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Relaxation of Hormonally Stimulated Smooth Muscular Tissues by the 8-Bromo Derivative of Cyclic GMP*

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Summary. Substances that cause contraction or relaxation of smooth muscle have been shown to increase intracellular levels of cyclic GMP. Because of the unclear role of cyclic GMP in the control of smooth muscle tone, cyclic GMP derivatives were exogenously applied to various smooth muscle preparations and their effects on tissue tone were studied.

Whereas the basal tone of the rat ductus deferens was not affected by exogenous cyclic GMP or its dibutyryl or 8-bromo derivatives, the contractile responses of this tissue to noradrenaline and acetylcholine were depressed by preincubation with 10 μM 8-bromo cyclic GMP (Br-cGMP). The 8-bromo derivatives of 2′:3′-cyclic GMP, 5′-GMP and guanosine were without effects. Cyclic AMP levels were not changed by Br-cGMP. The frequency of oxytocin-stimulated rat uteri was also depressed by Br-cGMP (10 μM). In helical strips of rat and rabbit aortae, Br-cGMP (1–100 μM) caused a concentration-dependent, rapid decrease in noradrenaline-stimulated tissue tension. Br-2′:3′-cyclic GMP was ineffective. Noradrenaline-stimulated strips from hog spleen arteries were less sensitive to Br-cGMP than aortic tissue. In ductus deferentes and aortic strips stimulated by K^+ at a depolarizing concentration, Br-cGMP caused less relaxation than under hormonal stimulation.

These findings support the concept that cyclic GMP is involved in the control of smooth muscle tone and that hormone- and drug-induced elevations of the cyclic GMP level can reduce contractile responses to neurotransmitters and hormones.

Key words: Cyclic GMP – 8-bromo cyclic GMP – Smooth muscle tone – Vascular smooth muscle – Ductus deferens.

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Abbreviations. Guanosine 3′:5′-monophosphate, cyclic GMP, cGMP; N^2, 2′-O-dibutyryl guanosine 3′:5′-monophosphate, dibutyryl cGMP; 8-bromo guanosine 3′:5′-monophosphate, Br-cGMP; 8-bromo guanosine 2′:3′-monophosphate, Br-2′:3′-cGMP; 8-bromo guanosine 5′-monophosphate, Br-GMP; 8-bromo guanosine, Br-guanosine, Br-Guo; adenosine 3′:5′-monophosphate, cyclic AMP, cAMP; N^6, 2′-O-dibutyryl adenosine 3′:5′-monophosphate, dibutyryl cAMP; 8-bromo adenosine 3′:5′-monophosphate, Br-cAMP.

Introduction

In numerous mammalian cell types, cyclic AMP has been shown to be an intracellular signal mediating the effects of β-adrenergic agonists and of many other hormonal factors (Robinson et al., 1971). Cyclic AMP is apparently involved in the relaxing effects of β-adrenergic agonists and of a few other endogenous factors on smooth muscle (Bär, 1974). This assumption is supported by the finding that the relaxing effects of β-adrenergic and other compounds can be mimicked by application of the dibutyryl derivative of cyclic AMP to intact tissues (Bär, 1974). Cyclic AMP is believed to affect the tissue tone by increasing the removal of calcium from the cytoplasm (Baudoin-Legros and Meyer, 1973; Andersson et al., 1975; Fitzpatrick and Szentivanyai, 1977; Nishikori et al., 1977; Bhalla et al., 1978).

The role of cyclic GMP in the control of cellular processes is generally obscure. In smooth muscle, various neurotransmitters and hormones that induce contraction increase the intracellular concentration of cyclic GMP (Schultz and Hardman, 1976; Goldberg and Haddox, 1977). Both events, contractile response and increased cyclic GMP level, appear to occur secondarily to an increase in cytoplasmic calcium

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concentration (Somlyo and Somlyo, 1970; Hurwitz and Suria, 1971; Schultz et al., 1973a, 1975). It was originally proposed that the increase in the cyclic GMP level may be causally involved in the contractile response (Lee et al., 1972; Dunham et al., 1974; Andersson et al., 1975). However, more recent studies indicate that quantitative and temporal correlations between increases in tissue tone and in the cyclic GMP level are poor (Schultz, 1977a, 1978; Diamond, 1978). In addition, various smooth muscle-relaxants are capable of increasing cyclic GMP levels (Diamond and Holmes, 1975; Diamond and Blisard, 1976; Schultz et al., 1977; Katsuki and Murad, 1977). On the basis of these observations it has been suggested that cyclic GMP may affect smooth muscle tone by reducing tissue excitation and excitability rather than promoting contraction (Schultz, 1977a, 1978; Schultz et al., 1977). In order to find out which if any of the proposed roles of cyclic GMP in the regulation of tissue tone is correct, we have studied the effects of exogenous cyclic GMP derivatives on the tone of various smooth muscular tissues. The observation that the 8-bromo derivative of cyclic GMP can relax the ductus deferens and vascular tissues favors the assumption that cyclic GMP may be involved in the control of smooth muscle tone and that this nucleotide may act as a factor reducing rather than promoting contractile responses.

