MOLECULAR PHARMACOLOGY, 23:424–430

Potentiation of the Effects of Sodium Nitroprusside and of Isoproterenol by Selective Phosphodiesterase Inhibitors

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Received August 27, 1982; Accepted November 18, 1982

SUMMARY

This study identified a series of alkylated xanthines and a papaverine analogue with a range of potencies and selectivities as inhibitors of phosphodiesterases isolated from bovine coronary arteries. The abilities of these inhibitors to potentiate the relaxant effects of sodium nitroprusside (SNP) and isoproterenol were predictable from the potencies to inhibit the calmodulin-sensitive and the cyclic AMP-specific forms of phosphodiesterase, respectively. Although the xanthines potentiated the SNP- and isoproterenol-induced increases in cyclic GMP and cyclic AMP, respectively, in manners that were consistent with the involvement of the respective cyclic nucleotides in the relaxation process, the papaverine analogue did not potentiate isoproterenol-induced increases in cyclic AMP levels. These data are consistent with the hypothesis that increases in cyclic GMP levels are responsible for the relaxation of coronary artery strips by SNP. In addition, the data indicate that the calmodulin-sensitive phosphodiesterase activity does not contribute significantly to the hydrolysis of cyclic AMP in the intact bovine coronary artery smooth muscle cells.

INTRODUCTION

There is considerable evidence to suggest that increases in intracellular cyclic AMP concentrations lead to smooth muscle relaxation (1), but this hypothesis, while widely accepted, does not appear to accommodate some observations. For example, there are reports that some agents have been found to raise cyclic AMP levels without causing relaxation of smooth muscle (2). It also has been suggested that cyclic GMP may be involved in processes leading to relaxation of smooth muscle (3, 4). As with cyclic AMP, however, evidence has been presented that increases in the concentration of cyclic GMP in some smooth muscles are not associated with relaxation of smooth muscle (5).

SNP[1] causes most smooth muscle tissues (including coronary arteries) to relax, and this pharmacological response, in most tissues, can be positively correlated with increases in the levels of cyclic GMP but not of cyclic AMP. Conversely, relaxation of smooth muscle tissue by isoproterenol is generally associated with increases in cyclic AMP levels but not in cyclic GMP levels. Thus, if cyclic nucleotides are involved in the mechanism of action of SNP and isoproterenol, selective inhibitors of cyclic GMP and cyclic AMP phosphodiesterase activities should selectively potentiate the abilities of these agonists to increase the levels of the appropriate cyclic nucleotide and to relax smooth muscle. We (6) and other (7) have reported phosphodiesterase inhibitors that selectively increase cyclic GMP levels (6, 7) or cyclic AMP levels (6) in coronary artery preparations. In this study we have investigated the abilities of potent phosphodiesterase inhibitors to inhibit the activity of the calmodulin-sensitive and the cyclic AMP-specific forms of phosphodiesterase from bovine coronary arteries, and to potentiate the effects of SNP and isoproterenol on contractility and cyclic nucleotide levels. We found that the xanthines potentiated the effects of isoproterenol and SNP in manners that were predictable from their abilities to inhibit the cyclic AMP-specific and the calmodulin-sensitive forms of phosphodiesterase, respectively. In addition, these data indicate that in intact bovine coronary artery strips the calmodulin-sensitive phosphodiesterase activity did not contribute significantly to the hydrolysis of cyclic AMP, even under conditions where intracellular cyclic AMP concentrations were increased by isoproterenol and Ca^{2+} concentrations were elevated (i.e., the calmodulin-sensitive phosphodiesterase should be fully active) (8).

EXPERIMENTAL PROCEDURES

Materials. Cyclic AMP and cyclic GMP (Sigma Chemical Company, St. Louis, Mo.) were prepared as stock solutions and used without further purification. Tritiated

This work was supported by United States Public Health Service Research Grants GM 21220 and HL 19325.

[1] The abbreviations used are: SNP, sodium nitroprusside; MIX, 1-methyl-3-isobutylxanthine; 8-MeOMeMIX, 1-methyl-3-isobutyl-8-methoxymethylxanthine; IIX, 1-isoamyl-3-isobutylxanthine; K-III-73, the 6-isopropoxy analogue of papaverine.

