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A New Enzymatic Assay for Guanosine 3′:5′-Cyclic Monophosphate and Its Application to the Ductus Deferens of the Rat

([^{32}P]GDP formation/cholinergic agents/adrenergic agents/phosphodiesterase inhibitors/smooth muscle)

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ABSTRACT **A sensitive enzymatic procedure has been developed for the determination of guanosine 3′:5′-cyclic monophosphate (cyclic GMP). It is based on the conversion of cyclic GMP to GMP by cyclic nucleotide phosphodiesterase and on the transfer of ^{32}P from [γ-^{32}P]ATP to GMP by the action of a specific ATP:GMP phosphotransferase (EC 2.7.4.8). The [^{32}P]GDP is separated from the remaining [^{32}P]ATP by enzymatic degradation of ATP by myosin and by precipitation of the $^{32}P_i$ formed. The reaction blank, which is mostly caused by the nucleotide content of the enzymes, is doubled by about 0.1 pmol of cyclic GMP. The procedure has advantages in speed and/or accuracy over other methods in current use.**

Cyclic nucleotide concentrations were studied in the ductus deferens of the rat; two agents were used, carbachol and norepinephrine, which cause contraction. Incubation with 0.1 mM carbachol caused a 3-fold increase in cyclic GMP content, which was maximal about 2 min after carbachol addition. Cyclic AMP concentrations were not significantly changed. Addition of 0.01 mM norepinephrine increased cyclic GMP content by about 25% within 1 min and by 40% within 3 min; cyclic AMP concentrations were only slightly increased. A 3-min incubation with the phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (0.1 mM) doubled the cyclic GMP content and increased cyclic AMP concentration by 50%.

While adenosine 3′:5′-cyclic monophosphate (cyclic AMP) has been established as an intracellular mediator in the action of various hormones (1), the role of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) is still unclear. This cyclic nucleotide, which was first discovered in rat urine (2), has been found in all mammalian tissues examined but in concentration much lower than those of cyclic AMP (3–5).

The present paper describes a new enzymatic assay for small amounts of cyclic GMP. The assay is based on the following enzymatic steps:

$$\text{Cyclic GMP} \xrightarrow{\text{phosphodiesterase}} \text{GMP} \quad [1]$$

$$\text{GMP} + [\gamma-{}^{32}\text{P}]\text{ATP} \xrightarrow{\text{GMP kinase}} [\beta-{}^{32}\text{P}]\text{GDP} + \text{ADP} \quad [2]$$

$$[\gamma-{}^{32}\text{P}]\text{ATP} \xrightarrow{\text{myosin}} \text{ADP} + {}^{32}\text{P}_i \quad [3]$$

After conversion of cyclic GMP to [^{32}P]GDP and degradation of the the remaining [^{32}P]ATP to ADP and $^{32}P_i$, the P_i is precipitated, and the amount of [^{32}P]GDP in the supernatant fluid is determined.

This assay has been applied to studies on the control of cyclic GMP content in the ductus deferens of the rat. Cyclic GMP concentrations were increased after incubation with carbachol or norepinephrine, agents that cause a contraction of this smooth muscular tissue.

MATERIALS AND METHODS

Enzymes. Cyclic nucleotide phosphodiesterase was purified from bovine hearts (6). The specific activity was 0.4–0.5 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ of protein if determined according to Beavo *et al.* (7) with 1 μM cyclic GMP as substrate. Protein was determined according to Lowry *et al.* (8). ATP:GMP phosphotransferase (GMP kinase, EC 2.7.4.8) was a commercial preparation from pig brain (specific activity 10 U/mg) by Boehringer Mannheim Corp. or was prepared from calf thymus (9) with a similar specific activity. Before use, the pig brain enzyme was dialyzed over night against 500–1000 volumes of 10 mM *N*-tris(hydroxymethyl)-methyl-2-amonimethane sulfonate (TES) buffer (pH 7.4). Myosin was prepared from rabbit skeletal muscle (10) with two additional precipitations. The enzyme was stored at −20° in a solution containing 0.25 M KCl and 50% (v/v) glycerol (11). When assayed as described by Perry (10), the preparations hydrolyzed at least 2 μmol of ATP per mg of protein in 5 min at 25°. The enzyme was diluted with 0.5 M KCl to an appropriate protein concentration just before use. 3-Phosphoglycerate kinase (EC 2.7.2.3) from yeast (180 U/mg) and glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) from rabbit muscle (80 U/mg) were purchased from Boehringer Mannheim Corp. The enzyme suspensions were centrifuged for 15 min at 40,000 × *g* and the pellets were taken up in 10 mM Tris·HCl (pH 7.5) and stored frozen until use.

