Epidermal growth factor stimulates phospholipase A₂ in vasopressin-treated rat glomerular mesangial cells

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Epidermal growth factor (EGF) enhances vasopressin- and ionophore-A23187-induced prostaglandin production and arachidonate release by rat glomerular mesangial cells in culture. The purpose of the present study was to delineate the phospholipid pathways involved in this effect. In cells labelled with [¹⁴C]arachidonate, EGF significantly enhanced the free arachidonate released in response to A23187 or vasopressin without enhancing the production of [¹⁴C]arachidonate-labelled diacylglycerol. EGF increased the [¹⁴C]arachidonate-labelled phosphatidic acid formed in response to vasopressin, but to a much smaller extent than it increased free arachidonate release. These results indicate that activation of phospholipase C is not sufficient to explain the increase in free arachidonate release observed on addition of EGF. To examine if EGF enhanced phospholipase A₂ activity, mesangial cells were labelled with [2-³H]glycerol and [¹⁴C]-arachidonate, and the formation of arachidonate-poor lysophospholipids was studied. When combined with vasopressin, EGF significantly enhanced the formation of arachidonate-poor lysophospholipids as compared with vasopressin alone. The fate of exogenously added lysophosphatidylcholine was not altered after stimulation with vasopressin plus EGF, indicating that decreased deacylation or reacylation of the lysophospholipids was not responsible for their accumulation. Taken together, these results indicate that EGF enhances free arachidonate release by activation of phospholipase A₂. The signalling mechanism responsible for the change in phospholipase A₂ activity is not known, but could conceivably involve phosphorylation of modulating proteins such as lipocortin or G-proteins.

INTRODUCTION

The mRNA for the EGF precursor protein preproEGF is produced in the kidney, and human urine has an EGF concentration of up to 10 nM [1,2]. Recent studies have been undertaken to define a physiological role for the EGF produced in the kidney. Our work and that of others has provided evidence that EGF modulates renal prostaglandin production [3,4]. Utilizing the rat renal glomerular mesangial cell as a model system [5], we found that EGF markedly enhanced the release of arachidonic acid and prostaglandins in response to Ca²⁺ ionophore and vasopressin [3]. This has physiological importance, because prostaglandins are critical in many aspects of renal function, from glomerular filtration to control of tubular function [6].

The exact mechanism by which EGF modulates prostaglandin production is not known. Some groups have found an increase in cyclo-oxygenase activity [7], but in mesangial cells EGF enhances arachidonate release [3]. At present the arachidonate release from phospholipids is considered to proceed by two pathways [8]. One involves the action of phospholipase C on phosphatidylinositol (PI), leading to the generation of arachidonate-rich diacylglycerol (DAG), which is then deacylated by DAG lipase to generate free arachidonic acid. The other pathway involves phospholipase A₂ action on

phospholipids, releasing arachidonate from the *sn*-2 position. In some cell lines, especially those that over-express EGF receptors, EGF has been shown to activate phospholipase C [9,10]. In contrast, Aoyagi *et al.* [11] demonstrated in epidermal cells that EGF enhanced arachidonate release from phosphatidylcholine (PC), but not from phosphatidylinositol (PI). Our previous studies in the mesangial cells demonstrated that EGF neither increased intracellular Ca²⁺ nor altered the Ca²⁺-signalling response to vasopressin, despite marked enhancement of arachidonate release [3]. These results suggest that EGF might modulate phospholipase A₂ activity, independently of effects on phospholipase C. Such an apparent increase in phospholipase A₂ activity is of great interest, because one substrate of the EGF receptor kinase is the putative phospholipase-A₂-inhibitory protein, lipocortin I [12,13]. Therefore the present studies were undertaken to define the phospholipid pathways utilized by EGF to enhance arachidonate release in mesangial cells.

EXPERIMENTAL

Materials

[2-³H]Glycerol and [1-¹⁴C]palmitoyl lysophosphatidylcholine (LPC) were obtained from NEN/DuPont

Abbreviations used: EGF, epidermal growth factor; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; DAG, diacylglycerol; NEFA, non-esterified fatty acid.

