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CALCIUM-DEPENDENT MODULATION OF GUANOSINE 3′,5′-MONOPHOSPHATE IN RENAL CORTEX

POSSIBLE RELATIONSHIP TO CALCIUM-DEPENDENT RELEASE OF FATTY ACID*

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Abstract—The effects of Ca^{2+} on cGMP accumulation in rat renal cortical slices were correlated with the effects on ^{14}C-fatty acid release in tissue prelabeled with ^{14}C arachidonate. Ca^{2+} in the presence and absence of ionophore A23187 exerted parallel effects on the release of labeled arachidonate from slices and on slice cGMP content. Thus, Ca^{2+} stimulated both arachidonate release and tissue cGMP accumulation 2 to 3-fold when added to slices of renal cortex previously deprived of Ca^{2+} and Mg^{2+}, whereas Mg^{2+} had no stimulatory effect on either arachidonate release or tissue cGMP content. In the presence of A23187, Ca^{2+} increased arachidonate release and tissue cGMP accumulation 4 to 6-fold. Tetracaine partially inhibited Ca^{2+}-induced arachidonate release and completely blocked Ca^{2+}-induced cGMP accumulation. Ca^{2+}-induced arachidonate release was unaffected by the absence of O_2. Addition of exogenous arachidonate to slices of renal cortex increased tissue cGMP content 2-fold. Linoleate exerted a lesser effect on tissue cGMP, while palmitate and oleate had no effect. Ca^{2+}- and arachidonate-induced cGMP contents in renal cortical slices were not additive, and both were abolished by exclusion of O_2. Since nitroprusside increased cGMP accumulation 10- to 15-fold in O_2-deprived slices, loss of the Ca^{2+} and arachidonate responses under these incubation conditions was selective. Ca^{2+}-induced cGMP accumulation was unaffected by indomethacin (100 μg/ml), but was abolished by 200 μM 5,8,11,14-eicosatetraynoic acid (TYA). The results are consistent with the possibility that the Ca^{2+}-dependent processes regulating cGMP in renal cortex include Ca^{2+}-dependent acyl hydrolase activity, which limits the availability of free polyunsaturated fatty acids. A role for fatty acid oxygenation products in the stimulation of cGMP is suggested, but not established, by the O_2 dependence of the actions of both Ca^{2+} and exogenous fatty acids. The failure of exogenous arachidonate or linoleate to mimic quantitatively the actions of Ca^{2+} on cGMP may reflect the involvement of other Ca^{2+}- and O_2-dependent processes in modulation of cGMP in this tissue or limited access of exogenous fatty acid to cGMP regulatory sites in the cell.

Previous studies in this laboratory have demonstrated that guanosine 3′,5′-monophosphate (cGMP)† content of intact renal cortex is modulated by mechanisms dependent on both extracellular Ca^{2+} and molecular O_2 [1–3]. Exclusion of extracellular Ca^{2+} from incubations of rat renal cortical slices results in a significant decline in tissue cGMP content [2, 3]. Tissue deprived of Ca^{2+} also fails to exhibit increased cGMP accumulation in response to the divalent cation ionophore A23187, carbamylcholine, histamine or bradykinin [1–3]. Addition of Ca^{2+} to Ca^{2+}-deprived tissue restores cGMP content. However, cGMP responses to Ca^{2+} and Ca^{2+}-dependent agonists occur only in the presence of molecular O_2 [3]. The effects of Ca^{2+} and O_2 deprivation on renal cortical slice cGMP are not due to GTP depletion or cell injury, since nitroprusside, nitrite and *N*-nitroso compounds produce clear cGMP increases in Ca^{2+}- and O_2-deprived tissues [1, 3, 4]. The modulation of cGMP by Ca^{2+}- and O_2-dependent processes has similarly been observed in umbilical artery [5] and renal medulla [3].

