

# Stimulation by epidermal growth factor of phospholipid methyltransferase in isolated rat hepatocytes

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Epidermal growth factor produces a time- and dose-dependent activation of phospholipid methyltransferase activity in hepatocytes isolated from juvenile and mature hepatectomized rats. This treatment however has no effect with hepatocytes isolated from mature or laparotomized rats. Dansylcadaverine ( $50\mu\text{M}$ ), an inhibitor of receptor-mediated internalization of epidermal growth factor, has no effect on basal phospholipid methyltransferase but inhibits the stimulation of this enzyme by epidermal growth factor.

These results indicate a possible role of phospholipid methylation during liver proliferation.

<i>Phospholipid methylation</i>	<i>Epidermal growth factor</i>	<i>Hepatocyte</i>
<i>Liver regeneration</i>	<i>Dansylcadaverine</i>	

## 1. INTRODUCTION

The binding of epidermal growth factor (EGF) to specific cell surface receptors originates a wide variety of effects which culminate with cell growth and division (reviews [1,2]). The early responses involve the phosphorylation of membrane proteins [3] and the internalization of bound EGF [4]. The effects of EGF on glycolysis and other metabolic events are also observed within a few minutes after the addition of the hormone [1,2]. We have studied the effect of EGF addition on phospholipid methyltransferase by hepatocytes isolated from juvenile, mature and mature hepatectomized rats. Addition to isolated rat hepatocytes of hormones that use  $\text{Ca}^{2+}$  or cAMP as a second messenger, activate phospholipid methyltransferase [5–7]. This paper shows a time- and dose-dependent activation of phospholipid methyltransferase by EGF in hepatocytes isolated from juvenile and mature hepatectomized rats but not in normal mature animals. This activation is observed about 5 min after stimulation indicating that it is one of the early cellular

responses triggered by EGF in hepatocytes. These results indicate a possible role of phospholipid methylation during liver proliferation.

## 2. METHODS

Juvenile (20–30 days, 60–80 g) and mature (60–90 days, 300–350 g) Wistar rats were used. Partial hepatectomy was performed as in [8] under ether anesthesia. All animals had free access to food and water. Hepatocytes were isolated as in [5,7]. Isolated hepatocytes were then shaken in stoppered 20 ml vials at  $37^{\circ}\text{C}$  in the presence of glucose as in [5,7]. Cells were stimulated after 30 min preincubation. EGF was dissolved in saline. At various times after the addition of EGF the hepatocytes were poured into precooled centrifuge tubes and immediately centrifuged at  $100 \times g$  for 20 s. The supernatant was then discarded and the pellet was homogenized and phospholipid methyltransferase assayed as in [5,7]. EGF was a generous gift of Dr Ira Pastan, NIH. *S*-[methyl- $^3\text{H}$ ]Adenosyl-L-methionine ( $15\text{ Ci/mmol}$ ) was from New England Nuclear.

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Table 1

Effect of EGF addition on phospholipid methyltransferase by rat hepatocytes isolated from juvenile, mature hepatectomized and mature laparotomized animals

Animal	% of control
Juvenile	159 $\pm$ 3
Mature	110 $\pm$ 4
Mature hepatectomized	163 $\pm$ 2
Mature laparotomized	108 $\pm$ 2

Hepatocytes were stimulated with 25 ng EGF/ml, and 3 min (juvenile rats) or 5 min later (mature rats) the activity phospholipid methyltransferase was assayed.

Results are the mean  $\pm$  SEM of 3 independent experiments in triplicate

### 3. RESULTS AND DISCUSSION

Treatment of hepatocytes isolated from juvenile rats with 25 ng EGF/ml, stimulates phospholipid methyltransferase (table 1). This treatment however has no effect with hepatocytes isolated from mature rats but is observed again with hepatocytes isolated from mature hepatectomized animals. This effect of EGF with hepatectomized rats is specific since it is not observed with hepatocytes isolated from laparotomized animals. EGF stimulation of phospholipid methyltransferase is maximal about 36 h after hepatectomy and it is not

observed with hepatocytes isolated 72 h after hepatectomy when the liver recovers its original cellular mass (not shown). These results indicate that the effect of EGF on phospholipid methyltransferase is specifically associated to liver growth and agree with the proposal that EGF plays a role during liver regeneration [9].

The time-dependent activation of phospholipid methyltransferase by EGF is shown in fig.1. In hepatocytes isolated from juvenile rats maximal activation is observed within 3 min of the addition of EGF and in hepatocytes isolated from hepatectomized rats maximal activation occurs about 5 min after stimulation. This pattern is similar to that reported for glucagon and vasopressin or angiotensin [5,7]. The dose-dependent activation of phospholipid methyltransferase by EGF with hepatocytes isolated from both juvenile and hepatectomized rats is shown in fig.2. In both cases the activation is maximal at about 25 ng EGF/ml. This concentration of EGF is similar to what has been shown to be effective for the phosphorylation of membrane proteins [3], enhancement of calcium uptake and phosphatidylinositol turnover [10]. The effect of EGF on phospholipid methyltransferase seems to be dependent on an influx of  $\text{Ca}^{2+}$ , since under conditions of  $\text{Ca}^{2+}$  deprivation (EGTA incubation of hepatocytes) phospholipid methyltransferase was not stimulated by the addition of EGF (not shown). These results agree with our previous observation showing that the addition to rat

