rapid communications

Arachidonic acid mobilizes calcium and stimulates prolactin secretion from GH_3 cells

RICHARD N. KOLESNICK, INES MUSACCHIO, COLETTE THAW, AND MARVIN C. GERSHENGORN
Endocrine Division, Department of Medicine, New York University Medical Center, New York, New York 10016

KOLESNICK, RICHARD N., INES MUSACCHIO, COLETTE THAW, AND MARVIN C. GERSHENGORN. *Arachidonic acid mobilizes calcium and stimulates prolactin secretion from GH_3 cells.* Am. J. Physiol. 246 (Endocrinol. Metab. 9): E458–E462, 1984.—Because arachidonic acid and/or its metabolites may be intracellular effectors of calcium-mediated secretion, we studied whether arachidonic acid added exogenously mobilizes calcium and stimulates prolactin secretion from GH_3 cells, cloned rat pituitary cells. Arachidonic acid caused efflux of ^{45}Ca from preloaded cells and stimulated prolactin secretion. The concentration dependencies of these effects were similar; stimulation was attained with 3 μM arachidonic acid. To determine indirectly whether these effects may be caused by arachidonic acid itself, not via conversion to metabolites, two experimental approaches were used. First, inhibitors of arachidonic acid metabolism, eicosatetraynoic acid and indomethacin, did not inhibit arachidonic acid-induced prolactin secretion. And second, α-linolenic acid, which cannot be converted to arachidonic acid, and linoleic acid, but not saturated fatty acids of equal chain length, stimulated ^{45}Ca efflux and prolactin secretion. These data demonstrate that arachidonic acid added exogenously causes Ca^{2+} mobilization and prolactin secretion from GH_3 cells and suggest that arachidonic acid itself, not via metabolism, may be a cellular regulator of prolactin secretion.

rat pituitary cells; cyclooxygenase/lipoxygenase inhibitors; fatty acids

ARACHIDONIC ACID is a naturally occurring cis-polyunsaturated fatty acid that is found primarily esterified to membrane lipids in mammalian cells. It has been suggested that stimulated release of arachidonic acid from lipids may be an early step in the mechanism of action of some secretagogues (23). Hence, arachidonic acid and/or its metabolites, namely prostaglandins, prostacyclins, thromboxanes, epoxides, and leukotrienes, may be cellular mediators of secretagogue action. Because the actions of certain secretagogues are associated with both accumulation of unesterified arachidonic acid and with changes in cellular calcium homeostasis (2), it has been proposed that arachidonic acid and/or one of its metabolites may act to mobilize calcium in order to mediate stimulus-induced secretion. In the majority of previous studies, arachidonic acid metabolites have been implicated as the biologically active factor(s) because inhibitors of arachidonic acid metabolism were shown to inhibit stimulated secretion, or administration exogenously of one or several metabolites was shown to reproduce the biological response (19). However, a number of studies have provided evidence in support of the notion that arachidonic acid itself may be biologically active and that it may act by mobilizing cellular and/or extracellular calcium (5, 22, 24). The role(s) of arachidonic acid and/or its metabolites in the regulation of secretion of hormones from the anterior pituitary gland is unclear (9).

In this report, we demonstrate that arachidonic acid added exogenously causes ^{45}Ca efflux and stimulates prolactin secretion from GH_3 cells, a cloned strain of rat pituitary tumor cells in which calcium has been shown to mediate secretion (7), and provide two lines of evidence that arachidonic acid itself, not via conversion to its metabolites, is biologically active.

MATERIALS AND METHODS

Fatty acids (Sigma Chemical, St. Louis, Missouri), as free acids, were dissolved in dimethyl sulfoxide and stored at −20°C under N_2; the final concentration of dimethyl sulfoxide added to the medium was 1%, which had no effect on prolactin secretion. [^{14}C]sucrose (3.6 mCi/mmol), [^{3}H]inulin (270 mCi/g), [^{3}H]arachidonic acid (90 Ci/mmol), and $^{45}CaCl_2$ (carrier-free) were purchased from New England Nuclear, Boston, MA. Liquid scintillation counting solution (Liquiscint) was purchased from National Diagnostics, Somerville, NJ. Reagents for prolactin radioimmunoassay were kindly supplied by the Pituitary Hormone Distribution Program of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases and Dr. A. F. Parlow, Baltimore, MD.