[γ-^{32}P]ATP was prepared by a modified enzymatic procedure (12, 13). To 100–150 μl of a solution containing 3–4 mCi of $^{32}P_i$ in 0.02 N HCl (International Chemical and Nuclear Corp.), 10–15 μl of 0.3 M Tris were added to adjust the pH to about 8. To this solution were added 50 μl of a mixture containing 200 nmol of 3-phosphoglycerate, 20 nmol of NAD^+, 1000 nmol of $MgCl_2$, 200 nmol of EDTA, 600 nmol of cysteine, 10–20 nmol of ATP, 0.3–1 μg of 3-phosphoglycerate kinase, and 0.3–1 μg of glyceraldehyde-3-phosphate dehydrogenase in 10 mM Tris·HCl (pH 8.0). After about 30 min of incubation at room temperature (25°), 70–80% of the P_i was incorporated into ATP. This was checked by addition of a few milligrams of Norit A to a small aliquot of the incubation medium that had been transferred into 1 ml of 0.5 M KH_2PO_4 in 0.1 N HCl and counting of aliquots before and after the charcoal addition (13). The incubation was stopped by application of a part (20–50 μl) of the incubation mixture in a 4-cm band to a thin-layer chromatography plate coated with polyethyleneimine cellulose (Serva, Heidelberg, Germany) (14); the rest was frozen, and aliquots were chromatographed as needed. The plates were developed at 2–4°

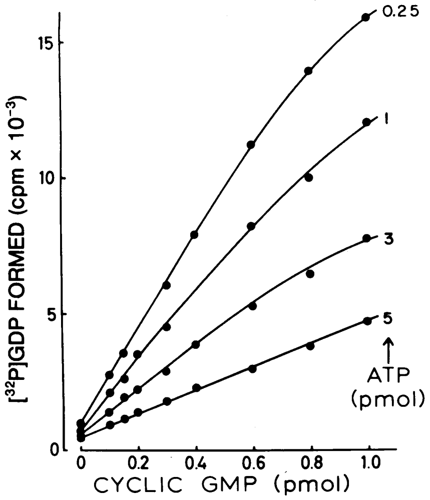

FIG. 1. Formation of [^{32}P]GDP from cyclic GMP with different amounts of ATP. 0.1–1 pmol of cyclic GMP was incubated with phosphodiesterase and thymus GMP kinase as described in the text. 0.25–5 pmol of ATP (37,000 cpm [^{32}P]ATP) were used per tube as indicated.

with 0.8–1 M LiCl solution as solvent. ATP (R_F about 0.1) was detected in a reference band by UV light. The coating material in the ATP band was scraped from the plate, and ATP was eluted by shaking successively with three 1-ml portions of a 2 M KCl solution. After addition of ethanol to a final concentration of 50%, the eluate was stored at −20°, where most of the KCl was precipitated. The supernatant was used in the assay after dilution and addition of cold ATP.

The specific activity of the [^{32}P]ATP varied between 100 and 200 Ci/mmol depending on the ratio $^{32}P_i$/ATP used and on the efficiency of phosphate incorporation. At least 99.7% of the incorporated ^{32}P could be split off by incubation with myosin and then could be precipitated as inorganic phosphate.

