Inhibitors of protein and RNA synthesis cause a rapid block in prostaglandin production at the prostaglandin synthase step

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ABSTRACT Inhibitors of protein or RNA synthesis prevented prostaglandin (PG) production in isolated skeletal muscles, brain, and spleen. Incubation of rat muscles with cycloheximide prevented the stimulation of PGE₂ production induced in vitro by the Ca^{2+} ionophore A23187 and in vivo by injection of endotoxin. Cycloheximide also inhibited the stimulation by arachidonic acid of PGE₂₆₀₆ and prostacyclin. These observations suggest that the block in prostaglandin production results from a loss of PG synthase activity (EC 1.14.99.1). These effects were detectable within 10 min after exposure of the muscle to cycloheximide. The degree of inhibition of PG production correlated with the degree of inhibition of protein synthesis. Other inhibitors of protein synthesis, puromycin and emetine, also prevented conversion of arachidonate into PGE₂ in these tissues, but they did not inhibit purified PG synthase. Exposure of muscles to actinomycin D for 20 min also reduced PGE₂ production from arachidonate by 90%. Thus, both the PG synthase and its mRNA appear to be inactivated rapidly (t₁/₂ < 10 min) in muscle and other mammalian tissues. The block in PG production induced by inhibitors of protein and RNA synthesis may account for their antipyrogenic actions and certain of their other physiological effects.

Inhibitors of protein synthesis, such as cycloheximide and puromycin, and inhibitors of RNA synthesis, especially actinomycin D, have been widely used to determine whether these processes are necessary for various physiological or cellular adaptations. If a biological response is prevented by such inhibitors, it is inferred that the response itself requires production of a new polypeptide and that other metabolic processes are not affected. In this communication we show that various inhibitors of translation and transcription rapidly block the synthesis of prostaglandins (PGs). These metabolites of arachidonic acid participate in a wide variety of pathological and physiological processes, including inflammation, hemostasis, initiation of fever, and the regulation of protein turnover (1–6) in skeletal muscle. In the course of our studies on the role of PGs in the activation of muscle proteolysis during fever (7, 8), we observed that muscles treated with cycloheximide synthesized little or no PGE₂. Other investigators (9–12) have noted that prolonged exposure of cultured cells to cycloheximide prevents the rise in PGs induced by platelet-derived growth factor, interleukin 1, or phorbol esters.

The present studies were undertaken to characterize further this unexpected effect of cycloheximide in muscle, to determine whether other metabolites of arachidonic acid and other mammalian tissues are affected similarly, and to explore the mechanism by which inhibitors of protein and RNA synthesis may reduce PG production. The present studies indicate that the rate-limiting enzyme in PG production and its mRNA are amongst the most short-lived in mammalian cells and that inhibitors of protein and RNA synthesis may elicit physiological effects indirectly by blocking PG production.

METHODS

Male CD rats (40–50 g), purchased from Charles River Breeding Laboratories, were housed in a temperature- and humidity-controlled environment with a 14-hr light/10-hr dark cycle for 2–4 days before each experiment. Rats were killed by cervical dislocation and the extensor digitorum longus muscles were excised, blotted, and weighed. In some experiments, spleen and brain were dissected out, washed in the incubation buffer, and sliced into four 1- to 3-mm sections. Tissues were incubated at 37°C for 30–60 min, then transferred to fresh media (1–2 ml) and incubated at 37°C for 1–3 hr as indicated. Incubations were carried out in Krebs–Henseleit buffer (pH 7.4) (13) equilibrated with O₂/CO₂ (19:1, vol/vol) supplemented with glucose (5 mM), insulin (0.1 unit/ml), leucine (0.85 mM), isoleucine (0.5 mM), and valine (1 mM). After incubation, the tissues were removed, the media were put on ice or frozen at −20°C, and the muscles were homogenized in 10% trichloroacetic acid.

