Proc. Nat. Acad. Sci. USA
Vol. 70, No. 12, Part II, pp. 3889-3893, December 1973

The Importance of Calcium Ions for the Regulation of Guanosine 3':5'-Cyclic Monophosphate Levels

(cyclic nucleotides/cholinergic agents/phosphodiesterase inhibitors/smooth muscle/salivary gland)

G. SCHULTZ*, J. G. HARDMAN†, K. SCHULTZ*, C. E. BAIRD, AND E. W. SUTHERLAND‡

Department of Physiology, Vanderbilt University, Nashville, Tennessee 37232

Contributed by E. W. Sutherland, August 23, 1973

ABSTRACT **Guanosine 3':5'-cyclic monosphosphate (cyclic GMP) levels in the ductus deferens of the rat were increased 2- to 3-fold by acetylcholine (10–1000 μM) or by 125 mM KCl, while adenosine 3':5'-cyclic monophosphate (cyclic AMP) levels were not changed. After incubation for 30 min in the absence of Ca^{++}, cyclic GMP control levels were decreased by 85% and were not affected by acetylcholine or KCl. The readdition of Ca^{++} (1.8 mM) for 3 min to Ca^{++}-deprived tissue partially restored basal cyclic GMP levels and the effects of acetylcholine and KCl. The addition of Sr^{++} (3.6 mM) or of Ba^{++} (1.8 or 10 mM) also caused an increase in basal cyclic GMP in Ca^{++}-deprived tissue. Cyclic AMP levels were not significantly changed under any of these conditions. The addition of the phosphodiesterase inhibitor, 1-methyl-3-isobutylxanthine (0.1 mM), to ductus deferentes increased the amount of cyclic AMP about 50% and that of cyclic GMP about 2-fold. The latter effect also depended on the presence of Ca^{++}. 1-Methyl-3-isobutylxanthine (0.1 mM) increased cyclic GMP and cyclic AMP levels in slices of rat submaxillary glands. Methacholine increased cyclic GMP if added in the presence of methyl isobutylxanthine. Cyclic GMP control levels and the effect of methyl isobutylxanthine were unchanged by Ca^{++} omission, but the effect of methacholine was abolished.**

These findings indicate that calcium ions are important for the control of cyclic GMP levels in these tissues.

The role of adenosine 3':5'-cyclic monophosphate (cyclic AMP) as an intracellular mediator in the action of various hormones has been well established (1). Hormones that increase cyclic AMP levels in intact cells can generally be shown to cause an increase in adenylate cyclase activity when added to broken cell preparations (1). While the physiological role of guanosine 3':5'-cyclic monophosphate (cyclic GMP) is still unclear, the concentration of this nucleotide is increased by cholinergic and other agents in many mammalian tissues (2).

The enzyme catalyzing the formation of cyclic GMP from GTP, guanylate cyclase, has been found in both particulate and high-speed supernatant fractions in many tissues (3). Neither of the forms of guanylate cyclase has consistently been affected in cell-free systems by agents that cause an increase in cyclic GMP levels in intact cells. Therefore, we have considered the possibility that the increase in intracellular cyclic GMP concentration in response to acetylcholine and other agents might be secondary to altered ion concentrations at the location of guanylate cyclase. The results presented in this paper show that calcium ions are involved in the regulation of cyclic GMP levels by cholinergic and other agents and that Ca^{++} may also be involved in the regulation of cyclic GMP formation under basal conditions.

MATERIALS AND METHODS

Materials and methods used were essentially the same as described (4). Acetylcholine chloride, methacholine chloride, and atropine sulfate were obtained from Sigma.

Segments of rat ductus deferens were incubated in an oxygenated balanced salt solution (5) which contained 30 μM EDTA and 1.8 mM Ca^{++} if not otherwise indicated. When high concentrations of K^+ were used, the NaCl (125 mM) in the medium was replaced by an equimolar amount of KCl. Cyclic nucleotides were extracted and purified as described (4).

