Inhibition of endothelin-mediated topoisomerase I activation by pertussis toxin

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Cultured rat mesangial cells contain high affinity endothelin (ET) receptors at high densities. Exposure of these cells to ET resulted in a transient activation of topoisomerase I extractable activity, which reached its maximum value at approximately 2 min and returned to basal value after approximately 10 min of treatment. The activation of this enzyme was dependent upon the concentration of ET added. Incubation of the cells with pertussis toxin inhibited ET-induced increases in topoisomerase I activity in a concentration-dependent manner, suggesting the involvement of pertussis toxin-sensitive GTP-binding protein in ET-mediated action. Endothelin had no detectable effect upon extractable topoisomerase II activity.

Endothelin; Topoisomerase I; Pertussis toxin; Mesangial cell

1. INTRODUCTION

Endothelin is a recently discovered 21 amino acid peptide with very potent vasoconstrictor properties [1]. It is isolated from the supernatant of cultured porcine endothelial cells. The action of ET has been shown to be mediated through voltage-dependent calcium channels as well as through elevation of intracellular calcium. Endothelin receptors have been identified in several types of tissue such as kidney, brain, heart, and cultured cells of various tissue origins [2-5]. Our laboratory has been interested in the signal transduction pathways mediated by ET receptors. We have used cultured rat renal mesangial cells as a model system to explore these pathways. The cells display high density, high affinity ET receptors that are coupled to the activation of phospholipase C and intracellular calcium release [6]. Activation of phospholipase C results in an increase in DAG levels and in the activation of protein kinase C which, in turn, leads to rapid expression of certain oncogenes such as c-myc and c-fos [7-9]. We have also shown that exposure of vascular smooth muscle cells to vasopressin results in rapid induction of c-fos oncoprotein [10] and transiently increases topoisomerase I activity [11].

Topoisomerase I (DNA topoisomerase; EC 5.99.1.2) and II are classes of enzymes which catalyze interconversions among topological isomers of DNA via transient single or double strand breaks [12,13]. They are believed to regulate DNA metabolism, e.g. replication and transcription [14-17]. In the present study, we demonstrate that the interaction of ET with cultured mesangial cells results in an increase in topoisomerase I activity.

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2. MATERIALS AND METHODS

Endothelin 1, 2 and 3 were from Peninsula Laboratories. Pertussis and cholera toxin were from List Biologicals (Campbell, CA). Collagenase was from Worthington Biochemical Corp. (Freehold, NJ). RPMI 1640 medium was from Gibco Labs (Grand Island, NY). Insulin, penicillin and streptomycin were from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was from Hazelton Labs (Logan, UT). All other chemicals were of the highest grade available. Human scleroderma antiserum was kindly provided by Dr Gerd Maul of the Wistar Institute (Philadelphia, PA).

2.1. *Cell culture*

Mesangial cells were cultured from glomeruli obtained from the kidney cortices of 55-70 g rats (Sprague-Dawley, Charles River). Glomeruli were isolated by a sequential sieving which removes tubules (300 μm, 150 μm), but retains glomeruli (63 μm). Isolated glomeruli were incubated for 10 min at 37°C in collagenase (750 μm/ml) and then plated in flasks in RPM 1640 medium supplemented with 0.6 U/ml insulin, 100 U/ml penicillin, 100 μg/ml streptomycin, and 15% fetal bovine serum. Cells were grown at 37°C in 5% CO_2, medium being changed two times per week. At confluency, cells were subcultured after washing with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline and incubated with trypsin supplemented with 20 mM EDTA. They were identified as mesangial cells by the following criteria: (i) a stellate morphology as determined by phase contrast microscopy; (ii) microfilaments and subplasmalemmal cytoplasmic densities as determined by transmission electron microscopy; (iii) insensitivity to puromycin aminonucleoside; and (iv) positive immunofluorescence staining for actin and desmin but negative for keratin and factor VIII antigens.

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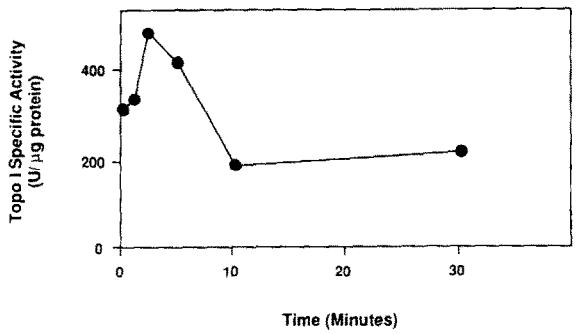

Fig. 1. Mesangial cells were treated with 100 nM ET for the indicated times and topoisomerase I activity contained in nucleus extracts of the cells was determined as described in section 2.