0026-895X/83/020424-07$02.00/0

cyclic nucleotides obtained from New England Nuclear Corporation (Boston, Mass.) were purified on Dowex-50 cation exchange resin columns (9). SNP and isoproterenol were obtained from Sigma Chemical Company and Breon Laboratories Inc., respectively. MIX, 8-MeOMeMIX, and IIX were prepared according to published procedures (10–12). The 6-isopropoxy analogue of papaverine (K-III-73) was prepared from 2-(3′-isopropoxy-4-methoxyphenyl)-2-methoxyethylamine by a modification of the Bischler-Napieralski scheme (13).

Tissue preparation. Bovine hearts were obtained from a local slaughterhouse. A segment of the right coronary artery was quickly dissected with the adhering fat and, within 1 hr of slaughter, placed in Krebs-Ringer bicarbonate buffer with 10 mM glucose and 1 mM pyruvate and bubbled with 95% O_2-5% CO_2 at pH 7.4. The adhering fat was removed, and four helical strips (approximately 3 × 25 mm each) were trimmed from each artery. Tissues were then stored in this buffer for 18–24 hr at 4° (the bicarbonate concentration was adjusted to maintain pH 7.4 at this temperature) before use.

Measurement of muscle tension responses. Coronary artery strips were mounted in separate organ baths in 25 ml of the incubation medium at 37° with 1 g of resting tension. Isometric tension was measured by a Statham strain gauge transducer connected to a Gould-Brush 2400 recorder. Tissues were preincubated for 2 hr. During this time the medium was changed at least three times and tension was periodically readjusted to 1 g until no more spontaneous relaxation was observed. Tissues were then further preconditioned by the addition of KCl (50 mM) to the incubation buffer for 1–2 min followed by equilibration in incubation buffer for 25 min, then exposure to 30 mM K^+ (about 30% of maximal stimulation) for 15 min, followed by equilibration in incubation buffer for 25 min. This preconditioning procedure helped to bring the tissues more quickly to a state of constant responsiveness to the contraction stimulus, as was reported for porcine coronary arteries (6). The phosphodiesterase inhibitors, SNP, isoproterenol, and the combinations of SNP or isoproterenol with the phosphodiesterase inhibitors were tested for their ability to cause relaxation of K^+-contracted strips in the following manner: The incubation buffer was replaced by buffer in which K^+ had been substituted for an equal concentration of Na^+ to give 30 mM total K^+. When developed tension reached a maximum, the phosphodiesterase inhibitor or vehicle (water or 0.2 N NaOH) (control tissue) was added. After the tissue had reached a lower, steady tension, SNP or isoproterenol was added. Control tissues consistently generated 8–10 g of tension and maintained this tension for 1–2 hr. Cumulative concentration-response relationships were determined for SNP and isoproterenol. The differences between the tension at the time of addition of SNP or isoproterenol and the tension after relaxation in response to the highest concentration of SNP (5 μM) or isoproterenol (30 μM) were taken as 100% relaxation.

Cyclic AMP and cyclic GMP levels. The tissues were mounted and preconditioned as described for measurement of tension responses. Four segments from one artery were used for each experiment and randomly used as (a) control tissue (no addition after K^+-induced contraction), (b) tissues that were treated with phosphodiesterase inhibitor only, (c) SNP or isoproterenol (agonist) only, and (d) tissues that were treated with both agonist and phosphodiesterase inhibitor. Phosphodiesterase inhibitors (when added) were added at the time the tissues attained peak contraction, and SNP or isoproterenol (when used) was added 5 min later. All tissues were frozen by methods previously described (6) 10 min after peak K^+-induced contraction. Frozen tissues were stored at −70° until assayed for cyclic nucleotide and protein content. Tissues were reduced to powder by placing the frozen sample in a plastic capsule containing a plastic pestle (all cooled in liquid nitrogen) and shaking the capsule and its contents in a Wig-L-Bug dental amalgamator (Cresent Dental Manufacturing Company, Lyons, Ill.) at maximal speed three times for 20 sec. The capsule was cooled in liquid nitrogen before and after each shaking. The resulting frozen powdered tissue was suspended in 3 ml of 6% trichloroacetic acid containing 5000 dpm of tritiated cyclic AMP and cyclic GMP. The radiolabeled cyclic nucleotides were added to monitor recoveries (about 40% for cyclic AMP and 75% for cyclic GMP). The mixture was suspended by agitation, and the suspension was centrifuged at 28,000 × *g* for 20 min. The cyclic AMP and cyclic GMP in the supernatant fraction were separated and purified by applying the trichloroacetic acid extract to alumina columns (2.5 × 0.74 cm) that had been washed with 2 ml of 0.2 M ammonium formate (pH 6.6) and 20 ml of water. The columns were then washed sequentially with water (10 ml), 0.6 N HCl in 95% ethanol (5 ml), 50% ethanol (5 ml) and water (2 ml). Cyclic nucleotides were then eluted from the alumina columns with 4 ml of 0.2 M ammonium formate (pH 6.6) onto Dowex 50 (chloride form) columns (4.5 × 0.74 cm). The cyclic nucleotides were eluted from the Dowex columns with water in separate 4-ml fractions. The fractions were lyophilized and the resulting residue was dissolved in 4 ml of water. Cyclic nucleotide concentrations were measured by radioimmunoassay (14). Control samples treated with phosphodiesterase showed no detectable amounts of cyclic AMP or cyclic GMP. All samples were assayed using three dilutions of the redissolved cyclic nucleotide samples, and cyclic nucleotide values were used only when they were consistent with the dilutions. Blanks were constructed by redissolving the residue after lyophilization of the appropriate fractions from columns to which 6% trichloroacetic acid containing tritiated cyclic nucleotides had been applied.