Submaxillary glands were obtained from male rats (150–200 g) which were killed by cervical dislocation. Slices of about 0.5-mm thickness were prepared with a Stadie–Riggs tissue slicer. The slices were incubated at 37° in O_2/CO_2 (95/5%)—gassed Krebs–Ringer bicarbonate buffer containing 3% bovine-serum albumin and 1.3 mM Ca^{++} if not otherwise stated. After 30 min of preincubation, the slices were transferred to fresh medium and agents were added as indicated. The slices were frozen between blocks of dry ice to terminate the incubation. The frozen slices (about 100–150 mg per sample) were homogenized in 3 ml of 50% ethanol containing 30 mM zinc acetate and tracer amounts of tritiated cyclic AMP and cyclic GMP at −20° with an Ultra-Turrax homogenizer (Jahnke and Kunkel, Staufen, Germany). Cyclic nucleotides were then purified by $ZnCO_3$-coprecipitation (4) and ion-exchange chromatography on two 0.62 × 30-cm Dowex-50 columns (6).

GTP Determination. For determination of GTP concentrations in ductus deferentes, segments of tissue (about 50 mg each) were incubated for 30 min in balanced salt solution (5) with or without Ca^{++}. The tissue was rapidly frozen, broken, and homogenized at −20° with a ground-glass homogenizer in 0.5 ml of 0.3 M perchloric acid in 50% ethanol containing about 80 nCi of [8-^{3}H]GTP (5.3 Ci/mmol, New England Nuclear Corp.). After centrifugation, the supernatant fluid was passed through a 0.4 × 3-cm column of cocoanut charcoal (50-200 mesh, Fisher) coated with Dextran 40 (Pharmacia, 1 part per 10 parts of charcoal) (7). The column was rinsed with water, and the nucleotides were eluted with 10 ml of 2 M NH_4OH in 40% ethanol (8). After evaporation, the dried

Abbreviation: SC-2964, 1-methyl-3-isobutylxanthine.

* Present address: Department of Pharmacology, University of Heidelberg, Heidelberg, Germany.
† To whom reprint requests should be sent.
‡ Present address: Department of Biochemistry, University of Miami, Miami, Florida.

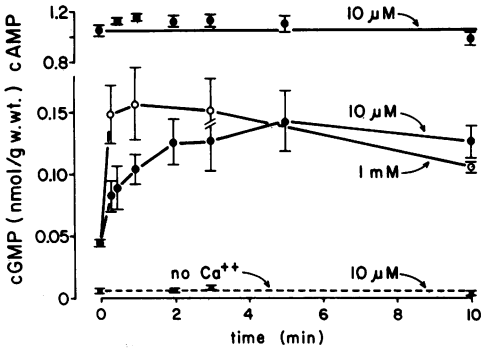

FIG. 1. Effect of acetylcholine on cyclic nucleotide levels in rat ductus deferens. After preincubation for 30 min in the presence (*solid lines*) or in the absence (*dotted line*) of Ca^{++}, acetylcholine (10 μM, ●, or 1 mM, ○) was added for various lengths of time. Values are means of 5–25 samples, and *vertical lines* are 2 SEM. *w.wt.*, wet weight.

eluate was taken up in water and applied in a 4-cm wide band to a thin-layer chromatography plate coated with polyethyleneimine cellulose. GTP was separated from GDP, GMP, ATP, ADP, and AMP by development with 1.2 M LiCl and eluted with 1 M KCl (9).

GTP was assayed in a system similar to that described for cyclic GMP (4). GTP (with and without addition of an internal standard) was converted to GDP by incubation with 20 μg of myosin for 60 min at 30°. After the sample was heated in a boiling-water bath for 5 min, 4 μg of pig-brain ATP:GMP phosphotransferase and about 15 nCi of [γ-^{32}P]ATP (44 Ci/mmol) were added. The samples were then treated essentially as described for the assay of cyclic GMP. Standard curves for GTP were linear between 0.5 and 30 pmol per tube.

RESULTS

Effects of Acetylcholine on Cyclic Nucleotides in Ductus Deferens. Cyclic GMP levels in rat ductus deferens were rapidly increased by addition of 10 μM acetylcholine, which is less than half-maximally effective in producing contraction (Fig. 1). A significant elevation of cyclic GMP was observed within 30 sec, and a maximal response of about 3-fold was found 2–5 min after acetylcholine addition. 1 mM acetylcholine, which is maximally effective for contraction, caused a more rapid but not greater increase in cyclic GMP than that observed with the lower dose. If the tissue was preincubated for 30 min in Ca^{++}-free medium, cyclic GMP control levels were decreased by about 85% and were not changed by acetylcholine added for various periods of time. Cyclic AMP levels were not significantly changed by acetylcholine (Fig. 1) or by omission of Ca^{++} (data not shown).

