Role of cyclic GMP in the mechanism of action of nitrovasodilators, endothelium-dependent agents and atrial natriuretic peptide

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The synthesis of cyclic GMP from GTP is catalysed by three isoenzyme forms of guanylate cyclase which include the soluble or cytosolic form, the particulate or membrane-associated form and a form associated with the cytoskeletal components (see reviews [1, 2]). These isoenzymes have different physiochemical, antigenic and kinetic properties and will undoubtedly have different primary structures. Each of these isoenzymes has different mechanisms for their regulation, which in our detailed studies described elsewhere, have proved to be rather complex (see reviews [1, 2]).

When we were characterizing and purifying these isoenzymes some years ago, we found that azide, hydroxylamine and nitrite activated the enzyme [3]. Since these were the first agents to activate the enzyme in cell-free preparations, we conducted a series of detailed studies to determine the mechanisms involved (see reviews [1, 2]). Fortunately at the time we were also examining cyclic GMP metabolism in tracheal smooth muscle preparations [4, 5]. We found that these and other agents such as nitroglycerin and nitroprusside activated guanylate cyclase, increased cyclic GMP synthesis and resulted in smooth muscle relaxation [4, 5]. We have proposed that these and other related agents can result in the formation of nitric oxide free radicals which can activate the enzyme [6, 7]. We have coined the term 'nitrovasodilators' for these agents that generate nitric oxide and through cyclic GMP accumulation result in the relaxation of various smooth muscle preparations, including tracheal, vascular and gastrointestinal smooth muscle (see reviews [8, 9]). These agents also increase cyclic-GMP-dependent protein kinase activity in vascular smooth muscle and other preparations [10] which is associated with the altered phosphorylation of numerous intracellular proteins [11, 12]. Accumulation of cyclic GMP is also associated with the dephosphorylation of myosin light chain [12, 13]. This could result from decreased activity of myosin light chain kinase and/or increased activity of the phosphatase. The recently described effects of cyclic GMP on cytosolic calcium concentrations and the activity of sarcolemmic calcium ATPase [14, 15] support the view that the dephosphorylation of myosin light chain is secondary to decreased concentrations of cytosolic calcium and myosin light chain kinase activity. However, additional studies are required. The cascade of events is illustrated in Fig. 1.

While the effects of nitrovasodilators are probably mediated through activation of soluble guanylate cyclase, effects on the particulate enzyme cannot be excluded. Indeed, in some preparations these agents may also activate the particulate isoenzyme, but these observations are somewhat controversial (see [1] and references therein).

Shortly after the description of endothelial-derived relaxant factor (EDRF) by Furchgott and his associates [16],

Fig. 1. Proposed mechanism of action of some vasodilators

EDRF and nitric oxide, which is derived from nitrovasodilators, activate the soluble isoenzyme form of guanylate cyclase. The cyclic GMP formed activates cyclic-GMP-dependent protein kinase and alters the phosphorylation of many smooth muscle proteins. Probably through decreased cytosolic Ca$^{2+}$ the phosphorylation of myosin light chain is decreased resulting in relaxation. The similar, if not identical, effects of nitrovasodilators and EDRF support the proposal that EDRF can be viewed as an equivalent to an ‘endogenous nitrate’. Atroiopeptins through specific receptors designated ANF-1 receptors result in activation of the membrane-associated isoenzyme form of guanylate cyclase. A similar cascade of events follows after cyclic GMP synthesis. The function of the ANF receptor (ANF-2) that is not coupled to cyclic GMP synthesis is unknown, nor is the effect of ANF on endothelium cells (from [8]).
we found that endothelium-dependent vasodilators also increased cyclic GMP accumulation in vascular preparations when the endothelium was intact [17]. These effects were also associated with the activation of cyclic GMP-dependent protein kinase [32] and altered phosphorylation of the same vascular smooth muscle proteins as observed with the nitrovasodilators [18, 19]. Thus, the pharmacological and biochemical effects of the nitrovasodilators and EDRF are very similar, if not identical, and EDRF might be viewed as the equivalent of an 'endogenous nitrovasodilator' or 'endogenous nitrate' [8]. The lability and properties of EDRF will be described in greater detail by others in this colloquium. While it has been suggested that EDRF may, in fact, be the nitric oxide free radical [20], this seems unlikely in view of the lability and reactivity of nitric oxide and the distance it must traverse from the endothelial cells to the smooth muscle cells to induce cyclic GMP synthesis. A more appealing hypothesis is that EDRF is a precursor of a free radical such as nitric oxide or another reactive species of oxygen. The lability of EDRF and its presumed low concentration in interstitial fluid have markedly hindered its purification and identification. It should be noted that nitric oxide concentrations of less than micromolar are sufficient to activate guanylate cyclase [7, 21] and presumably EDRF will be present at concentrations similar, if not identical, and EDRF might be viewed as the nitrovasodilators and ANP also increase cyclic GMP synthesis and accumulation in a variety of other cell types and tissues and have assisted us and others in defining other functions of cyclic GMP.