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(Mississauga, Ont., Canada), and [^{14}C]arachidonate was from Amersham (Oakville, Ont., Canada). EGF was purified from mouse submaxillary glands [14] and was a gift from Dr. J. Kudlow (University of Toronto). Tissue-culture supplies were from GIBCO (Burlington, Ont., Canada), and all other chemicals were from Sigma (St. Louis, MO, U.S.A.).

Cell culture

Homogeneous cultures of renal glomerular mesangial cells were derived from collagenase-treated glomeruli isolated from Sprague–Dawley rats, and were established and characterized as previously described [15]. For these studies, cloned populations of cells derived from single colonies were maintained in RPMI medium with 20% (v/v) fetal-calf serum in a 5%-CO₂ incubator at 37 °C, and utilized between passages 30 and 50.

Phospholipid extraction

Mesangial cells were plated at 300 000 cells per 60 mm-diam. dish, and grown for 72 h until confluent, with a change of medium at 40 h. In experiments utilizing glycerol labelling, 15 μCi of [^3H]glycerol was added when cells were initially plated, and then again at 40 h. [^{14}C]Arachidonate was added at 40 h only. After 72 h in culture, cells were washed with 4 $\times$ 4 ml of Hepes-buffered Dulbecco's Modified Eagle Medium (30 mM-Hepes, pH 7.5) with 5 mg of defatted albumin/ml. Cells were then incubated with the Hepes-buffered medium with 1 mg of defatted albumin/ml for 40 min at 30 °C, after which the medium was replaced with 2 ml of medium with or without 10 nM-EGF and the dishes were returned to the incubator for another 20 min. The medium was then removed, and cells were stimulated with 1 μM -A23187 or 1 μM -vasopressin with or without 10 nM-EGF in Hepes-buffered medium. After 5 min at 30 °C, the medium was removed and 2 ml of ice-cold methanol was added to the dish. The dish was then scraped free of cells into a total of 5 ml of methanol, and this was added to the previously collected medium. A modified Bligh–Dyer [16] extraction was then performed. After the initial extraction with 5 ml of chloroform and 2.5 ml of 0.88% KCl, the lower phase was kept and the upper phase was re-extracted with 5 ml of chloroform and 50 μl of conc. HCl. The pooled lower phases were dried under N₂, resuspended in chloroform/methanol (2:1, v/v) and spotted on t.l.c. plates.

In one set of experiments, the fate of exogenously added LPC was examined. Cells were grown for 72 h as above, but no radiolabels were added. Cells were then washed twice with Hepes-buffered medium with 1 mg of defatted albumin/ml and incubated for 40 min at 30 °C. At 40 min, 10 nM-EGF was added to half the dishes as described above, and the dishes were incubated for another 20 min. Then 0.5 μCi of [^{14}C]palmitoyl LPC was added with or without 1 μM -vasopressin plus 10 nM-EGF. After further incubations for 5 or 30 min, cells and medium were extracted and separated by two-dimensional t.l.c. as outlined below.

Phospholipid separation

Phospholipids were separated and identified by modifications of previously described techniques [17,18]. For separation of DAG and non-esterified fatty acid (NEFA), a one-dimensional system of heptane/isopropyl ether/acetic acid (20:40:3, by vol.) was utilized. For the

separation of phospholipids and lysophospholipids, Merck silica-gel plates were spotted with samples dissolved in 25 μl of chloroform/methanol (2:1, v/v) containing 2.5 μg each of the authentic standards phosphatidic acid (PA), lysophosphatidyl-inositol (LPI), -choline (LPC) and -ethanolamine (LPE), and developed in the first dimension with chloroform/methanol/conc. NH₃ (130:70:11, by vol.). Plates were then dried at 40 °C for 1 h, turned through 90°, and redeveloped in chloroform/methanol/formic acid (11:5:1, by vol.). This system allows separation of NEFA and all phospholipids and lysophospholipids. After separation of the lipids, and observation under u.v. light [17], samples were scraped into vials with 1.5 ml of water and 15 ml of scintillant. The samples were counted for radioactivity in a Beckman LS 7500 liquid-scintillation counter by using the dual-labelling formula which yields d.p.m. of ^{14}C and of ^3H , and the ratio of ^3H to ^{14}C counts.