TABLE 1. *Effect of potassium chloride on cyclic nucleotide levels in rat ductus deferens*

	Cyclic GMP (pmol/g of wet weight)	Cyclic AMP
Control	48.3 ± 3.0 (24)	901 ± 32 (26)
KCl		
20 sec	103 ± 14.2 (6)	851 ± 77 (4)
1 min	109 ± 7.9 (6)	912 ± 42 (5)
2 min	96.4 ± 17.8 (5)	968 ± 36 (4)
3 min	92.0 ± 6.3 (14)	957 ± 42 (12)
Atropine 3.5 min	51.0 ± 3.5 (13)	904 ± 30 (21)
KCl 3 min + atropine	91.3 ± 9.5 (11)	992 ± 35 (6)
Acetylcholine 3 min	76.5 ± 4.8 (9)	897 ± 47 (13)
Acetylcholine + atropine	39.9 ± 7.3 (9)	901 ± 31 (5)

Pairs of ductus deferentes were incubated for the time indicated in regular medium or in medium that contained 125 mM KCl but no NaCl. Atropine (10 μM) was added 30 sec before addition of KCl or acetylcholine (1 μM). Results are means ±SEM of the number of tissue samples indicated in parentheses.

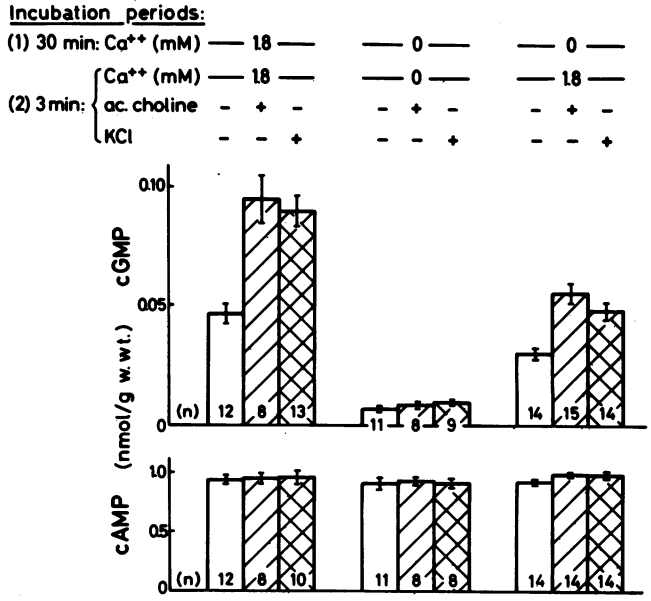

FIG. 2. Influence of Ca^{++} omission and readdition on the effects of acetylcholine or KCl on cyclic nucleotide levels in rat ductus deferens. Tissue was preincubated for 30 min in the presence (1.8 mM) or absence of Ca^{++} and then transferred for 3 min to fresh medium with or without addition of Ca^{++}, acetylcholine (10 μM; *ac. choline*), or KCl (125 mM) substituted for NaCl. *Bars* represent means and *vertical lines* 2 SEM. (*n*), no. of samples.

Effects of KCl on Cyclic Nucleotides in Ductus Deferens. Like acetylcholine, a high concentration of KCl induces a contraction of the ductus deferens. When the NaCl in the incubation medium was replaced by KCl, cyclic GMP levels were increased about 2-fold within 20 sec to 3 min, while cyclic AMP levels were not significantly affected (Table 1). While the effect of 1 μM acetylcholine on cyclic GMP was blocked by simultaneous addition of 10 μM atropine, the effect of KCl on cyclic GMP was not altered by atropine. This finding indicates that the effect of KCl on cyclic GMP is not caused by a potassium-induced release of endogenous acetylcholine.