Materials and Methods

a) Contraction Studies. The following tissues were used: Segments (2–3 cm) of rat ductus deferens; uterine horns of rats pretreated by injection of diethylstilbestrol (0.1 mg/kg, s.c. injected 24 h prior to excision); helical strips (length 2–3 cm, width 2 mm) of rat and rabbit aortae and of hog spleen arteries. The physiological salt solution used had the following composition (all values in mM): NaCl 128.0, KCl 4.7, $NaHCO_3$ 11.9, NaH_2PO_4 1.2, $MgCl_2$ 1.2, $CaCl_2$ 2.5, EDTA 0.026, glucose 10.0 (Kreye et al., 1975).

The medium was gassed with 95% O_2 and 5% CO_2 and was kept at 37° C. In a multi-channel organ bath with 10 ml individual bath volume, isometric contractions were recorded by means of microforce displacement transducers (type Grass FT.03), amplifiers and servo chart recorders, essentially as previously described (Kreye et al., 1975). Initial tension applied to the tissue was 1,200 dyn and 2,000 dyn for vascular and other preparations, respectively. After a relaxation period of 120 and 30 min for vascular and other tissues, respectively, the recording systems were electronically zeroed, and the experiments were started. In all statistical analyses, the null hypothesis was tested at the 5% level of significance.

b) Cyclic AMP Determination. The tissue cyclic AMP concentration was determined essentially as previously described (Schultz et al., 1973b). Tissue was preincubated for 30 min at 37° C and then transferred to fresh medium containing Br-cGMP for specified times.

c) Materials. Br-cGMP, dibutyryl cGMP, dibutyryl cAMP and Br-cAMP were obtained from Boehringer Mannheim. Br-guanosine was purchased from Pharma Waldhof GmbH, Düsseldorf. Cyclic GMP was obtained from Boehringer Mannheim and Pharma Waldhof. Br-2′:3′-cGMP and Br-GMP were prepared according to general procedures of purine bromination (Chantot and Guschlbauer, 1969; Michal et al., 1974). The identities and the purities were checked by thin layer chromatography on cellulose plates with butanol: acetic acid: water (60:15:25) as solvent and by IR and UV spectroscopies. Acetylcholine chloride and (–)-noradrenaline bitartrate were from Serva Biochemica, Heidelberg. Oxytocin was obtained from Hoechst AG, Frankfurt, diethylstilbestrol dipropionate from Bayer AG, Leverkusen, and A-23187 was from Eli Lilly, Indianapolis.

Results

1. Rat Ductus Deferens

When cyclic GMP or its more lipophilic dibutyryl and 8-bromo derivatives at concentrations up to 0.1 mM were added to unstimulated segments of rat ductus deferens, the basal tissue tone was not affected. In contrast, the contractile response to noradrenaline was reduced by Br-cGMP. When segments of ductus deferens were preincubated with 10 μM Br-cGMP for 20–25 min, the response to 1 μM noradrenaline was decreased by about 50% (Fig. 1). This effect of Br-cGMP depended on the preincubation time with the nucleotide. Much smaller depression of the response to noradrenaline was seen with preincubation times less than 10 min, and the effect was fully developed after 20–25 min exposure to Br-cGMP. The depressant effect of Br-cGMP on the contractile response to noradrenaline was reversed within 30 min.

The effect on noradrenaline-induced contraction was specific for the 8-bromo derivative of cyclic GMP (Table 1). Cyclic GMP itself, its dibutyryl derivative and Br-2′:3′-cGMP (10 μM each) were uneffective. Br-cAMP (10 μM) was also without effect, whereas dibutyryl cAMP was a potent relaxant as previously shown (Kreye and Schultz, 1972). Sodium bromide at 1 μM to 1 mM concentrations had no effect on tissue tone.

