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Review

Endothelin$_A$-endothelin$_B$ receptor cross-talk and endothelin receptor binding

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Abstract

Background and objective The magnitude of inhibition of an endothelin (ET)-1 response by selective blockade of the ET_A or ET_B receptors can be limited by apparent compensation mediated by the unblocked receptor. While the mechanism underlying this functionally defined interaction, or 'cross-talk', is not clear, binding studies suggest an interaction between the ET receptor subtypes.

Key findings These binding studies are reviewed and suggest that, in general, they support the hypothesis that ET_A and ET_B receptor activation of intracellular signalling pathways influence ET-1 binding to these receptor subtypes.

Summary However, the relationship of these binding studies to functional effects and, thus, functional ET_A-ET_B receptor cross-talk, remains largely untested.

Keywords endothelin$_A$; endothelin$_B$; endothelin-1; receptor binding; receptor cross-talk

Introduction

Defining the relative roles of endothelin$_A$ (ET_A) versus endothelin$_B$ (ET_B) receptor activation in the mediation of responses to endothelin-1 (ET-1) can be complicated by an interaction between the ET_A and ET_B receptors.[1] This interaction, generally termed 'cross-talk', refers to a functional scenario in which the inhibitory effect of selective ET receptor blockade is limited by apparent compensation mediated by the unblocked receptor.[1]

Although the mechanism underlying functional ET_A-ET_B receptor cross-talk is not clear, binding studies of the displacement of radiolabelled ET-1 by selective ET_A and ET_B receptor ligands provide some potential mechanistic insights. Indeed, recent review articles that discuss the mechanisms and illustrate in detail the numerous pathways potentially responsible for functional ET_A-ET_B receptor cross-talk,[2,3] refer to one of these studies.[4] This review contrasts studies in which occupation of one ET receptor subtype influences radiolabelled ET-1 displacement by a selective ET receptor ligand for the other receptor subtype and, additionally, contrasts these findings with studies in which an influence was not observed. The relationship between the ET receptor binding studies and functional ET_A-ET_B receptor cross-talk is also examined.

^{125}I-ET-1 Displacement by ET_B Receptor Ligand Requires ET_A Receptor Occupation

Evidence that ET_A receptor occupation is required for ET_B receptor ligand displacement of ET-1 is supported by experiments in human Girardi heart cells, a cell type that expresses ET_B, but essentially lacks ET_A receptors (Table 1).[5–7] BQ788 and BQ3020 (selective ET_B receptor antagonists) monophasically displaced ^{125}I-ET-1 with IC50 values of 1.2 nM and 0.62 nM, respectively, in human Girardi heart cells.[5,7] Transfection of human Girardi heart cells with human ET_A receptor cDNA, which yielded a 4 : 6 ratio of ET_A : ET_B receptors determined by Scatchard analysis, increased the IC50s for displacement of ^{125}I-ET-1 by BQ788 and BQ3020 to 0.85 μM and 2.8 μM, respectively.[7] Furthermore, BQ123 (selective ET_A receptor antagonist) prevented the increased IC50s for ET_B receptor ligand displacement of ^{125}I-ET-1.[7]

In contrast to intact human Girardi heart cells in which ET_A receptors were experimentally expressed, membranes prepared from these same cells did not display decreased

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Table 1 Displacement of ^{125}I-endothelin-1 by endothelin$_A$ and endothelin$_B$ receptor ligands

Preparation	^{125}I-ET-1 displacement by ET_A and ET_B receptor ligands[a]	Reference
Human Girardi heart cells, ET_A receptor transfected	A	Ozaki *et al*.[7]
Rat anterior pituitary	A	Harada *et al*.[4] Himeno *et al*.[13]
Rat primary astrocytes	A, B	Hasselblatt *et al*.[14] Jensen *et al*.[15] Ehrenreich *et al*.[16]
Human astrocytoma cell line	C	Jensen *et al*.[15]
Rat trachea	C	Henry[17]
Mouse trachea	C	Henry & Goldie[18] Carr *et al*.[19]
HEK293 cells, ET_A and ET_B receptor transfected	C	Evans & Walker[20]