The trichloroacetic acid-insoluble pellet was resuspended in 5 ml of 1 M sodium hydroxide and heated for 30 min. The mixture was centrifuged at 28,000 × *g* for 20 min and the supernatant fluid was assayed for protein by the method of Lowry *et al.* (15).

Enzyme preparation. The separated, partially purified phosphodiesterases from bovine right coronary arteries were prepared essentially as described before for porcine coronary arteries (16). Briefly stated, the right coronary arteries were freed of fat and connective tissue and minced. The minced artery was homogenized in 4 ml of buffer (20 mM Tris-HCl, 2 mM Mg^{2+} and 1 mM dithiothreitol, pH 7.5) per g of tissue (wet weight). After centrifugation at 48,000 × *g* for 30 min, the supernatant

fraction was fractionated on DEAE-cellulose as described before.

Phosphodiesterase assay. The agents were assayed for inhibition of the hydrolysis of 1 μM cyclic nucleotide by two forms of cyclic nucleotide phosphodiesterase eluted from DEAE-cellulose. The assay procedure has been reported (16, 17). Assays were performed with 1 μM substrate at 30° for 30 min at enzyme dilutions that gave 10–20% hydrolysis of substrate in the absence of inhibitors. Product accumulation was linear for at least 40 min with enzyme dilutions used in this study. Agents were dissolved in water or 0.2 mM NaOH, and 25 μl of the solution were added to the assay. Controls (no inhibitor) were constructed using 25 μl of water or 0.2 mM NaOH. The presence or absence of calmodulin had no significant effect on the potency of the agents used in this study to inhibit the calmodulin-sensitive phosphodiesterase fraction (Peak I). The data reported were obtained from assays in the presence of saturating calmodulin. The agents had no effect on the efficacy of the nucleotidase step or subsequent steps in the phosphodiesterase assay. Concentrations of the agents that inhibited by 50% the hydrolysis of 1 μM substrate (I_{50}) were determined from concentration-percentage inhibition curves, utilizing concentrations of the compounds from 0.1 μM to 100 μM.

Statistical methods. All cyclic nucleotide levels were evaluated on the basis of paired data with equal-sized groups utilizing Student's *t*-test. Relaxation data were evaluated at each concentration of SNP and isoproterenol by Duncan's new multiple-range test.

RESULTS

Separation of phosphodiesterase activities by DEAE-cellulose chromatography. The supernatant fraction contained greater than 80% of the cyclic AMP and cyclic GMP hydrolytic activity of the whole homogenate of bovine coronary arteries when assayed at 1 μM substrate (data not shown). Two fractions (Peaks I and II) of phosphodiesterase activity were resolved by DEAE-cellulose chromatography of the 48,000 × *g* supernatant fraction (Fig. 1) using an ammonium sulfate gradient. This chromatography system has been shown to resolve the calmodulin-sensitive phosphodiesterase from calmodulin, which is eluted at higher ammonium sulfate concentrations than either of the two phosphodiesterase forms (16). Peak I and Peak II phosphodiesterase activities isolated from bovine coronary arteries were similar to activities reported for the calmodulin-sensitive and the cyclic AMP-specific phosphodiesterases, respectively, isolated from porcine coronary arteries (16) and many other mammalian tissues (18). Peak I hydrolyzed both cyclic GMP and cyclic AMP, but it displayed a much greater activity with cyclic GMP as substrate at 1 μM concentrations. Peak II phosphodiesterase activity displayed good activity against cyclic AMP but very low activity with cyclic GMP as substrate. Total phosphodiesterase activity recovered with either cyclic nucleotide as substrate amounted to about 85% of that applied to the column.