The effect of Br-cGMP on the contractile response to noradrenaline was studied at various concentrations of this agonist by recording cumulative concentration-response-curves with 0.1 μM to 1 mM noradrenaline (Fig. 2). The relaxing effect of Br-cGMP depended on the concentration of the nucleotide added to the medium. A significant effect of 1 μM Br-cGMP was seen only at low concentrations of noradrenaline (0.1 μM), whereas 10 and 100 μM Br-cGMP depressed the contractile response to noradrenaline over a wide range of noradrenaline concentrations. In control experiments with Br-guanosine and Br-GMP (1–100 μM), no effects on the contractile response to noradrenaline between 0.1 μM and 1 mM were observed (not shown).

Besides α-adrenergic agonists, acetylcholine causes contraction of the ductus deferens. The maximal response to this stimulus is smaller than that observed with α-adrenergic stimuli. The contraction of the

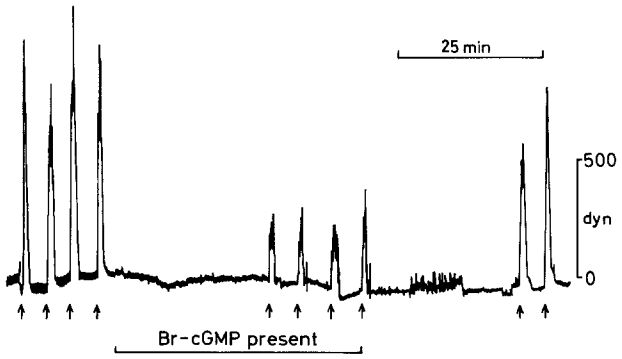

Fig. 1. Effect of Br-cGMP on the contractile response of the rat ductus deferens to noradrenaline. Tissue was stimulated by 1 μM noradrenaline added at the time points indicated by arrows. The medium was changed when the maximal phasic response was reached, recovery periods were 3 min. After recording of 4 control responses to noradrenaline, 10 μM Br-cGMP was added to fresh medium for 25 min, and repeated contractile responses to noradrenaline were again recorded with Br-cGMP readded when the medium was changed after each stimulation. After a recovery period of about 30 min in the absence of Br-cGMP, another series of control responses to noradrenaline was recorded. The figure shows the recording of a representative example

Table 1. Effects of various cyclic nucleotide derivatives on the contractile response of the rat ductus deferens to noradrenaline

Nucleotide added	Tension (dyn)	
	Before addition	After addition
None	978 ± 59	1,178 ± 67 (120%)
Dibutyryl cAMP	914 ± 83	131 ± 16 (14%)
Br-cAMP	869 ± 74	962 ± 70 (111%)
Cyclic GMP	734 ± 60	850 ± 68 (116%)
Dibutyryl cGMP	900 ± 78	957 ± 91 (106%)
Br-cGMP	959 ± 96	430 ± 57 (45%)
Br-2′:3′-cGMP	844 ± 93	876 ± 98 (104%)

Contractile responses to 1 μM noradrenaline were recorded by repeated addition (generally 4 times), with changes of medium after having reached the maximum of the phasic response as indicated in Fig. 1. After 20–25 min of incubation with nucleotide added at 10 μM, contractile responses to noradrenaline were again recorded; nucleotide was readded when the medium was changed after each stimulation. Data are given as mean of the average responses to repeated stimulation ± S.E.M. Experimental data expressed as percent of the values observed before nucleotide addition are given in parentheses. The number of tissue pieces was 8–13 per group

ductus deferens caused by acetylcholine was also reduced by Br-cGMP (Table 2). The depressing effect of Br-cGMP on the acetylcholine-induced response occurred more rapidly and was more pronounced than the effect on the response to noradrenaline. Br-cGMP (10 μM) caused about 75% reduction of the contractile response to 0.1 mM acetylcholine, which is a maximally effective concentration, when the nucleotide was added