Recent work in subcellular preparations has demonstrated that oxygenated derivatives of unsaturated fatty acids activate guanylate cyclase from platelets [6], spleen [7, 8] and lung [9], and it has been suggested that these species might act as endogenous modulators of cGMP [8]. In intact cells, the activity of lipase on cellular lipids might be a key determinant of the availability of polyunsaturated fatty acid for oxygenation [10, 11]. These considerations are of interest with regard to Ca^{2+}- and O_2-dependent regulation of cGMP, since the activity of at least some tissue lipases exhibits Ca^{2+} dependence in subcellular preparations [10, 12–15]. Recent studies also support the existence of hormone-responsive phospholipase activity in kidney [11, 16].

Accordingly, in the present study we examined

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† Abbreviations used: cGMP, guanosine 3′5′-monophosphate; TYA, 5,8,11,14-eicosatetraynoic acid; KRBG, Krebs–Ringer bicarbonate medium with glucose; EGTA, ethyleneglycol-bis (β-amino ethyl ether) *N*,*N*′-tetra-acetate; MIX, 1-methyl-3-isobutylxanthine; 16:0, sodium palmitate, sodium hexadecanoate; 18:1 ω9, oleate, sodium 9-octadecenoate; 18:2 ω6, linoleate, sodium, 9,12-octadecadienoate; and 20:4 ω6, arachidonate, sodium 5,8,11,14-eicosatetraenoate.

the possibility that Ca^{2+}-dependent cGMP accumulation in rat renal cortex might be related to Ca^{2+}-induced unsaturated fatty acid release and the subsequent oxygenation of these fatty acids.

MATERIALS AND METHODS

Materials. Arachidonic acid was purchased from NuChek Prep, Elysian, MN. [1-^{14}C]Arachidonic acid and ACS scintillation-counting solution were obtained from the Amersham Searle Co., Arlington Heights, IL. A23187 was a gift from Dr. Robert L. Hamill of the Lilly Research Laboratories, Indianapolis, IN. TYA was provided by Dr. W. E. Scott of Hoffmann-LaRoche Inc., Nutley, NJ. Other materials were obtained from sources cited in previous publications [1–3].

Experimental animals and tissue preparation. Female Sprague–Dawley rats weighing 300–400 g were obtained from the Zivic-Miller Laboratories, Pittsburgh, PA. Animals were anesthetized with sodium pentobarbital (50 mg/kg). The kidneys were excised and placed in ice-cold saline. After removal of the renal capsule, cortical slices were prepared with a Stadie–Riggs microtome and kept at 4° on filter paper saturated with 0.9% NaCl until use (within 1 hr).

Incubation of slices and cGMP determination. For determination of cGMP, slices were incubated in an atmosphere of 95% O_2 plus 5% CO_2 at 37° in 2 ml Ca^{2+}- and Mg^{2+}-free KRBG containing 0.5 mM EGTA, 2 mM 1-methyl-3-isobutylxanthine (MIX) and 1 mg/ml fatty acid-free bovine serum albumin (Sigma Chemical Co., St. Louis, MO). Ionophore A23187 was present at a concentration of 10 or 20 μM, where indicated. When anaerobic conditions were imposed, flasks were gassed vigorously with 95% N_2 plus 5% CO_2 10 min prior to addition of cGMP agonists and again at the time of agonist addition. Indomethacin, fatty acids and A23187 were dissolved in ethanol (final ethanol concentration, 0.5%). Controls received an equivalent amount of ethanol. Incubations were terminated by transferring tissues slices to 50 mM socium acetate (pH 4.0) at 95°. cGMP was extracted and then determined by radioimmunoassay after acetylation [17], as described previously [2, 3, 18].

Measurement of the release of [^{14}C]*arachidonate from slices of renal cortex.* A total of 140–160 mg of renal cortex was placed in 2 ml of Ca^{2+}- and Mg^{2+}-free KRBG containing 2 mM EGTA and 0.25 μCi [1-^{14}C]arachidonate, in the presence or absence of 10 μM A23187. Preincubations were conducted at 37° in an atmosphere of 95% O_2-5% CO_2 for 30 min. Labeled slices were then subjected to a 5-min wash in 2 ml of Ca^{2+}- and Mg^{2+}-free KRBG containing 0.5 mM EGTA and 5 mg/ml fatty acid-free bovine serum albumin in the presence or absence of 20 μM A23187. Slices were then transferred to a medium identical to the wash medium, and incubation was continued, with periodic monitoring of radioactivity released into the incubation medium. Fresh medium of composition similar to that being sampled was added back at the time of each aliquot (50 μl). The rate of the release of arachidonate is expressed as a per cent of total radioactivity (tissue plus medium) per min in order to provide a measure of label release which is independent of variables such as the total uptake of radioactivity during the prelabeling period and minor variations in the ratio of tissue weight to medium volume.

