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Effect of prostaglandin A_1 in the induction of stress proteins in *Aedes albopictus* cells

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Abstract

Prostaglandins are natural fatty acid derivatives with diverse physiological effects, including immune function and the control of cell growth. While the action of prostaglandins in the induction of stress proteins in vertebrate cells is well documented, their functions in invertebrate cells have been poorly investigated. The purpose of the present study was to investigate the effect of prostaglandin A_1 (PGA_1; 0.25, 1.25 and 12.5 µg/ml) on protein synthesis during the growth of *Aedes albopictus* cells. We found that PGA_1 stimulates the synthesis of several polypeptides with molecular masses of 87, 80, 70, 57, 29, 27 and 23 kDa in *Aedes albopictus* cells. When the proteins induced by PGA_1 and those induced by heat treatment were compared by polyacrylamide gel electrophoresis, PGA_1 was found to induce the stress proteins. The HSP70 family and the low-molecular weight polypeptides (29 and 27 kDa, respectively) were induced by PGA_1 in the lag phase. We also observed that PGA_1 is able to induce a 23-kDa polypeptide independently of the growth phase of the cell.

Key words
- Prostaglandin
- Stress proteins
- *Aedes albopictus* cells

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Prostaglandins (PGs) are a class of naturally occurring cyclic 20 carbon fatty acids synthesized from polyunsaturated fatty acid precursors by most types of eukaryotic cells. These compounds have been shown to function as microenvironmental hormones and intracellular signal mediators, and to participate in the regulation of a large variety of physiological and pathological processes (1). PGs of the A series and related compounds, which share a common cyclopentenone structure, present a remarkable inhibitory effect on the replication of several viruses (2).

Several PGs inhibit the rate of cell proliferation in animal and human tumor systems *in vitro* and *in vivo*. Type A and J PGs are the most active in controlling cell proliferation. The antiproliferative activity of PGs may be associated with the induction of heat-shock proteins (HSPs). The mechanism by which PGs can control cell proliferation, however, is still mostly unknown (1).

HSPs are a set of proteins synthesized by prokaryotic and eukaryotic cells in response to heat treatment or other environmental stress conditions. The structure of the major HSP (the 70-kDa family) has been widely conserved through evolution, from bacteria to man, indicating an important role in the survival of the organism (3). Several PGs in-

duce heat-shock proteins. PG A_1 and J_2 induce the synthesis of a 74-kDa protein that was identified as a heat-shock protein related to the major 70-kDa heat-shock protein group (4,5).

PGs and other active derivatives of polyunsaturated fatty acids have been detected in a large number of invertebrate species (6). Recently, Petzel et al. (7) reported the presence of arachidonic acid and PG E_2 in Malpighian tubules of *Aedes aegypti*. Our group reported (8) that PGA_1 inhibits replication of Mayaro virus in *Aedes albopictus* cells. The presence of these molecules has been described in some insects since the early 70s, and their biological significance is related to reproduction, cellular defense mechanisms and ion and water transport (6). In the present report, we study the action of PGA_1 in the induction of stress proteins during the growth of *Aedes albopictus* (mosquito) cells, clone C6/36. This clone was isolated by Igarashi (9) and is sensitive to the growth of several arboviruses.

This cell line was a gift from the Arbovirus Research Unit, Yale University, USA. The cells were grown in 60-cm^2 glass bottles at 28°C in medium consisting of Dulbecco's modified Eagle medium supplemented with 0.2 mM non-essential amino acids, 2.25% $NaHCO_3$, 2% fetal calf serum, penicillin (500 U/ml), streptomycin (100 µg/ml) and amphotericin B (Fungizone, 2.5 µg/ml). For subcultures, confluent monolayers containing 1.5×10^7 cells/bottle were gently washed with Dulbecco's phosphate-buffered saline (PBS) and, after brief trypsinization, suspended in culture medium. The monolayers grown in scintillation vials were incubated at 28°C in an atmosphere of 5% CO_2. The growth curve of *Aedes albopictus* cells was determined by counting aliquots in a hematocytometer. Monolayers containing 10^5 cells/vial (10 h after seeding) were considered to be in the lag phase, whereas those containing 5×10^5 cells/vial (52 h after seeding) were considered to be in the exponential phase. A totally confluent monolayer was observed for the stationary phase with 3×10^6 cells/vial (96 h after seeding).

Prostaglandin A_1 (Sigma Chemical Co, St. Louis, MO) was stored as a 100% ethanol stock solution (1 mg/ml) at -20°C and diluted to the indicated concentrations. Control medium contained the same concentration of ethanol diluent, which was shown not to affect cell growth.