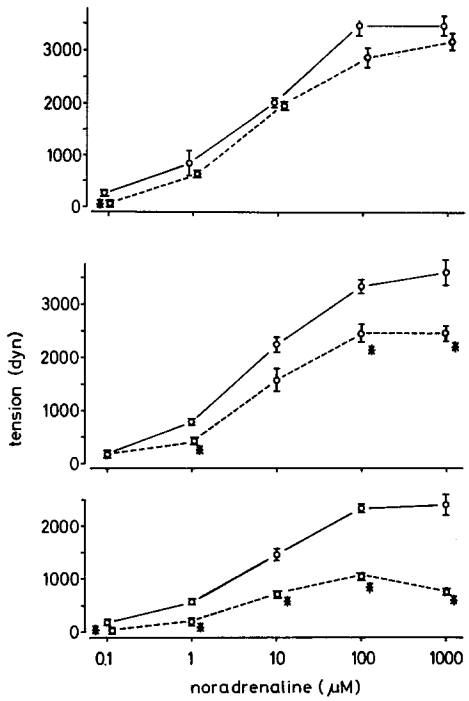

Fig. 2. Effect of Br-cGMP on the contractile response of the rat ductus deferens to noradrenaline. Concentration-response curves to noradrenaline were obtained by adding the agonist at increasing concentrations at peak response without intermediate rinsing. Cumulative dose-response-curves to noradrenaline were repeated after 20 min incubation with Br-cGMP. The Br-cGMP concentration was 1, 10 and 100 μM in the experiments shown on the upper, middle and lower panel, respectively. Data are given as means ± S.E.M. of 4 to 5 tissue pieces per group. * indicates a significant difference to the control group in the Wilcoxon test. Solid lines represent control responses, interrupted lines indicate the presence of Br-cGMP

for 5 min prior to the addition of acetylcholine. An apparent increase in the effectiveness of Br-cGMP with 10 min preincubation compared to the 5 min point is probably due to the fact that the contractile response to acetylcholine was reduced when the tissue was stimulated repeatedly by this agonist. Br-GMP (10 μM) was without effect.

The effects of Br-cGMP on K^+-induced contractions of the ductus deferens was relatively small. When Br-cGMP was added at 10 and 100 μM concentration after the tonic response to K^+ had developed, tissue tension was decreased by 20 and 30%, respectively (not shown).

The effect of Br-cGMP on the Ca^{2+}-induced increase in tissue tone was studied in K^+-depolarized ductus deferentes (Fig. 3). After preincubation in the absence of added Ca^{2+}, the tissue was stimulated by 35 mM K_2SO_4 and Ca^{2+} was added at increasing concentrations. Br-cGMP (10 μM) reduced the Ca^{2+}-induced increase in tissue tone at the tested Ca^{2+} concentration between 0.1 and 10 mM. In contrast, under the same experimental conditions, Br-GMP and

Table 2. Effects of Br-cGMP and Br-GMP on the contractile response of the rat ductus deferens to acetylcholine

Nucleotide added	Relative tension	
	5 min	10 min
None	0.96 ± 0.08	0.78 ± 0.07
Br-cGMP	0.23 ± 0.04[a]	0.12 ± 0.02[a]
Br-GMP	–	0.76 ± 0.07

Contractile responses to 100 μM acetylcholine were recorded before and after 5 or 10 min incubation with Br-cGMP or Br-GMP (10 μM each) added after the first stimulation. Data indicate maximal tension developed and are expressed relatively to the primary response to acetylcholine (about 400 dyn) as mean ± S.E.M. The number of tissue pieces was 5 to 13 per group

[a] Indicates a significant difference to the corresponding control group in the Wilcoxon test

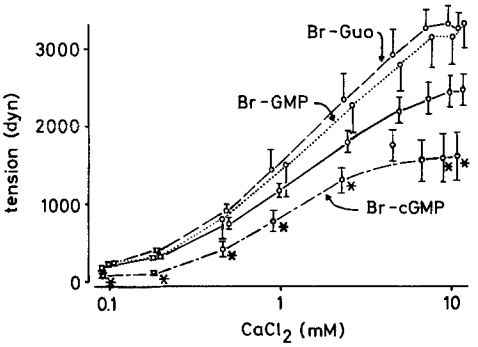

Fig. 3. Effects of Br-cGMP, Br-GMP and Br-guanosine on the response of the K^+-depolarized ductus deferens to calcium. Rat ductus deferentes were kept for 60 min in medium without added calcium. The medium was changed in 5 min-intervals. After 20 min preincubation with 10 μM Br-cGMP, Br-GMP or Br-guanosine, 70 mM K^+ (35 mM K_2SO_4) and 0.1 mM $CaCl_2$ were added. When the effect of Ca^{2+} on tissue tone was fully developed, i.e. after about 15 min, the Ca^{2+} concentration was increased stepwise up to 10 mM as indicated on the abscissa. Data are means ± S.E.M., the number of experiments was 29, 14, 5 and 5 for controls, Br-cGMP, Br-GMP and Br-guanosine, respectively; connecting lines are —— for controls, ·–·–· for Br-cGMP, ····· for Br-GMP, and ––––– for Br-guanosine. * indicates a significant difference to the control group in the Wilcoxon test

Br-guanosine (10 μM each) caused small increases in the response to Ca^{2+}. The reason for this finding is not clear. A small contraction-promoting component may also be involved in the overall relaxant effect of Br-cGMP.