Other Materials. [*8*−^{3}H]Guanosine 3′:5′-cyclic monophosphate (24.6 Ci/mmol) and [*8*−^{3}H]adenosine 3′:5′-cyclic monophosphate (28.8 Ci/mmol) were purchased from ICN and were repurified on thin-layer chromatography plates coated with polyethyleneimine cellulose (14). The eluted cyclic nucleotides were stored in 50% ethanol at −20°. Other nucleotides were obtained from Boehringer Mannheim Corp., Sigma, or Nutritional Biochemical Corp. Dowex-50 was bought from Bio-Rad Laboratories as AG-50×8, 100–200 mesh, H$^+$ form. QAE-Sephadex A-25 was purchased from Pharmacia in the Cl$^-$ form and converted to the formate form by successive treatments with 0.2 N NaOH until Cl$^-$-free and then with 0.2 N formic acid until the pH of the effluent was 2.5. The phosphodiesterase inhibitor 1-methyl-3-isobutylxanthine (SC-2964) was a gift of G. D. Searle and Co. Carbachol and *l*-norepinephrine bitartrate were purchased from Sigma.

Preparation and Incubation of the Tissue. Male albino rats weighing 250–450 g were decapitated and ductus deferentes (100–120 mg of tissue from one rat) were removed and carefully freed of sperm, fat, and connective tissue. After weighing, the tissue was incubated with shaking for 30 min at 36.5° in 10 ml of an O_2-gassed, balanced salt solution (15). The tissue was then transferred for the time indicated to flasks containing fresh medium with additions as indicated.

Extraction and Purification of Cyclic Nucleotides. The tissue was fixed by freezing in liquid nitrogen-cooled aluminum clamps. The frozen tissue was broken into small pieces in a N_2-cooled mortar and then homogenized in a conical ground-glass homogenizer (Duall size 23, Kontes Glass Comp.) in 2 ml of 50% ethanol cooled to −20° to which were added 200 μl of a solution containing 1 M zinc acetate and tracer amounts of tritiated cyclic AMP and cyclic GMP (about 15 nCi of each). The homogenate was transferred to a centrifuge tube, and the homogenizer was rinsed with 2 ml of 50% ethanol which are then added to the homogenate. After centrifugation for 10 min at 50,000 × *g*, 100 μl of 2 M Na_2CO_3 were added to the supernate. While most of the 5′-nucleotides are coprecipitated with the $ZnCO_3$ formed (16), little if any cyclic AMP and only about 15% of the cyclic GMP are precipitated. After centrifugation for 10 min at 50,000 × *g*, the supernatant (followed by 6 ml of H_2O) was filtered through a 0.7 × 1.5-cm column of neutral Dowex-50 (in the NH_4^+ form) to remove all Zn^{++}.

The cyclic nucleotides were further purified on a 0.7 × 4-cm column of QAE-Sephadex A-25 in the formate form. The application of the sample was followed by 10 ml of H_2O and then by 6 ml of 0.1 M ammonium formate adjusted to pH 9.0 with NH_4OH. The eluting fluid was then changed to 0.1 M ammonium formate adjusted to pH 6.0 with formic acid, and the first 5.5 ml, containing cyclic AMP, were collected. The following 3 ml were discarded, and the next 5.5 ml of the eluate, containing cyclic GMP, were collected. The cyclic nucleotide fractions were lyophilized, and the dried samples were taken up in 0.5–1 ml of H_2O or dilute buffer. The overall recoveries, which are 60–70% for cyclic AMP and 40–50% for cyclic GMP, were determined by counting aliquots of the samples in "tT-21" scintillation fluid (17) in a liquid scintillation spectrometer. Cyclic AMP was determined by a modified procedure (4) using a phosphate-generating, enzymatically cycling system (18).

General Procedure for Cyclic GMP Determination. Cyclic GMP was hydrolyzed by 0.7–1 μg of phosphodiesterase to GMP

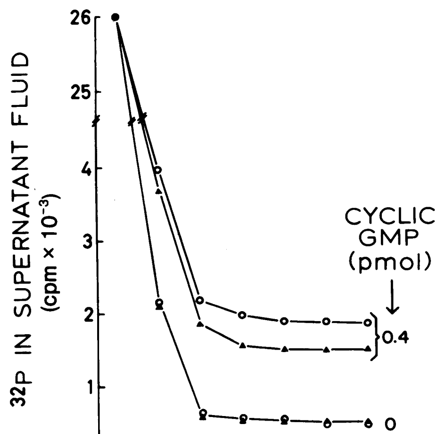

FIG. 2. Effect of myosin on ^{32}P measured in the supernatant fluid after P_i precipitation. 0 or 0.4 pmol of cyclic GMP were incubated with phosphodiesterase and pig-brain GMP kinase and 5 pmol (26,000 cpm) of [^{32}P]ATP as described in the text. 10 mM Ca^{++} was included (O) or omitted (▲) for the final incubation, which was performed with different amounts of myosin.