Extraction of lipids from tissue and medium. At the end of the experiment, tissue was removed and homogenized in 20 vol. of $CHCl_3$–CH_3OH (2:1) containing 1 mg of butylated hydroxytoluene/liter. Medium for lipid analysis (1.8 ml) was mixed with 0.1 ml of 0.1 M EDTA and 5 μl of 0.1 M butylated hydroxytoluene and centrifuged. Radioactivity in tissue and the 10,000 *g* supernatant fraction of medium were determined by liquid scintillation counting in ACS. Quenching was estimated by use of [1-^{14}C]benzoic acid (New England Nuclear, Boston, MA) as an internal standard. The medium supernatant fraction was extracted with $CHCl_3$–CH_3OH essentially by the method applied to platelet incubation mixtures by Bills *et al.* [19]. Lipid extracts of tissue and medium were made up in $CHCl_3$ and loaded on 0.5 g columns of Unisil (100–200 mesh, Clarkson Chemical Co., Williamsport, PA). Successive elutions with 5 ml $CHCl_3$–CH_3OH (19:1), 6 ml CH_3OH and 4 ml CH_3OH–H_2O (99:1) yielded fractions termed neutral lipid, prostaglandin and phospholipid (last two solvents), respectively [19].

Assessment of fatty acid uptake. Uptake of fatty acid from the medium was assessed under conditions nearly identical to those pertaining during measurement of Ca^{2+}-induced fatty acid accumulation in the incubation medium. Slices were preincubated for 30 min in Ca^{2+}- and Mg^{2+}-free KRBG containing EGTA and A23187. They were then transferred to Ca^{2+}- and Mg^{2+}-free KRBG containing 0.5 mM EGTA, A23187, and 5 mg/ml fatty acid-free bovine serum albumin. After a 40-min incubation, Ca^{2+} or arachidonate was added, followed in 30 sec by 0.05 μCi [1-^{14}C]arachidonate. Slices were removed after 3 or 7 min of incubation with labeled arachidonate, washed twice in medium containing albumin, and homogenized in counting solution. Rates of uptake were determined from the differences in tissue radioactivity between 3 and 7 min for quadruplicate slices at each time point. Under these conditions the rate of uptake was observed to be proportional to arachidonate added over the concentration range 1–200 μM.

Data presentation. Results are presented as means ± S.E. Where indicated, the statistical significance of differences between mean values from three or more separate experiments was assessed by the *t*-test for unpaired values. In some instances results from a representative experiment repeated twice are shown.

RESULTS

Comparison of Ca^{2+}*-induced cGMP accumulation and arachidonate release in slices of renal cortex.* When incubated with [1-^{14}C]arachidonate, renal cortical slices incorporated most of the label into lipids. In a typical incubation of 0.25 μCi arachidonate with 150 mg of renal cortex, 40 per cent of the added label could be recovered from the tissue after 30 min. The total radioactivity in medium plus tissue