RESULTS

Fig. 1 demonstrates the [^{14}C]arachidonate present as DAG and as NEFA in control, A23187-treated and A23187 + EGF-treated cells. The DAG was not increased above control in either of the stimulated groups, indicating that phospholipase C was not measurably activated. In contrast, A23187 stimulated the release of [^{14}C]arachidonate as NEFA, and this effect was significantly enhanced with EGF treatment. EGF alone had no effect on DAG production or arachidonate release, as demonstrated in Fig. 2. It has been previously shown that vasopressin activates phospholipase C in these cells, resulting in an increase in cytosolic Ca²⁺ [19,20]. As shown in Fig. 2, this is also associated with production of DAG and release of free arachidonic acid. EGF was synergistic with vasopressin in arachidonate release, but had no effect on vasopressin-induced DAG formation.

Fig. 1. Effects of EGF and A23187 on [^{14}C]arachidonate-labelled DAG and NEFA

Mesangial cells were labelled with 0.5 μCi of [^{14}C]arachidonate for 32 h. Lipids were extracted from control cells, cells stimulated with A23187 (1 μM), and cells stimulated with A23187 + EGF (10 nM). After separation by t.l.c, the silica-gel bands with DAG (■) and NEFA (▨) were scraped into scintillation vials and counted for radioactivity. Results are means $\pm$ S.E.M. for three dishes: * $P < 0.05$ versus control (paired t -test analysis).

Fig. 2. Effects of EGF and vasopressin on [¹⁴C]arachidonate-labelled DAG and NEFA

Mesangial cells were labelled with 0.5 μ Ci of [¹⁴C]-arachidonate for 32 h. Lipids were extracted from control cells and from cells stimulated with 10 nM-EGF (E), 1 μ M-vasopressin (VP) or vasopressin + EGF (VP + EGF). After separation by t.l.c, the silica-gel bands with DAG (■) and NEFA (▨) were scraped into scintillation vials and counted for radioactivity. Results are means $\pm$ S.E.M. for four dishes: * $P < 0.05$ versus control, † $P < 0.05$ versus vasopressin alone (paired *t*-test analysis).

Since these data suggested a predominant effect of EGF to enhance phospholipase A₂ activation, further experiments were undertaken to measure the formation of the labile lysophospholipid products of phospholipase A activity. The assessment of phospholipase A₂ activity requires the specific measurement of the formation of 1-acyl-2-lysophospholipids. Phospholipase A₁, which may have significant activity in some cells [21], will form 2-acyl-1-lysophospholipids, but not free arachidonate. Additionally, because 1-lysophospholipids can be formed during acid extraction of alkenylphospholipids [22], measurements were required which would distinguish between the formation of these two classes of lysophospholipids.

Table 1 shows the results of experiments in which cells were dually labelled for 72 h with [2-³H]glycerol and then for an additional 32 h with [¹⁴C]arachidonate and the formation of lysophospholipids was examined. There was a significant increase in glycerol-labelled LPI, LPE and LPC in the vasopressin + EGF-treated cells compared with the control, EGF-treated or the vasopressin-only-treated groups. This was not accompanied by corresponding changes in the arachidonate label in the lysophospholipids. Consequently, the ratio of radiolabelled glycerol to arachidonate d.p.m. rose significantly in all three lysophospholipids in the vasopressin + EGF-treated cells. These results demonstrate the formation of arachidonate-poor lysophospholipids, which would be produced specifically by the action of phospholipase A₂.