Inhibition of Peak I and Peak II phosphodiesterase activities. Potencies of the agents to inhibit the hydrolysis of 1 μM cyclic AMP by Peak II and 1 μM cyclic GMP by the Peak I phosphodiesterase activity are presented in Table 1. MIX was a potent, but relatively nonselective, inhibitor of both forms of phosphodiesterase. On the other hand, IIX and 8-MeOMeMIX showed selective

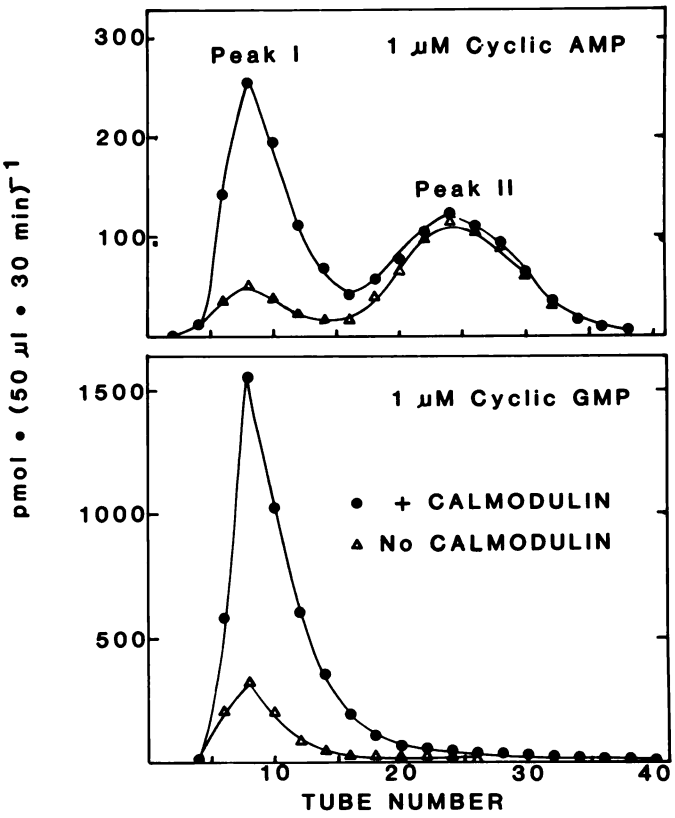

FIG. 1. *DEAE-cellulose chromatography of the 48,000 × g supernatant fraction from bovine coronary arteries*

The supernatant fraction (8 ml) prepared as described before (16) was applied to a column of DEAE-cellulose (10 × 0.9 cm). The column was washed with homogenizing buffer and then developed with an exponential gradient of $(NH_4)_2SO_4$ as described by Wells *et al.* (16). The flow rate was 0.5 ml/min and 8-ml fractions were collected in tubes containing 8 mg of bovine serum albumin in 0.2 ml of water. Phosphodiesterase activity (*Y* axis) was determined using 1 μM cyclic AMP (●, ○) or 1 μM cyclic GMP (▲, △) in the presence (●, ▲) or absence (○, △) of excess calmodulin. Results are means of duplicate determinations and are representative of three preparations.

TABLE 1

Inhibition of bovine coronary artery phosphodiesterase activities

Compound	I_{50}[a]	
	Peak I (1 μM cyclic GMP)	Peak II (1 μM cyclic AMP)
	μM	μM
MIX	3.5 ± 0.1	6.7 ± 0.4
IIX[b]	50[c]	1.2 ± 0.2
8-MeOMeMIX	4.2 ± 1.0	68.7 ± 7.0
K-III-73	50. ± 4.0	4.0 ± 0.4

[a] I_{50} = the concentration of the agent required to inhibit by 50% the hydrolysis of cyclic GMP (1 μM) by the isolated Peak I fraction or of cyclic AMP (1 μM) by the isolated Peak II fraction. Values are means ± standard error of the mean from three different preparations.

[b] IIX was not soluble at concentrations above 50 μM.