GH_3 cells, obtained from the American Type Culture Collection, Rockville, MD, were grown as monolayer cultures in Ham's F-10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum at 37°C as described (20) and were harvested with 0.02% ethylene-

0193-1849/84$1.50

diaminetetraacetate. Before an experiment, the cells were centrifuged at 180 *g* for 5 min and resuspended in a balanced salt solution (BSS): 135 mM NaCl, 4.5 mM KCl, 0.5 mM $MgCl_2$, 5.6 mM glucose, and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4 containing 1.5 mM $CaCl_2$ and then incubated for at least 20 min. BSS without added Ca^{2+} contained 3.0 ± 0.2 μM Ca^{2+} as measured by a metallochromic dye, arsenazo III (20).

For ^{45}Ca efflux experiments, cells were resuspended in BSS containing 1.5 mM $^{45}CaCl_2$ (3–5 μCi/ml; 1.0×10^6/0.15 ml) and stirred gently in a beaker for 45 min, at which time isotopic steady-state was attained (6). The cells were centrifuged at 180 *g* for 5 min and resuspended in fresh BSS containing no added $CaCl_2$ and 33 μM ethylene glycol-bis-(β-aminoethylether)-*N,N,N'N'*-tetraacetic acid (EGTA); the calculated free Ca^{2+} concentration was 0.02 μM, using an apparent dissociation constant for Ca^{2+}-EGTA of 0.15 μM (4). After 10 min, fatty acids were added and the cells separated from the medium after an additional 10 min by centrifugation through silicone oil at 8,000 *g* for 45 s. Radioactivity in the pellet was determined by liquid scintillation counting.

For measurement of prolactin secretion, cells were incubated in BSS containing 1.5 mM Ca^{2+} ($0.5–1.5 \times 10^6$/ml) without (control) or with fatty acids, at 37°C. After 20 min, cells were separated from the medium by centrifugation and the mediums stored at −20°C until assayed. Prolactin was measured by radioimmunoassay. For experiments with 30 μM eicosatetraynoic acid (ETYA) and/or 100 μM indomethacin, cells were preincubated with these agents for 5 min prior to fatty acid addition.

To determine whether arachidonic acid affected plasma membrane integrity nonspecifically, its effects on permeability of GH_3 cells to Na^+, inulin, sucrose, and trypan blue were tested. After incubation of cells in BSS containing 1.5 mM Ca^{2+} and $^{22}Na^+$ (0.05 mCi/ml) for 30 min, cells were exposed to varying concentrations of arachidonic acid, and the cell-associated ^{22}Na radioactivity was determined after an additional 5 min. Cellular ^3H_2O volume was measured simultaneously. Arachidonic acid did not affect cellular $^{22}Na^+$ at concentrations of 60 μM or less. One hundred micromoles per liter of arachidonic acid increased the concentration of ^{22}Na in the cell H_2O from 22 ± 1.1 to 32 ± 5.0 mM. Also, arachidonic acid at 60 μM or less did not increase plasma membrane permeability to [^{3}H]inulin, [^{14}C]sucrose, or trypan blue; 100 μM arachidonic acid increased the permeability to all these agents.

Metabolism of arachidonic acid by GH_3 cells was assessed by incubating cells for 1–5 min in BSS containing 0.5 nM or 5 μM [^{3}H]arachidonic acid. After the incubation, the cells were separated from the medium by centrifugation and the cellular lipids extracted with $CHCl_3$:methanol:HCl (100:100:1, vol/vol). The ^{3}H-labeled lipids were analyzed by thin-layer chromatography on Silica Gel-G using the upper phase of ethyl acetate:2,2,4-trimethylpentane:acetic acid:H_2O (90:50:20:100, vol/vol) (12).