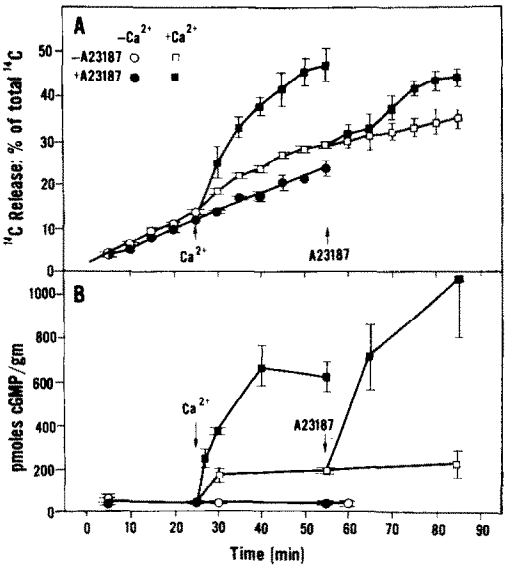

Fig. 1. Panel A: accumulation of label in medium bathing renal cortical slices prelabeled with [1-^{14}C]arachidonate: effects of Ca^{2+} and ionophore A23187. Slices of renal cortex weighing 150 ± 10 mg were incubated in 2 ml KRBG (no Ca^{2+}, no Mg^{2+}) containing 0.5 mM EGTA and 0.25 μCi [1-^{14}C]arachidonate. Gas phase: 95% O_2 + 5% CO_2. After 30 min, the slices were washed for 5 min in KRBG containing 0.5 mM EGTA and 5 mg/ml fatty acid-free bovine serum albumin. They were then transferred to fresh medium identical to the wash, and the appearance of ^{14}C in the medium was monitored. A23187 was absent from some samples (open symbols) and present at 20 μM in others (closed symbols). Ca^{2+} was absent (circles) or added at 2 mM at the indicated time (squares). ^{14}C appearing in the medium with time is expressed as a per cent of total radioactivity (tissue plus medium). A representative experiment is shown. Each value is the mean ± S.E. for duplicate incubates. Panel B: response of renal cortical slice cGMP to Ca^{2+} and ionophore A23187. Conditions of incubation were identical to those of panel A, except that slices weighed 50 ± 10 mg. A single representative experiment is shown. Each value is the mean ± S.E. for triplicate slices.

during a subsequent 1-hr incubation in fresh medium remained constant. One-third of the tissue radioactivity was present as unesterified fatty acid (1.5 ± 0.2 per cent, determined on four 150-mg pools of renal cortex). Ninety per cent or more of the label present in the incubation media was unesterified fatty acid, with at least 85 per cent of this identified chromatographically as arachidonate.

When tissue which had been prelabeled with arachidonate was incubated in the presence of fatty acid-free albumin (5 mg/ml), radioactivity was gradually transferred from the tissue into the medium (Fig. 1A). The rate of basal accumulation of radioactivity in the medium was not affected by excluding Mg^{2+} from the Krebs–Ringer buffer or by incubation with divalent-cation ionophore A23187 plus EGTA (not shown). A marked increase in the rate of accumulation of radioactivity in the medium occurred upon addition of Ca^{2+} (Fig. 1A). This effect of Ca^{2+} was most pronounced in the presence of A23187 and in the absence of Mg^{2+} (Fig. 1A). Increased accumulation of arachidonate label in the medium in response to Ca^{2+} was not prevented by the absence of oxygen or by the presence of a 2 mM concentration of the cyclic nucleotide phosphodiesterase inhibitor MIX (not shown). No enhancement of arachidonate label accumulation in the medium was elicited by 1.5 mM Mg^{2+}. The uptake of [^{14}C]arachidonate under conditions identical to those prevailing during measurement of net accumulation (5 mg/ml albumin) was negligible both before and after addition of Ca^{2+}, indicating that the effect of Ca^{2+} of increasing [^{14}C]arachidonate accumulation in the media was due to increased release of the fatty acid from the tissue. Accumulation of radioactivity in the incubation medium of renal cortical slices labeled with [^{14}C]arachidonate was also stimulated by addition of A23187 subsequent to Ca^{2+}.

The effects of Ca^{2+} or Ca^{2+} plus A23187 on the release of [^{14}C]arachidonate from tissue slices to the incubation medium (Fig. 1A) correlated with the effects of these agents on cGMP content of slices incubated under identical conditions (Fig. 1B). Thus, renal cortical slice cGMP showed a much greater increase in the presence of Ca^{2+} plus A23187 than in the presence of Ca^{2+} alone. Furthermore, addition of A23187 subsequent to Ca^{2} elicited an increase in renal cortical slice cGMP, whereas its addition prior to Ca^{2+} did not.