Since Br-cGMP is a poor substrate of cyclic nucleotide phosphodiesterases (Michal et al., 1974), we considered the possibility that Br-cGMP could act as a competitive inhibitor of the enzymatic degradation of endogenous cyclic AMP and, thereby, may lead to an intracellular accumulation of this nucleotide and hence to tissue relaxation. We have studied the effect of Br-cGMP on cyclic AMP levels in the ductus deferens (Table 3). Incubation of tissue segments for 10 and 20 min with 10 μM Br-cGMP did not affect the cyclic AMP concentrations so that an indirect relaxing effect of Br-cGMP, involving increased accumulation of cyclic AMP, is unlikely.

Table 3. Effect of Br-cGMP on the cyclic AMP level in rat ductus deferens

	cAMP (nmol/g wet weight)
Control	1.12 ± 0.03 (7)
Br-cGMP, 10 min	0.99 ± 0.04 (8)
Br-cGMP, 20 min	1.07 ± 0.08 (8)

After 30 min preincubation, 10 μM Br-cGMP was added for the times indicated. Data are means ± S.E.M. with the number of experiments in parentheses

Table 4. Effects of Br-cGMP and Br-GMP on uterine frequency

Nucleotide added	Relative uterine frequency	
	10 μM	100 μM
None	0.97 ± 0.08	0.95 ± 0.12
Br-cGMP	0.59 ± 0.05[a]	0.41 ± 0.05[a]
Br-GMP	0.92 ± 0.08	0.82 ± 0.07

Tissue was preincubated with 0.2 μM oxytocin. After a constant frequency had been reached for more than 30 min (about 15 per 30 min), Br-cGMP or Br-GMP were added at 10 μM for 30 min and at 100 μM for another 30 min period. The observed uterus frequencies are given relative to the control frequency observed prior to nucleotide addition. Data are means ± S.E.M. of 7 to 10 tissue pieces

[a] Indicates a significant difference to the correponding control group in the Wilcoxon test

2. Rat Uterus

The effect of Br-cGMP on uterine frequency was studied in oxytocin-stimulated uterus horns from estrogen-pretreated rats (Table 4). Br-cGMP added at 10 and 100 μM reduced the uterine frequency by 40 and 60 %, respectively. In contrast, Br-GMP had no effect. The tension developed was not changed by Br-cGMP.

3. Vascular Tissues

Br-cGMP was a potent relaxant of vascular tissues. When rat aortic strips were stimulated by noradrenaline (10 nM) and Br-cGMP was added after the plateau of the tonic contraction was reached, Br-cGMP (1 – 100 μM) caused a concentration-dependent relaxation which occurred after a very short lag phase (Fig. 4). Dibutyryl cGMP (up to 100 μM, not shown)

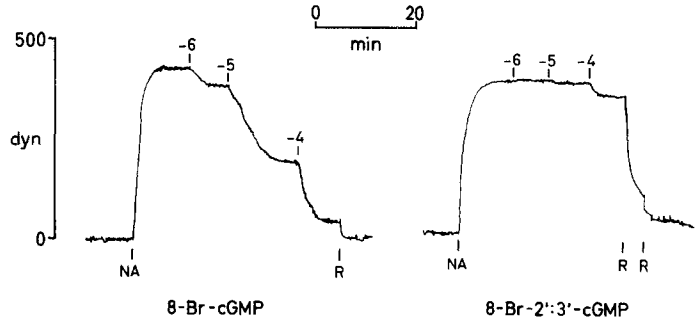

Fig. 4
Effects of Br-cGMP and Br-2′:3′-cGMP on noradrenaline-induced contraction of rat aorta. Rat aortae were stimulated by 10 nM noradrenaline (NA). After having reached the plateau of the tonic response, nucleotide derivatives were added at increasing concentrations ($10^{-6}-10^{-4}$ M) as indicated in logarithmic form. Representative examples of the effects of Br-cGMP and Br-2′:3′-cGMP are shown in the left and right panel of the figure, respectively. *R* indicates a change of the medium

