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cGMP and cAMP inhibit tension development in skinned coronary arteries

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Abstract. The effects of physiological concentrations of cGMP and cAMP on tension development in skinned coronary arteries (Triton X-100) were studied. cGMP inhibited tension elicited at intermediate Ca^{2+} concentrations at pH 7.0 but not at more acidic or alkaline pH values. cAMP, on the other hand, decreased submaximal tension development independent of pH (from pH 6.5 to pH 7.2). Neither nucleotide affected tension development at maximally activating Ca^{2+} concentrations.

Key words: cGMP – cAMP – Skinned coronary arteries

Introduction

Vascular smooth muscle relaxation induced by nitrogen oxide-containing compounds such as nitroglycerin and nitroprusside is associated with an increase in tissue levels of cGMP (Schultz et al. 1977; Kukovetz et al. 1979; Janis and Diamond 1979). The increase in the concentration of cGMP depends on the concentration of the compound (Kukovetz et al. 1979; Keith et al. 1982) and the duration of its application (Galvas and DiSalvo 1983). It could also be shown that these compounds activate guanylate cyclase derived from bovine coronary arteries (Gruetter et al. 1979; Gerzer et al. 1981) and the relaxant effect on smooth muscle is mimicked by 8-bromo-cGMP, a potent activator of cGMP-dependent protein kinase (Schultz et al. 1979).

However, little is known about the mechanisms by which cGMP relaxes vascular smooth muscle. In analogy to cAMP, cGMP may affect Ca^{2+} transport (Ives et al. 1980; Scheid et al. 1978) and/or may have a direct effect on the contractile apparatus by phosphorylation of specific proteins (Conti and Adelstein 1980; Rüegg and Paul 1982; Rüegg et al. 1983; Silver and DiSalvo 1979). Detergent skinned fiber preparations which are devoid of functional Ca^{2+}-stores and of a sarcolemmal diffusion barrier offer an excellent model to test whether or not cGMP directly influences contractility. In an earlier study (Pfitzer et al. 1982) performed at pH 6.7 we were not able to demonstrate a direct influence of cGMP on contractility. We now show for the first time that cGMP inhibits contraction of skinned coronary arteries in concentrations which have been obtained in vivo in the presence of acetylcholine and the phosphodiesterase inhibitor M & B 22,498 (Holzmann 1982). Recalculation of these in vivo data using correction values of Casteels (1969) and Jones et al. (1973) indicate that the in vivo cGMP concentrations can be at least as high as 2 μM. Inhibition by cGMP has a very sharp pH optimum of 7.0 which distinguishes the inhibitory effect of cGMP from that of cAMP.

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Methods

Chemically skinned smooth muscle bundles were prepared from porcine coronary arteries and tension was recorded as described by Rüegg et al. (1983). Tissues were bathed in 0.3 ml Lucite chambers maintained at 27° C by a circulating water bath. Fibers were relaxed in a solution containing 30 mM imidazole, 4 mM EGTA, 7.5 mM ATP, 10 mM $MgCl_2$, 1 mM NaN_3, 2 mM dithioerytritol (DTE), 6 mM KH_2PO_4, 0.2 μM calmodulin and an ATP regenerating system consisting of 10 mM creatine phosphate and 150 U/ml creatine phoshokinase (Boehringer Mannheim, FRG) (Schneider et al. 1981). In bathing solutions used for eliciting contraction EGTA was replaced by 4 mM Ca-EGTA. In experiments performed to test for the presence of functional Ca^{2+}-stores, EGTA was lowered to 0.2 mM. The experimental protocol was identical to the one described by Saida (1982).

Free $[Ca^{2+}]$ was calculated (Portzehl et al., 1964) using the following apparent dissociation constants: 1.8×10^{-7} M (pH 7.2), 4.2×10^{-7} M (pH 7.0), 1.6×10^{-6} M (pH 6.7), and 3.9×10^{-6} M (pH 6.5). These constants have been corrected for the fact that pH conventionally refers to activity rather than to the concentration of protons (Thomas 1982). The pH of the solutions was titrated with KOH and ionic strength was adjusted to about 0.09 M with KCl. Homogeneous preparations of cGMP-dependent protein kinase (Flockerzi et al. 1978) and calmodulin (Scharma and Wang 1979) were prepared from bovine lung and porcine brain.

The data are expressed as the mean ± standard error of the mean (SEM). Statistical evaluation was performed with a two tailed Student's *t*-test, and $P < 0.05$ was taken as indicative of statistical significance.