In some experiments [U-¹⁴C]phenylalanine (0.05 mM, 0.1 mCi/mmol; 1 Ci = 37 GBq) was added and rates of protein synthesis were estimated from the amount of phenylalanine incorporated into muscle protein during the incubation as described previously (14). The muscle homogenates were centrifuged (10 min at 2000 × g), and the pellet was washed twice with 10% trichloroacetic acid and then once with diethyl ether. The washed pellets were solubilized, and the amount of [U-¹⁴C]phenylalanine incorporation was determined by liquid scintillation counting.

The release of PGE₂, PGF₂α, and 6-keto-PGF₁α into the incubation medium was measured by radioimmunoassay. Aliquots of the incubation media of muscles were used in the radioimmunoassay without solvent extraction. PGs in the incubation media of brain and spleen were extracted (to prevent binding to cellular debris) with 3 vol of ethyl acetate after the pH had been decreased to 3.5 with glacial acetic acid. The ethyl acetate in the upper organic phase containing PGs was evaporated, and the remaining residue was dissolved in Krebs–Henseleit buffer. Amounts of PG in the samples were compared with a standard curve in which PG standards were prepared in the incubation buffer containing the additions used in the experiment. This was important because the addition of cycloheximide, puromycin, or emetine to the incubation medium at micromolar to millimolar concentrations was found to prevent complete separation of the free radiolabeled PG from the antibody-bound PG. In the radioimmunoassay, dextran-coated charcoal is

Abbreviation: PG, prostaglandin.
used to bind free $[^3H]PGE_2$, and in control studies, these inhibitors of protein and RNA synthesis were found to bind also to the charcoal.

PG synthase (EC 1.14.99.1) was purified from ram seminal vesicle microsomes (15) and assayed by L. Marnett. The purified enzyme, which ran as a single band on NaDodSO$_4$/polyacrylamide gel electrophoresis, exhibited cyclooxygenase and peroxidase activities in the presence of hemin (15).

Arachidonic acid oxidation by the purified PG synthase was determined after a 3-min incubation of the purified enzyme (150 nM) in 0.1 M sodium phosphate (pH 7.8) containing hematin (400 nM) and phenol (500 µM), stirred at 37°C, in the presence or absence of the inhibitors of protein synthesis (5–50 µM). Assays were performed in triplicate. Arachidonic acid (10 µM) was then added, and the initial velocity of oxygen uptake was determined by using a Clark electrode (Yellow Springs Instrument).

**Materials.** Cycloheximide, puromycin, emetine, and actinomycin D were purchased from Sigma. Porcine insulin was a gift of Eli Lilly. [U-¹⁴C]Phenylalanine, [5,6,8,9,11,12,14,15-H(N)]PGE$_2$, and [5,6,8,9,11,12,14,15-H(N)]PGF$_2$α were purchased from New England Nuclear. 16,16-DimethylPGE$_2$ and 16,16-dimethylPGF$_2$α standards were obtained from Upjohn. Rabbit anti-PGE and rabbit anti-PGF were obtained from Miles-Yeda (Rehovot, Israel). A 6-keto$[^3H]$PGF$_1$α radioimmunoassay kit was purchased from Seragen (Boston, MA). Arachidonic acid was purchased from Nuc Check Prep and Ca$^{2+}$ ionophore A23187 from Calbiochem-Behring. Lipopolysaccharide (Escherichia coli J5, rough strain) was purchased from List Biological Laboratories (Campbell, CA).

**RESULTS.** Addition of cycloheximide to incubated extensor digitorum longus muscles from normal rats inhibited, almost completely, the basal production of PGE$_2$ (Table 1, Exp. 1). Skeletal muscles from rats injected with *E. coli* endotoxin show an enhanced production of PGE$_2$, and this effect is associated with the acceleration of muscle protein breakdown (7, 8).

