STIMULATORY EFFECT OF BRADYKININ ON THE ACTIVITY OF CYCLOOXYGENASE AND PROSTAGLANDIN PRODUCTION IN CULTURE FIBROBLASTS

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ABSTRACT

The effect of bradykinin on cyclooxygenase (COX) activity and Prostaglandin (PGE₂) production were investigated in cultured fibroblasts. Bradykinin significantly induced PGE₂ synthesis in mouse fibroblast in a dose-dependent manner. The bradykinin stimulated PGE₂ release was apparent at concentrations of 0.5 µM, 1µM and 2 µM. The bradykinin concentration achieving a maximum effect at 1µM. We next examined which of the COX enzymes was expressed in bradykinin-stimulated fibroblasts by using western blotting analyses. As a result, western blotting manifested activation of COX-1 was more potent than COX-2 polypeptide at position of 70 kDa in response to 1µM bradykinin. The present study suggests that mechanism of bradykinin increase PGE₂ production in culture fibroblast is mainly attributed to the action of COX-1 and COX-2, respectively.

Keywords: Bradykinin, cyclooxygenase, prostaglandin, fibroblast

INTRODUCTION

The cell damage associated with inflammation acts on cell membrane to cause leukocyte to release lysosomal enzymes; arachidonic acid is then liberated from precursor compound and various eicosanoids are synthesized such as prostaglandin. Prostaglandin has a variety of effect on blood vessels, on nerve ending, and on cell involved in inflammation. Recent evidence suggest that cyclooxygenase isozyme (COX-2) responsible for prostaglandin production by cells involved in inflammation is not identical to the cyclooxygenase present in most other cells in the body (COX-1). Bradykinin and neuropeptides are also released at the site of tissue injury. It has been reported that cyclooxygenase isozyme (COX-2) responsible for prostaglandin production by cells involved in inflammation is not identical to the

cultured in Dulbecco’s modified Eagle’s medium (DMEM; ICN Biomedicals, Costa Mesa, CA, USA) supplemented with 10 % fetal calf serum (FCS; Gibco BRL, Grand Island, NY, USA), 100 u/ml of penicillin (Meiji Seika). The cells were cultured at 37 °C in a humidified atmosphere of 5 % CO₂/ 95 % air.

Measurement of PGE₂ production

Fibroblast cells were incubated with 0.5 µM, 1µM and 2 µM bradykinin in DMEM containing 10 % FBS in 48 well culture plate at 37 °C for 24 hr prior to washing 3 times with serum-free DMEM. Fibroblast cells incubated at 37 °C for 72 hr without changing the medium before rinsing with serum-free DMEM were designated as non-stimulated cells. On completion of the final incubation in all experiments, the medium was collected and stored at −20 °C before determination of PGE₂ content by specific enzyme immunoassay (Cayman chemical, Ann Arbor, MI, USA).

Western blot analyses

Fibroblast cells in culture flasks were incubated in DMEM containing 10 % FBS with and without 1µM Bradykinin at 37 °C for 24 hr. On decantation of the medium, the cells were rinsed 3 times with ice-cold PBS and scraped into 1.5 ml samples of 20 mM Tris-HCl (pH 7.4) containing 150 mM NaCl, 10 mM EDTA, 1 mM diethyldithiocarbamate, 1 mM phenylmethylsulfonyl fluoride, 0.2 mM leupeptin, 50 uM pepstatin A and 1 % triton X-100, COX polypeptide were solubilized by sonication of cell samples for 5 sec, and the sonicate was centrifuged at 15,000 x g for 30 min to remove insoluble contaminants. Protein contents in the supernatant were determined by DC protein assay (Bio-Rad Laboratories, Hercules, CA, USA) using BSA
as a standard. Solubilized proteins, precipitated by adding 3 vol of cold acetone (-20 °C), were dissolved in 80 mM Tris HCl (pH 8.0) containing 0.3 mM diethyldithiocarbamate and 0.1 % Tween 20 to afford a 20 mg/ml solution. Dissolved protein were then heated at 100 °C for 1 min with gel-loading buffer (50 mM Tris HCl pH 6.8 containing 10 % glycerol, 2 % SDS, 5 % 2-mercaptoethanol and 2 mg/ml bromophenol blue) in a ratio of 1 : 1 (v/v). Sample proteins (160 and 20 µg/lane for COX-1 and COX-2 analyses, respectively) were separated by SDS-polyacrylamide gel electrophoresis on 7.5 % polyacrylamide gel and transferred electrophoretically (2 mA/cm, 1 hr) to polyvinylidene fluoride membranes (immobilon-P; Millipore, Bedford, MA, USA) with a transfer buffer containing 25 mM Tris and 192 mM glycine. After blocking the membranes with 20 mM Tris-HCl (pH 7.4) containing 1550 mM NaCl and 10 % non fat dry milk, the membrane was incubated overnight at 4 °C with either 1:100 rabbit anti COX-1 antibody (Oxford Biomedical Research, Oxford, MI, USA) or 1:500 rabbit anti COX-2 antibody (Cayman, Chemical, Ann Arbor, MI, USA) in TBS containing 0.05 % Tween 20 (TBST) and 3 % BSA. The membranes, washed with TBST and incubated for 30 min at room temperature with 1:20000 goat anti rabbit IgG-alkaline phosphatase (Jackson Immunoresearch Laboratories, West Grove, PA, USA), were then incubated with CDP-Star (Tropix, Bedford, MA, USA), an enzyme substrate, before exposing them to X-ray film for chemiluminescence. Purified COX-1 (100 ng/lane) and COX-2 (80 ng/lane) polypeptides, derived respectively from ram seminal vesicles and sheep placentae (Cayman Chemical, Ann Arbor, MI, USA), were used as positive controls.