2.2. *Preparation of nucleus extracts: assay for topoisomerase I activity*

Topoisomerase I activity contained in nucleus extracts was determined according to Nambi et al. [11]. Protein was determined by the method of Bradford [18].

2.3. *Gel electrophoresis and immunoblotting*

SDS-polyacrylamide gels (1.5 mm thick; 4% stacking gel and 7% resolving gel) were electrophoresed on a Minigel apparatus (8 × 9 cm) at 20 mA. The proteins were transferred from the gel onto nitrocellulose (Schleicher and Schuell, Keene, NH) by electroblotting in 5 mM 3-(cyclohexylamino)-1-propanesulfonic acid, pH 11, at 300 mA overnight using a BioRad transfer apparatus. Blots were blocked with 1% BSA in TBST (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, 0.1% Tween 20) and probed with human scleroderma antiserum appropriately diluted into TBST. Blots were incubated with antibody for 2 h at room temperature. Following three 2-min washes in TBST, the blots were incubated with ^{125}I-Protein A (1 μCi/ml in TBST) for 1 h at room temperature. The blots were then washed 3 times (2 min each wash) in TBST, air-dried, and autoradiographed with preflashed Kodak X-omat AR film. Autoradiograms were scanned with a Beckman DU-8B spectrophotometer. Band intensities on the autoradiograms were converted to relative amounts of topoisomerase I.

3. RESULTS

Incubation of rat renal mesangial cells in culture with ET resulted in an increase in extractable topoisomerase I activity. This increase was transient, with maximum activity observed in the first 5 min after hormone addition (Fig. 1). Between 10 and 30 min after ET was added to the cultures, extractable topoisomerase I activity returned to basal level (Fig. 1). Under the same conditions, there was no detectable change in extractable topoisomerase II activity in response to ET (not shown). Immunoblot analysis demonstrated that neither the cellular content of topoisomerase I nor its extractability by 0.35 M NaCl was altered by 2 or 5 min incubation with 100 nM ET (Fig. 2); thus, the observed increase in topoisomerase I specific activity was likely

TREATMENT	TOPOISOMERASE I CONTENT† OF			FRACTION OF TOPOISOMERASE I EXTRACTED
	0.35M NaCl Extract	Extracted Nuclei	Total	
None	1.3	0.6	1.9	.68
100 nM ET				
2 min	1.1	0.8	1.9	.58
5 min	1.1	0.6	1.7	.65

† density units x 10^{-7}

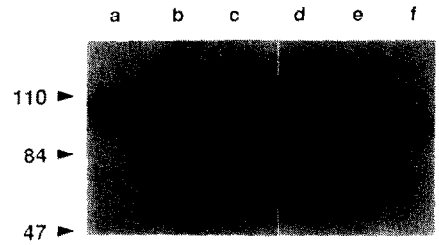

Fig. 2. Topoisomerase I content of 0.35 M NaCl nucleus extracts (a–c) and corresponding extracted nuclei (d–f) was assayed by immunoblotting with human scleroderma antiserum. Equal amounts (10 μg) of protein were loaded onto each lane of the gel. (a,d) untreated; (b,e) 100 nM ET-1, 2 min; (c,f) 100 nM ET-1, 5 min.

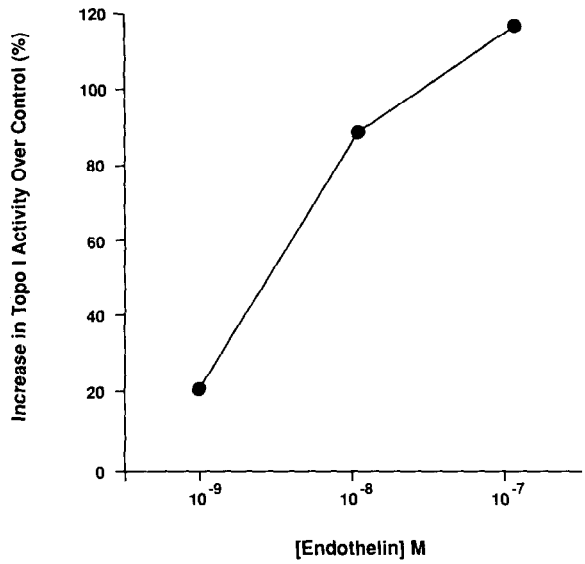

Fig. 3. Mesangial cells were incubated with increasing concentrations of ET for 2 min, and the topoisomerase I activity in the nucleus extracts was assayed as described. Data are from one experiment representative of two.

due to activation of enzyme that was present at the time of treatment. The increase in topoisomerase I activity also depended on the concentration of ET used (Fig. 3). The EC_{50} for ET was approximately 6.0 nM. Next, we tested the involvement of GTP binding protein in ET-mediated topoisomerase I activation. Cells were treated with increasing concentrations of pertussis toxin for 12 h and then challenged with ET for 2 min. As shown in Fig. 4, increasing concentrations of pertussis toxin inhibited ET-stimulated topoisomerase I activity, and at 1 μg/ml of pertussis toxin, the activation of topoisomerase I by ET was totally abolished. Pertussis toxin treatment did not affect basal topoisomerase I activity (not shown).