Table 1. Effects of cycloheximide on production of PGE$_2$ and prostacyclin by muscle

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>PGE$_2$ Without CX</th>
<th>PGE$_2$ With CX</th>
<th>6-Keto-PGF$_2$α Without CX</th>
<th>6-Keto-PGF$_2$α With CX</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>66 ± 11</td>
<td>8 ± 2</td>
<td>163 ± 34</td>
<td>9 ± 3</td>
</tr>
<tr>
<td></td>
<td>Endotoxin i.p.</td>
<td>ND</td>
<td>ND</td>
<td>320 ± 16</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>ND</td>
<td>ND</td>
<td>320 ± 16</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>+ A23187</td>
<td>18 ± 4</td>
<td>2 ± 0</td>
<td>39 ± 9</td>
<td>2 ± 1</td>
</tr>
<tr>
<td></td>
<td>+ arachidonic acid</td>
<td>66 ± 8</td>
<td>5 ± 1</td>
<td>136 ± 12</td>
<td>27 ± 11</td>
</tr>
</tbody>
</table>

Values represent the mean ± SEM of eight muscles from eight animals. ND, not detectable. PG production by contralateral extensor digitorum longus muscles was determined during a 2-hr incubation (after a 30-min preincubation) in the presence or absence of 0.25 mM cycloheximide (CX). In Exp. 2, animals were injected intraperitoneally with 0.4 ml of 0.9% NaCl or *E. coli* J5 lipopolysaccharide (5 µg of lipopolysaccharide per 100 g of body weight) 12 hr prior to the experiment (7, 8). In Exp. 2 and 3, extensor digitorum longus muscles from normal animals were preincubated and incubated with or without the Ca$^{2+}$ ionophore A23187 (10 µM) or arachidonic acid (20 µM). A23187 was first solubilized in dimethyl sulfoxide and in control tissues this solvent was added at the same concentration (usually <0.1%).

This increased synthesis of PGE$_2$ by muscles from endotoxin-treated rats could also be completely inhibited by the addition of cycloheximide to the incubation medium (Table 1, Exp. 1). Incubation of muscle from normal rats with arachidonic acid, the precursor of the PGs, or with the Ca$^{2+}$ ionophore A23187, also stimulates PGE$_2$ and PGF$_2$α production (1, 2). Exposure to cycloheximide blocked completely this accelerated production of PGE$_2$ induced by either arachionate or the ionophore (Table 1, Exp. 2 and 3).

Synthesis of both PGE$_2$ and PGF$_{2α}$ decreased very rapidly after addition of cycloheximide (Fig. 1 and data not shown). When muscles exposed for 30 min to arachidonic acid (10 µM) were transferred to fresh medium containing arachidonate with or without cycloheximide (0.1 mM), the controls produced PGE$_2$ and PGF$_2$α at a linear rate for 1 hr, but those exposed to cycloheximide did not synthesize significant amounts of either PGE$_2$ or PGF$_{2α}$ (Fig. 1 and data not shown). In fact, within only 10–20 min after exposure to cycloheximide, production of both PGE$_2$ and PGF$_2$α ceased. In addition, this agent blocked almost completely the synthesis of prostacyclin (PGI$_2$) from arachidonic acid. This process was determined by measuring the release of the stable metabolite of PGI$_2$, 6-keto-PGF$_{1α}$, which fell by 95% after addition of cycloheximide (Table 1). In addition, cycloheximide prevented any significant rise in PGE$_2$ production above control levels when arachidonic acid was added to stimulate PGI$_2$ synthesis. Since the marked fall in production of PGE$_2$, PGF$_{2α}$, and PGI$_2$ was also observed after addition of arachidionate, the block appears to be at the conversion of arachidionate to PGI$_2$, the common precursor of prostacyclin, thromboxane, and the prostaglandins (16). These effects of cycloheximide appear similar to those of cyclooxgenase inhibitors — e.g., aspirin and indomethacin (17) — and therefore cycloheximide, either indirectly or directly, must be blocking the cyclooxygenase and/or the peroxidase activity (18) of the PG synthase.

To examine whether similar effects of cycloheximide occur in other tissues as well, we incubated slices of brain and spleen with this agent and measured PG production. In both tissues, cycloheximide almost completely inhibited PGE$_2$ synthesis from arachidonic acid, as was seen in muscle (Table