Statistical analyses

The statistical significance of differences between the control and bradykinin-treated group was assessed by analysis of variance followed by t-test.

RESULTS

Effect of bradykinin-stimulated PGE2 production from fibroblast

As shown in Fig. 1, Bradykinin significantly induced PGE2 synthesis in mouse fibroblast in a dose-dependent manner. The bradykinin stimulated PGE2 release was apparent at concentrations of 0.5 µM (3.2 ± 0.3), 1µM (6.2 ± 0.5) and 2 µM (6.7 ± 0.4). The bradykinin concentration achieving a maximal effect at 1µM and enhanced the PGE2 release to a level of more than 5 times in only 1 hr of culture, as compared with the basal levels (1.3± 0.2). These results agree closely with the previously reported findings (Tanaka et al., 1992). We next attempted to examine which of the COX-1 and COX-2 enzymes was expressed in bradykinin-stimulated fibroblasts.

![Fig.1. Effect of bradykinin on PGE2 production in cultured fibroblasts.](image-url)
COX activity in bradykinin-stimulated fibroblasts

To further elucidate the inhibitory effect of curcumin on COX activity in fibroblast was measured by using western blot analyses. The contents of COX-1 and COX-2 proteins were determined by Western blots using polyclonal antibodies for COX-1 and COX-2. Purified COX-1 polypeptide (100 ng/lane) from ram seminal vesicles and COX-2 polypeptide (80ng/lane) from sheep placentae were used as positive controls.

Figure 2A shows western blotting manifested induction of COX-1 polypeptide at a position of 70 kDa in response to bradykinin at concentration of 1 µM detectable at 1 hr, 5 hr, 10 hr and 20 hr post incubation. While figure 2B, COX-2 polypeptide in fibroblast stimulated with 1 µM bradykinin was detectable at 5 hr post incubation, displayed the maximum content at 10 hr post incubation, and it remained elevated thereafter for up to 20 hr.

A) COX-1

B) COX-2

Fig.2. Western blot analyses Of COX-1 (A) and COX-2 (B) polypeptide in cultured fibroblast were incubated without (lane 1) and with 1µM Bradykinin for 1 hr (lane 2), 5 hr (lane 3), 10 hr (lane 4) and for 20 hr (lane 5). Purified COX-1 polypeptide (100 ng/lane) from ram seminal vesicles and COX-2 polypeptide (80 ng/lane) from sheep placentae were used as the positive controls (lane 6).

DISCUSSION

In several studies, prostaglandin is produced from arachidonic acid by mean of the activity of cyclooxygenase enzyme. Its expression is enhanced at sites of inflammation in soft tissues, and it performs various biological activities. In the present study reported that bradykinin significantly induced PGE2 synthesis in mouse fibroblast in a dose-dependent manner. These results agree closely with the previously reported findings (Tanaka et al., 1992).

Cyclooxygenase is a rate-limiting enzyme in prostanoids (PGs) biosynthesis (Needleman et al, 1986). The 2.8-kilobase complementary DNA (cDNA) of COX was cloned in mammalian cells (Yokoyama and Tanabe, 1989; DeWitt et al., 1990). Recently, a mitogen inducible form of COX encoded by a 4.1-kilobase mRNA has been discovered in mice (Kujubu et al., 1991), Human (Hla and Neilson, 1992) and chickens (Xie et al., 1991). This isoform is designated as COX-2, and the first discovered form is designated as COX-1. The two isoform show about 60 % homology in both their nucleic acid and amino acid structures. In contrast, COX-1 and COX-2 are pharmacologically distinct. For
instance, although COX-1 is constitutively expressed enzyme that is present in most tissue (Simmons et al., 1991), COX-2 is inducible enzyme present especially in inflammatory (Crofford et al., 1994) and mitogen-stimulated cells (Kujubu et al., 1991)

Mouse fibroblast has been reported to induce COX mRNA expression when subjected to phorbol esters (Kujubu et al., 1991), interleukin-1 (Romero et al., 1989) and platelet-derived growth factor (Lin et al., 1989). Bradykinin potentiates the PGs production in fibroblsts by increasing the release of arachidonic acid in mediating the activation of the phospholipase A2 (Burch and Axelrod, 1987) or phospholipase C pathway (Clark et al., 1986), as has been previously suggested. However, COX-1 and COX-2 induction by this bradykinin has not yet been demonstrated. To assess the COX-1 and COX-2 activity was determined by using western blot analyses. As result, COX-1 activity was detected more potent than COX-2 activity in the fibroblast were stimulated with 1 µM bradykinin. Western blotting manifested induction of COX-1 polypeptide at a position of 70 kDa in response to bradykinin at concentration of 1 µM detectable at 1 hr, 5 hr, 10 hr and 20 post incubation. While COX-2 activity was detected which was dependent on the time of incubation with 1 µM bradykinin. COX-2 polypeptide in fibroblast stimulated with 1 µM bradykinin was detectable at 5 hr post incubation, displayed the maximum content at 10 hr post incubation, and it remained elevated thereafter for up to 20 hr. These results suggest that stimulatory effect of bradykinin on COX-1 activity was more potent than COX-2 activity in cultured fibroblast.

**CONCLUSION**

In conclusion, the present study demonstrated that mechanism of bradykinin increase PGE2 production in culture fibroblast is mainly attributed to the action of COX-1 and COX-2 enzymes, respectively. In addition, the cellular COX-2 activity was dependent on the time of incubation with bradykinin.

**REFERENCES**


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