Calcium channels in smooth muscle cells

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The importance of calcium ions for contraction of muscle began to be appreciated when attempts were made to construct physiological salt solutions suitable for maintaining contraction of muscle tissues in vitro (e.g. Ringer, 1896). Much later, experiments on smooth muscles were carried out which created some confusion concerning the mechanism of tension generation in this muscle type: recordings with microelectrodes showed a good correlation between the level of membrane potential, frequency of action potential discharge, and tension in phasic visceral smooth muscle (Bulbring, 1955), but it was soon demonstrated in phasic uterine smooth muscle (Evans et al., 1958) that it could contract well to acetylcholine in depolarizing high-potassium solutions. This paradox was resolved by the suggestion that calcium may enter the smooth muscle cell both via channels gated by potential ('potential-sensitive channels') and through channels gated by receptor ('receptor-operated channels') (Bolton, 1979; van Breeman et al., 1979). A further problem was the variable degree to which contractions to receptor activation (e.g. by acetylcholine via muscarinic receptors in visceral muscle or by noradrenaline via α-receptors in vascular muscle) were resistant to calcium-free conditions (Edman & Schild, 1962). Some smooth muscle responses are very resistant (e.g. Tomita et al., 1985). We now believe this is caused by the sequestration of calcium in storage sites within the smooth muscle cell from where it can be efficiently and rapidly released following receptor activation.

Potential-sensitive calcium channels seem to be ubiquitously present on smooth muscle cells, although their density is lower if the smooth muscle type is electrically inexcitable (e.g. many vascular muscles do not generate action potentials very readily either spontaneously or in response to electrical depolarization) (Bolton et al., 1988). These calcium channels open in response to depolarization of the membrane and are responsible for the upstroke of the action potential where this is seen in excitable smooth muscles. In non- or less excitable smooth muscle, depolarization causes the opening of these channels also, but action potentials do not occur. Probably at least two types of potential-sensitive calcium channel exist (Aaronson et al., 1986); the exact contributions of these to the total inward calcium current is in doubt as is the sensitivity of individual channel types to calcium-entry blocking drugs: the dihydropyridines (Worley et al., 1986; Yatani et al., 1987).

Receptor-operated channels may also admit calcium, although the amounts probably vary depending on the smooth muscle and the receptor involved. The main function of receptor-operated channels is probably to shift the membrane potential into (or out of, in the case of relaxant substances) the potential range where potential-sensitive calcium channels operate. Smooth muscle depolarized by high-potassium solution can admit calcium when carbachol, a muscarinic-receptor stimulant, is applied (Durbin & Jenkinson, 1961). Single-cell studies show that ATP receptor-operated channels can admit divalent cations including calcium (Benham et al., 1987) and single-channel studies have shown that appreciable amounts of calcium may enter through ATP-receptor-operated channels (Benham & Tsien, 1987). We find that muscarinic-receptor-operated channels behave in a similar way.

In single smooth muscle cells the importance of release of calcium-stores can be amply demonstrated. In vascular muscle noradrenaline can release stored calcium, and in intestinal smooth muscle, acetylcholine can have a very similar effect (Benham et al., 1985; Benham & Bolton, 1986). However, the importance of this release for the contractile response of the whole muscle to low concentrations of stimulants needs to be established; it is likely that the release of calcium in response to receptor activation involves inositol 1,4,5-trisphosphate production from phosphatidylinositols 4,5-bisphosphate by the action of phospholipase C (Itoh et al., 1988).