Table 1 also displays the percentage of label from

[³H]glycerol and [¹⁴C]arachidonate in each of the separated phospholipid fractions, including PA. There were significant decreases in glycerol- and arachidonate-labelled PI and significant increases in arachidonate- and glycerol-labelled PA in vasopressin-treated and vasopressin + EGF-treated cells. It should be noted that the increase in PA formed from prelabelled phospholipids may provide a more sensitive index of phospholipase C activation, since DAG formed by the activation of phospholipase C may be quickly converted into PA [8]. Therefore, as expected, vasopressin markedly increased formation of [¹⁴C]arachidonate-labelled PA [19,23]. However, the slight further increase in this response owing to EGF addition is insufficient to account for the much more marked enhancement of free arachidonate release. There was also a decrease in the glycerol/arachidonate ratio in PA in the vasopressin-treated groups. This is expected, owing to the increased contribution to PA made by the arachidonate-rich DAG originating in PI upon stimulation of phospholipase C by vasopressin. In contrast, there was no alteration in the glycerol/arachidonate ratios in PC, PE or PI, as was the case for the corresponding lysophospholipids, indicating that the alterations seen in the lysophospholipids were specific.

Studies were also undertaken to evaluate the possibility that EGF might slow the reacylation or further deacylation of lysophospholipids formed by the action of vasopressin [24]. For these studies, the fate of [1-¹⁴C]-palmitoyl LPC was examined in control and vasopressin + EGF-stimulated mesangial cells, in two experiments performed as outlined in the Experimental section. After 30 min, no decline was observed in the formation of labelled PC {1.61% and 1.46% for control, versus 1.54% and 1.43% for vasopressin + EGF (% of recovered [¹⁴C]LPC)} or the release of free palmitate {0.58% and 0.84% for control, versus 0.61% and 1.13% for vasopressin + EGF (% of recovered [¹⁴C]LPC)}. Similarly, there was no difference in the rate of conversion of [¹⁴C]LPC into these products when the process was studied with a 5 min incubation. Although the addition of exogenous lysophospholipids does not completely simulate their intracellular production, these data suggest that EGF does not slow the metabolism of lysophospholipids.

DISCUSSION

The purpose of the present study was to examine the mechanism whereby EGF enhances the release of arachidonate and the formation of prostaglandins. There have been detailed reports of EGF action in cells where EGF stimulates phospholipase C and initiates a rise in intracellular Ca²⁺ concentration [9,10], yet in many cell lines EGF does not stimulate phospholipase-C-mediated hydrolysis of polyphosphoinositides [25,26]. There has not been a detailed report on the phospholipid pathways involved in cell lines where EGF has been reported to enhance prostaglandin production. One report has demonstrated that EGF enhances cyclo-oxygenase activity [7], but this required incubations longer than 2 h, and most studies to date also report enhancement of arachidonate release [3,11,27]. In the only study where phospholipids were measured, EGF induced release of arachidonate from PC and not from PI, which is suggestive, but not proof, of phospholipase A₂ activation

Table 1. Effects of EGF and vasopressin (VP) on mesangial-cell phospholipids

Cells were labelled with [2-³H]glycerol for 72 h and with [¹⁴C]arachidonate for 32 h, and then stimulated as described in the Experimental section with EGF (10 nM), VP (1 μ M) or VP+EGF. Results are presented as percentages of total labelled lipid ([2-³H]glycerol total d.p.m. = 861 013 $\pm$ 60 227; [¹⁴C]arachidonate total d.p.m. = 470 369 $\pm$ 14 604; means $\pm$ S.E.M for six dishes): * P < 0.05 versus control, † P < 0.05 versus VP, control, or EGF (paired t test).