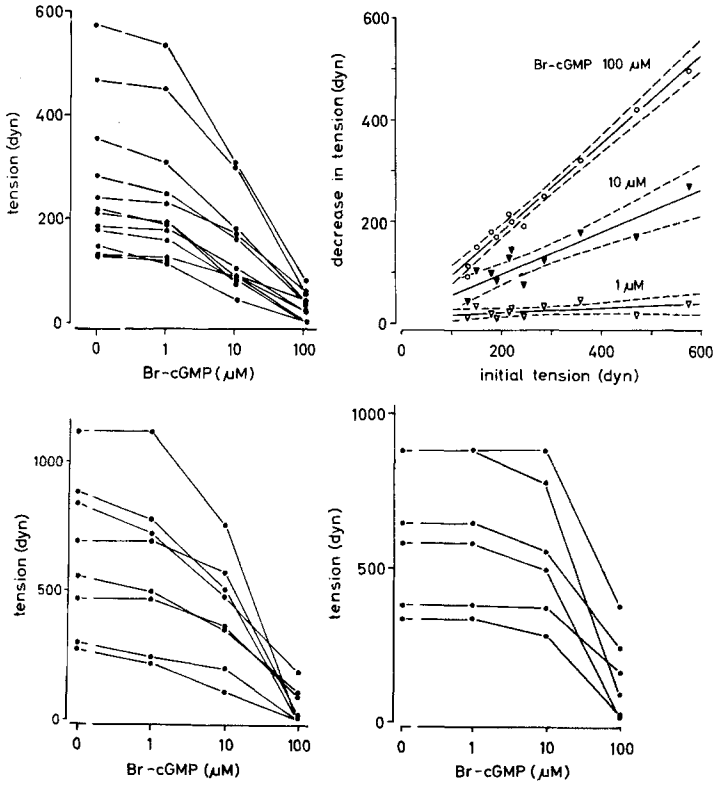

Fig. 5
Effect of Br-cGMP on the tension of the noradrenaline-stimulated vascular tissues. After preincubation for 120 min, vascular strips were stimulated by 10 nM noradrenaline. The upper left panel shows the initial maximal tensions developed by individual rat aortic strips after noradrenaline addition and the tensions reached after subsequent additions of Br-cGMP at the concentrations indicated on the abscissa. In the upper right panel, decreases from the initial noradrenaline-stimulated tension as caused by 1, 10 and 100 μM Br-cGMP are plotted against the initial tensions. The solid lines indicate the regression lines of the decrease in tension as a function of the initial tension, the interrupted lines give the confidential intervals of the regression lines. Individual noradrenaline-induced initial tensions and tensions reached after Br-cGMP addition for rabbit aorta and hog spleen artery strips are shown in the lower left and right panel, respectively. Rabbit aortic and spleen arterial strips were stimulated by 10 and 100 μM noradrenaline, respectively

had no significant effect. Similarly ineffective was Br-guanosine (not shown). Br-GMP and Br-2′:3′-cGMP (see Fig. 4) had small relaxing effects at 100 μM. Whereas Br-cGMP at 1 μM had a relaxant effect only in some of the arterial strips, at 10 and 100 μM the nucleotide caused potent relaxation in all preparations (Fig. 5).

The extent of the Br-cGMP-induced decrease in tissue tension appeared to depend on the initial tension of the noradrenaline-stimulated tissue. Regression analyses of the data obtained with 1, 10 and 100 μM Br-cGMP confirmed this impression; regression coefficients with 95% confidence intervals were 0.412 ± 0.140 and 0.862 ± 0.089 for the data obtained with 10 and 100 μM Br-cGMP, respectively, which values were both significantly different from zero. The mean tension of the noradrenaline-stimulated rat aortic strips was decreased by 9, 47 and 89% by 1, 10 and 100 μM Br-cGMP, respectively (Table 5).

Noradrenaline-stimulated rabbit aortae were similarly sensitive to Br-cGMP (Fig. 5). Analyses performed in a similar way as with rat aortae revealed 8, 35 and 92% decreases in the mean tension by 1, 10 and 100 μM Br-cGMP, respectively (Table 5).

The effect of Br-cGMP on noradrenaline-stimulated hog spleen arteries was less pronounced than on aortic strips (Fig. 5). Decreases in mean tissue tension were 10 and 76% with 10 and 100 μM Br-cGMP, respectively (Table 5).