Dependence of Acetylcholine and KCl on Ca^{++}. The effects of both acetylcholine and potassium on cyclic GMP levels depend on the presence of calcium ions in the medium. When ductus deferentes were incubated for 30 min in the presence of the usual 1.8 mM Ca^{++}, as shown above, the addition of 10 μM acetylcholine or of 125 mM KCl caused an increase of about 2-fold in cyclic GMP after 3 min but did not affect cyclic AMP levels (Fig. 2). After a 30-min incubation in the absence of Ca^{++}, cyclic GMP levels were decreased to about 15% of the control levels while cyclic AMP was not changed. When acetylcholine or KCl was added to tissues incubated in the

absence of Ca^{++}, they did not increase cyclic GMP. When 1.8 mM Ca^{++} was added back to the Ca^{++}-deprived tissue for 3 min, cyclic GMP control levels were partially restored, and the effects of both acetylcholine and KCl, which were added for the same period of time, were also partially restored.

Lack of Effect of Ca^{++} Omission on GTP Levels. Decreased cyclic GMP levels after incubation in Ca^{++}-free medium do not appear to be due to decreased levels of GTP. When ductus deferentes were incubated for 30 min with omission of Ca^{++}, GTP levels (55.9 ± 5.4 nmol/g wet weight, $n = 6$) were not changed compared with controls (54.3 ± 7.5 nmol/g, $n = 5$) incubated in the presence of Ca^{++}.

Effects of 1-Methyl-3-isobutylxanthine on Cyclic Nucleotides in Ductus Deferens. The concentrations of both cyclic AMP and cyclic GMP in the ductus deferens are elevated by incubation with the cyclic nucleotide phosphodiesterase inhibitor SC-2964, which is 1-methyl-3-isobutylxanthine (4). A 50% increase in cyclic GMP was observed 1 min after the addition of this compound, and a maximal increase of about 3-fold was found after 3 min (Table 2). No further increase in cyclic GMP was observed 10 and 30 min after addition of SC-2964. The relative increase in cyclic AMP caused by SC-2964 was smaller than that in cyclic GMP.

The effect of the phosphodiesterase inhibitor on cyclic GMP in the ductus deferens also depends on the presence of Ca^{++}. When tissue was incubated with 1.8 mM Ca^{++}, the addition of 0.1 mM SC-2964 caused a 2.5-fold increase in cyclic GMP and a 50% increase in cyclic AMP after 3 min (Fig. 3). However, after 30-min incubation of the tissue in Ca^{++}-free medium, there was no significant effect of the phosphodiesterase inhibitor on cyclic GMP while the effect on cyclic AMP levels was unchanged. Readdition of 1.8 mM Ca^{++} for 3 min to Ca^{++}-deprived tissue caused a partial restoration of the effect of SC-2964 on cyclic GMP levels.

Effects of Sr^{++} and Ba^{++} on Cyclic Nucleotides in Ductus Deferens. Sr^{++} can substitute for Ca^{++} in Ca^{++}-deprived ductus deferens to restore the contractile responses to various agents, although somewhat higher concentrations of Sr^{++} than of Ca^{++} are required. To study the effect of Sr^{++} on cyclic GMP levels, segments of ductus deferens were incubated for 30 min in Ca^{++}-free buffer (Table 3). The addition of 3.6 mM Sr^{++} for 3 min to the Ca^{++}-deprived tissue increased cyclic GMP control levels to a degree similar to that with 1.8 mM Ca^{++}. With Sr^{++}, as with Ca^{++}, acetylcholine caused a further increase in cyclic GMP. Cyclic AMP levels were not significantly changed under any of these conditions.

Ductus deferentes presumably depleted of Ca^{++} by preincubation in the absence of Ca^{++} with addition of 4 mM

TABLE 2. *Effect of the phosphodiesterase inhibitor SC-2964 (0.1 mM) on cyclic nucleotide levels in rat ductus deferens*

	Cyclic GMP (pmol/g of wet weight)	Cyclic AMP (pmol/g of wet weight)
Control	48 ± 5 (9)	1020 ± 50 (12)
SC-2964		
1 min	77 ± 3 (3)	not determined
3 min	132 ± 9 (15)	1470 ± 60 (14)
10 min	166 ± 3 (3)	1700 ± 120 (3)
30 min	146 ± 28 (3)	1560 ± 40 (3)