	Control	EGF	VP	VP + EGF	
[³ H]Glycerol (%)	58.68 $\pm$ 2.16	58.52 $\pm$ 2.22	57.41 $\pm$ 1.78	57.16 $\pm$ 1.56	PC
	19.07 $\pm$ 1.79	19.04 $\pm$ 1.42	19.09 $\pm$ 0.94	18.39 $\pm$ 0.94	PE
	10.67 $\pm$ 0.22	10.53 $\pm$ 0.41	9.92 $\pm$ 0.35*	9.52 $\pm$ 0.28†	PI
	5.81 $\pm$ 0.30	5.84 $\pm$ 0.38	6.21 $\pm$ 0.35*	6.17 $\pm$ 0.42	PS
	0.90 $\pm$ 0.08	0.82 $\pm$ 0.06	1.89 $\pm$ 0.07*	2.24 $\pm$ 0.09†	PA
	0.34 $\pm$ 0.03	0.31 $\pm$ 0.02	0.38 $\pm$ 0.02	0.47 $\pm$ 0.03†	LPC
	0.11 $\pm$ 0.02	0.10 $\pm$ 0.01	0.13 $\pm$ 0.02	0.20 $\pm$ 0.03†	LPE
	0.26 $\pm$ 0.06	0.28 $\pm$ 0.04	0.27 $\pm$ 0.03	0.47 $\pm$ 0.03†	LPI
[¹⁴ C]Arachidonate (%)	38.07 $\pm$ 2.45	37.37 $\pm$ 2.18	36.61 $\pm$ 1.6	35.68 $\pm$ 1.56	PC
	30.84 $\pm$ 2.87	30.92 $\pm$ 1.99	31.61 $\pm$ 1.50	30.25 $\pm$ 1.22	PE
	18.37 $\pm$ 0.29	18.82 $\pm$ 0.68	16.76 $\pm$ 0.58*	15.96 $\pm$ 0.80*	PI
	4.58 $\pm$ 0.11	4.70 $\pm$ 0.25	4.65 $\pm$ 0.18	4.81 $\pm$ 0.25	PS
	0.71 $\pm$ 0.05	0.67 $\pm$ 0.06	1.95 $\pm$ 0.04*	2.33 $\pm$ 0.06†	PA
	0.71 $\pm$ 0.04	0.81 $\pm$ 0.03	1.38 $\pm$ 0.03*	3.84 $\pm$ 0.09†	NEFA
	0.18 $\pm$ 0.02	0.15 $\pm$ 0.02	0.20 $\pm$ 0.03	0.16 $\pm$ 0.02	LPC
	0.09 $\pm$ 0.03	0.12 $\pm$ 0.03	0.06 $\pm$ 0.02	0.08 $\pm$ 0.01	LPE
	0.26 $\pm$ 0.07	0.29 $\pm$ 0.05	0.22 $\pm$ 0.03	0.28 $\pm$ 0.04	LPI
Glycerol (d.p.m.)/ arachidonate (d.p.m.)	2.80 $\pm$ 0.32	2.86 $\pm$ 0.31	2.83 $\pm$ 0.31	2.89 $\pm$ 0.32	PC
	1.10 $\pm$ 0.10	1.10 $\pm$ 0.09	1.07 $\pm$ 0.09	1.09 $\pm$ 0.09	PE
	1.03 $\pm$ 0.09	1.01 $\pm$ 0.09	1.05 $\pm$ 0.10	1.05 $\pm$ 0.08	PI
	2.28 $\pm$ 0.28	2.27 $\pm$ 0.28	2.39 $\pm$ 0.30	2.31 $\pm$ 0.30	PS
	2.18 $\pm$ 0.25	2.21 $\pm$ 0.22	1.72 $\pm$ 0.18*	1.68 $\pm$ 0.14*	PA
	3.14 $\pm$ 0.39	3.39 $\pm$ 0.25	3.45 $\pm$ 0.50	4.63 $\pm$ 0.38†	LPC
	1.90 $\pm$ 0.22	2.04 $\pm$ 0.42	3.28 $\pm$ 0.64	4.02 $\pm$ 0.71†	LPE
	1.84 $\pm$ 0.27	1.74 $\pm$ 0.14	2.08 $\pm$ 0.26	2.85 $\pm$ 0.23†	LPI