Cells cultured in scintillation vials were exposed to growth medium with or without PGA_1 for 12 h. Thereafter, the medium was replaced with methionine-free Eagle's medium in the absence of serum and cells were preincubated for 30 min at 28°C or at 37°C (heat-shock treatment). After this period, the medium was supplemented with ^{35}S-methionine (20 µCi/ml) and the incubation continued. One hour later, the medium was removed and the cellular proteins were analyzed by SDS-PAGE using the SDS buffer system of Laemmli (10). Equal numbers of cells were applied to each gel lane and the dried gels were exposed to Kodak X-OMAT (YAR-S) film. The molecular weights of proteins were determined by electrophoresis of standard proteins (Pharmacia).

When cell cultures of *A. albopictus* growing at 28°C in the lag phase were transferred to 37°C, we observed the induction of heat-shock protein members of the HSP70 gene family and a group of low-molecular weight (29 and 27 kDa) heat-shock proteins (Figure 1a, lane B). Comparing lanes A and B, we also observed that the synthesis of normal cellular proteins is inhibited by the heat treatment. This phenomenom was not observed in cells in the exponential or stationary phase (Figure 1b and 1c).

In most of the cell cultures studied thus far, the synthesis of heat-shock proteins was paralleled by a strong reduction in the synthesis of most proteins synthesized before the thermal shock (3). Storti et al. (11), working with *Drosophila*, showed that during the heat shock only the heat-shock mRNAs plus

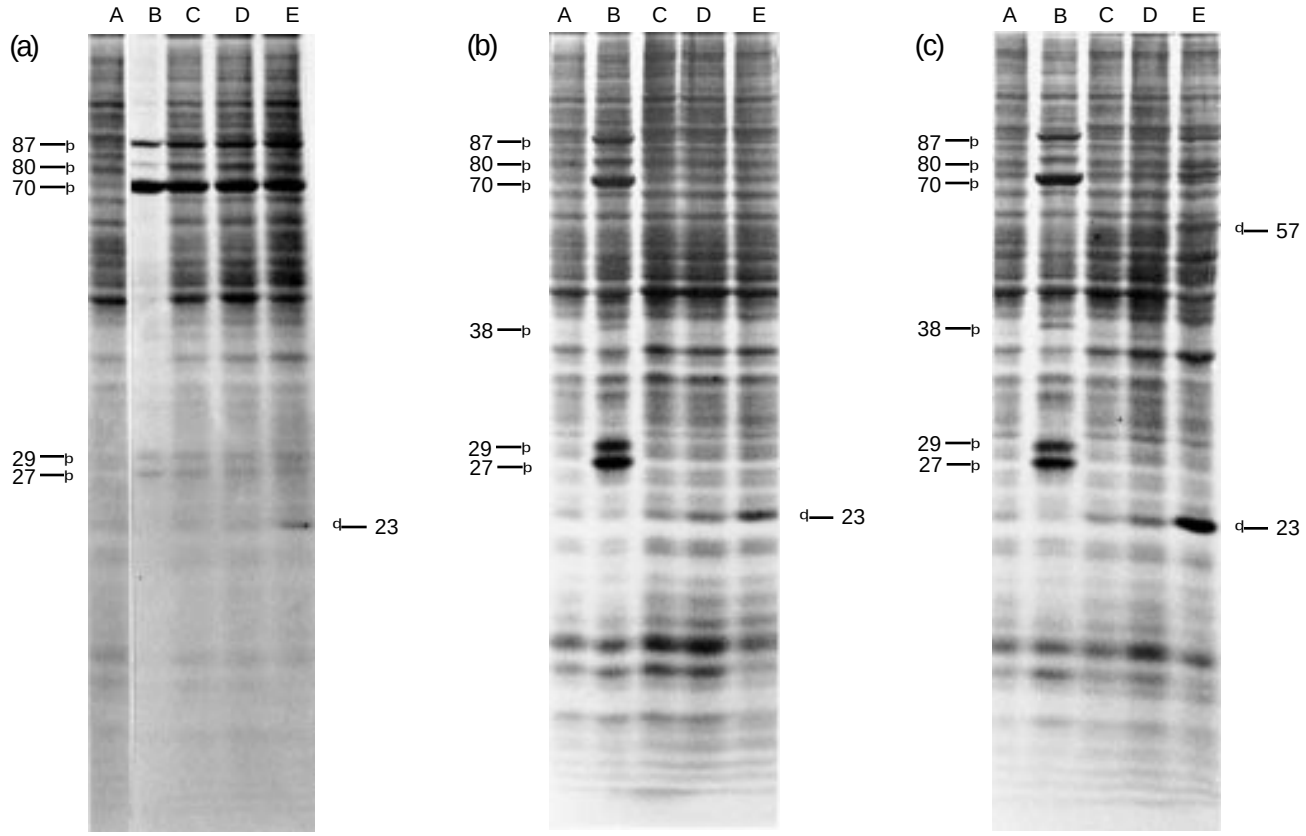

Figure 1 - Effect of PGA_1 on protein synthesis in growing *A. albopictus* cells in the lag phase (a), exponential phase (b), and stationary phase (c). Cell monolayers were labeled with ^{35}S-methionine and the proteins subjected to SDS-PAGE as described. Lane A represents untreated control cells maintained at 28ºC, lane B, heat-shocked cells, and lanes C, D and E, cells treated with PGA_1 at the concentrations of 0.25, 1.25 and 12.5 µg/ml, respectively.