In all three vascular tissues studied, Br-cGMP (up to 1 mM) had very little relaxant effect (10 – 20%) when the arteries were contracted by the addition of 35 mM

Table 5. Effect of Br-cGMP on the tension of some noradrenaline-stimulated vascular tissues

Tissue	*N*	Mean initial tension	Decreases in mean initial tension by Br-cGMP		
			1 μM	10 μM	100 μM
Rat aorta	12	263	25 ± 8 (9%)	122 ± 19 (47%)	235 ± 12 (89%)
Rabbit aorta	8	644	49 ± 44 (8%)	225 ± 65 (35%)	593 ± 66 (92%)
Hog spleen artery	6	617	0	58 ± 53 (10%)	467 ± 151 (76%)

The mean of the noradrenaline-stimulated initial tensions and of the Br-cGMP-induced decreases in this mean value are derived from the data shown in Fig. 5. Mean initial tensions and decreases in tension are given in dyn; decreases in tension are indicated as means with their 95% confidential intervals; the decrease in tension expressed as % of the initial tension is given in parentheses. Values for rabbit aorta and hog spleen artery have been derived from the data shown in Fig. 5 and were calculated as described for rat aorta

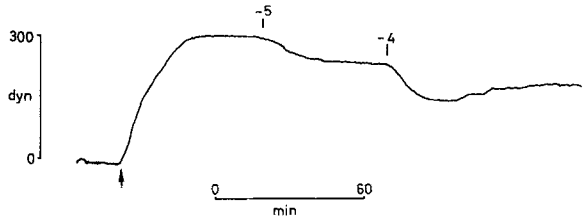

Fig. 6. Effect of Br-cGMP on the rat aorta stimulated by the divalent cation ionophore, A-23187. Aortic strips were contracted by addition of 10 μM A-23187 at the time point indicated by the arrow. After a tonic contractile response had developed, Br-cGMP was added at 10 and 100 μM as indicated in logarithmic form

K_2SO_4 (not shown). Dibutyryl cAMP was similarly ineffective in K^+-depolarized arteries (not shown).

In rat aortic strips contracted by the divalent cation ionophore, A-23187, the addition of Br-cGMP (10–100 μM) caused relaxation, which was far less pronounced, however, than the effect on the noradrenaline-stimulated tissue (Fig. 6). In most of the A-23187-stimulated strips, dibutyryl cAMP was also a weak relaxant (not shown).

Discussion

The present studies clearly indicate that the 8-bromo derivative of cyclic GMP is capable of relaxing various smooth muscular preparations especially when these were contracted by hormonal stimuli. These findings are in contrast with earlier observations indicating that exogenous cGMP derivatives can promote smooth muscle contraction (Puglisi et al., 1971; Lewis et al., 1973; Takayanagi and Takagi, 1973; Wikberg and Andersson, 1978). In some of these studies (Puglisi et al., 1971; Lewis et al., 1973), however, the contracting effects of exogenous cyclic GMP derivatives were blocked by atropine and, therefore, were most likely indirect. Relaxation by exogenously applied derivatives of cyclic GMP have previously been reported in guinea pig ileum (Lewis et al., 1973) and trachea (Szaduykis-Szadurski et al., 1972a, b; Lewis et al., 1973). Since control experiments were inadequate or high nucleotide concentrations were used in the studies mentioned above, the specificity of these nucleotide effects has not been clear.

Concentrations of exogenous Br-cGMP required to cause half-maximal relaxation of the ductus deferens and of the aorta were about 10 μM. This concentration, which is 100- to 1,000-fold higher than basal intracellular cyclic GMP levels, appears to be reasonably low to be specific. The assumption that Br-cGMP is a specific exogenous substitute of cyclic GMP is supported by the results of control experiments applying Br-2′:3′-cGMP, Br-GMP and Br-guanosine. It is not clear why cyclic GMP itself and dibutyryl cGMP were ineffective. The unmodified cyclic nucleotide may poorly penetrate the cell membranes. Dibutyryl cGMP, like Br-cGMP, is more lipophilic and more resistent to enzymatic inactivation by cyclic nucleotide phosphodiesterases than cyclic GMP itself (Michal et al., 1974). Dibutyryl cyclic GMP may sufficiently permeate the cellular membranes but may not be effectively deacylated to become an active stimulant of the cyclic GMP-sensitive system and may not induce accumulation of endogenous cyclic nucleotides as shown with dibutyryl cyclic AMP in bone (Heersche et al., 1971).