[11]. In the continuously passaged rat mesangial cell, EGF alone is not a potent enhancer of prostaglandin production. However, in agreement with results of Rigas & Levine [28] in the C-9 liver cell, we observed marked synergism of EGF with vasopressin. This large release of arachidonate allowed delineation of the specific phospholipid pathways involved in this aspect of EGF action. The data in the present paper demonstrate that the major effect of EGF is to enhance phospholipase A₂ activity in rat glomerular mesangial cells stimulated with vasopressin. Definitive demonstration of phospholipase A₂ activation was accomplished by utilizing a dual-labelling technique to demonstrate the enhanced formation of arachidonate-poor lysophospholipids. It is possible that some of the increase in arachidonate release in response to vasopressin + EGF was due to the action of DAG lipase on DAG released by phospholipase C. For several reasons it is unlikely that this accounts for a large percentage of arachidonate release. Firstly, there was no change in the formation of labelled DAG when EGF was combined with A23187 or vasopressin. Although there was a significant increase in labelled PA in the cells treated with vasopressin + EGF compared with vasopressin alone, this represented only 0.35% of the total label in arachidonate. In contrast, 3.84% of the arachidonate label was released as NEFA in the vasopressin + EGF-treated cells, compared with 1.38% in re-

sponse to vasopressin alone. Thus the addition of EGF to vasopressin resulted in approx. 7-fold increase in the label appearing as NEFA compared with PA. Furthermore, the increase in PA could well result from phospholipase D action, and this would not serve as a source for free arachidonate release. Taken together, it is very unlikely that an increase in phospholipase C activity alone could result in the increase in release of free arachidonate observed when EGF was added to vasopressin. It is possible that a marked enhancement of DAG lipase activity in combination with an enhanced phospholipase C activity could explain the observed results. However, Schlondorff [29] has failed to find evidence of DAG lipase action in the mesangial cell after angiotensin II treatment. In summary, the enhancement of phospholipase A₂ activity appears to be predominantly responsible for the enhanced arachidonate release observed in response to EGF.

The mechanism whereby EGF enhances phospholipase A₂ activity is not known. One factor important in controlling phospholipase A₂ is intracellular Ca²⁺ [8]. Our previous work demonstrated that increasing the intracellular Ca²⁺ markedly enhanced the ability of EGF to increase prostaglandin production, but that EGF did not mediate its effect through modulation of cell Ca²⁺ [3]. Also, we have demonstrated that EGF does not exert its effect via protein kinase C, as EGF is effective in protein-

kinase-C-down-regulated cells [3]. This is consistent with the present data, which demonstrate that EGF does not alter DAG contents. The results are also in agreement with preliminary reports by Knauss *et al.* [4], who demonstrated that EGF alone enhanced prostaglandin production, but did not increase inositol trisphosphate, DAG or intracellular Ca²⁺ in the mesangial cell. Finally, EGF effectively increased prostaglandin production in cells in which the intracellular pH was clamped at either pH 7.0 or 7.4, by using nigericin in a KCl medium (B. L. Margolis & K. L. Skorecki, unpublished work; [30,31]). Thus, although intracellular pH is known to modulate phospholipase A₂ activity, this is not the mechanism whereby EGF exerts its action.

Other potential mechanisms whereby EGF could enhance phospholipase A₂ activity are intriguing, but remain speculative. One possibility involves EGF-receptor-mediated phosphorylation of lipocortin I. This protein was identified by Fava & Cohen as one of the major substrates of the EGF receptor kinase, and was later found to be identical with one of a group of phospholipase-A₂-inhibitory proteins previously identified [12,32]. At present, it remains unclear whether EGF can alter phospholipase A₂ activity via phosphorylation of lipocortins [33–35]; however, the current results motivate the use of the mesangial cell as a model system for such studies. Furthermore, it has been suggested recently that phospholipase A₂ activity is controlled by GTP-binding proteins [36,37], and GTP-binding proteins have been postulated to explain the ability of EGF to stimulate phospholipase C action in other cells [10]. Therefore it will also be of interest to evaluate the potential intermediacy of a GTP-binding protein in the stimulation of phospholipase A₂ activity by EGF reported in the current study.

