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Short communication

[^{125}I]Endothelin-1 binding to renal brush border and basolateral membranes

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Abstract

[^{125}I]Endothelin-1 bound with high affinity to a single site on both brush border membranes ($K_d = 192 \pm 26$ pM, $B_{max} = 314 \pm 49$ fmol/mg) and basolateral membranes ($K_d = 94.7 \pm 3.4$ pM, $B_{max} = 612 \pm 107$ fmol/mg) isolated from rat renal cortex. Competition binding experiments using subtype selective ligands revealed that the proportion of ET_B to ET_A receptors was 80:20 and 60:40 in the brush border membrane and the basolateral membrane, respectively. The results demonstrate that endothelin-1 binds to brush border membranes, and that endothelin ET_B receptors may be involved in the previously described effects of endothelin-1 on brush border membrane Na^+ transport. © 1998 Elsevier Science B.V.

Keywords: Endothelin; Brush border membrane; Basolateral membrane; Proximal tubule; Kidney

1. Introduction

The renal proximal tubule is a target for a number of hormones such as angiotensin II (Morduchowicz et al., 1991) and parathyroid hormone (Kaufmann et al., 1994). The traditional view of hormone action in this polarized epithelium localized the receptors for these and other hormones to the basolateral membrane of the cell. However, recent studies have shown that binding sites for various hormones including angiotensin II (Morduchowicz et al., 1991), parathyroid hormone (Kaufmann et al., 1994), dopamine (Felder et al., 1989) and vasoactive intestinal peptide (Kniaz et al., 1991) are present not only on the basolateral membrane but also on the apical or brush border membrane. In some cases (e.g., angiotensin II (Morduchowicz et al., 1991) and dopamine (Sheikh-Hamad et al., 1993)), these binding sites have been shown to elicit functional responses and therefore, can be classified as true receptors.

Physiological concentrations of endothelin-1 have been shown to stimulate Na^+ transport in intact proximal tubules when present on the basolateral side of the tubule (Garcia and Garvin, 1994). Likewise, endothelin-1 has been reported to stimulate Na^+ transport when added directly to proximal tubule brush border membrane vesicle preparations (Eiam-Ong et al., 1992). These studies, therefore, suggest that endothelin-1 receptors are present on both the basolateral and brush border membranes of the proximal tubule in a manner similar to the above mentioned hormones. However, to our knowledge, endothelin-1 binding sites have not previously been reported for the brush border membrane. Accordingly, the purpose of this study was to determine if endothelin-1 binds to renal brush border membranes in addition to basolateral membranes.

2. Materials and methods

2.1. Membrane preparation

Brush border and basolateral membranes were prepared from the renal cortex of male Sprague–Dawley rats (200–250 g) by the previously described Mg^{2+} precipitation and Percoll density gradient centrifugation methods, respectively (Edwards et al., 1997). The final membrane pellets were resuspended in binding buffer consisting of 50 mM Tris and 5 mM $MgCl_2$. The purity of the membrane preparations was assessed by measuring the relative enrichment over that found in the homogenate of the basolat-

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eral membrane marker enzyme Na^+,K^+-ATPase, and the brush border membrane marker, alkaline phosphatase. For the basolateral membrane, these factors were 14.2 ± 2.3 and 2.1 ± 1.1 and for the brushborder membrane, 0.91 ± 0.3 and 13.1 ± 1.5 for Na^+,K^+-ATPase and alkaline phosphatase, respectively. Protein was measured by the Lowry method.

2.2. Binding assays

Binding assays were performed in triplicate in a final volume of 150 μl of binding buffer containing 4–10 μg membrane protein, various concentrations of [^{125}I]endothelin-1 and other competing ligands, 1 μM phosphoramidon, 100 μM phenylmethylsulfonyl fluoride, 10 μg/ml bestatin, 10 μg/ml leupeptin and 0.05% bovine serum albumin. Incubations were performed for 60 min at 25° and were terminated by filtration and washing using a Skatron cell harvester. The filters were subsequently counted in a gamma counter. In both saturation and competition binding studies specific binding was defined as total binding minus binding in the presence of 1 μM unlabeled endothelin-1 and was always less than 10% of total binding. Saturation binding experiments were performed over a range of 25–600 pM [^{125}I]endothelin-1. Competition binding experiments were performed with 100 pM [^{125}I]endothelin-1 and various concentrations of competing ligands. Saturation and competition binding experiments were analyzed by nonlinear regression using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). Single and two site models were statistically compared using a F test ($P < 0.05$) to determine the best fit. Other statistical analyses were performed with Student's t-test.

2.3. Materials

[^{125}I]Endothelin-1 (2200 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Unlabeled endothelin-1, BQ-123 (cyclo-(D-Asp-Pro-D-Val-Leu-D-Trp) and sarafotoxin 6c were obtained from Phoenix Pharmaceuticals (Mountain View, CA, USA). (±)-SB 209670 ([(1*RS*-2*SR*,3*RS*)-3-(2-carboxymethoxy-4-methoxyphenyl)-5-(prop-1-yloxy)indane-2-carboxylic acid]) was synthesized in the Department of Medicinal Chemistry, SmithKline Beecham Pharmaceuticals (King of Prussia, PA, USA).