Because arachidonic acid obtained from commercial sources may be contaminated with products that may have biological activity and because arachidonic acid can be oxidized to products that may be biologically active (18), the effects of freshly dissolved arachidonic acid, repurified arachidonic acid, and arachidonic acid exposed to air on ^{45}Ca efflux were compared. Stock arachidonic acid was freshly dissolved in dimethyl sulfoxide or in ethanol containing butylated hydroxytoluene. A portion of the stock solution was placed in a beaker and was exposed to air for 24 h prior to the experiment. Another portion of the stock was repurified by thin-layer chromatography on Silica Gel-G using the upper phase of ethyl acetate:2,2,4-trimethylpentane:acetic acid:H_2O (90:50:20:100, vol/vol) (12); recovery was assessed by comparison with recovery of [^{3}H]arachidonic acid added to the sample prior to chromatography. The effects of the samples were qualitatively similar but quantitatively different. In three experiments performed in triplicate, all three preparations of arachidonic acid caused $^{45}Ca^{2+}$ efflux; the order of potency was that air-oxidized arachidonic acid was slightly more potent than the commercial preparation, and both were more potent than repurified arachidonic acid. A typical result was efflux of ^{45}Ca during 10 min of 0.62 ± 0.007 (mean ± SE), 0.58 ± 0.001, and 0.41 ± 0.047 ($P < 0.001$ versus both other preparations) caused by 60 μM concentrations of air-oxidized, commercially prepared, and freshly repurified arachidonic acid, respectively. Because oxidation products of arachidonic acid appeared to enhance the potency of a preparation of arachidonic acid to mobilize Ca^{2+}, arachidonic acid was repurified before use.

Statistical analyses were performed by *t* test.

RESULTS

The effect of arachidonic acid was to cause ^{45}Ca efflux and prolactin secretion as shown in Fig. 1. One micromole per liter of arachidonic acid caused efflux of 15 ± 3% (mean ± SE, $P < 0.001$) of residual cellular ^{45}Ca at 10 min from cells prelabeled to isotopic steady state (Fig. 1*A*). This effect was concentration dependent; 60 μM arachidonic acid caused efflux of 70 ± 4% of residual cellular ^{45}Ca. The concentration-dependent effect of arachidonic acid in stimulating prolactin secretion from cells incubated in medium containing 1.5 mM $CaCl_2$ was similar (Fig. 1*B*). Three micromoles per liter of arachidonic acid increased prolactin in the medium to 130 ± 9.0% of control ($P < 0.005$), whereas 30 μM increased prolactin secretion maximally to 470 ± 82% of control. Arachidonic acid also stimulated prolactin secretion from cells incubated in medium containing approximately 0.02 μM Ca^{2+} (data not shown). Hence, the effects of arachidonic acid in causing ^{45}Ca efflux and prolactin secretion from GH_3 cells demonstrated similar concentration dependencies.

Two different experimental approaches were used in attempts to determine whether the effects of arachidonic acid in causing ^{45}Ca efflux and prolactin secretion were due to arachidonic acid itself or to metabolites. First, the effects of inhibitors of arachidonic acid metabolism, eicosatetraynoic acid (ETYA) and indomethacin, on arachidonic acid-induced prolactin secretion were studied