[c] A concentration of 50 μM caused 40% inhibition.

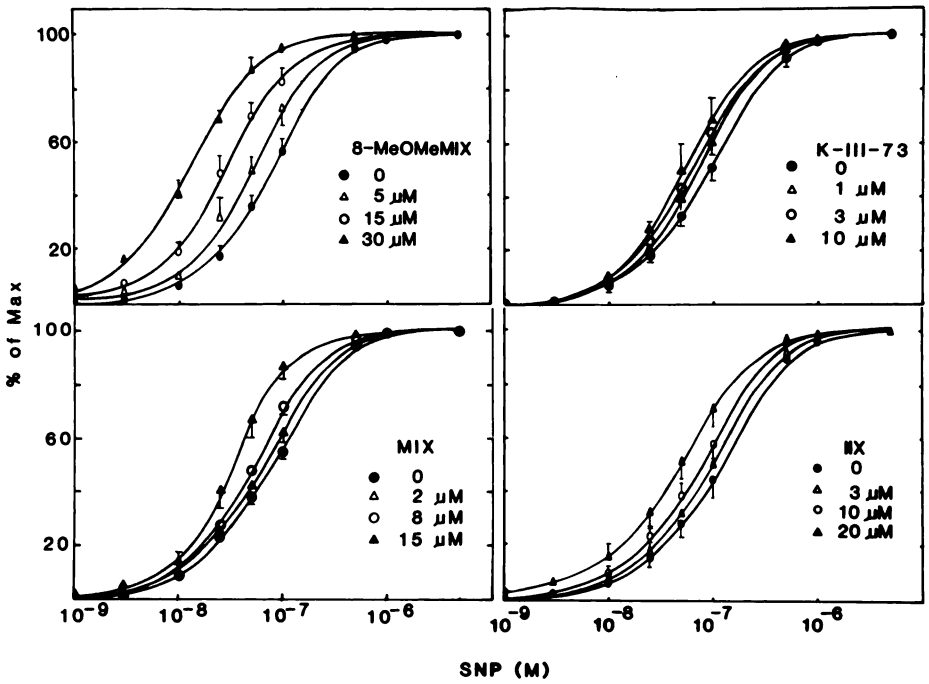

FIG. 2. *Relaxation of bovine coronary artery strips by SNP in the presence and absence of phosphodiesterase inhibitors*

Strips were caused to contract with 30 mM KCl as described under Experimental Procedures. Cumulative concentration-response curves for SNP-induced relaxation of the tissue were generated in the absence (●) or presence (△, ○, ▲) of the indicated concentrations of the phosphodiesterase inhibitors. Vertical bars show standard errors of the means of five experiments.

potency for inhibition of one of the two forms. IIX was a potent inhibitor of Peak II activity but had much less potency to inhibit Peak I activity, whereas 8-MeOMeMIX was equipotent with MIX as an inhibitor of Peak I activity but was a relatively weak inhibitor of Peak II activity. The only agent studied that was not a xanthine was K-III-73. K-III-73 was also a potent inhibitor of Peak II activity but was a relatively weak inhibitor of Peak I activity.

Relaxation of K^+-contracted bovine coronary artery strips. Both isoproterenol and SNP caused concentration-dependent relaxation of the artery strips (Figs. 2 and 3). The concentration-response curve for SNP was shifted to the left by 5, 15, or 30 μM MeOMeMIX. The