Results

Detergent skinned coronary smooth muscle fibres are relaxed in a Ca^{2+}-free ATP-salt solution. Incubation with caffeine (20 mM) after preloading the fibers with Ca^{2+} induced no contraction suggesting that the functional Ca^{2+}-stores were destroyed by the detergent. Increasing $[Ca^{2+}]$ to 1.7 μM induced approximately 50% of the tension that

Table 1. Ca^{2+}-dependence and pH-dependence of tension responses (%) to cGMP and cAMP

pH	$[Ca^{2+}]$ (μM)	1st contraction	2nd contraction in the presence of:		
			IBMX (0.1 mM)	cGMP (5 μM) + IBMX	cAMP (5 μM) + IBMX
7.2	1.7	76.95 ± 1.76 (19)	74.49 ± 3.55 (6)	73.41 ± 2.88 (7)	62.57 ± 4.09 (6)
7.0	1.7	56.5 ± 2.0 (36)	57.3 ± 11.04 (7)	36.78 ± 6.9 (6)*	36.86 ± 6.34 (5)*
6.7	2.3	83.05 ± 1.2 (27)	84.73 ± 3.24 (4)	82.3 ± 2.5 (7)	66.84 ± 3.53 (6)*
6.5	1.7	51.4 ± 2.11 (28)	63.61 ± 3.19 (7)	51.61 ± 4.6 (8)	36.55 ± 3.48 (5)*
7.2	10	100	94.64 ± 2.66 (6)	100.23 ± 2.2 (7)	98.52 ± 1.2 (6)
7.0	15	100	96.59 ± 2.91 (7)	88.19 ± 4.06 (6)	86.08 ± 3.74 (5)

Tension at submaximal $[Ca^{2+}]$ is expressed as percent of the tension attained at maximally activating $[Ca^{2+}]$ which is a measure of the Ca^{2+}-responsiveness. For maximally activating $[Ca^{2+}]$ tension development of the first stimulation is taken as 100% and the subsequent tension developments at maximally activating $[Ca^{2+}]$ are expressed in percent of the first contraction. Values are the mean ± SEM, numbers in brackets indicate number of fibers used. * Indicates significant differences ($P < 0.05$) compared to the response in the presence of IBMX alone

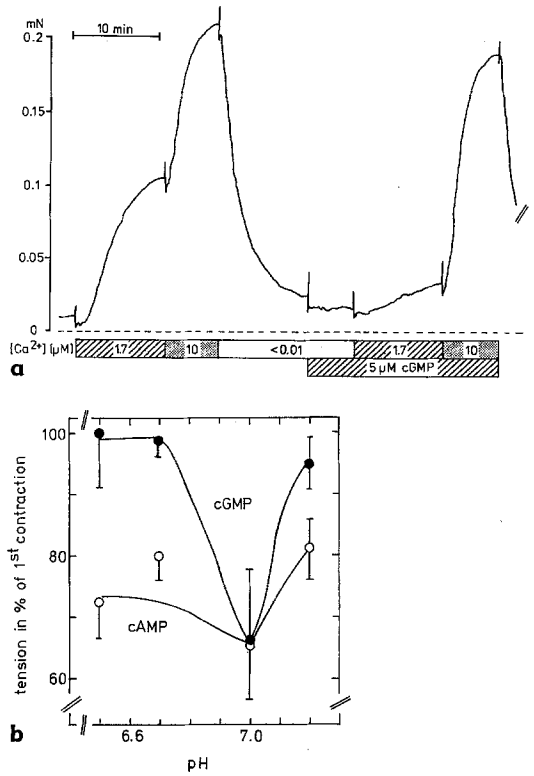

Fig. 1. a Tension recording of a chemically skinned coronary artery. Control contractions were elicited by raising Ca^{2+} to 1.7 μM and 10 μM (pH 7.0). Relaxation was induced by lowering $[Ca^{2+}]$ to less than 0.01 μM. After a short preincubation period in relaxing solution fibers were contracted a second time in the presence of cGMP (5 μM). Note that contraction at submaximal $[Ca^{2+}]$ is inhibited while maximal contraction is little affected. A similar protocol was used to test for the effects of cAMP and IBMX on the tension development. **b** pH-dependence of cGMP- and cAMP-induced inhibition of submaximal tension development. Experimental protocol as in Fig. 1a. The responses to cGMP and cAMP (5 μM) are expressed as percent of the first submaximal contraction (cf. Table 1). Values are the mean ± SEM for 4–7 different fiber preparations. Each fiber was used only once

could be induced with $\geqslant 10$ μM Ca^{2+} (cf. Table 1). Decreasing $[Ca^{2+}]$ to 0.1 μM caused the fibers to relax fully with a half time of 3–5 min. When stimulated a second time the maximal tension developed was 96.6 ± 3% ($n = 6$, pH 7.0) and 94.6 ± 2.7% ($n = 6$, pH 7.2) of the first maximal tension attained in the first contraction. However, at more acidic conditions the second contraction was 75.7 ± 4.7% ($n = 4$, pH 6.7) and 69.2 ± 2.8% ($n = 7$, pH 6.5) of the first contraction. Figure 1a shows that incubation with 5 μM cGMP reduces tension developed at submaximal $[Ca^{2+}]$ whereas the response to maximal $[Ca^{2+}]$ is little affected (cf. Table 1). Since the ratio of the responses obtained at submaximal (1.7 μM Ca^{2+}) and maximal concentration of Ca^{2+} (10 μM Ca^{2+}) can be taken as a measure of Ca^{2+} responsiveness, the findings strongly suggest that cGMP decreases the Ca^{2+} reponsiveness of the fiber bundles (Fig. 1a, Table 1). It is particularly noteworthy that cGMP is as effective as cAMP in inhibiting tension development in the presence of submaximal Ca^{2+} at pH 7.0 (Fig. 1 b). In these experiments, the phosphodiesterase inhibitor 3-isobutylmethylxanthine (IBMX, 0.1 mM) was included in the incubation medium to prevent enzymatic hydrolysis of either cGMP or cAMP. Ca^{2+} responsiveness was not affected by IBMX (cf. Table 1).