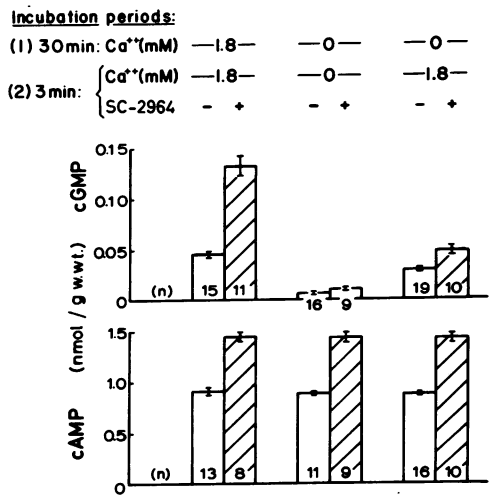

FIG. 3. Influence of Ca^{++} omission and readdition on the effects of 1-methyl-3-isobutylxanthine (SC-2964) on cyclic nucleotide levels in rat ductus deferens. Tissue was preincubated for 30 min in the presence (1.8 mM) or absence of Ca^{++} and then transferred for 3 min to fresh medium with or without addition of Ca^{++} and SC-2964.

EGTA are contracted by Ba^{++}. Cyclic GMP levels lowered by the Ca^{++}-depletion were increased about 2-fold by 1.8 mM Ba^{++} and about 4-fold by 10 mM Ba^{++} within 3 min (Fig. 4). Similar effects on cyclic GMP levels were observed when 1.8 or 10 mM Ca^{++} was added for 3 min. While acetylcholine caused an increase in cyclic GMP in the presence of Ca^{++} (1.8 and 10 mM), there was no acetylcholine effect in the presence of Ba^{++} (1.8 or 10 mM). Cyclic AMP levels were not significantly changed under these conditions (data not shown).

Effects of Methacholine and SC-2964 on Cyclic Nucleotides in Submaxillary Gland. Cholinergic agents stimulate secretion in salivary glands, and this effect depends on the presence of Ca^{++} (10). The effect of the cholinergic agent, methacholine, was studied in slices of rat submaxillary glands (Fig. 5). The addition of 0.1 mM methacholine did not affect cyclic GMP levels after 3 min. However, in the presence of 0.1 mM SC-2964, which increased cyclic GMP by about 50%, methacholine caused a 3-fold increase in cyclic GMP levels. Cyclic AMP levels were increased by SC-2964 by about 50%, but were not affected by methacholine.

TABLE 3. *Effect of acetylcholine on cyclic nucleotide levels in rat ductus deferens after a 30-min preincubation in the absence of Ca^{++}*

Cation (mM)	Acetylcholine	Cyclic GMP (pmol/g of wet weight)	Cyclic AMP (pmol/g of wet weight)
No cation	−	5.9 ± 0.9 (19)	907 ± 52 (11)
	+	6.9 ± 1.0 (16)	928 ± 35 (8)
Ca^{++} (1.8)	−	30.1 ± 2.5 (14)	909 ± 19 (14)
	+	55.4 ± 4.4 (14)	960 ± 17 (11)
Sr^{++} (3.6)	−	30.9 ± 2.0 (19)	979 ± 21 (18)
	+	45.7 ± 5.5 (20)	971 ± 25 (20)

Acetylcholine (10 μM) was added for 3 min in the absence of added divalent cation or with 1.8 mM Ca^{++} or 3.6 mM Sr^{++} added for the same period of time.