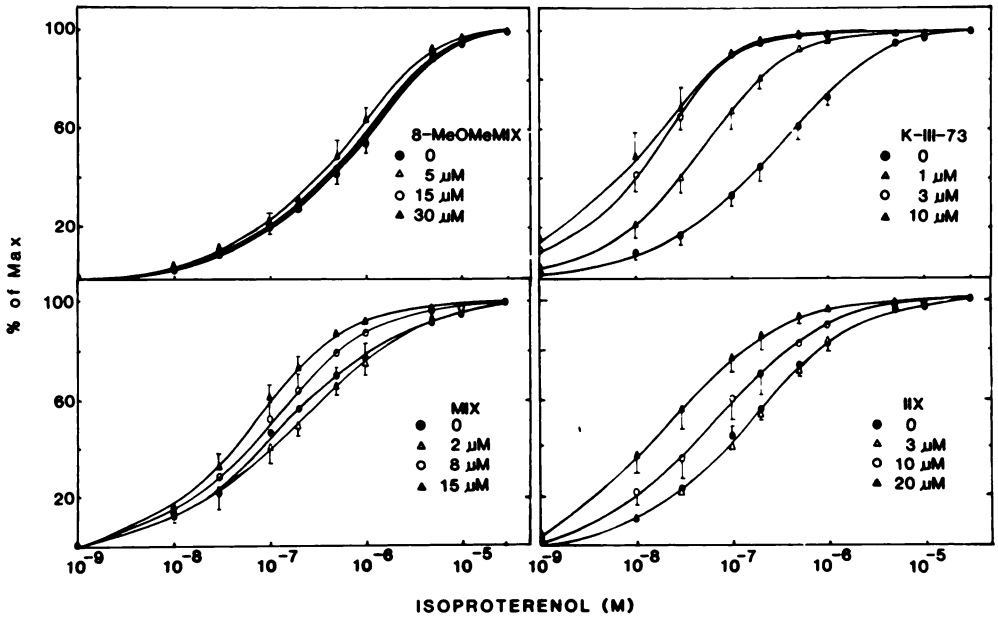

FIG. 3. *Relaxation of bovine coronary artery strips by isoproterenol in the presence and absence of phosphodiesterase inhibitors*

Cumulative concentration-response curves for relaxation of KCl-contracted strips were generated in the absence (●) or presence (△, ○, ▲) of phosphodiesterase inhibitors as described under Experimental Procedures. Vertical bars indicate standard errors of the means of five experiments with 8-MeOMeMIX, K-III-73, and MIX, and seven experiments with IIX.

concentration-response curve for SNP was also shifted to the left when 15 μM MIX was present in the tissue bath but was not significantly altered in the presence of 2 or 8 μM MIX. Although 20 μM IIX did not cause a statistically significant change in the extent of relaxation to any individual concentration of SNP, it did appear to cause a leftward shift of the SNP concentration response curve. The presence of K-III-73, on the other hand, did not alter the concentration-response relationship of SNP.

The concentration-response curve to isoproterenol was not affected by 8-MeOMeMIX (the most potent agent to alter the SNP concentration-response curve). Conversely, K-III-73 (1 and 3 μM) shifted to the left the concentration-response curve to isoproterenol in a dose-dependent manner, but 10 μM K-III-73 did not shift the curve beyond that with 3 μM K-III-73. IIX at 10 and 20 μM caused dose-dependent shifts in the isoproterenol concentration-response curve. MIX (15 μM) caused an apparent leftward shift of the isoproterenol concentration-response curve, but the increased relaxation was not statistically significant.

Cyclic AMP and cyclic GMP levels. Four strips from one bovine coronary artery were used in each experiment. The four strips were mounted in tissue baths and were preconditioned as described under Experimental Procedures. The tissues were randomly assigned the following treatments: (a) control (KCl contracted but no further additions were made to the bath), (b) inhibitor alone (a phosphodiesterase inhibitor was added when peak tension had been attained), (c) agonist alone (SNP or isoproterenol was added 5 min after peak tension had been attained), and (d) a combination of a phosphodiesterase inhibitor and an agonist (a phosphodiesterase inhibitor was added when peak tension had been attained and then 5 min later SNP or isoproterenol was added). All tissues were quick-frozen 10 min after peak tension had been attained. Tissues treated with agonist alone had relaxed 69–75% (isoproterenol) and 45–60% (SNP) when the tissues were quick-frozen.

The concentrations of the phosphodiesterase inhibitors used in these studies were the highest concentrations used in the contraction studies (Figs. 2 and 3). The concentrations of SNP (0.2 μM) and isoproterenol (0.9 μM) used in the levels studies were those that caused 70% relaxation of 30 mM K^+-contracted tissues in the absence of any phosphodiesterase inhibitor. SNP alone did not cause a significant rise in cyclic AMP levels in the coronary artery strips (Table 2), but did cause a dramatic increase in cyclic GMP levels above those of the control tissues. The phosphodiesterase inhibitors each appeared to increase cyclic GMP levels above their paired control, but only 8-MeOMeMIX did so in a statistically significant fashion. In the presence of SNP, only 8-MeOMeMIX and MIX raised cyclic GMP levels above those that would be expected if the effects of SNP and the inhibitors on cyclic GMP levels were additive. Neither K-III-73 nor IIX in combination with SNP significantly raised cyclic GMP levels above those predicted by additivity.