The inhibitory response to cGMP could be distinguished from that to cAMP by variation of pH. cAMP reduced submaximal tension development (Fig. 1b) and Ca^{2+}-responsiveness (Table 1) independent of pH from 6.5 to 7.2. In contrast cGMP decreased submaximal tension development at pH 7.0 only (Fig. 1 b and Table 1). Maximal tension development was little affected by cGMP or cAMP (cf. Table 1). The dependence of cGMP and cAMP on pH and free $[Ca^{2+}]$ is summarized in Table 1.

Since cGMP-dependent protein kinase might be lost from the fibers during the skinning procedure, several experiments were performed in the presence of added purified cGMP-dependent protein kinase (0.1 μM). The added enzyme neither enhanced the inhibitory effect of cGMP at pH 7.0 nor induced inhibition of tension at pH 6.7. Therefore it seems unlikely that the lack of responsiveness to cGMP at the acidic pH is due to loss of cGMP-dependent protein kinase from the fibers.

At pH 7.0 it was also tested whether lower concentrations of cGMP and cAMP (0.75 μM and 1 μM) would inhibit submaximal tension development. At 1 μM cGMP a small inhibition of tension (about 12%) was observed. However, the level of significance was only reached when 5 μM of either cGMP or cAMP was applied.

Discussion

Skinned coronary smooth muscle preparations contract reversibly in response to micromolar concentrations of Ca^{2+}. Since they are devoid of a functional Ca^{2+}-storage system and a sarcolemmal diffusion barrier they are especially useful for studying possible direct effects of intracellular second messengers on the contractile apparatus.

Incubation of these preparations with in vivo observed concentrations of either cGMP (Holzmann 1982) or cAMP at pH 7.0 inhibits development of tension at low, but not at high concentrations of Ca^{2+}. Since membrane-associated Ca^{2+}-storage sites are not present in skinned arterial preparations, the inhibitory effect of the cyclic nucleotides cannot be ascribed to an influence on transport mechanisms that lower the concentration of Ca^{2+}. Instead, the data strongly suggest that the inhibitory effect of both nucleotides is mediated at the level of the contractile apparatus. Conceivably, such a mechanism might involve cyclic nucleotide-dependent phosphorylation of myosin light chain kinase which reduces its enzymic activity (Conti and Adelstein 1981) and subsequent actin-myosin interaction (Rüegg and Paul 1982; Rüegg et al. 1983; Silver and DiSalvo 1979). In this context, it is noteworthy that cAMP-induced phosphorylation of myosin light chain kinase at low Ca^{2+} inhibits the latter enzyme, whereas no inhibition is manifest when phosphorylation occurs at high Ca^{2+} (Conti and Adelstein 1981). Our findings that cGMP and cAMP inhibit development of tension at low but not at high concentrations of Ca^{2+} are consistent with the operation of such a mechanism in the skinned fiber preparation.

However, unlike cAMP, the inhibitory effect of cGMP exhibits a marked dependence on pH, showing an optimum at pH 7.0 and a sharp decline at either lower or higher pH values. This sharp pH dependency of the cGMP response is not likely to be attributable to excessive loss of cGMP-dependent protein kinase from the fiber because addition of the purified enzyme to the incubation medium did not promote responsiveness of the preparation to cGMP at either pH 6.7 or pH 7.0. It is possible that the pH dependency of the inhibitory response to cGMP reflects the pH optimum for phosphorylation of a specific protein (i.e., myosin light chain kinase) by cGMP-dependent protein kinase, or that other factors present in the skinned arterial preparation limit activation of the kinase at low or high pH. Although further studies are required to test these possibilities, the distinct dependence on pH suggests that cGMP-induced inhibition may also involve a mechanism which is different from that of cAMP induced inhibition.

Recently it has been shown that endothelium-dependent relaxation of vascular smooth muscle and relaxation induced by nitrogeneoxide containing compounds are associated with identical patterns of protein phosphorylation (Rapoport et al. 1983). Since under both conditions there is an increase in cGMP levels (Schultz et al. 1977; Kukovetz et al. 1979; Janis and Diamond 1979; Furchgott 1983; Rapoport et al. 1983), the data presented in this communication suggest that the relaxation may be partly mediated by a direct effect of cGMP on the contractile apparatus. This mechanism could operate in concert with other effects of nitrates such as those involved in altering the intracellular concentration of free Ca^{2+} (Itoh et al. 1983).

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