![Fig. 1. Extensor digitorum longus muscles were first washed in incubation medium for 15 min at 37°C, then transferred to fresh medium containing arachidonic acid (10 µM) for a 30-min preincubation. Arachidonic acid was stored in a sealed ampule under nitrogen at −20°C and was freshly prepared in absolute ethanol before addition to the incubation buffer. At zero time, muscles were transferred to fresh medium containing arachidonic acid (10 µM) but in the presence or absence of cycloheximide (0.1 mM) or actinomycin D (10 µM) and incubated for up to 1 hr. Values represent the mean ± SEM for eight muscles.](attachment:image)
Table 2. Inhibition by cycloheximide of arachidonic acid-stimulated PGE2 production by rat spleen and brain slices

<table>
<thead>
<tr>
<th></th>
<th>PGE2 production, pg/hr per mg of muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No inhibitor</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>19.4 ± 1.5</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>30.6 ± 3.7</td>
</tr>
<tr>
<td>Stimulation</td>
<td>57%*</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>4.8 ± 0.5</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>9.6 ± 1.1</td>
</tr>
<tr>
<td>Stimulation</td>
<td>99%*</td>
</tr>
</tbody>
</table>

Slices of spleen and brain were preincubated for 30 min and incubated for 2 hr at 37°C as described in the text. Arachidonic acid (50 μM) (prepared as described for Fig. 1) and cycloheximide (0.25 mM) were added to the preincubation and incubation media where indicated. In tissues not exposed to arachidonic acid, absolute ethanol was added at the same concentration (usually <0.1%). Values represent the mean ± SEM of eight samples from eight animals. NS, no significant inhibition or stimulation. *P < 0.05 by the paired Student’s t test.

2). Other investigators have also noted a marked reduction of PGE2 production when protein synthesis was inhibited by cycloheximide in various experimental systems, including dermal fibroblasts stimulated by interleukin 1 (9) or human platelet-derived growth factor (12) and in cultured hepatocytes and kidney cells treated with tumor promoters (10, 11). Thus, similar effects of cycloheximide seem to occur in a variety of mammalian cells.

To test whether the blockage of PGE2 production was due to the inhibition of protein synthesis or was a specific effect of cycloheximide on the synthase, we incubated muscles with other inhibitors of protein synthesis (Table 3). Puromycin (50 μM) and emetine (50 μM) (19), like cycloheximide, inhibited the incorporation of [U-14C]phenylalanine into muscle proteins by 80–90% and also markedly inhibited the production of PGE2 (by 72–85%) (Table 3). The degree of inhibition of PGE2 production by these agents was thus similar to the percent fall in protein synthesis. Furthermore, lower concentrations of cycloheximide (0.5 μM), which reduced protein synthesis partially (by 57%), caused only a 25% inhibition of PGE2 production (Table 3, Exp. 2). At 0.005 μM, no significant inhibition of protein synthesis occurred and no effect on PGE2 synthesis was detected.

The similar effects seen with the different inhibitors of protein and RNA synthesis (puromycin, emetine, and cycloheximide), which have quite different structures, makes it unlikely that these compounds act by inhibiting the PG synthase and argue strongly that these agents act by preventing synthesis of this enzyme. Further evidence supporting this conclusion was obtained in muscles treated with emetine, which, unlike cycloheximide, inhibits protein synthesis irreversibly (19). In muscles extensively washed free of emetine and incubated for an additional 2 hr, protein synthesis was still inhibited by 82% and production of PGE2 stimulated with the Ca2+ ionophore A23187 was still inhibited by 87% (data not shown).

To test whether inhibitors of protein synthesis influence the PG synthase directly, L. Marnett exposed the enzyme purified from ram seminal vesicle microsomes to these inhibitors and measured the conversion of arachidonic acid to PGH2. Addition of these inhibitors to the purified PG synthase had no effect on the ability of this enzyme to oxidize arachidonic acid. In control incubations, the initial rate of arachidonic acid oxidation was 182 ± 8 μM O2 per min and 182 ± 2 μM O2 per min with 50 μM emetine, 196 ± 8 μM O2 per min with 50 μM puromycin, and 171 ± 9 μM O2 per min with 50 μM cycloheximide added. Under the same conditions, the PG synthase inhibitor indomethacin (3 μM) abolished oxygen uptake. Thus, inhibitors of protein synthesis do not act as simple inhibitors of the PG synthase. It is therefore likely that the PG synthase is subject to rapid turnover, and, in the absence of continued protein synthesis, this enzymatic activity is lost very rapidly through irreversible inactivation, proteolytic degradation, or both.