[a]^{125}I-ET-1 displacement by A: ET_B receptor ligand requires ET_A receptor ligand; B: ET_A receptor ligand requires ET_B receptor ligand; C: ET_A and ET_B receptor ligand does not require ET_B and ET_A receptor ligand, respectively.

affinity for ET_B receptor ligand.[7] The lack of decreased affinity for ET_B receptors in the membrane preparations was attributed to disruption of an ET_A receptor-mediated intracellular signalling pathway responsible for ET_B receptor desensitization.[7] The identity of the proposed ET_A receptor-mediated intracellular signalling pathway responsible for ET_B receptor desensitization[7] is not known, although possibilities could include the phosphorylation of the ET_B receptor through protein kinase C.[8,9] Thus, although both ET_A and ET_B receptor ligands displace ^{125}I-ET-1 in cell membranes from, for example, rat renal microvasculature and lung, and human bronchial smooth muscle,[10–12] whether ET_A (or ET_B) receptor ligands displace ^{125}I-ET-1 in intact cells from these tissues remains to be tested.

The requirement of ET_A receptor occupation for ET_B receptor ligand displacement is supported by ^{125}I-ET-1 displacement studies in intact rat anterior pituitary gland (Table 1).[4,13] First, saturation binding experiments with ^{125}I-ET-1 established that ET_A and ET_B receptor ligands have high and low binding affinity/capacity, respectively.[4,13] That is, the K_d values in the absence of other ligand and in the presence of 1 μM BQ123 and 10 nM sarafotoxin S6c (selective ET_B receptor agonist) were 71, 8.3 and 72 nM, respectively, and the B_{max} values were 129, 8 and 110 fmol/mg, respectively.[4,13]

Subsequent ^{125}I-ET-1 displacement studies in intact rat anterior pituitary gland demonstrated that sarafotoxin S6c and ET-3 (selective ET_B receptor agonist), IRL1620 and BQ788 (selective ET_B receptor antagonists), displaced ^{125}I-ET-1 essentially only in the presence of BQ123.[4,13] Specifically, the K_i values for sarafotoxin S6c, IRL1620 and BQ788 in the absence of BQ123 were greater than 1 μM and for ET-3 the K_i was 0.11 μM.[4,13] In the presence of 1 μM BQ123, the K_i values for sarafotoxin S6c, IRL1620, BQ788 and ET-3 decreased to 0.14 nM, 0.35 nM, 14 nM and 18 pM, respectively.[4,13]

Additionally, it was proposed that the requirement for ET_A receptor occupation for ET-1 binding to the ET_B receptor was due to ET_A-ET_B receptor heterodimerization, the result of ET-1 acting as a bivalent ligand.[4,13] It should be noted that numerous G protein coupled receptors heterodimerize as well as homodimerize.[2,3]

^{125}I-ET-1 Displacement by ET_A Receptor Ligand Requires ET_B Receptor Occupation and Displacement by ET_B Receptor Ligand Requires ET_A Receptor Occupation