Table 1. Effects of tetracaine on Ca^{2+}-induced cGMP accumulation and arachidonate release by renal cortical slices incubated with or without ionophore A23187*

Initial additions		cGMP (pmoles/g wet wt)			Rate of ^{14}C release (% of total ^{14}C/min)		
1 mM Tetracaine	10 μM A23187	$-Ca^{2+}$	$+Ca^{2+}$	+/−	$-Ca^{2+}$	$+Ca^{2+}$	+/−
−	−	60 ± 2	135 ± 13†	2.3 ± 0.3	0.26 ± 0.01	0.65 ± 0.03†	2.5 ± 0.2
+	−	63 ± 11	63 ± 10‡	1.0 ± 0.1‡	0.28 ± 0.03	0.50 ± 0.04†‡	1.8 ± 0.1‡
−	+	47 ± 4§	367 ± 86†§	7.8 ± 1.8§	0.35 ± 0.07	1.55 ± 0.94†‡	4.4 ± 0.1§
+	+	36 ± 5‡§	37 ± 11‡§	1.0 ± 0.3‡	0.22 ± 0.09	0.45 ± 0.13‡	2.2 ± 0.5‡

* Slices were incubated as described in the legend of Fig. 1. When cGMP analyses were to be performed, reactions were teminated 5 min after the addition of 2 mM Ca^{2+}. Rates for the release of fatty acid label represent those for the first 10 min following addition of 2 mM Ca^{2+}. The rate of [^{14}C]arachidonate release was calculated as the per cent of total radioactivity (tissue plus medium) appearing in the medium. Values represent means ± S.E. for six incubates.

† $P < 0.05$ vs value without Ca^{2+}.

‡ $P < 0.05$ vs corresponding value in the absence of tetracaine.

§ $P < 0.05$ vs value in the absence of A23187.

Tetracaine, which has been shown previously to block Ca^{2+}- and Ca^{2+} plus A23187-induced cGMP accumulation in renal cortex [2], also inhibited Ca^{2+}-induced release of ^{14}C from slices labeled with arachidonate (Table 1). Tetracaine, at a concentration of 1 mM, completely blocked Ca^{2+}-induced cGMP accumulation, both in the absence and in the presence of A23187. In the presence of tetracaine, a 10-fold greater increase in renal cortical cGMP was routinely elicited by 10 mM nitroprusside, thus demonstrating that the effects of tetracaine on Ca^{2+}-induced cGMP accumulation were selective. Tetracaine also inhibited a significant proportion of Ca^{2+}-induced accumulation of [^{14}C]arachidonate in the incubation medium (Table 1). Indeed, in the experiment shown, 1 mM tetracaine abolished the increment of Ca^{2+}-induced accumulation of label observed in the presence of A23187.

Comparison of Ca^{2+} and arachidonate-induced cGMP accumulation of renal cortex. Of the fatty acids tested, only the polyunsaturated fatty acids, linoleate and arachidonate, detectably enhanced cGMP content of renal cortical slices deprived of Ca^{2+} (Table 2). A nearly 2-fold increase in cGMP was elicited by exogenous arachidonate under aerobic conditions. Under anaerobic conditions, no significant cGMP response to arachidonate or linoleate occurred (Table 2). Addition of Ca^{2+} under aerobic conditions resulted in a greater cGMP increase than did arachidonate. The cGMP increase in response to Ca^{2+} also failed to occur in the absence of O_2 (Table 2). In contrast to arachidonate and Ca^{2+}, 10 mM nitroprusside increased cGMP content of renal cortex deprived of O_2 (Table 2). However, in the presence of 1 mM tetracaine, arachidonate failed to increase cGMP accumulation (not shown).