(Table 1). In these experiments, arachidonic acid (60 μM) increased the level of prolactin in the medium from 18 ± 5.8 ng/10^6 cells (mean ± SE) to 78 ± 13 ($P < 0.005$) after 20 min. ETYA (30 μM) and indomethacin (100 μM) or both agents added simultaneously (3, 8, 13) (see below) had no effect on basal prolactin secretion or on secretion induced by 60 μM arachidonic acid. In another experiment (data not shown), neither ETYA nor indomethacin affected prolactin secretion induced by 20 μM arachidonic acid. Hence, inhibitors of the conversion of arachidonic acid by lipoxygenase and cyclooxygenase enzymes did not inhibit arachidonic acid-induced prolactin secretion. We attempted to assess the efficacy of ETYA and indomethacin by monitoring their effects on the generation of metabolites of arachidonic acid in GH_3 cells. Cells were incubated with 0.5 nM or 5 μM [^{3}H]-arachidonic acid for 1 and 5 min, after which the cells were separated from the medium and the cellular lipids were extracted. Although significant quantities of [^{3}H]-arachidonic acid were incorporated into phospholipids and neutral lipids at these times, there was no measurable radioactivity in the areas of the chromatograms where the polar metabolites of arachidonic acid migrate (data not shown). We must conclude, therefore, that GH_3 cells do not convert arachidonic acid into its polar metabolites under these conditions. This finding is consistent with the observation of Osborne and Tashjian (16) who could not detect prostaglandins E_2 and F_2 in the incubation medium from GH_4C1 cells, another subclone of GH cells. Hence, although this approach is not definitive, as we could not demonstrate that complete inhibition of arachidonic acid metabolism was achieved, it has been used successfully by previous investigators. In other cell types (3, 8, 13), similar concentrations of ETYA and indomethacin have been shown to inhibit maximally the biological effects specifically stimulated by similar concentrations of exogenously added arachidonic acid.

A second and more definitive approach compared the effects of arachidonic acid on ^{45}Ca efflux and prolactin secretion with a series of related fatty acids, some of which (α-linolenic, oleic, stearic, and eicosanoic acids) cannot be converted to arachidonic acid or its metabolites in mammalian cells (15) (Fig. 2). The effects of arachidonic acid (20:4), a 20-carbon fatty acid with four double bonds, α-linolenic acid (18:3), an 18-carbon fatty

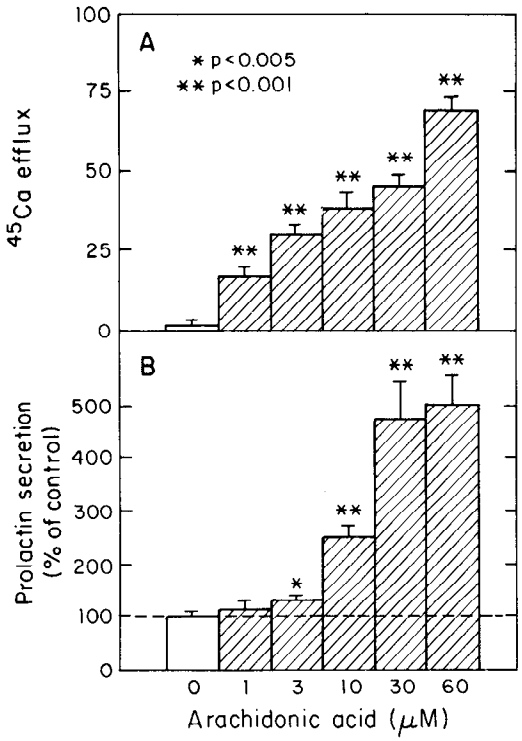

FIG. 1. Concentration dependence of arachidonic acid-stimulated ^{45}Ca efflux and prolactin secretion from GH_3 cells. *A*: cells ($0.5–1 \times 10^6$/0.15 ml) were labeled in medium with 1.5 mM $^{45}Ca^{2+}$ (3–5 μCi/ml) for 45 min and resuspended in Ca^{2+}-free medium containing 33 μM EGTA. After 10 min, portions were separated into individual test tubes containing arachidonic acid or diluent. After an additional 10 min, cells were separated from medium by centrifugation at 8,000 *g*, and cellular ^{45}Ca was measured. Stimulated ^{45}Ca efflux was calculated as percent difference between residual ^{45}Ca in control and arachidonic acid-exposed cells, i.e., 100 × (control − arachidonic acid-exposed) cell ^{45}Ca/control cell ^{45}Ca. *Bars*: means ± SE of triplicate determinations from 4 experiments. *B*: cells (1×10^6/ml) were incubated for 20 min at 37°C in medium containing arachidonic acid or diluent. Prolactin levels in medium of control incubations were 30 ± 5.0 ng/10^6 cells. *Bars*: means ± SE of triplicate determinations from 3 experiments.