Similar to intact rat anterior pituitary and human Girardi heart cells expressing ET_A receptors along with endogenous ET_B receptors,[4,7,13] potency and maximal ET_B receptor ligand displacement of ^{125}I-ET-1 were increased by ET_A receptor ligand in rat cortical primary astrocytes.[14–16] Specifically (percent displacement derived by graphical approximation from the figures), the following results were reported. (1) IRL1620 alone at a concentration of 1 μM maximally displaced ^{125}I-ET-1 by <10%.[15] In the presence of 0.1 μM BQ123, 1 μM IRL1620 completely displaced ^{125}I-ET-1 and the IC50 for displacement was 10 nM.[15] (2) ET-3 induced a biphasic displacement curve, with the high affinity phase accounting for 20% of the total bound ^{125}I-ET-1 and the radioligand was maximally displaced by 0.1 μM/ET-3.[15] In the presence of 0.1 μM BQ123 or 0.1 μM LU135252 (selective ET_A receptor antagonist), ET-3 induced a monophasic displacement curve, with 50% and complete displacement of ^{125}I-ET-1 at 3 nM and 30 nM/ET-3, respectively.[15] (3) BQ788 displaced ^{125}I-ET-1 with a K_i of 2.5 μM, with 0.1 μM and 2 μM BQ788 displacing <10% and 50%, respectively.[14,16] In the presence of 0.1 μM BQ123, BQ788 displaced ^{125}I-ET-1 with a K_i of 0.25 μM, with 0.1 μM BQ788 displacing 50%.[14,16] In another report by the same group, however, the effect of BQ788 on displacement of ^{125}I-ET-1 was apparently similar in the presence and absence of 0.1 μM BQ123, with 50% displacement by 0.1 μM BQ788.[15] It is possible that different experimental conditions resulted in these contrasting findings.[14,15] For example, displacement studies were performed for 2 h at 37°C[14] and for 20 h at 4°C.[15] It should be noted, however, that the IC50s for ET-1 displacement of ^{125}I-ET-1 were similar under both experimental conditions.[14,15]

In contrast to the displacement of ^{125}I-ET-1 by ET_A receptor ligand in rat anterior pituitary and human Girardi heart cells expressing ET_A receptors,[4,7,13] in rat cortical primary astrocytes ^{125}I-ET-1 was not displaced by even 10 μM BQ123 and was displaced <10% by 0.1 μM LU135252 (Table 1).[14–16] Furthermore, the K_i for BQ123 displacement of ^{125}I-ET-1 was decreased by ~10-fold in the presence of 0.1 μM BQ788.[14]

The requirement of ET_B receptor occupation for ET_A receptor ligand displacement of ^{125}I-ET-1 is further supported by studies in rat cortical primary astrocytes containing an ET_B receptor null mutation. In wildtype astrocytes, 0.1 μM LU135252 and 0.1 μM BQ788 displaced ^{125}I-ET-1 by <10%, while the combined antagonists at these concentrations elicited 50% displacement.[16] However, in astrocytes containing

an ET_B receptor null mutation, in which 0.1 μM BQ788 only slightly displaced ^{125}I-ET-1, 3 nM and 0.1 μM LU135252 resulted in complete and 50% displacement, respectively.[16] Consistent with the null mutation of the ET_B receptor, LU135252 displacement of ^{125}I-ET-1 was unaltered by BQ788.[16] To explain these findings, it was also proposed that ET-1 acts as a bivalent ligand at an ET_A-ET_B receptor hybrid or heterodimer.[14–16]

^{125}I-ET-1 Dsplacement by ET_A/ET_B Receptor Ligand Independent of ET_B/ET_A Receptor Occupation

In contrast to the weak displacement of ^{125}I-ET-1 by ET_A and ET_B receptor ligand in rat cortical primary astrocytes,[14–16] in a human astrocytoma cell line LU135252 and ET-3 displaced ^{125}I-ET-1, that is 1 μM LU135252 and 0.1 μM/ET-3 resulted in ~67% and ~97% displacement, respectively (Table 1).[15] If intracellular signalling pathways regulate ET receptor subtype desensitization,[7] the ^{125}I-ET-1 displacement by ET_A and ET_B receptor ligand in the human astrocytoma cell line could reflect changes in these pathways. Also, in intact rat and mouse trachea, sarafotoxin S6c, BQ3020 and BQ123 partially displaced ET-1 binding as determined autoradiographically (Table 1).[17–19] In rat trachea, 1 μM BQ123 and 30 nM sarafotoxin displaced ^{125}I-ET-1 by 43 and 41%, respectively.[17] In mouse trachea, 1 μM BQ123, 0.3 μM BQ3020 and 0.1 sarafotoxin S6c displaced ^{125}I-ET-1 by 36, 44 and 47%, respectively.[18] In another report with mouse trachea, 1 μM BQ123 and 0.1 μM sarafotoxin displaced ^{125}I-ET-1 by 49 and 51%, respectively.[19] The displacement of ^{125}I-ET-1 by ET_A and ET_B receptor ligand was not due to displacement from different cell types, since the majority of the ET-1 binding was restricted to the smooth muscle band, that is, essentially from smooth muscle cells.[17–19]