Table 3. Effect of inhibitors of protein and RNA synthesis on PGE2 production stimulated by arachidonic acid in muscle

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Inhibitor</th>
<th>Arachidonic acid</th>
<th>PGE2 production</th>
<th>Protein synthesis</th>
<th>% inhibition</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pg/hr per mg of muscle</td>
<td></td>
<td>pmol [14C]Phe</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>incorporated per hr</td>
<td>per mg of muscle</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>None</td>
<td>-</td>
<td>34 ± 4</td>
<td>158 ± 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>+</td>
<td>62 ± 5</td>
<td>160 ± 8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Puromycin (50 μM)</td>
<td>+</td>
<td>9 ± 2</td>
<td>32 ± 2</td>
<td>80*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Emetine (50 μM)</td>
<td>+</td>
<td>17 ± 4</td>
<td>16 ± 2</td>
<td>90*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>-</td>
<td>22 ± 5</td>
<td>157 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>+</td>
<td>46 ± 3</td>
<td>166 ± 8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cycloheximide (50 μM)</td>
<td>+</td>
<td>7 ± 2</td>
<td>16 ± 1</td>
<td>90*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cycloheximide (0.5 μM)</td>
<td>+</td>
<td>34 ± 3</td>
<td>71 ± 8</td>
<td>57*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cycloheximide (0.005 μM)</td>
<td>+</td>
<td>47 ± 4</td>
<td>151 ± 11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>-</td>
<td>24 ± 2</td>
<td>154 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Actinomycin D (10 μM)</td>
<td>-</td>
<td>2 ± 0</td>
<td>111 ± 5</td>
<td>28†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>+</td>
<td>53 ± 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Actinomycin D (10 μM)</td>
<td>+</td>
<td>5 ± 1</td>
<td></td>
<td>90†</td>
<td></td>
</tr>
</tbody>
</table>

Extensor digitorum longus muscles were preincubated for 1 hr and then incubated for 2 hr (PGE2 was measured in the first hour and protein synthesis was determined in the subsequent hour) at 37°C in the absence or presence of arachidonic acid (10 μM) and cycloheximide, puromycin, emetine, or actinomycin D at the indicated concentrations. Values represent the mean ± SEM of eight muscles per group. NS, not significant.

*P < 0.005 by the unpaired Student’s t test.
†P < 0.005 by the paired Student’s t test.
Because of these large effects of protein synthesis inhibitors, we tested whether an inhibitor of RNA synthesis, actinomycin D (20), also inhibited PGE₂ production in muscle. The addition of actinomycin D (10 μM) to both the preincubation and incubation medium reduced PGE₂ synthesis by 93% in the isolated rat extensor digitorum longus muscles (Table 3, Exp. 2). The acceleration of PGE₂ production induced by arachidonic acid (10 μM) was also inhibited by 90% by actinomycin D. However, actinomycin D (5–50 μM) did not affect the activity of the purified PG synthase (data not shown). Thus, this agent, like cycloheximide, puromycin, and emetine, seems to affect the PG synthase step. With time, exposure to actinomycin D causes an increasing inhibition of protein synthesis (21), presumably because of the loss of more short-lived mRNA under these conditions. PG synthase activity decreased almost completely within the first hour, even though by the second hour overall protein synthesis had fallen by only 28%. Thus inhibition of transcription with actinomycin D seems to inhibit differentially the synthesis of this enzyme. Therefore, the mRNA for the PG synthase, like the enzyme activity, appears to be very short-lived. In fact, the 90% loss of enzyme activity within 10–20 min after addition of actino-
mycin D (Fig. 1) would suggest an mRNA half-life of less than 10 min.