TABLE 1. *Effects of ETYA and indomethacin on basal and arachidonic acid-induced prolactin secretion from GH_3 cells*

	Prolactin Secretion, ng·10^6 cells^{-1}·20 min^{-1}	
	Basal	Arachidonic acid
Control	18 ± 5.8	78 ± 13*
ETYA	24 ± 8.0	78 ± 10*
Indo	23 ± 6.7	74 ± 12*
ETYA + Indo	31 ± 10	67 ± 12*

Values are means ± SE of quadruplicate determinations from two experiments. ETYA, eicosatetraynoic acid; Indo, indomethacin. * $P < 0.005$.

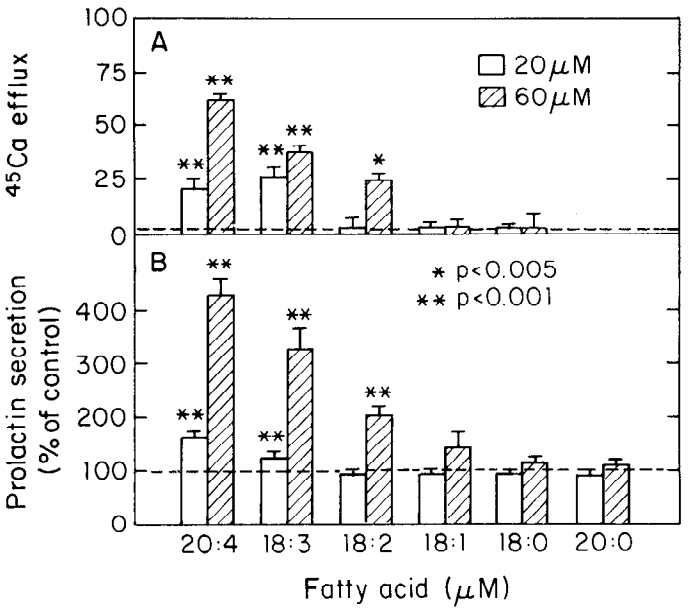

FIG. 2. Effects of arachidonic (20:4), α-linolenic (18:3), linoleic (18:2), oleic (18:1), stearic (18:0), and eicosanoic (20:0) acids on ^{45}Ca efflux and prolactin secretion from GH_3 cells. *A*: performed and analyzed as in Fig. 1*A*. *Bars*: means ± SE triplicate determinations from 4 experiments. *B*: performed and analyzed as in Fig. 1*B*. *Bars*: means ± SE of triplicate determinations from 3 experiments.

acid with three double bonds, linoleic acid (18:2), an 18-carbon fatty acid with two double bonds, oleic acid (18:1), an 18-carbon fatty acid with one double bond, and stearic acid (18:0), an 18-carbon saturated fatty acid, on ^{45}Ca efflux from GH_3 cells are illustrated in Fig. 2*A*. Arachidonic and α-linolenic acids caused ^{45}Ca efflux at both 20 and 60 μM concentrations, whereas linoleic acid caused ^{45}Ca efflux at 60 μM only. Oleic and stearic acids did not increase ^{45}Ca efflux from GH_3 cells under these conditions. The ability of these fatty acids to stimulate prolactin secretion displayed a similar order of activity (Fig. 2*B*). Arachidonic acid (20:4) stimulated prolactin secretion at 20 and 60 μM to 160 ± 7% of control (mean ± SE; $P < 0.001$) and 430 ± 30% ($P < 0.001$), respectively. α-Linolenic acid (18:3), which cannot be metabolized to arachidonic acid, had similar effects, but linoleic acid (18:2), which can be metabolized to arachidonic acid in mammalian cells (15), stimulated prolactin secretion at 60 μM only. Oleic acid (18:1) and two saturated fatty acids, stearic (18 carbons) and eicosanoic (20 carbons) acids, did not cause prolactin secretion. Hence, the effect of a fatty acid in this series in stimulating both ^{45}Ca efflux and prolactin secretion correlated with the number of its double bonds and not with whether it could be converted to arachidonic acid or its metabolites.