In HEK293 cells transfected with ET_A and ET_B receptors tagged with cyan/yellow fluorescent protein or fluorescein arsenical hairpin for fluorescence resonance energy transfer studies, 1 μM BQ123 and 1 μM BQ788 displaced ^{125}I-ET-1 by 66 and 34%, respectively, consistent with the proportion of ET_A and ET_B receptor expressed (Table 1).[20] Even though ET_A or ET_B receptor activation increased intracellular $Ca^{[2+]}$ in transfected HEK293 cells,[20,21] the absence of coupling to a particular signalling pathway or incomplete activation of the pathway may underlie the lack of influence of ET_A/ET_B receptor ligand on ET-1 displacement by ET_B/ET_A receptor ligand in these cells (Table 1).[20,21]

^{125}I-ET-1 displacement studies were also performed in membranes from HEK293 cells singly or co-expressing ET_A or ET_B receptors, in which the receptors were combined with a fusion protein for fluorescence resonance energy transfer measurements.[22] The K_i values for BQ123 displacement of ^{125}I-ET-1 were similar in membranes prepared from cells co-expressing both receptors and expressing only ET_A receptors (Table 1).[22] Also, the K_i values for ET-3 displacement of ^{125}I-ET-1 were similar in membranes prepared from cells co-expressing both receptors and expressing only ET_B receptors (Table 1).[22] Again, however, preparation of membranes may disrupt an intracellular signalling pathway responsible for interaction between ET_A and ET_B receptors.[7]

^{125}I-ET-1 Displacement by ET_A and ET_B Receptor Ligand and Functional ET_A-ET_B Receptor Cross-Talk

The relationship between the above studies of ET_A and ET_B receptor ligand binding in intact cells and functional ET_A-ET_B receptor cross-talk is largely unknown due to differences in experimental paradigms. For example, in cultured cells from the rat anterior pituitary, BQ123 inhibited the ET-1 induced changes in hormone release,[23,24] consistent with BQ123 displacement of ^{125}I-ET-1 from intact rat anterior pituitary.[4,13] However, while ET_B receptor ligand displaced ^{125}I-ET-1 in the presence of BQ123,[4,13] the functional effect of ET_B receptor ligand in the presence of BQ123 on hormone release from rat anterior pituitary cells remains unknown.

In rat cortical primary astrocytes, while there is apparent qualitative agreement between the relative effectiveness of BQ123 and BQ788 to displace ^{125}I-ET-1 and inhibit phospholipase D,[14,15,25] the effects of combined BQ123 and BQ788 on phospholipase D activation remain to be investigated. However, ET_B receptor down-regulation decreased ET-1 stimulated phospholipase D activity and the remaining activity was abolished by BQ123.[25] Thus, functional ET_A-ET_B receptor cross-talk may be absent at least with respect to phospholipase D activation in rat cortical primary astrocytes. Whether or not the lower ET-1 (^{125}I-ET-1) concentration used in the binding studies in the astrocytes (50 pM)[14,15] as compared with the ET-1 concentration in the functional ET_B receptor down-regulation studies (1 nM)[25], influenced the results should also be considered, since different ET-1 binding characteristics, including BQ123 displacement of ET-1 are observed at pM versus nM ET-1 (^{125}I-ET-1) concentrations.[26]

A complex relationship between ^{125}I-ET-1 displacement by selective ET receptor antagonist and functional response was observed in intact rat and mouse trachea.[17–19] In intact rat trachea, 1 μM BQ123 displaced 43% of 0.5 nM ^{125}I-ET-1.[17] However, 0.5 nM/ET-1 did not elicit contraction and, while 10 μM BQ123 did not inhibit contraction to 3–30 nM/ET-1, contraction to 0.1 μM and 0.3 μM/ET-1 was inhibited.[17] Similarly, in intact mouse trachea, 1 μM BQ123 and 1 μM BQ788 each displaced approximately 50% of 0.2–0.3 nM ^{125}I-ET-1.[18,19] However, 3 nM/ET-1 elicited threshold contraction, while neither 3 μM BQ123 nor 1 μM BQ123 antagonist inhibited ET-1 contraction.[18,19] Again, the significantly lower ET-1 concentrations used in the binding studies as compared with the contraction studies may have influenced these apparently disparate findings.[18,19,25]