Isoproterenol alone did not cause significant increases in cyclic GMP levels above those found in the control tissues but did cause approximately a 2-fold increase in cyclic AMP levels above those of paired control tissues. Increases in cyclic AMP levels of tissues treated with both isoproterenol and IIX were about 2-fold higher than would be expected if the increases in tissues treated with isoproterenol and the increases in tissues treated with IIX were additive. MIX caused a less dramatic, but nonetheless significant, increase in cyclic AMP levels above that predicted based on the additivity of the isoproterenol and the MIX elicited increases. The presence of 8-MeOMeMIX or K-III-73 did not alter the isoproterenol-induced increases in cyclic AMP.

DISCUSSION

This study addressed the question of whether or not the ability of an agent to inhibit relatively selectively one form of phosphodiesterase activity *in vitro* would be reflected by the ability of the agent to potentiate the relaxant effects of SNP or isoproterenol on intact bovine coronary artery strips. It is widely assumed that the effect of isoproterenol to relax smooth muscle is caused by an increase in cyclic AMP levels. Cyclic AMP is hydrolyzed, and thereby inactivated, by the cyclic AMP phosphodiesterase activities of the smooth muscle cell. Either of the two forms of phosphodiesterase isolated from the soluble fraction of homogenates of bovine coronary arteries would appear to be capable of regulating the levels of cyclic AMP (Fig. 1). It is therefore not clear whether only the cyclic AMP-specific or both enzyme activities contribute to the hydrolysis of cyclic AMP in the intact cell.

We have presented data that indicated that in the basal state (i.e., in the absence of adenylate cyclase activation) of the porcine coronary arteries the cyclic AMP-specific form (Peak II-type) of phosphodiesterase had sufficient activity to control the cyclic AMP levels (6). That is, inhibitors of the calmodulin-sensitive phosphodiesterase that were weak inhibitors of Peak II-type activity caused increases in cyclic GMP levels but did not alter cyclic AMP levels. Alternatively, a relatively selective inhibitor of the Peak II-type phosphodiesterase activity increased cyclic AMP levels but did not alter cyclic GMP levels. In the present study 8-MeOMeMIX, a potent inhibitor of the calmodulin-sensitive form but a weak inhibitor of the cyclic AMP-specific form of phosphodiesterase, did not cause cyclic AMP levels to increase in the bovine coronary artery strips. In addition, this agent did not potentiate the relaxant effects of isoproterenol or the ability of isoproterenol to increase cyclic AMP levels.

These data indicate that the calmodulin-sensitive form of phosphodiesterase does not participate to a significant degree in the hydrolysis of cyclic AMP in bovine coronary arteries even under conditions where the calmodulin-sensitive phosphodiesterase should be fully active (8) and cyclic AMP levels are elevated by isoproterenol. The relaxation of most smooth muscles by SNP has been associated with increases in cyclic GMP levels. This apparent association between the relaxant effects of SNP and its ability to increase intracellular cyclic GMP concentrations has led some to suggest that increases in cyclic GMP levels are responsible for the relaxant effects of SNP (3, 4, 7, 19, 20). This hypothesis is not universally accepted (5). While the data presented here do not prove

TABLE 2

Effects of phosphodiesterase inhibitors, SNP, and isoproterenol on cyclic AMP and cyclic GMP contents of bovine coronary artery strips

Cyclic nucleotide contents are mean values ± standard error of the mean of artery strips treated as described under Experimental Procedures.