A maximal cGMP response to 200 μM arachidonate occurred within 5–10 min of arachidonate addition to slices of renal cortex (Fig. 2A). In this incubation system (1 mg/ml albumin) concentrations of arachidonate from 100 to 200 μM were most effective in elevating slice cGMP content (Fig. 2B). Longer periods of incubation (40 min) and higher arachidonate concentrations (0.5–1 mM) resulted in diminished cGMP responses to arachidonate (Fig. 2). The cGMP responses to arachidonate also occurred in the absence of MIX. Five minutes after the addition of 200 μM arachidonate to renal cortical slices in the absence of MIX, tissue cGMP rose from 9 ± 1 to a maximum level of 13 ± 1 pmoles/g ($P < 0.05$ based on three experiments). The cGMP of renal cortex deprived of Ca^{2+} increased in response to Ca^{2+} to a degree significantly greater than in response to arachidonate (Table 2 and Fig. 3). Addition of arachidonate to Ca^{2+}-replete slices had no further effect on cGMP (Fig. 3). It is of note that 200 μM exogenous arachidonate significantly inhibited nitroprusside-responsive cGMP. By contrast, Ca^{2+}had no significant inhibitory effect on the cGMP response to nitroprusside (Fig. 3).

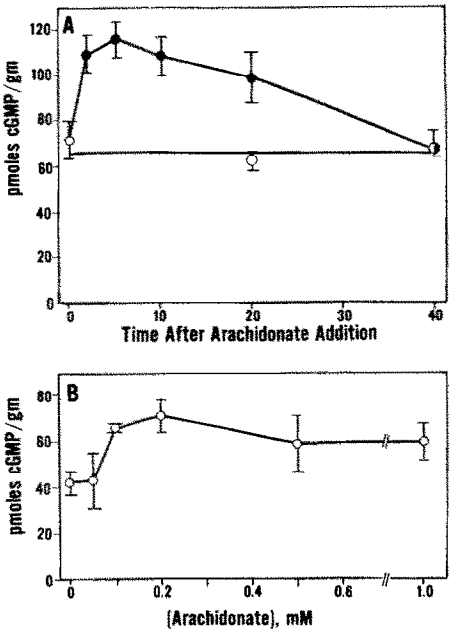

Fig. 2. Panel A: time course for renal cortical cGMP with (●) or without (○) 200 μM arachidonate. After a preincubation for 30 min in Ca^{2+}-free KRBG plus 0.5 mM EGTA, 2 mM MIX and 1 mg/ml fatty acid-free bovine serum albumin, arachidonate was added as its sodium salt in 40% ethanol (final ethanol concentration in control and arachidonate samples was 0.5%). Slices were removed at timed intervals thereafter. Panel B: renal cortical cGMP as a function of arachidonate concentration. Conditions of the experiment were as described in panel A and in the legend for Table 2. Values represent the means ± S.E.M. of six slices.

Table 2. Effects of fatty acids on cGMP content of renal cortical slices in the presence and absence of oxygen*

Incubation conditions	Final addition: None	16:0	18:1 ω9	18:2 ω6	20:4 ω6	Nitroprusside	Ca^{2+}
95% O_2, 5% CO_2	63 ± 2	60 ± 9	73 ± 6	77 ± 6†	97 ± 8†	1860 ± 220†	173 ± 34†
95% N_2, 5% CO_2	48 ± 4‡			49 ± 4‡	47 ± 3	620 ± 100†‡	41 ± 4‡

* Slices were incubated for 30 min in 2 ml of Ca^{2+}-free KRBG containing 1 mg fatty acid-free bovine serum albumin/ml, 0.5 mM EGTA and 2 mM MIX. Gas phase: 95% O_2, 5% CO_2. Fatty acids were then added as sodium salts in 40% ethanol for a final concentration of 200 μM (final ethanol concentration, 0.5%) and incubations were continued for an additional 5 min. Where shown, anaerobic conditions were imposed 10 min prior to fatty acid addition. Ca^{2+} (2.0 mM) and nitroprusside (10 mM) were also added for the final 5 min of the incubation, where indicated. Values represent the means ± S.E. of nine to fifteen slices.

† $P < 0.05$ vs no addition.

‡ $P < 0.05$ vs value in the presence of O_2.

