amounts of NO that form endogenously. This property of YC-1 may explain why Chien et al. (2003) found, in drug-treated brain tissue, that moderate rates of neuronal firing induced maximal amounts of synaptic plasticity. This sensitizing action is explained by YC-1 preventing release of NO from sGC (Fig. 1). Under normal conditions, the release of NO from sGC (and associated enzyme deactivation) has a half-life of ~4 s; this is increased to more than 10 min in the presence of YC-1 (Russwurm et al., 2002).

Even more dramatic is the interaction of YC-1 with carbon monoxide (CO) (Friebe et al., 1996), the other endogenous gaseous regulator of sGC (Verma et al., 1993). Although heme oxygenase produces endogenous CO in neurons and in other cells, physiological levels of CO only modestly increase sGC activity (~4-fold stimulation). Carbon monoxide does not dramatically activate purified sGC because CO binding to the heme iron does not break the histidine-to-iron bond necessary for allosteric regulation (Fig. 1). However, in the presence of YC-1, CO binding does rupture the histidine-to-iron bond, and this activates sGC enzyme activity to the same level as does NO binding (Makino et al., 2003). Although Chien et al. (2003) found that endogenous NO (rather than CO) works together with YC-1 to augment hippocampal LTP, it is possible that CO conspires with YC-1 in other brain regions or tissues. Whether an endogenous YC-1–like molecule might regulate sGC to make the enzyme a potent CO sensor also remains uncertain.

In addition to facilitating physiological studies of the NO-sGC pathway, YC-1–like drugs have the potential to serve as novel therapeutics. The vasodilator actions of YC-1 suggest possible roles in treatment of hypertension. In fact, BAY 41-2272, a more potent compound that acts like YC-1, lowered blood pressure and decreased mortality in a rat model of hypertension (Stasch et al., 2001). Furthermore, these compounds may also be beneficial in treatment of male erectile dysfunction, because YC-1 and a related compound, A-350619, promote penile erection in rats (Miller et al., 2003). Finally, by enhancing synaptic plasticity, these drugs could improve cognitive performance or have other roles in neurological disorders. Future studies of YC-1 and related drugs will clarify the utility of this novel class of sGC regulators in treatment of a variety of disorders associated with deficient NO signaling.

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