DISCUSSION

Arachidonic acid and its metabolites appear to be important intracellular mediators of many biological processes, including stimulated secretion from a number of secretory cells (23). Because arachidonic acid and its metabolites have been shown to affect Ca^{2+} homeostasis in several cell types (2), it is possible that secretion stimulated by arachidonic acid or its metabolites is mediated by Ca^{2+}. In this report, we demonstrate that arachidonic acid added exogenously to GH_3 cells, a cloned strain of rat pituitary tumor cells, stimulates efflux of ^{45}Ca and secretion of prolactin. Morever, we have presented two lines of evidence that suggest that these actions of arachidonic acid are mediated by arachidonic acid itself and not by conversion to its metabolites. First, inhibitors of arachidonic acid metabolism by cyclooxygenase and lipoxygenase pathways did not affect prolactin secretion. Second, other polyunsaturated fatty acids, in particular, α-linolenic acid, which cannot be metabolized to arachidonic acid or its metabolites, stimulated $^{45}Ca^{2+}$ efflux and prolactin secretion. It was of interest that there was a good correlation between the potency of a fatty acid to stimulate ^{45}Ca efflux and prolactin secretion and the number of its double bonds, a finding similar to that made for superoxide production by human neutrophils (1).

A trivial explanation for the association of stimulated ^{45}Ca efflux and prolactin secretion is that the ^{45}Ca efflux represents loss of calcium from the secretory granule during exocytosis of prolactin. Against this notion is the observation that the kinetics of these effects are different; greater than 95% of the ^{45}Ca efflux occurs during the 1st min after stimulation, whereas prolactin secretion begins during the 1st min and continues at a significant rate for 20 min (data not shown). The action of arachidonic acid of stimulating prolactin secretion from GH_3 cells may be mediated by Ca^{2+}, as efflux of ^{45}Ca presumably reflects mobilization of cellular Ca^{2+}; alternatively, these events may be simultaneous but not causally related effects of arachidonic acid. A role for arachidonic acid itself in mobilizing Ca^{2+} from isolated cellular organelles, for example, from rat kidney and liver mitochondria (22) and from pig longissimus dorsi sarcoplasmic reticulum (5), has been demonstrated, and it was suggested that arachidonic acid may be an intracellular mediator of Ca^{2+} mobilization from mitochondria in stimulated hepatocytes (24). Hence, arachidonic acid may mediate, at least in part, the loss of Ca^{2+} from mitochondria and/or endoplasmic reticulum in GH_3 cells during stimulated prolactin secretion. Alternatively, arachidonic acid may directly stimulate secretion (exocytosis) by increasing the rate of fusion of the membranes of the secretory granule and the plasmalemma as suggested by Pollard and his colleagues (17).