**DISCUSSION**

The finding that inhibitors of protein and RNA synthesis can rapidly block the production of PG may influence the interpretation of various biological experiments using these inhibitors. These concerns are especially important when investigating physiological responses in which metabolites of arachidonic acid are known to play a role. For example, the initiation of fever involves production of PGE₂ from arachidonic acid in the hypothalamus (22). It has been reported that cycloheximide and actinomycin D can reduce fever in human patients with Hodgkin disease and other malignancies (23) and that cycloheximide and other inhibitors of protein synthesis inhibit fever in animals injected with viruses or endogenous pyrogen (22–27). These effects on body temperature thus resemble those seen with cyclooxynase inhibitors (e.g., aspirin and indomethacin). Furthermore, other unexplained effects of these inhibitors [e.g., their ability to influence rates of protein breakdown under certain conditions (28)] may also result from the fall in PGs or thrombox-
ane or indirectly from increased production of other metab-
olites of arachidonic acid due to the block in the cyclooxy-
genase pathway (e.g., leukotrienes).

Our findings indicate that inhibitors of transcription and translation prevent production of PGE₂ by causing a block at the PG synthase step. This enzyme, which displays both cyclooxygenase and peroxidase activities, is responsible for the conversion of arachidonate to PGG₂ and PGH₂, the common precursor for prostacyclin, thromboxane, and the prostaglandins. Effects on later enzymatic steps could not account for the simultaneous fall in PGE₂, PGF₂α, and PGI₂ from arachidonate. The results presented here cannot be explained by rapid turnover of the phospholipase as has been suggested (10). Inhibition at the PG synthase step has also been noted recently by Habenicht et al. (12) in fibroblasts and Daniel et al. (11) in kidney cells. Nevertheless, it is possible that other enzymes in this pathway, in addition to the PG synthase, are also affected (see below).

A concern of particular interest is the rapidly reversible by which the inhibitors of protein and RNA synthesis block the production of PGs. Exposure to cycloheximide and actinomycin D for as little as 10–20 min prevented almost completely the subsequent production of PGE₂ from arachidonate in muscle. These various inhibitors seem to prevent synthesis of the PG synthase and do not influence its activity directly when added to the purified enzyme. Therefore, as shown in Fig. 1 and Table 2, the enzyme, as well as the mRNA encoding it, may be inactivated, degraded, or both very rapidly in vivo; enzyme activity was lost with a half-life of 5–10 min or less, and mRNA half-life was also of 5–10 min. These values would make the enzyme and its mRNA among the most short-lived species thus far reported in mammalian cells. Very few enzymes or polypeptides (either cytosolic or membrane-bound) have been reported in cells with functional half-lives of less than 1 hr (28–33), and in rat skeletal muscle the bulk of proteins seem to have half-lives of several days or longer (28).

Rapid turnover of a protein, such as the PG synthase, means that its level can rise or fall quickly in response to changes in its rate of synthesis. The other short-lived proteins in mammalian cells seem to catalyze rate-limiting steps in metabolic pathways (like the PG synthase) (28, 34) or may be crucial in the regulation of cell growth (30–33). A short half-life of the mRNA for this enzyme would also make possible large adaptive changes in its rate of synthesis. Several other rapidly degraded, inducible enzymes have also been found to have short-lived mRNA (35). Accordingly, a rapid rise in the activity of PG synthase has been reported in a variety of cells in response to stimulation by hormones and growth factors (11, 12, 36–41).

It remains to be established whether the PG synthase undergoes irreversible inactivation, complete degradation, or both after inhibition of its synthesis. In cell extracts, a rapid irreversible autoinactivation of the PG synthase (42) has been reported for both the cyclooxygenase (43, 44) and the peroxidase (45, 46) activity. The enzyme is thought to be inactivated by oxidants that are released during the enzymatic reduction of the hydroperoxide on PGG₂ (45, 46), and the enzyme could be protected in extracts by agents that scavenge oxygen radicals (44–49). The oxidant responsible for the inactivation of the prostaglandin synthase was also found to inactivate the PGI₂ synthase (50, 51) as well as a variety of other enzymes (52). Presumably, this irreversible inactivation seen in extracts also occurs in vivo; therefore continuous replacement of the PG synthase by de novo synthesis would be necessary. Although autoinactivation of the enzyme cannot explain the rapid loss of its mRNA, such a mechanism would account nicely for the rapid disappearance of the enzyme activity after protein synthesis was blocked.

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Physiological Sciences: Fagan and Goldberg