by incubation of 50 μl of sample with 50 μl of a solution containing 7.8 mM $MgCl_2$, 260 mM KCl, 0.52 mM EDTA, and 130 mM TES buffer (pH 7.5), and 10 μl of H_2O or of cyclic GMP standard solution. After 30 min of incubation at 30°, the tubes were stoppered and heated for 3 min in a boiling water bath. Then 10 μl of diluted GMP kinase (4 μg of the pig brain enzyme or about 2 μg of the calf-thymus enzyme) and 10 μl of a solution containing 5 pmol of carrier ATP and 15–20 nCi of [^{32}P]ATP were added. After incubation for two more hours, 20 μl of a diluted myosin solution (about 10 μg of protein) including 80 mM $CaCl_2$ were added and the tubes were incubated at 30° for 30 min. The incubation was stopped by the addition of 500 μl of a solution containing 2 mM KH_2PO_4 and 1 μM GDP, and the tubes were placed in an ice bath. P_i was precipitated according to the procedure of Sugino and Miyoshi (19) by addition of 500 μl of a freshly prepared mixture consisting of 1 volume of 1.2 N perchloric acid containing 40 mM ammonium molybdate and 1 volume of 60 mM triethylamine adjusted to pH 5 by HCl. After low-speed centrifugation, 1 ml of the supernatant fluid was transferred to another tube. The precipitation was repeated by addition of 200 μl of 2 mM KH_2PO_4 and 1 μM GDP, and the tubes were centrifuged. 1 ml of the supernatant fluid was added to 15 ml of a 0.1% aqueous solution of 7-amino-1,3-naphthalene-disulfonate (20) and the Čerenkov-radiation of the [^{32}P] GDP was detected in a liquid scintillation counter.

Each purified sample was incubated in duplicate tubes without phosphodiesterase, with phosphodiesterase, and with phosphodiesterase plus an internal standard. The variation of values obtained for a given sample repeatedly assayed was generally under 10%.

RESULTS

Assay Sensitivity. Under regular conditions, with 5 pmol of ATP, the blank counting rate is doubled by 0.1 pmol of cyclic GMP; with 0.25 pmol of ATP about 0.05 pmol of cyclic GMP causes a doubling of the basal counting rate (Fig. 1). Standard curves are virtually linear between 0.1 and 1 pmol of cyclic GMP per tube when 5 pmol of ATP are used, but are nonlinear with lower amounts of ATP. The percent of cyclic GMP converted to GDP also depends on the amount of ATP used. In the presence of 5 pmol of ATP, 60–70% of the cyclic GMP is converted to GDP. By reducing the amount of unlabeled ATP while keeping [^{32}P]ATP constant, the amount of ^{32}P incorporated into GDP is increased, although the percent of cyclic GMP converted to GDP is reduced. Thus, by increasing the specific activity of the ATP and by using parabolic standard curves, the sensitivity of the assay can be increased.

TABLE 1. *Influence of various nucleotides on the GMP kinase reaction*

	0 pmol of cyclic GMP	0.2 pmol of cyclic GMP
Control	336 ± 5	926 ± 22
AMP (100 pmol)	650 ± 1	1203 ± 15
IMP (100 pmol)	369 ± 8	981 ± 22
XMP (100 pmol)	426 ± 3	988 ± 7
CMP (100 pmol)	314 ± 5	915 ± 9
UMP (100 pmol)	472 ± 4	1023 ± 8
TMP (100 pmol)	337 ± 8	924 ± 11
dAMP (100 pmol)	316 ± 3	922 ± 21
NAD^+ (100 pmol)	284 ± 10	932 ± 10
NADH (100 pmol)	298 ± 18	877 ± 27
$NADP^+$ (100 pmol)	312 ± 11	991 ± 38
CoA (100 pmol)	1212 ± 2	1859 ± 11
GTP (100 pmol)	1430 ± 33	2402 ± 115
GDP (20 pmol)	7053 ± 292	7134 ± 104
ADP (20 pmol)	255 ± 15	231 ± 2