The role(s) of arachidonic acid and/or metabolites in the regulation of secretion of hormones from the anterior pituitary gland is unclear (9). However, two previous studies have demonstrated that release of arachidonic acid from lipids occurs rapidly after secretagogue interaction with anterior pituitary cells (11, 14). In pituitary cells in vitro derived from immature female rats (14), Naor and Catt demonstrated that gonadotropin-releasing hormone (GnRH) caused release of arachidonic acid from phospholipids. They also showed that inhibitors of arachidonic acid release inhibited GnRH-induced luteinizing hormone secretion and that inhibition of the metabolism of unesterified arachidonic acid did not affect GnRH- or arachidonic acid-induced luteinizing hormone secretion, and suggested that arachidonic acid itself may mediate luteinizing hormone secretion. We (11) have shown that thyrotropin-releasing hormone (TRH) caused a rapid increase in the concentration of unesterified arachidonic acid in pituitary cells in vitro derived from mouse tumors that secrete thyrotropin (thyroid-stimulating hormone, TSH). Because exogenously added arachidonic acid mobilized cellular calcium and stimulated TSH secretion from these cells, we suggested that arachidonic acid and/or its metabolites may mediate, at least in part, TRH-induced TSH secretion. In contrast to our findings in thyrotropic cells, we (21) could not demonstrate an increase in unesterified arachidonic acid during TRH stimulation of prolactin secretion from mammotropic GH_3 cells, and it therefore appears unlikely that arachidonic acid mediates TRH-stimulated prolactin secretion. However, other prolactin secretagogues may stimulate arachidonic acid release from lipids in GH_3 cells and arachidonic acid may be an intracellular messenger of prolactin secretion by these secretagogues.

In summary, arachidonic acid itself, not via conversion to its metabolites, appears to cause ^{45}Ca efflux and prolactin secretion from GH_3 cells. These findings suggest that arachidonic acid may serve, under some circumstances, as an intracellular messenger to mobilize Ca^{2+} and mediate prolactin secretion.

The authors thank Susanna Lodato and Emelda Peck for expert preparation of this manuscript.

These studies were supported by National Institutes of Health Grants AM-20249 and AM-30162. M. C. Gershengorn is the recipient

of National Institutes of Health Research Career Development Award AM-00566 and of an award from the Irma T. Hirschl-Monique Weill-Caulier Trust.

Present address: Div. of Endocrinology and Metabolism, Dept. of Medicine, Cornell Univ. Medical College, 1300 York Ave., New York, NY 10021.

Received 14 November 1983; accepted in final form 26 January 1984.