Cyclic GMP-stimulated protein kinases have been demonstrated in various mammalian tissues and are assumed to be the primary target system controlled by this cyclic nucleotide (Lincoln and Corbin, 1978; Kuo et al., 1978). Br-cGMP is at least as potent as cyclic GMP itself as an activator of cyclic GMP-dependent protein kinases from various sources, whereas dibutyryl cGMP is essentially inactive and N^2-butyryl cyclic GMP is only a weak stimulant (Kuo et al., 1978). Fifty times or even higher concentrations of cyclic GMP or Br-cGMP than of cyclic AMP are necessary to stimulate type II cyclic AMP-dependent protein kinase from beef heart (F. Hofmann, personal communication). Although the physiological substrates of cyclic GMP-dependent protein kinases are unknown, increased

phosphorylation of specific protein substrates ist most likely involved in the effects of cyclic GMP and exogenous Br-cGMP. There are no findings that would indicate an effect of cyclic GMP on the contractile apparatus. On the other hand, cyclic GMP stimulated the phosphorylation of some membrane proteins in several smooth muscular tissues (Casnellie and Greengard, 1974; Wallach et al., 1978). An inhibitory effect of cyclic GMP on calcium binding by microsomal fractions from intestinal smooth muscle has been reported (Nilsson and Andersson, 1977). However, the significance of this finding for tissue function is unclear.

The present findings with Br-cGMP are consistent with earlier observations indicating that several smooth muscle relaxants are powerful stimulants of cyclic GMP levels (Diamond and Holmes, 1975; Diamond and Blisard, 1976; Schultz et al., 1977; Katsuki and Murad, 1978) and that quantitative and temporal relations between increases in tissue tone and cyclic GMP levels are poor (Schultz, 1977a, 1978; Diamond, 1978). The observations with Br-cGMP favor the concept that hormone- and drug-induced elevations of cyclic GMP can reduce smooth muscle responses to neurotransmitters and hormones (Schultz, 1977a, 1978; Schultz et al., 1977). It has been proposed that cyclic GMP may act as an inhibitor of calcium influx into cytoplasm (Schultz et al., 1977). This assumption appears to be supported by the present findings that Br-cGMP reduced the increase in tone of Ca^{2+}-depleted, K^+-depolarized ductus deferentes when the extracellular calcium concentration was stepwise elevated and that Br-cGMP was a poor inhibitor of ionophore-induced contractions. This concept is additionally supported by the observations that various smooth muscle relaxants that are assumed to act as "calcium-antagonists" (Fleckenstein et al., 1971) increased the cyclic GMP level in the rat ductus deferens (Schultz et al., 1977) and that Br-cGMP reduced the ^{45}Ca flux in an insect salivary gland (Berridge and Lipke, 1978).

However, there are several observations that are not consistent with the notion that cyclic GMP inhibits calcium influx into cytoplasm. Br-cGMP was a very poor relaxant of K^+-contracted ductus deferentes and aortae. Sodium nitroprusside and hydroxylamine, agents which belong to the most potent stimulants of guanylate cyclase (Arnold et al., 1977; Böhme et al., 1978) and cyclic GMP levels in smooth muscle (Schultz et al., 1977; Katsuki and Murad, 1978), were similarly poor relaxants of the K^+-depolarized tissues in comparison with their strong relaxant effects on the noradrenaline-stimulated tissues (Kreye et al., 1975; Schultz et al., 1978). The reason for the different effectiveness of Br-cGMP and of the above smooth muscle relaxants against various stimuli is not clear. One possible explanation is that cyclic GMP has different effects on the influx of calcium from extra- and intracellular sources which are assumed to contribute to contractile responses to various stimuli to different degrees.

The situation is especially complicated by several findings with sodium nitroprusside. This potent vasodilator had no relaxant effect on the noradrenaline-stimulated rat ductus deferens (Kreye et al., 1975), whereas it was the most powerful stimulant of cyclic GMP levels in this tissue (Schultz et al., 1977). Recent observations indicate that sodium nitroprusside, which can cause hyperpolarization of vascular tissues (Häusler and Thorens, 1976), has no effect on membrane calcium flux (Häusler and Thorens, 1976; Kreye and Lüth, 1976; Fermum et al., 1976) or may increase calcium efflux (Zsotér et al., 1977) in vascular tissues. On the other hand, sodium nitroprusside and nitroglycerin have been shown to decrease the noradrenaline-stimulated ^{36}chloride efflux from the rabbit aorta (Kreye et al., 1977). Therefore, it is possible that cyclic GMP affects tissue excitation and excitability by changing a membrane function other than calcium permeability. The cyclic GMP-controlled cellular function, which still needs to be defined, may contribute to different degrees to the membrane potential and the excitation process in various smooth muscular tissues when stimulated by hormonal and non-hormonal factors.

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