^{32}P-transfer from [^{32}P]ATP to myosin-stable products was determined in the absence or in the presence (0.2 pmol) of cGMP. The incubation with GMP kinase from calf thymus and phosphodiesterase was performed with 2 pmol of ATP (8600 cpm). GDP and GTP were purified by thin-layer chromatography before use. The values are the counts remaining in the supernatant fluid after precipitation of the $^{32}P_i$ released during the incubation with myosin (mean ± SEM of three determinations).

TABLE 2. *Influence of carbachol and choline on cyclic nucleotide content in ductus deferens of the rat*

	Cyclic GMP	Cyclic AMP
	pmol/g of wet weight	
Control	51.1 ± 1.9 (19)	870 ± 37 (19)
Carbachol 0.5 min	108.2 ± 2.9 (3)*	910 ± 81 (3)
Carbachol 1 min	122.1 ± 4.4 (3)*	909 ± 132 (3)
Carbachol 2 min	144.4 ± 10.3 (15)*	833 ± 37 (15)
Carbachol 5 min	131.3 ± 8.6 (3)*	771 ± 86 (3)
Carbachol 10 min	106.9 ± 7.3 (4)*	838 ± 83 (4)
Choline 2 min	56.8 ± 8.6 (4)	895 ± 24 (5)

Pairs of ductus deferentes were incubated as described under *Methods* for the time indicated in medium containing no additions (control), 0.1 mM carbachol, or 0.1 mM choline. Results are given as means ± SEM with the number of samples in parentheses.

* $P < 0.05$ if compared to control.

Assay Blank. The sensitivity of the assay is limited by the blank that is measured in the absence of cyclic GMP. The most troublesome source of blank is the nucleotide content of the GMP kinase preparations. The blank caused by the GMP kinase preparations from pig brain is generally substantially higher than that caused by the calf-thymus enzyme preparations. The blank contributed by phosphodiesterase is very small if the samples are boiled before the addition of GMP kinase and [^{32}P]ATP. With some phosphodiesterase preparations, substantial blanks were observed if phosphodiesterase, GMP kinase, and ATP were simultaneously added. The blank contributed by nonmyosin-degradable contaminants of the [^{32}P]ATP is very small if ATP is prepared as described above. Commercial preparations of [^{32}P]ATP generally yielded troublesome blanks. An increased sensitivity will be possible when further purification of the enzymes is obtained. Attempts to reduce the nucleotide content of GMP kinase preparations have so far not been successful. These attempts have included treatment of the enzyme preparations with charcoal and anion-exchange resins.

Reversibility of the GMP Kinase Reaction. Myosin effectively degrades ATP, but does not attack GDP detectably under the conditions of the assay. Nevertheless, the amount of [^{32}P]GDP finally counted can be affected by the myosin because the GMP kinase present during the final myosin step can catalyze the backward reaction [^{32}P]GDP + ADP → GMP + [^{32}P]ATP, and this reaction is favored by the

TABLE 3. *Influence of norepinephrine and SC-2964 on cyclic nucleotide content in ductus deferens of the rat*

	Cyclic GMP	Cyclic AMP
	pmol/g of wet weight	
Control	55.2 ± 3.9 (19)	982 ± 36 (18)
Norepinephrine 0.33 min	56.7 ± 3.9 (4)	1107 ± 34 (4)
Norepinephrine 1 min	71.0 ± 6.1 (8)*	1037 ± 47 (8)
Norepinephrine 3 min	80.6 ± 8.0 (11)*	1087 ± 45 (12)*
Norepinephrine 10 min	80.3 ± 7.1 (8)*	1139 ± 42 (7)*
SC-2964 3 min	128.6 ± 8.7 (8)*	1503 ± 31 (6)*

Pairs of ductus deferentes were incubated for the time indicated in medium with no addition (control), with 0.01 mM norepinephrine, or with 0.1 mM SC-2964. The results are given as means ± SEM with the number of samples in parentheses.