a small number of preexisting mRNAs are translated, while most of the other messages are stored and can be reactivated upon returning the cells to their normal temperature.

In Figure 1a, lanes C, D and E, we compared the proteins induced by PGA_1 with those induced by thermal treatment. As shown in Figure 1, PGA_1 is able to induce, in the lag phase, all the proteins induced by heat (HSPs 87, 80, 70, 29 and 27 kDa). In cells derived from exponential and stationary phases (Figure 1b and 1c, lanes C, D and E) PGA_1 induced the synthesis of the HSP70 family less intensely and failed to induce the group of low-molecular weight HSPs (p38, p29 and p27). PGA_1 induced the synthesis of two other proteins (p57 and p23), which were not observed in heat-shocked cells. These results provide evidence that these proteins represent stress proteins whose expression is primarily regulated by PGA_1 but not by hyperthermia. Protein p57 is visible only in the stationary phase (Figure 1c, lane E) but p23 is found in all of the three growth phases. In the stationary phase (Figure 1c), however, the synthesis of p23 seems to be pronounced and dose dependent.

The induction of the HSP70 family by PGs has been well documented in several mammalian cell lines. This phenomenom has been correlated to an inhibition of cellular proliferation, although a causal relationship between HSP70 expression and growth arrest has not been clearly established nor

has the mechanism of protein induction by PGs been determined (1).

Holbrook et al. (12), studying the effect of PGA_2 on HeLa cells, demonstrated that PGA_2 induces high levels of HSP70 mRNA, which results from an increase in the rate of transcription of the HSP70 genes. This induction is dependent upon protein synthesis and occurs through the interaction of heat-shock transcription factor (HSF) with a specific DNA sequence, with the heat-shock element (HSE) in the promoter regions of the HSP genes increasing their rates of transcription (13).

The induction of heat-shock proteins during the growth of *Aedes albopictus* cells has been previously reported (14). Heat-shock treatment of these cells produces a drastic alteration in the pattern of protein synthesis which is a function of cellular growth.

The present results show that the induction of HSP70 by PGA_1 is highly dependent on the growth state of the cells, occurring in proliferating but not in confluent cells. In the lag phase PGA_1 induces the same proteins induced by heat in *A. albopictus* cells except for the 23-kDa protein.

A vast amount of literature has described the antiproliferative activity of several PGs in a large number of experimental models. However, the mechanism by which some PGs can control cell proliferation is still mostly unknown (1). Santoro et al. (15) reported that type A PGs totally suppress the proliferation of the human erythroleukemic cell line K562 at doses that do not affect cell viability. This action is reversible depending on the duration of treatment and is accompanied by a partial inhibition of protein synthesis and glycosylation, and by the synthesis of a 74-kDa protein. PGs that do not inhibit cell proliferation, such as PGB_2 and PGE_1, did not produce any significant change in protein metabolism and did not induce p74 synthesis (1). Contrary to the induction of high-molecular mass proteins by heat shock, some authors have noted the induction of low-molecular mass proteins in cells submitted to stress conditions (16).

Koizumi et al. (17) found that PGD_2 and PGJ_2 stimulated porcine aortic endothelial cells to synthesize a 31-kDa protein. Comparing the molecular mass of proteins induced by PGA_1 with those induced by heat treatment we observed that PGA_1 induces not only the HSP70 family and the low molecular mass HSPs, but also stress proteins designated as p57 and p23. Numerous studies on heat-shock protein synthesis have revealed that a polypeptide of approximately 70 kDa exhibiting enhanced synthesis following heat shock occurs in virtually every organism that has been examined. A possible role for the product of the hsp70 gene in the control of cell growth has been suggested by several authors (18-20). In summary, the present results show that PGA_1 induces heat-shock proteins and stress proteins during the different growth phases of *A. albopictus* cells. Our data, however, do not support evidence concerning a correlation between the presence of these proteins and cell proliferation.

In view of these results and the role of PGs in insect physiology, the study of the relationship between PGs and protein induction could provide insights into the understanding of a possible link between PGs and the control of *A. albopictus* cell proliferation.

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