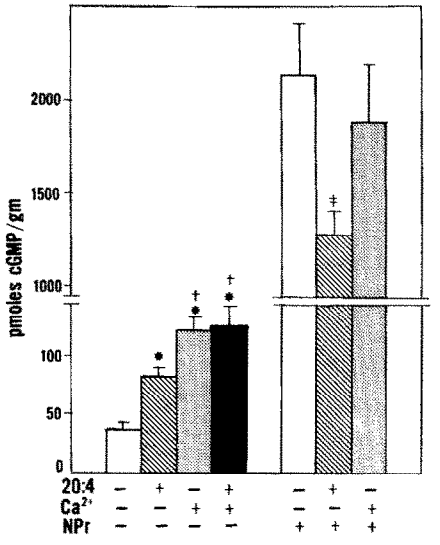

Fig. 3. Effects of arachidonate, Ca^{2+} and nitroprusside (NPr) on cGMP in renal cortical slices. Experiments were performed essentially as described in Table 2 except that 200 μM arachidonate and 2 mM Ca^{2+} were added 15 and 5 min, respectively, prior to the termination of the incubation. Values represent the means ± S.E.M. of nine slices. The asterisk (*) indicates $P < 0.05$ vs control without Ca^{2+} or fatty acid. The single dagger (†) indicates $P < 0.05$ vs value with arachidonate only. The double dagger (‡) indicates $P < 0.05$ vs value with NPr but no arachidonate.

Indomethacin, employed at a concentration previously shown to inhibit renal medullary PGE synthesis [20], failed to inhibit Ca^{2+}-induced cGMP accumulation (Table 3). Similarly, meclofenamate (50 μg/ml), a structurally distinct cyclo-oxygenase inhibitor [21], also did not suppress Ca^{2+}-induced increases in cGMP (not shown). By contrast, 200 μM TYA abolished the cGMP response to Ca^{2+}, whereas the action of nitroprusside on cGMP was diminished but still clearly expressed in the presence of TYA. Significant arachidonate-induced increases in cGMP accumulation were not evident in the presence of either indomethacin or TYA. Both indomethacin and TYA produced modest but variable increases in basal cGMP. These increases were not of statistical significance. Nevertheless, they may be related to the loss of a demonstrable action of arachidonate on cGMP in the presence of indomethacin or TYA, since absolute cGMP accumulation remained higher than control (no addition) values under these conditions (Table 3).

DISCUSSION

The results of the present study are consistent with the suggestion that mobilization of tissue polyunsaturated fatty acids may be one mode of Ca^{2+}-dependent regulation of renal cortical cGMP, but they do not provide direct evidence in support of this contention. They also emphasize the complexity of cGMP regulation. Clear correlations were evident between the effects of Ca^{2+}, A23187 plus Ca^{2+} and tetracaine on mobilization of tissue arachidonate and on tissue cGMP content (Fig. 1 and Table 1). Furthermore, of several fatty acids tested, arachidonate had the greatest effect on cGMP in renal cortex (Table 2). This action of arachidonate was not additive with that of Ca^{2+} (Fig. 2), and both Ca^{2+} and arachidonate failed to increase cGMP in the absence of O_2. The specificity for arachidonate or linoleate as compared to palmitate or oleate in eliciting an increase in renal cortical cGMP would suggest a role for fatty acid oxygenation systems [22], as opposed to the system of β-oxidation of fatty acids [28]. The oxygen requirement for Ca^{2+}- and arachidonate-dependent stimulation of cGMP probably does not relate to an obligatory β-oxidation of fatty acid for maintenance of ATP and GTP levels in renal cortex, since nitroprusside elicits an increase in cGMP in tissue deprived of O_2 (Table 2). The data are compatible with a role for Ca^{2+}-dependent arachidonate release and the subsequent oxygenation of this fatty acid in the expression of Ca^{2+}-induced increases in cGMP accumulation. TYA, an inhibitor of fatty acid hydroperoxide synthesis and prostaglandin synthesis [24], blocked Ca^{2+}-induced cGMP accumulation. By contrast, indomethacin, an inhibitor of prostaglandin synthesis alone [21], failed to block this action of Ca^{2+}. The differential effects of TYA and indomethacin suggest that oxygenation of released fatty acids to hydroperoxides could mediate Ca^{2+}- and O_2-dependent regulation of