There are several potentially important physiological implications for the phospholipase-A₂-stimulatory activity of EGF in the kidney. The kidney is a rich source of EGF, which appears in the urine of mammalian species [2]. Possible sources of EGF in the kidney include production by renal tubules [1], and possibly arterioles [38], as well as production by infiltrating mononuclear cells and platelets in inflammatory conditions [39,40]. Prostaglandin production in response to vasoactive hormones at key sites (mesangial cell and renomedullary interstitial cell) has been shown to regulate haemodynamic and hydro-osmotic responses in the kidney, and thus form the potential basis for modulation of these responses by EGF. Indeed, in preliminary reports, EGF has been shown to modulate both glomerular filtration and water reabsorption [41,42], and in published reports prostaglandin-dependent haemodynamic effects of EGF on vascular smooth muscle have been demonstrated [43]. Finally, in animal models of obstructive uropathy, an unidentified factor released by macrophages enhances prostaglandin production by renal cells in response to vasoactive hormones, and this is thought to be the basis for some of the perturbations in renal function characteristic of this lesion [44].

Irrespective of these potential physiological implications, the findings in the current study provide a clear demonstration of the modulation of phospholipase A₂ activity by EGF in a manner which is independent of alterations in phospholipase C, Ca²⁺, and protein kinase C signalling responses. Further studies in this model

system are likely to provide additional insights regarding the mechanism for this important signalling action of EGF.

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REFERENCES

1. Bell, G. I., Fong, N. M., Stempien, M. M., Wormstead, M. A., Caput, D., Ku, L., Urdea, M. S., Rall, L. B. & Sanchez-Pescador, R. (1986) *Nucleic Acids Res.* **14**, 8427–8446.
2. Starkey, R. H. & Orth, D. N. (1977) *J. Clin. Endocrinol. Metab.* **45**, 1144–1153.
3. Margolis, B. L., Bonventre, J. V., Kremer, S. G., Kudlow, J. E. & Skorecki, K. L. (1988) *Biochem. J.* **249**, 587–592.
4. Knauss, T. C., Mene, P., Kester, M. & Abboud, H. E. (1988) *Kidney Int.* **33**, 162 (abstr.)
5. Schlondorff, D. (1987) *FASEB J.* **1**, 272–281.
6. Schlondorff, D. (1986) *Am J. Med.* **81**, Suppl. **2B**, 1–11.
7. Yokota, K., Kusaka, M., Oshima, T., Yamamoto, S., Kurihara, N., Yoshino, T. & Kumegawa, M. (1986) *J. Biol. Chem.* **261**, 15410–15415.
8. Abdel-Latif, A. A. (1986) *Pharmacol. Rev.* **38**, 227–272.
9. Pike, L. J. & Eakes, A. T. (1987) *J. Biol. Chem.* **262**, 1644–1651.
10. Johnson, R. M. & Garrison, J. C. (1987) *J. Biol. Chem.* **262**, 17285–17293.
11. Aoyagi, T., Suya, H., Kato, N., Nemoto, O., Kobayashi, H. & Miura, Y. (1985) *J. Invest. Dermatol.* **84**, 168–171.
12. Pepinsky, R. B. & Sinclair, L. K. (1986) *Nature (London)* **321**, 81–84.
13. Flower, R. J. (1987) *Adv. Prostaglandin, Thromboxanes, Leukotriene Res.* **17**, 577–580.
14. Savage, C. R., Jr. & Cohen, S. (1972) *J. Biol. Chem.* **247**, 7609–7611.
15. Harper, P. A., Robinson, J. M., Hoover, R. L., Wright, T. C. & Karnovsky, M. J. (1984) *Kidney Int.* **26**, 875–880.
16. Bligh, E. G. & Dyer, W. J. (1959) *Can. J. Biochem. Physiol.* **37**, 911–919.
17. Holub, B. J. & Skeaff, C. M. (1987) *Methods Enzymol.* **141B**, 234–244.
18. Mahadevappa, V. G. & Holub, B. J. (1986) *Biochem. Biophys. Res. Commun.* **134**, 1327–1333.
19. Pfeilschifter, J., Kurtz, A. & Bauer, C. (1984) *Biochem. J.* **223**, 855–859.
20. Bonventre, J. V., Skorecki, K. L., Kreisberg, J. I. & Cheung, J. Y. (1986) *Am. J. Physiol.* **251**, F94–F102.
21. Martin, T. W. & Wysolmerski, R. B. (1987) *J. Biol. Chem.* **262**, 13086–13092.
22. Christie, W. W. (1982) *Lipid Analysis*, 2nd edn., p. 19, Pergamon Press, Toronto.
23. Troyer, D. A., Kreisberg, J. I., Schwertz, D. W. & Venkatachalam, M. A. (1985) *Am. J. Physiol.* **249**, F139–F147.
24. Schlondorff, D., Perez, J. & Satriano, J. A. (1985) *Am. J. Physiol.* **248**, C119–C126.
25. Chambard, J. C., Paris, S., L'Allemain, G. & Pouyssegur, J. (1987) *Nature (London)* **326**, 800–803.
26. Tsuda, T., Hamamori, Y., Fukumoto, Y., Kaibuchi, K. & Takai, Y. (1986) *J. Biochem. (Tokyo)* **100**, 1631–1635.
27. Takasu, N., Yamada, T. & Shimizu, Y. (1987) *FEBS Lett.* **225**, 43–47.
28. Rigas, A. & Levine, L. (1984) *J. Pharmacol. Exp. Ther.* **231**, 230–235.
29. Schlondorff, D., DeCandido, S. & Satriano, J. A. (1987) *Am. J. Physiol.* **253**, C113–C120.