Considering that the preparation of cell membranes may disrupt ET_A-ET_B receptor heterodimerization and/or possible relevant signalling pathways that regulate ET receptor ligand binding,[4,7,13] disparities between ET_A and ET_B receptor ligand displacement experiments of ET-1 performed in cell membranes and functional response experiments are not entirely unexpected. Examples of this disparity are illustrated in smooth muscle cell membrane preparations versus contraction of intact cells. In membrane preparations of human bronchial smooth muscle, 0.1 μM BQ123 displaced 10 pM

^{125}I-ET-1 by 40%.[27] However, despite that 100 pM/ET-1 elicited threshold contraction in human bronchus, 10 μM BQ123 did not even partially inhibit ET-1 contraction.[27] In membrane preparations of rabbit pulmonary artery, 1 μM BQ123 displaced 10 pM ^{125}I-ET-1 by 20%.[28] However, contraction to the lowest ET-1 concentration tested (1 nM) remained uninhibited by 10 μM BQ123.[28]

A potential association between displacement of ^{125}I-ET-1 by ET_A and ET_B receptor ligand and functional ET_A-ET_B receptor cross-talk was observed with respect to the inhibition of ET-1 uptake in rat cortical primary astrocytes.[14] BQ123 (1 μM) and 1 μM BQ788 were without effect and partially inhibited ET-1 uptake, respectively, while combined BQ123 and BQ788 (1 μM each) greatly inhibited uptake.[14] Similarly, as discussed above, 1 μM BQ123 and 1 μM BQ788 did not displace and partially displaced ^{125}I-ET-1, respectively, while combined BQ123 and BQ788 (each 1 μM) caused near complete displacement.[14] It should be noted, however, and as discussed by the authors, increased ET-1 synthesis following combined BQ123 and BQ788 exposure may have partially influenced the uptake findings.[14]

Conclusions

Taken together, these studies support the conclusion that occupation of one ET receptor subtype by a selective ET receptor ligand can influence displacement of ET-1 by ligand for the other receptor subtype. Whether ET_A and/or ET_B receptor occupation wields this influence depends on the cell type and may reflect different intracellular signalling pathways.

However, the relationship between the binding studies to functional ET_A-ET_B receptor cross-talk is largely unknown due to differences in experimental paradigms. To provide optimal insight into the relationship between ET receptor binding and functional responses, two conditions should be met.

The first condition is that the concentrations of ET receptor ligand for binding studies should be similar to those required to yield a functional response. In most cases, meeting this condition would require dilution of the high specific activity ligand with unlabelled (but chemically identical) peptide. Specifically, the relatively lower ET-1 concentrations used in the binding experiments as compared with the functional experiments may reflect only high affinity binding and, thus, not the intended response or even a readily identifiable functional effect.[26] Similarly, the concentrations of ET receptor antagonists used in the binding experiments should be those that demonstrate inhibitory effects on the ET-1 functional response.

The second condition is that the cell/tissue preparation used in the binding experiments should be as close or identical to that adopted in the functional study. Specifically, caution needs to be used when utilizing cultured cells, cell lines, or cells transfected with ET receptors, as these conditions may alter relevant signal transduction processes relative to those of normal cells.

The mechanisms underlying ET_A-ET_B receptor cross-talk are complex and undoubtedly varied. Clearly, binding studies have provided considerable insight into relevant underlying mechanisms. However, further insight can be achieved through the use of at least reasonably similar conditions in the binding studies as those of the functional studies. In this way, the mechanisms underlying ET_A-ET_B receptor cross-talk can be further elucidated. Thus, rational approaches can be adopted to advance the therapeutic efficacy of ET receptor antagonists.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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