Table 3. Effects of TYA and indomethacin on Ca^{2+}- and arachidonate-induced cGMP accumulation*

Initial addition	cGMP (pmoles/g wet wt) Final addition: None	2 mM Ca^{2+}	200 μM 20:4	10 mM Nitroprusside
None	64 ± 7	172 ± 17†	107 ± 7†	2570 ± 240†
Indomethacin (100 μg/ml)	99 ± 14	219 ± 46†	100 ± 11	2220 ± 200†
TYA (100 μM)	77 ± 7	158 ± 24†	95 ± 10	2260 ± 150†
(200 μM)	80 ± 14	90 ± 15‡	90 ± 11	810 ± 260†‡

* Experiments were conducted as described in the legend of Table 2. TYA or indomethacin was added in ethanolic solution (final ethanol concentration, 0.5%) at the start of the incubation. Control flasks received ethanol only. Arachidonate, Ca^{2+} or nitroprusside was added at 30 min for the final 5 min of incubation. Values represent means ± S.E. of six to nine slices.

† $P < 0.05$ vs corresponding value without a final addition.

‡ $P < 0.05$ vs corresponding value without an initial addition.

cGMP in renal cortex, a possibility consistent with the action of these fatty acid oxygenation products in stimulating guanylate cyclase directly in broken cell preparations [6–9]. However, other interpretations are clearly not excluded. It must be emphasized that the presence of lipoxygenase activity in renal cortex had not been demonstrated, and that the inhibitory effects of 200 μM TYA were not entirely specific for Ca^{2+}-induced cGMP accumulation, since the cGMP response to nitroprusside was also reduced by this agent (Table 3). Moreover, exogenous arachidonate and tetracaine had complex actions on renal cortical cGMP content. These included (a) the inability of exogenous arachidonate to elicit a tissue cGMP response comparable in magnitude to that resulting from addition of Ca^{2+}; (b) the apparent inhibitory influence by exogenous arachidonate on nitroprusside-responsive cGMP; and (c) an inhibition by tetracaine of the cGMP response to arachidonate. In part, these results may reflect a limited access of exogenous arachidonate to tissue cGMP regulatory sites. They may also reflect multiple actions of exogenous arachidonate and tetracaine. For example, tetracaine inhibits both arachidonate release and cGMP accumulation in response to Ca^{2+}, and has been shown to inhibit tissue phospholipase A_2 activity [25, 26]. Accordingly, tetracaine has been postulated to inhibit the generation of oxygenation products of polyunsaturated fatty acids by limiting free fatty acid availability [25]. Inhibition of Ca^{2+}-dependent acyl hydrolase might be directly related to suppression of Ca^{2+}-induced cGMP accumulation by tetracaine in renal cortex. However, tetracaine has other effects on Ca^{2+} homeostasis and cell metabolism which also could be involved in the expression of its inhibitory effects on cGMP [27–29]. This is suggested by tetracaine inhibition of the increases in cGMP induced by exogenous arachidonate, an effect not readily attributable to limited arachidonate availability.

Thus, the relationship between Ca^{2+}-induced release of free fatty acids and Ca^{2+}-induced increases in renal cortical cGMP accumulation remains uncertain. It is of interest, however, that a relationship between these two actions of Ca^{2+} has also been suggested by observations in other tissues. In the rat ductus deferens, mepacrine and TYA, putative inhibitors of phospholipase and lipoxygenase activities, respectively, have been shown to suppress Ca^{2+}-induced cGMP accumulation [30]. In platelets, A23187 increases the release of arachidonate from phospholipase [31]. This is correlated with A23187- and arachidonate-induced increases in platelet cGMP [32, 33], although in platelets it has been proposed that the action of arachidonate on cGMP may be expressed through mobilization of intracellular Ca^{2+} from the dense tubule system by an arachidonate oxygenation product [33]. It is evident that further studies are needed to define the role of fatty acid release and oxygenation in Ca^{2+}-induced cGMP accumulation in renal cortex and other tissues.

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