30. Grinstein, S., Cohen, S. & Rothstein, A. (1984) *J. Gen. Physiol.* **83**, 341–369
31. Margolis, B. L., Kremer, S., Holub, B. & Skorecki, K. L. (1988) *Kidney Int.* **33**, 274 (abstr.)
32. Fava, R. A. & Cohen, S. (1984) *J. Biol. Chem.* **259**, 2636–2645
33. Davidson, F. F., Dennis, E. A., Powell, M. & Glenney, J. R., Jr. (1987) *J. Biol. Chem.* **262**, 1698–1705
34. Touqui, L., Rothhut, B., Shaw, A. M., Fradin, A., Vargaftig, B. B. & Russo-Marie, F. (1986) *Nature (London)* **321**, 177–180
35. Schlaeper, D. D. & Haigler, H. T. (1987) *J. Biol. Chem.* **262**, 6931–6937
36. Murayama, T. & Ui, M. (1985) *J. Biol. Chem.* **260**, 7226–7233
37. Burch, R. M., Luini, A. & Axelrod, J. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7201–7205
38. Olsen, P. S., Nexø, E., Poulsen, S. S., Hansen, H. F. & Kirkegaard, P. (1984) *Regul. Pept.* **10**, 37–45
39. Kurobe, M., Tokida, N., Furukawa, S. & Hayashi, K. (1986) *Biochem. Int.* **13**, 729–733
40. Kasselberg, A. G., Orth, D. N., Gray, M. E. & Stahlman, M. T. (1985) *J. Histochem. Cytochem.* **33**, 315–322
41. Badr, K. & Harris, R. C. (1987) *Clin. Res.* **35**, 542 (abstr.)
42. Breyer, M. D., Jacobsen, H. R. & Breyer, J. (1988) *Kidney Int.* **33**, 255 (abstr.)
43. Maramatsu, I., Hollenberg, M. D. & Lederis, K. (1985) *Can. J. Physiol. Pharmacol.* **63**, 994–999
44. Thomasson, D. L., Zenser, T. V. & Davis, B. B. (1988) *Kidney Int.* **33**, 71–76

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