REFERENCES

1. BADWEY, J. A., J. T. CURNUTTE, AND M. J. KARNOVSKY. Cis-polyunsaturated fatty acids induce high levels of superoxide production by human neutrophils. *J. Biol. Chem.* 256: 12640–12643, 1981.
2. BARRITT, G. J. A proposal for the mechanism by which α-adrenergic agonists, vasopressin, angiotensin and cyclic AMP induce calcium release from intracellular stores in the liver cell: a possible role for metabolites of arachidonic acid. *Cell Calcium* 2: 53–63, 1981.
3. BASS, D. A., J. T. O'FLAHERTY, P. SZEJDA, L. R. DECHATELET, AND C. E. MCCALL. Role of arachidonic acid in stimulation of hexose transport by human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* 77: 5125–5129, 1980.
4. CARAFOLI, E., AND M. CROMPTON. The regulation of intracellular calcium. *Curr. Top. Membr. Transp.* 10: 151–216, 1978.
5. CHEAH, A. M. Effect of long chain unsaturated fatty acids on the calcium transport of sarcoplasmic reticulum. *Biochim. Biophys. Acta* 648: 113–119, 1981.
6. GERAS, E., M. J. REBECCHI, AND M. C. GERSHENGORN. Evidence that stimulation of thyrotropin and prolactin secretion occur via different calcium-mediated mechanisms: studies with verapamil. *Endocrinology* 110: 901–906, 1982.
7. GERSHENGORN, M. C., Thyrotropin-releasing hormone. A review of the mechanisms of acute stimulation of pituitary hormone release. *Mol. Cell. Biochem.* 45: 163–179, 1982.
8. HANDWERGER, S., J. BARRETT, S. BARRY, E. MARKOFF, P. ZEITLER, B. CWIKEL, AND M. SIEGEL. Stimulation of human placental lactogen release by arachidonic acid. *Mol. Pharmacol.* 20: 609–613, 1981.
9. HEDGE, G. A. Roles for the prostaglandins in the regulation of anterior pituitary secretion. *Life Sci.* 20: 17–34, 1977.
10. KLAUSNER, R. D., D. K. BHALLA, P. DRAGSTEN, R. L. HOOVER, AND M. J. KARNOVSKY. Model for capping derived from inhibition of surface receptor capping by free fatty acids. *Proc. Natl. Acad. Sci. USA* 77: 437–441, 1980.
11. KOLESNICK, R. N., I. MUSACCHIO, C. THAW, AND M. C. GERSHENGORN. Thyrotropin-releasing hormone decreases phosphatidylinositol and increases unesterified arachidonic acid in thyrotropic cells. Possible early events in stimulation of TSH secretion. *Endocrinology* 114: 671–676, 1984.
12. LAPETINA, E. G., AND P. CUATRECASAS. Stimulation of phosphatidic acid production in platelets precedes the formation of arachidonate and parallels the release of serotonin. *Biochim. Biophys. Acta* 573: 394–402, 1979.
13. NACCACHE, P. H., H. J. SHOWELL, E. L. BECKER, AND R. I. SHA'AFI. Arachidonic acid induced degranulation of rabbit peritoneal neutrophils. *Biochem. Biophys. Res. Commun.* 87: 292–299, 1979.
14. NAOR, Z., AND K. J. CATT. Mechanism of action of gonadotropin-releasing hormone. *J. Biol. Chem.* 256: 2226–2229, 1981.
15. NAUGHTON, J. M. Supply of polyenoic fatty acids to the mammalian brain. *Int. J. Biochem.* 13: 21–32, 1981.
16. OSBORNE, R., AND A. H. TASHJIAN, JR. Tumor-promoting phorbol esters affect production of prolactin and growth hormone by rat pituitary cells. *Endocrinology* 108: 1164–1170, 1981.
17. POLLARD, H. B., C. E. CREUTZ, V. FOWLER, T. SCOTT, AND C. J. PAZOLES. Calcium-dependent regulation of chromaffin granule movement, membrane contact, and fusion during exocytosis. *Cold Spring Harbor Symp. Quant. Biol.* 46, part 2: 819–834, 1982.
18. PRYOR, W. A., J. P. STANLEY, AND E. BLAIR. Autooxidation of polyunsaturated fatty acids. II. A suggested mechanism for the formation of TBA-reactive materials from prostaglandin-like endoperoxides. *Lipids* 11: 370–379, 1976.
19. PUTNEY, J. W., JR. Recent hypotheses regarding the phosphatidylinositol effect. *Life Sci.* 29: 1183–1194, 1981.
20. REBECCHI, M. J., R. H. GERRY, AND M. C. GERSHENGORN. Thyrotropin-releasing hormone causes loss of cellular calcium without calcium uptake by rat pituitary cells in culture. Studies with arsenazo III for direct measurement of calcium. *J. Biol. Chem.* 257: 2751–2753, 1982.
21. REBECCHI, M. J., R. N. KOLESNICK, AND M. C. GERSHENGORN. Thyrotropin-releasing hormone stimulates rapid loss of phosphatidylinositol and its conversion to 1,2-diacylglycerol and phosphatidic acid in rat mammotropic pituitary cells. *J. Biol. Chem.* 258: 227–234, 1983.
22. ROMAN, I., P. GMAJ, C. NOWEICKA, AND S. ANGIELSKI. Regulation of Ca^{2+} efflux from kidney and liver mitochondria by unsaturated fatty acids and Na^+ ions. *Eur. J. Biochem.* 102: 615–623, 1979.
23. SAMUELSSON, B. Prostaglandins, thromboxanes and leukotrienes: formation and biological roles. *Harvey Lect.* 75: 1–40, 1981.
24. WHITING, J. A., AND G. J. BARRITT. On the mechanism by which hormones induce the release of Ca^{2+} from mitochondria in the liver cell. *Biochem. J.* 206: 121–129, 1982.