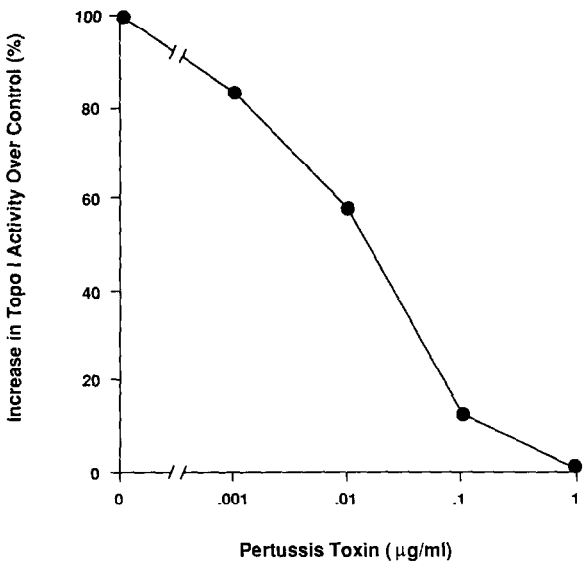

Fig. 4. Mesangial cells were treated with various concentrations of pertussis toxin for 12 h and then challenged with 100 nM ET for 2 min. Topoisomerase I activity in the nucleus extracts was assayed as described. Data are from one experiment representative of two.

4. DISCUSSION

Activation of topoisomerases I and II have been demonstrated to be associated with induced gene activity [15,16]. By the use of a variety of agonist-responsive cells, it has been shown that binding of hormones to specific cell surface receptors activates phosphatidylinositol (PI) turnover, which results in the elevation of cytosolic calcium and diacylglycerol levels. The latter, in turn, activate protein kinase C [19,20]. These events induce rapid expression of certain oncogenes such as c-myc and c-fos [7–10]. It has been reported recently that the calcium ionophore, A23187, induces c-fos in A431 cells and increases the frequency of camptothecin-induced topoisomerase I-linked DNA strand breakage in the induced gene [21]. Cellular content of c-fos mRNA and cellular topoisomerase I activity (camptothecin-induced breaks) were kinetically coupled, reaching maximum levels 10 min after induction before decreasing. We have previously demonstrated that exposure of vascular smooth muscle cells or human monocytic leukemia cells (U937) to hormones (vasopressin, thrombin) or cytokines (leukotrienes) results in the transient activation of nuclear topoisomerase I activity [11,22] as well as PKC [23]. Thus, release of intracellular Ca^{2+} and activation of protein kinase C by a number of hormone-dependent or -independent pathways appear to induce specific genes and concomitantly activates topoisomerase I.

Endothelin has been reported to stimulate the formation of inositol phosphates and mobilization of calcium in a variety of cells, DNA synthesis in vascular smooth muscle cells, Swiss 3T3 cells, and c-fos in rat mesangial cells [24–27]. In addition, inhibitors of PKC activation have been shown to obliterate the physiological effects of brief treatments with endothelin [28], suggesting that PKC stimulation is affected by ET. Here, we report that exposure of rat mesangial cells to ET likewise resulted in a transient stimulation of topoisomerase I activity. Since there was no change in either the cellular content or extractability of topoisomerase I when the cells were exposed to ET, the observed increase in topoisomerase I activity appears to be due to the activation of the enzyme. This activation depended on the concentration of ET used and the time of exposure of the cells to the hormone. Subsequent to the spike of enhanced extractable topoisomerase I activity induced by ET was a decrease to sub-basal specific activity. Because this decline was observed in 2 of 2 experiments with ET and has been reported in experiments with vasopressin [11], we believe that it is real. Prior treatment of the cells with

pertussis toxin completely abolished ET-mediated stimulation of topoisomerase I activity. Thus, ET, whose topoisomerase I effect is completely PT-sensitive, is distinguishable from hormones such as thrombin, whose effect is partially sensitive, or vasopressin, whose effect is insensitive to PT [11]. It is of interest that in rat mesangial cells, ET-mediated Ca^{2+} mobilization has been determined to be PT-sensitive [6]. Thus, the results reported here provide additional evidence for the interrelationship, via specific G-protein coupling, of inositol-phosphate turnover, activation of nuclear enzymes such as topoisomerase I, and physiological effects of the hormone.

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