Agent	Concentration	Cyclic GMP	Cyclic AMP	n^a
	μM	*pmoles/mg protein*		
Control[b]	—	0.08 ± 0.02	3.1 ± 1.0	
K-III-73	10	0.17 ± 0.02	4.2 ± 0.8	4
SNP	0.2	0.76 ± 0.20	3.9 ± 0.9	
K-III-73 + SNP		1.02 ± 0.27	4.3 ± 0.6	
Control[b]	—	0.04 ± 0.01	2.8 ± 0.5	
8-MeOMeMIX	30	0.10 ± 0.04	3.7 ± 0.6	4
SNP	0.2	0.42 ± 0.08	3.4 ± 0.5	
8-MeOMeMIX + SNP		1.25 ± 0.10[c]	3.9 ± 0.6	
Control[b]	—	0.07 ± 0.01	3.3 ± 0.5	
MIX	15	0.12 ± 0.02	4.5 ± 0.6	8
SNP	0.2	0.26 ± 0.03	3.3 ± 0.4	
MIX + SNP		0.64 ± 0.10[d]	5.2 ± 0.8	
Control[b]	—	0.07 ± 0.01	2.9 ± 0.2	
IIX	20	0.10 ± 0.02	4.3 ± 0.5	8
SNP	0.2	0.33 ± 0.06	2.9 ± 0.5	
IIX + SNP		0.49 ± 0.07	5.3 ± 0.7	
Control[b]	—	0.07 ± 0.01	3.2 ± 0.4	
K-III-73	10	0.08 ± 0.01	3.5 ± 0.2	4
isoproterenol	0.9	0.05 ± 0.01	7.6 ± 0.8	
K-III-73 + isoproterenol		0.12 ± 0.02	8.3 ± 0.7	
Control[b]	—	0.10 ± 0.04	3.8 ± 0.1	
8-MeOMeMIX	30	0.23 ± 0.06	4.6 ± 0.7	4
Isoproterenol	0.9	0.07 ± 0.02	8.0 ± 1.0	
8-MeOMeMIX + isoproterenol		0.11 ± 0.01	8.7 ± 0.8	
Control[b]	—	0.05 ± 0.01	2.6 ± 0.2	
MIX	15	0.10 ± 0.03	4.1 ± 0.3	8
Isoproterenol	0.9	0.09 ± 0.02	5.5 ± 0.4	
MIX + isoproterenol		0.14 ± 0.02	9.9 ± 0.7[d]	
Control[b]	—	0.17 ± 0.09	3.7 ± 0.6	
IIX	20	0.17 ± 0.04	6.1 ± 1.1	8
Isoproterenol	0.9	0.10 ± 0.02	7.8 ± 1.4	
IIX + isoproterenol		0.13 ± 0.03	16.2 ± 2.0[e]	

[a] Number of experiments using paired tissues.

[b] No additions were made after the tissue was caused to contract with 30 mM KCl.

[c] $p < 0.001$ as compared with the values calculated if changes in levels elicited by 8-MeOMeMIX and SNP in paired tissues were additive.

[d] $p < 0.01$ as compared with the values calculated assuming that changes elicited in paired tissues by MIX and by SNP or isoproterenol were additive.

[e] $p < .005$ as compared with the values calculated by assuming that changes elicited in paired tissues by IIX and by isoproterenol were additive.

that cyclic GMP is involved in the mechanism of the relaxant effect of SNP, they are consistent with this hypothesis. 8-MeOMeMIX, a potent inhibitor of the calmodulin-sensitive form of phosphodiesterase, significantly increased cyclic GMP levels by itself, potentiated the relaxant effects of SNP, and potentiated the SNP-induced increases in cyclic GMP. The potentiation of both SNP effects does not prove that cyclic GMP is responsible for the relaxant effects, since an effect of the xanthine on some unrecognized process other than cyclic GMP metabolism that is also affected by SNP could lead to potentiation. This possibility seems unlikely, however, since the ability of the three xanthines to potentiate the relaxant effect of SNP appeared to be predictable from their relative abilities to inhibit Peak I cyclic GMP phosphodiesterase activity. That is, IIX was the least potent xanthine to inhibit Peak I activity and also appeared to have the least effect on the SNP concentration-response relationship.

The data presented also demonstrate the dangers inherent in assuming that a phosphodiesterase inhibitor will produce a pharmacological effect by increasing the levels of a cyclic nucleotide (21). K-III-73 (the 6-isopropoxy analogue of papaverine) was a potent inhibitor of the cyclic AMP-specific phosphodiesterase activity (Peak II). In addition, this agent potentiated the relaxant effects

of isoproterenol on the coronary artery strips. This potentiation does not appear to have involved cyclic AMP, however, since 10 μM K-III-73 neither increased cyclic AMP levels nor potentiated the isoproterenol-induced increases in cyclic AMP. The mechanism by which K-III-73 potentiated the relaxant effects of isoproterenol is unclear, but it does not appear to have involved inhibition of phosphodiesterase activity, at least as reflected in detectable changes in cyclic AMP or cyclic GMP levels in the coronary artery strips.

ACKNOWLEDGMENTS

The authors wish to thank Dana Tigani for conducting some of the phosphodiesterase assays. We also wish to thank Drs. Joel G. Hardman, George Kramer, and Jerry Miller for valuable discussion and constructive criticism.

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