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Et-1 and Et-3 Actions Mediated by Cloned ETA Endothelin Receptors Exhibit Different Sensitivities to BQ-123

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Et-1 and Et-3 activate phospholipase C in fibroblasts expressing cloned ETA receptors of bovine, rat and human origins. BQ-123 competitively antagonizes both responses but Et-3 actions are 10 times more sensitive to BQ-123 than Et-1 actions. It is suggested that differential sensitivity to BQ-123 is an intrinsic property of Et-1 and Et-3 activated ETA receptors and that there is no need to postulate the existence of new ETA receptor isoforms to account for singular actions of BQ-123.

Endothelins (Et) are 21-amino acid peptides that have important cardiovascular actions. They include 3 endogenous isoforms in mammals (Et-1, Et-2 and Et-3) and snake venom toxins, the sarafotoxins (1, 2). Two forms of endothelin receptors have been cloned from mammalian genomes. The ETA receptor subtype is more selective for Et-1 and Et-2 than for Et-3. It is selectively antagonized by BQ-123. The ETB receptor does not discriminate between the different isoforms of endothelin (3, 4).

A number of vascular preparations exhibit an agonist order of potency of Et-1 > Et-3 which would imply the involvement of an ETA receptor subtype. Yet, in some preparations, BQ-123, a selective antagonist of ETA receptors inhibits actions of Et-3 more potently than those of Et-1 (5-12). The differential sensitivity to BQ-123 has led to the suggestion that different ETA receptors that have different sensitivities to BQ-123 might exist and determine contractile actions of Et-1 and Et-3 (13). All these studies have been performed on isolated organ preparations and similar results have not yet been reported in isolated cell preparations. In this study we investigated the antagonist potency of BQ-123 on Et-1 and Et-3 induced activations of phospholipase C in fibroblast cell lines that expressed cloned ETA receptors of rat, bovine and human origins.

MATERIALS AND METHODS

A bovine ETA receptor cDNA clone was kindly provided by Dr. S. Nakanishi. Stable transfectant CC139 fibroblasts were kindly provided by Dr. J. Pouyssegur. Rat and human ETA receptors were PCR cloned from rat uterus and human placenta respectively. Stable transfectant CHO cells were prepared by the calcium phosphate precipitation method and neomycin (G418) selection. Cells were grown into Dulbecco's modified Eagle's medium (DMEM) supplemented with 7.5 % fetal bovine serum, 100 U/ml of penicillin and 100 μg/ml of streptomycin.

Cells (40,000/well) were seeded into 96 well tissue culture clusters in DMEM supplemented with 7.5 % fetal bovine serum and allowed to grow for 24 hours. Culture media were then changed to a serum free culture medium supplemented with 2 μCi/ml [^{3}H]inositol (19 Ci/mol, Amersham). After 24 hours of incubation, cells were rinsed and further incubated into an Hepes buffered DMEM supplemented with 13 mM LiCl, 0.2 % lysozyme, Et-1, Et-3 or BQ-123. After 15 minutes of incubation at 37°C, cells were extracted with 10 mM formic acid and the cell extract was applied onto Dowex AG1X8 anion exchange columns (Biorad). Columns were sequentially eluted with 3 mM NH4OH, 40

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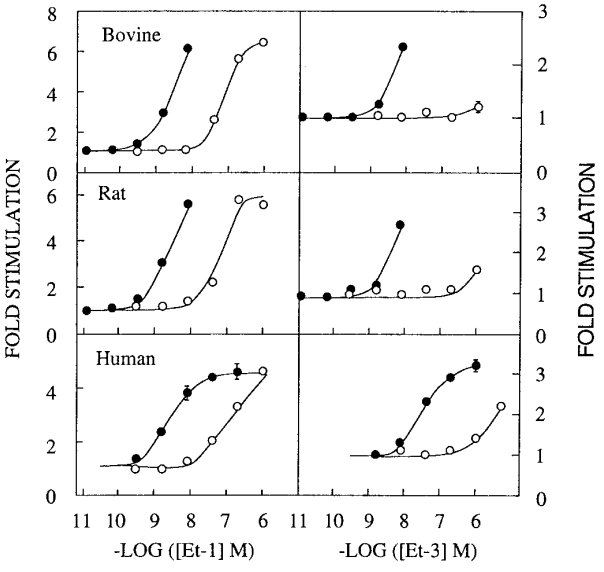