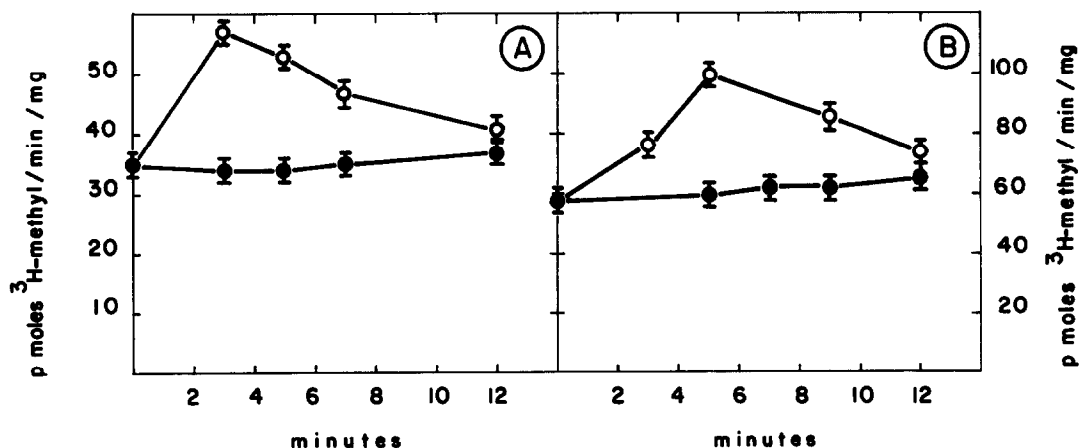


Fig.1. Time course of the effect of EGF on phospholipid methyltransferase activity in isolated rat hepatocytes from juvenile (A) and hepatectomized (B) rats: (○) EGF (25 ng/ml)-treated cells; (●) control hepatocytes. At zero time EGF was added. Values are means  $\pm$  SEM from 3 independent experiments in triplicate.

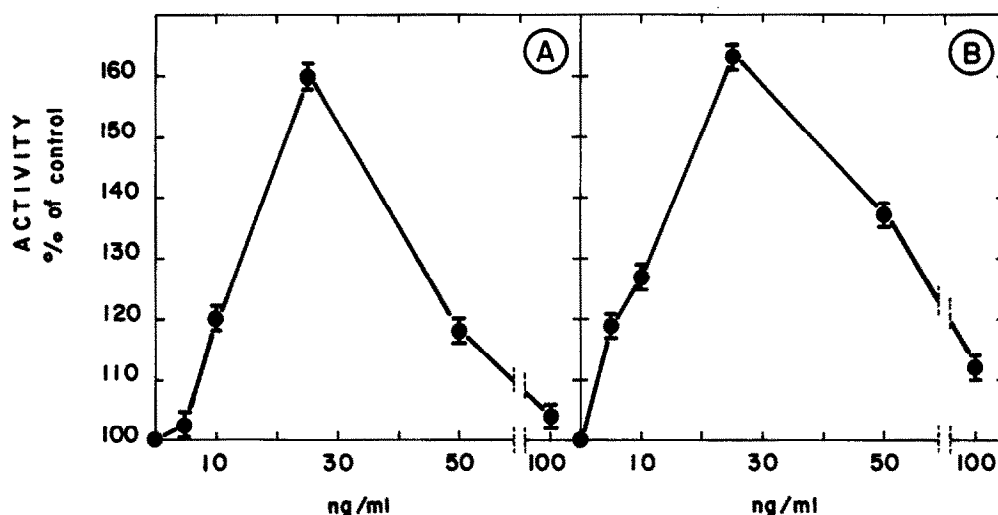


Fig.2. Effect of various concentrations of EGF on phospholipid methyltransferase activity of isolated rat hepatocytes from juvenile (A) and hepatectomized (B) rats. Aliquots of hepatocytes for enzyme assay were taken 3 min (A) and 5 min (B) after the addition of EGF. Values are means  $\pm$  SEM from 3 independent experiments in triplicate.

hepatocytes of hormones whose actions are mediated by  $\text{Ca}^{2+}$  or the cationophore A23187 activate phospholipid methyltransferase [5].

Dansylcadaverine, an inhibitor of receptor-mediated internalization of EGF [11], has no effect on basal phospholipid methyltransferase but inhibits the stimulation by EGF of this enzyme (table 2). Since internalization of bound EGF is also a rapid process, these results are compatible with a role of receptor internalization in the activation of phospholipid methyltransferase by EGF.

Many signals which start their biological effects

by binding to specific cell surface receptors activate phospholipid methylation (review [12]). Although it is not yet clear what is the function of phospholipid methylation, these results strongly suggest that this process is involved in signal transduction. These data add EGF to the list of signals that modulate phospholipid methylation. The significance of the transient stimulation by EGF of phospholipid methyltransferase to the biological effects elicited by EGF has yet to be determined. However, it is of interest that the addition of nerve growth factor, another potent growth factor, to growing rat neuritis also induces a transient stimulation of phospholipid methylation [13] and that the addition of insulin, also a growth factor, induces a transient stimulation of phospholipid methylation in chicken embryo [14]. Although it is tempting to suggest that phospholipid methylation mediates the effects elicited by growth factors, before making any generalization it would be important to know if other growth factors also modulate phospholipid methylation.

Table 2

Effect of dansylcadaverine (DACAD) on the stimulation by EGF of phospholipid methyltransferase in hepatocytes isolated from juvenile rats

Addition	% of control
EGF (25 ng/ml)	168 $\pm$ 0.3
DACAD (50 $\mu\text{M}$ )	107 $\pm$ 5
EGF (25 ng/ml) + DACAD (50 $\mu\text{M}$ )	103 $\pm$ 2

Hepatocytes were stimulated with EGF 10 min after the addition of dansylcadaverine. The activity phospholipid methyltransferase was assayed 3 min after stimulation.

Results are the mean  $\pm$  SEM of 3 independent experiments in triplicate

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