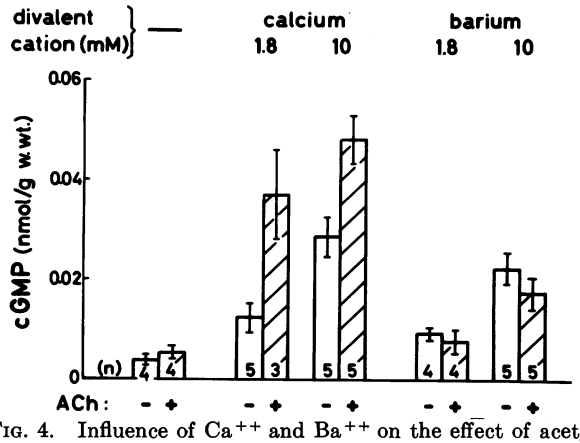

FIG. 4. Influence of Ca^{++} and Ba^{++} on the effect of acetylcholine on cyclic GMP levels in Ca^{++}-deprived rat ductus deferens. Tissue was preincubated for 30 min in Ca^{++}-free medium with 4 mM EGTA added and then transferred for 3 min to fresh medium containing Ca^{++} (1.8 or 10 mM) or Ba^{++} (1.8 or 10 mM) and acetylcholine (0.3 mM; *Ach*) as indicated in the figure.

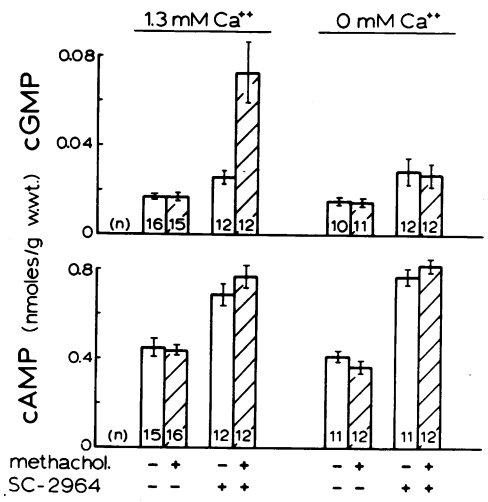

FIG. 5. Effects of methacholine and 1-methyl-3-isobutylxanthine (SC-2964) on cyclic nucleotide levels in slices of rat submaxillary glands. Tissue was preincubated for 30 min in the presence (1.3 mM) or absence of Ca^{++} and then transferred for 3 min to fresh medium with or without addition of Ca^{++}, methacholine (0.1 mM), and SC-2964 (0.1 mM).

In contrast to the ductus deferens, basal cyclic GMP levels in submaxillary glands were not significantly lowered when the tissue was incubated for 30 min in the absence of Ca^{++}. The effect of the phosphodiesterase inhibitor on cyclic GMP was also unchanged by the omission of Ca^{++}. However, as was the case with acetylcholine in the ductus deferens, there was no effect of methacholine on cyclic GMP in the submaxillary gland in the absence of Ca^{++}. The omission of Ca^{++} did not alter basal cyclic AMP levels or the effect of SC-2964 on these.

DISCUSSION

An increase in cytoplasmic free calcium is believed to be involved in the physiological response of smooth-muscular tissues (11) and salivary glands (10) to stimulation by cholinergic and other active agents. The present data show that Ca^{++} is also a very important factor for the regulation of cyclic GMP levels in a smooth-muscular and a secretory tissue. Cyclic GMP levels are markedly decreased in Ca^{++}-deprived ductus deferens and are increased under conditions thought to be associated with increased intracellular calcium concentrations. Cyclic AMP levels are not changed under the same conditions.

A reduction of cyclic GMP in Ca^{++}-deprived tissue may be due to several possible Ca^{++} effects on GTP or cyclic GMP metabolism. A decreased GTP content of the tissue causing a reduced formation of cyclic GMP can be excluded. Ca^{++} has been shown to inhibit cyclic nucleotide phosphodiesterase under certain conditions. However, there is no reason to expect that cyclic GMP degradation would be increased in Ca^{++}-deprived tissue while cyclic AMP hydrolysis would not be affected, since the hydrolysis of both nucleotides by phosphodiesterases from smooth-muscular tissues seems to be similarly inhibited by Ca^{++} (J. N. Wells, C. E. Baird, and J. G. Hardman, manuscript in preparation). The activity of soluble and particulate guanylate cyclase preparations obtained from various tissues can be influenced by calcium ions. When the enzymatic activity is measured with certain concentrations of GTP and Mn^{++}, Ca^{++} is capable of stimulating cyclic GMP formation (3, 12). The exact mechanism by which Ca^{++} affects cyclic GMP formation is not known.