FIG. 1. Inhibition by BQ-123 of Et-1 and Et-3 induced activation of phospholipase C. Dose response curves for Et-1 and Et-3 actions were established in the absence (●) or the presence (○) of BQ-123. Upper panel: bovine ETA receptor expressing CC139 fibroblasts. Middle panel: rat ETA receptor expressing CHO cells. Lower panel: human ETA receptor expressing CHO cells. BQ-123 was used at 5 μM in the upper two panels and at 1 μM in the lower panel. Means ± SE (n = 4) are represented. Identical results were obtained in 2-3 other experiments.

mM ammonium formate and 2 M ammonium formate. The last fraction that corresponds to total inositol phosphates, was counted.

RESULTS AND DISCUSSION

Fibroblasts expressing cloned ETA receptors of human, rat and bovine origins responded to Et-1 by 4 to 6 fold activations of phospholipase C. Concentrations of Et-1 that produced half maximum activations (EC50) were 2.3 ± 0.4 nM (n = 5), 1.2 ± 0.2 nM (n = 3) and 3.3 ± 0.6 nM (n = 3) for bovine, rat and human receptors respectively. Fibroblasts also responded to Et-3 by 3 to 4 fold activations of phospholipase C. EC50 values for Et-3 actions were 9.0 ± 1.6 nM (n = 5), 28.3 ± 7.3 (n = 3) and 27.0 ± 2.0 nM (n = 5) for bovine, rat and human receptors respectively. The 10 fold higher potency of Et-1 was characteristic of an ETA receptor subtype.

Figure 1 shows that BQ-123 shifted the dose response curve for Et-1 action to larger concentrations as expected for a competitive type of inhibition. pA2 values for BQ-123 action against Et-1 were 6.88, 7.00 and 7.52 for bovine, rat and human receptors respectively. Very similar values have been reported in isolated vessel preparations (5-12). Figure 1 further shows that actions of Et-3 were prevented by BQ-123 but that they were much more sensitive to the inhibitor than Et-1 responses. pA2 values for BQ-123 actions against Et-3 were 8.30, $>$ 8.0 and 8.15 for bovine, rat and human receptors respectively. Untransfected fibroblasts did not respond to either Et-1 or Et-3.

Thus cloned ETA receptors of three different species exhibit the same 10 fold greater sensitivity of Et-3 actions to BQ-123 as that reported for various vascular preparations (5-12). This clearly indicates that differential sensitivity of Et-1 and Et-3 responses to BQ-123 is an intrinsic property of ETA receptors and that there is no need to postulate the existence of different receptor subtypes to account for these actions of BQ-123. Indeed molecular evidence indicates that human genomes have only one type of ETA receptor (14).

Different hypotheses may account for the differential sensitivity to BQ-123 of Et-1 and Et-3 actions mediated by ETA receptors. First, it should be noted that mathematical calculations used to define pA2 values make the implicit assumption that ligands bind reversibly and that binding equilibrium is achieved. Et-1 is an unusual ligand in that it binds almost irreversibly to its receptors. BQ-123 binds reversibly (15). In competition experiments, the action of the less reversible ligand being favored, a lower efficacy of BQ-123 is expected when it is tested against Et-1. Et-3 actions are antagonized by BQ-123 with pA2 values (> 8.30) that correspond to an inhibitory constant of a few nM. This value being close to the equilibrium dissociation constant of BQ-123 ETA receptor complexes (15), true competition probably determines the sensitivity of Et-3 actions of BQ-123.

Another possibility is that as reported for NK1 tachykinin receptors (16), Et-1 and Et-3 bind to different subdomains of the same ETA receptor and that the properties of interaction of BQ-123 with these different subdomains differ. This possibility is supported by recent site selected mutagenesis experiments (17). Finally more complex mechanisms involving selective trafficking of ETA receptors to different G proteins (18) may also be considered.

In conclusion, Et-1 and Et-3 actions mediated by ETA receptors exhibit different sensitivities to BQ-123. This is an intrinsic property of the receptor and there is no need to postulate the existence of multiple ETA receptor subtypes to account for singular actions of BQ-123 in isolated vessel preparations. Other singular actions of BQ-123 such as the fact that it acts as a competitive or a non competitive antagonist depending on its mode of application to the cells have been considered previously (19).

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