* $P < 0.05$ if compared to control.

myosin-induced formation of ADP. This reaction appears to be inhibited by Ca^{++}, since Ca^{++} completely inhibited the degradation of purified [β-^{32}P]GDP that was incubated with GMP kinase, ADP, unlabeled ATP, and myosin under otherwise usual assay conditions. No degradation of [^{32}P]-GDP could be detected if GMP kinase was omitted. The inclusion of 10 mM Ca^{++} for the final myosin step did not affect the blank measured in the absence of cyclic GMP, but it did increase by almost 50% the amount of [^{32}P]GDP recovered after reaction of 0.4 pmol of cyclic GMP and 5 pmol of ATP (Fig. 2).

Assay Specificity. The specificity of the assay is assured by chromatographic purification of samples to be assayed and by the high substrate specificities of the cyclic nucleotide phosphodiesterase and of the ATP:GMP phosphotransferase. The possibilities for interference by other nucleotides was tested as follows. Several nucleoside monophosphates were included in the assay system in high amounts (100 pmol per tube), with 2 pmol of [^{32}P]ATP in the absence and presence of cyclic GMP (0.2 pmol per tube) (Table I). Despite the high excess of the monophosphates, none of these compounds yielded a labeled, myosin-stable product containing more than 4% of the available terminal phosphate group of the [^{32}P]ATP. The conversion of cyclic GMP to [^{32}P]GDP was not affected by any of the monophosphates. NAD^+, $NADP^+$, and NADH did not yield myosin-stable radioactive products or affect the conversion of cyclic GMP to [^{32}P]GDP. With coenzyme A (100 pmol per tube), about 10% of the terminal phosphate of the [^{32}P]ATP was incorporated into a myosin-stable product. When 100 pmol of GTP were added, a small ^{32}P incorporation was observed although the compound had been purified by thin-layer chromatography on polyethyleneimine cellulose (14). None of the compounds yielding phosphorylated, myosin-stable products required the presence of phosphodiesterase for ^{32}P incorporation. The small effects observed with all the above compounds are probably due to small side activities of the GMP kinase for other nucleoside monophosphates or to contaminations with other nucleotides (e.g., of the GTP and perhaps of the CoA).

Two other compounds directly affected the GMP kinase reaction. Addition of GDP (20 pmol per tube, also purified by thin-layer chromatography) resulted in the transfer from [^{32}P]ATP of about 80% of the labeled phosphate, most likely due to an exchange reaction of the terminal phosphate groups between ATP and GDP catalyzed by the GMP kinase. ADP (20 pmol per tube), however, reduced the counting rate observed in the absence of cyclic GMP as well as a complete prevention of the conversion of cyclic GMP to [^{32}P]GDP. This apparent inhibition of GMP phosphorylation by ADP could also be observed with much lower amounts of ADP.

Cyclic Nucleotide Concentrations in Rat Ductus Deferens. Cyclic nucleotide concentrations were measured in segments of rat ductus deferentes. Basal cyclic GMP concentrations were about 55 pmol/g of wet weight; those of cyclic AMP were 900–1000 pmol/g. When the tissue was incubated with 0.1 mM carbachol—a concentration that is almost maximally effective with regard to stimulating contraction—cyclic GMP was rapidly increased. The content of this cyclic nucleotide was doubled 30 sec after addition of carbachol and reached a maximum within about 2 min with a subsequent slow decline (Table 2). Cyclic AMP concentrations were not significantly affected. Choline (0.1 mM), which does not induce a contraction of the ductus deferens, did not change the cyclic nucleotide content measured 2 min after its addition.

The effect of norepinephrine on cyclic nucleotide content was also studied in this tissue (Table 3). Norepinephrine was added in a 0.01 mM concentration, which is approximately half maximally effective with respect to contraction. Cyclic GMP concentrations were increased by 25% after 1 min and by 40% after 3 and 10 min. Consistent small elevations of cyclic AMP concentrations were observed with this concentration of norepinephrine.

Incubation of the tissue for 3 min with the phosphodiesterase inhibitor SC-2964 (0.1 mM) more than doubled cyclic GMP content, but increased cyclic AMP concentrations by only 50% (see Table 3).