The finding that an elevation of cellular cyclic GMP in response to cholinergic agents and a depolarizing concentration of K^+ occurs only in the presence of extracellular Ca^{++} suggests that this increase in cyclic GMP is a secondary event brought about by an increased cytoplasmic Ca^{++} concentration due to increased inflow of Ca^{++} from the extracellular space or to release of Ca^{++} from intracellular storage sites. The importance of Ca^{++} for the response in cyclic GMP to acetylcholine, histamine, and K^+ has also been shown in longitudinal smooth muscle from guinea-pig small intestine (13). While this manuscript was being prepared, Ferrendelli *et al.* (14) reported that various depolarizing agents raised both cyclic GMP and cyclic AMP concentrations in brain in a Ca^{++}-dependent manner. It is possible that Ca^{++} is involved in the effect of most, if not all, agents known to increase cyclic GMP levels, since most of these agents are thought to elevate cytoplasmic Ca^{++}. These compounds include, in addition to the agents already discussed, α-adrenergic agents (15, 16), NaF (17, 18), and phytohemagglutinin (18, 19). That Sr^{++} and Ba^{++} can substitute for Ca^{++} to restore cyclic GMP levels in Ca^{++}-deprived tissue indicates either that these ions may act by a mechanism similar to that of Ca^{++} on cyclic GMP formation or that they can release an intracellular store of Ca^{++}.

In Ca^{++}-deprived ductus deferentes, a phosphodiesterase inhibitor did not cause an increase in cyclic GMP content. This finding indicates that under these conditions the rate of formation of cyclic GMP must be very small. In contrast to these findings in the ductus deferens, incubation in a Ca^{++}-free medium did not affect cyclic GMP control levels or the effect of the phosphodiesterase inhibitor on cyclic GMP in slices of submaxillary glands. This finding indicates that the basal turnover of cyclic GMP is not effectively changed by incubation of this tissue in Ca^{++}-free medium. It is possible that this tissue is not as easily depleted of Ca^{++} as the ductus deferens, but it is also conceivable that the basal formation of cyclic GMP in the salivary gland is less dependent on the presence of Ca^{++} than is that in the ductus deferens.

The observation that cyclic GMP levels are elevated under conditions that are accompanied by a contraction of smooth-muscular tissues and increased secretion of salivary glands may suggest a regulatory role of cyclic GMP in these processes. However, such an assumption would be premature considering other findings. For example, incubation of smooth-muscular tissues with phosphodiesterase inhibitors causes a relaxation of contracted tissue which is accompanied by an increase in cyclic GMP levels that usually is at least as large (on a relative basis) as the increase in cyclic AMP. While addition of cyclic AMP or dibutyryl cyclic AMP to the incubation medium leads to a relaxation of smooth-muscular tissues (1), no direct effect of exogenous cyclic GMP or of its dibutyryl derivative to cause contraction of smooth muscle has been published. In other cell types, however, recent studies have shown effects of 8-bromo- or dibutyryl cyclic GMP on cell function that are similar to those observed after cholinergic or α-adrenergic stimulation (20, 21). Since the stimulation of cyclic AMP-dependent protein kinase (from skeletal muscle) has been reported to be partially antagonized by low concentrations of cyclic GMP (22), an antagonistic action of the two cyclic nucleotides on one regulated system is possible in certain tissues. This view cannot be generalized, however, since cholinergic agents and agents that elevate cyclic AMP produce similar effects in other tissues, e.g., in thyroid (23, 24) and pancreatic islets (25, 26). An independent action of cyclic GMP on a separate system not influenced by cyclic AMP has to be considered. Cyclic GMP-dependent protein kinases have been described in some tissues (especially in lower animals), but their physiological substrates are not known (27).

A control function for cyclic GMP in the tissues examined has not been established. It is possible that cyclic GMP acts as a comediator with Ca^{++} in certain cellular functions of these tissues. On the other hand, the nucleotide may act as a negative feedback signal to accelerate Ca^{++}-removal from an extracellular compartment. More work will be required to establish which, if either, of these possibilities is true.

We thank Dr. E. V. Newman, who as Principal Investigator of Program Project Grant HL-08195 made available laboratory space and some items of equipment used in conducting these studies. This work was supported by NIH Grants GM-16811, HL-08332, HL-13996, and AM-07462. A preliminary report of this work was presented in ref. 13. We acknowledge the expert technical assistance of Mrs. Marvist A. Parks, Mr. James W. Davis, and Miss Sherry Burnitt. E.W.S. is Career Investigator of the American Heart Association; G.S. was a recipient of a Research Fellowship of the Deutsche Forschungesgemeinschaft and a Visiting Scientist Award of the American Heart Association.

1. Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1971) *Cyclic AMP* (Academic Press, New York and London).
2. Goldberg, N. D., O'Dea, R. F. & Haddox, M. K. (1973) *Advan. Cycl. Nucl. Res.* **3**, 155–223.
3. Hardman, J. G., Chrisman, T. D., Gray, J. P., Suddath, J. L. & Sutherland, E. W. (1973) *Proc. 5th Internat. Congr. Pharmacol* (S. Karger, Basel), Vol. 5, 134–145.
4. Schultz, G., Hardman, J. G., Schultz, K., Davis, J. W. & Sutherland, E. W. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1721–1725.
5. Hurwitz, L. & Joiner, P. D. (1970) *Amer. J. Physiol.* **218**, 12–19.
6. Hardman, J. G. & Sutherland, E. W. (1969) *J. Biol. Chem.* **244**, 6363–6370.
7. Herbert, V., Lau, K.-S., Gottlieb, C. W. & Bleicher, S. J. (1965) *J. Clin. Endocrin. Metab.* **25**, 1375–1384.
8. Tsuboi, K. K. & Price, T. D. (1959) *Arch. Biochem. Biophys.* **81**, 223–237.
9. Böhme, E. & Schultz, G. (1974) in *Methods in Enzymology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic Press, New York and London), in press.
10. Douglas, W. W. & Poisner, A. M. (1962) *J. Physiol.* **162**, 385–392.
11. Hurwitz, L. & Suria, A. (1971) *Annu. Rev. Pharmacol.* **11**, 303–326.
12. Hardman, J. G., Beavo, J. A., Gray, J. P., Chrisman, T. D., Patterson, W. D. & Sutherland, E. W. (1971) *Ann. N.Y. Acad. Sci.* **185**, 27–35.
13. Schultz, G., Hardman, J. G., Hurwitz, L. & Sutherland, E. W. (1973) *Fed. Proc.* **32**, 773 abstr.
14. Ferrendelli, F. A., Kinscherf, D. A. & Chang, M. M. (1973) *Mol. Pharmacol.* **9**, 445–454.
15. Ball, J. H., Kaminsky, N. I., Hardman, J. G., Broadus, A. E., Sutherland, E. W. & Liddle, G. W. (1972) *J. Clin. Invest.* **51**, 2124–2129.
16. Glass, D. B., White, J., Haddox, M. K. & Goldberg, N. D. (1973) *Pharmacologist* **15**, 156 abstr.
17. Yamashita, K. & Field, J. B. (1972) *J. Biol. Chem.* **247**, 7062–7066.
18. Whitney, R. B. & Sutherland, R. M. (1972) *Cell. Immunol.* **5**, 137–147.
19. Hadden, J. W., Hadden, E. M., Haddox, M. K. & Goldberg, N. D. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 3024–3027.
20. Kaliner, M., Orange, R. P. & Austen, K. F. (1972) *J. Exp. Med.* **136**, 556–567.
21. Ignarro, L. J. & Colombo, C. (1973) *Science* **180**, 1181–1183.
22. Goldberg, N. D. (1972) *5th International Congr. Pharmacol.* Abstracts of Invited Presentations, 229–230.
23. Pastan, I., Herring, B., Johnson, P. & Field, J. B. (1961) *J. Biol. Chem.* **236**, 340–342.
24. Altman, M., Oka, H. & Field, J. B. (1966) *Biochim. Biophys. Acta* **116**, 586–588.
25. Mayhew, D. A., Wright, P. H. & Ashmore, J. (1969) *Pharmacol. Rev.* **21**, 183–212.
26. Burr, I. M., Taft, H. P., Stauffacher, W. & Renold, A. E. (1971) *Ann. N.Y. Acad. Sci.* **185**, 245–262.
27. Kuo, J. F., Wyatt, G. R. & Greengard, P. (1971) *J. Biol. Chem.* **246**, 7159–7167.