DISCUSSION

Within the last few years several methods have been described for determination of cyclic GMP concentration. In their principles, these assays are very similar to methods that are in use for determination of cyclic AMP concentration. The assays for cyclic GMP are based on enzymatically cycling systems (3, 4, 18), activation of cyclic GMP-stimulated protein kinases (21), or competition for binding by a protein kinase (22) or by a specific antibody (23). The principle of the assay described in the present paper is similar to that of a method that has been described by Turtle and Kipnis (24) for the determination of cyclic AMP, but that has not found wide application.

Only two of the assays mentioned above, the cycling system described by Goldberg *et al.* (3) and the radioimmunoassay of Steiner *et al.* (23) allow the determination of amounts as small as 0.1 pmol of cyclic GMP per tube. With regard to sensitivity, the assay described in this paper is comparable to these two assays. It is probably faster and less laborious than methods involving enzymatic cycling. While it requires more purification of tissue extracts than is necessary for radioimmunoassay or protein-binding assay, the assay presented here has the advantage of a linear standard curve, and it probably allows the detection of smaller changes.

The specificity of the assay for cyclic GMP is assured by the ion-exchange chromatographic separation of this cyclic nucleotide, by including tubes of each sample not treated with phosphodiesterase, and by the substrate specificity of the GMP kinase. The inclusion of internal standards of cyclic GMP with each sample gives additional reassurance that inhibitory substances such as ADP or heavy metals are excluded by the chromatographic purification of the samples. The method, however, does not allow the differentiation between cyclic GMP and deoxyguanosine 3′:5′-cyclic monophosphate. The deoxy-derivative must be assumed to cochromatograph with cyclic GMP and to react like cyclic GMP in the enzymatic procedure. There is, however, no indication yet for the natural occurrence of deoxy-derivatives of cyclic AMP and cyclic GMP.

The principle of this method appears to be applicable to determinations of other nucleoside monophosphates. ATP: NMP phosphotransferases are not only available with relatively high specificity for AMP and GMP, but also for pyrimidine nucleotides. Since GDP has also been shown to incorporate ^{32}P by enzymatic exchange of the terminal phosphates with labeled ATP, the principle of the method can also be applied for a sensitive determination of GDP and, with use of other nucleoside monophosphate kinases, of other nucleoside diphosphates.

Cyclic GMP concentrations have recently been found to be altered by cholinergic agents in several tissues. Stimulation of muscarinic cholinergic receptors caused a rapid elevation of cyclic GMP content in perfused hearts (25), heart and brain slices (21), intestinal smooth muscle (26, 27), and slices of thyroid (28) and submaxillary glands (27), while cyclic AMP concentrations were not affected or were only slightly decreased. In the present work, contraction-producing cholinergic stimulation of the ductus deferens also led to an increase of cyclic GMP content.

Low concentrations of norepinephrine also caused a significant increase in cyclic GMP content of rat ductus deferens while cyclic AMP concentrations were only slightly increased. In some experiments atropine has caused a partial reduction of the effect of norepinephrine on cyclic GMP content while having no effect on the contraction produced by this hormone. Thus, it is not clear yet if the effect of norepinephrine on cyclic GMP content is a direct one or if it is mediated by the release of endogenous acetylcholine from nerve endings in the tissue. As conditions involving increased concentrations of intracellular free calcium are connected with increased cyclic GMP content (27), it is conceivable that α-adrenergic stimulation of the ductus deferens does cause a Ca^{++}-mediated increase in cyclic GMP. Whether or not increased cyclic GMP content—in combination with increased intracellular Ca^{++} concentration—is involved in the contractile response of the tissue to hormonal stimulation is unknown.

Incubation of the ductus deferens for 3 min with the phosphodiesterase inhibitor SC-2964 caused increases in the concentrations of both cyclic nucleotides. The effect on cyclic GMP, however, was much more pronounced than that on cyclic AMP. Unlike acetylcholine and norepinephrine, SC-2964 causes relaxation of the contracted ductus deferens. The observation that agents causing either contraction or relaxation of the ductus deferens can raise cyclic GMP concentration suggests that if this cyclic nucleotide plays a role in smooth muscle function, it does so by interacting with other regulatory factors, e.g., with cyclic AMP and Ca^{++}.

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