3. Results

Under the conditions used, [^{125}I]endothelin-1 binding reached equilibrium in 45 min and remained stable for at least 90 min in both membrane preparations. Scatchard analyses of typical saturation binding experiments are shown in Fig. 1. Specific binding to both the brush border and basolateral membranes was saturable and consistent

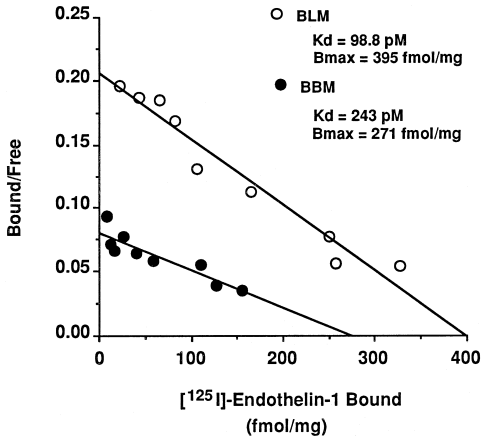

Fig. 1. Scatchard transformation of specific binding of ^{125}I-endothelin-1 from saturation binding experiments in basolateral membranes (BLM) and brush border membranes (BBM). Results from a representative experiment are shown.

with a single binding site. The average dissociation constant, K_d, and maximum number of binding sites, B_{max}, obtained from four different membrane preparations were 192 ± 26 pM and 314 ± 49 fmol/mg for brush border membrane and 94.7 ± 3.4 pM and 612 ± 107 fmol/mg for the basolateral membrane, respectively. The brush border membrane displayed both a lower affinity ($P = 0.01$) and a lower number of binding sites ($P = 0.04$) when compared to the basolateral membrane.

In brush border membranes the endothelin ET_B selective ligand, sarafotoxin 6c (Williams et al., 1991), displaced 89.4 ± 0.3% of specifically bound [^{125}I]endothelin with an IC_{50} of 1.2 ± 0.2 nM (Fig. 2). In contrast, the endothelin ET_A selective peptide, BQ 123 (Ihara et al., 1992), displaced only 21.9 ± 5.7% of endothelin-1 binding with an IC_{50} of 16 nM ± 1.2 nM (Fig. 2). The nonpeptide endothelin ET_A/ET_B receptor antagonist, SB209670 (Ohlstein et al., 1994), completely displaced [^{125}I]endothelin-1 with an IC_{50} of 1.4 ± 0.3 nM (Fig. 2). Analysis of the competition curves revealed that all three ligands interacted at only one binding site. Therefore, the results suggest that in the brush border membrane, ET_B receptors account for approximately 80% of the total endothelin binding sites with the remaining 20% having characteristics of the ET_A receptor.

In basolateral membranes as in the brush border membranes, BQ 123 displaced only a small fraction (18 ± 3.5%) of [^{125}I]endothelin-1 with an IC_{50} (14 ± 1.0 nM) similar to that seen in the brush border membranes. However, analysis of the sarafotoxin 6c and SB209670 competition curves revealed that both ligands were interacting at two sites. Sarafotoxin 6c displayed a high affinity ($IC_{50} = 0.4 \pm 0.2$ nM) for 56 ± 0.1% of the sites and a lower affinity ($IC_{50} = 36 \pm 2.3$ nM) for the remaining 44% of the binding sites. Likewise, SB209670 had a high affinity ($IC_{50} = 34 \pm 19$ pM) for 39.8 ± 1.0% of the sites and a lower affinity ($IC_{50} = 2.3 \pm 0.7$ nM) for the remaining 60.2% of

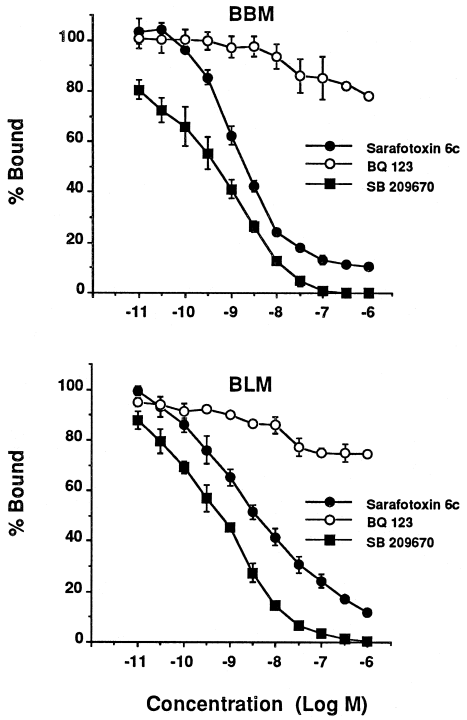

Fig. 2. Competition of specific ^{125}I-endothelin-1 binding to brush border (BBM) and basolateral membranes (BLM) by sarafotoxin 6c, BQ 123 and SB 209670. Results are expressed as a percent of ^{125}I-endothelin-1 binding in the absence of competing ligands. Each point is the mean ± S.E.M. of three to four separate preparations.

the sites. Since SB209670 has a higher affinity for the ET_A receptor than for the ET_B receptor (Ohlstein et al., 1994), these results together with the sarafotoxin 6c data suggest that the basolateral membrane contains a higher proportion (40%) of ET_A receptors than does the brush border membranes.

Since brush border membranes spontaneously forms vesicles during preparation (Murer and Gmaj, 1986), experiments were performed to differentiate between binding and the potential uptake of [^{125}I]endothelin-1 into the brush border membrane vesicles. For these experiments, [^{125}I]endothelin-1 binding and ^{32}P uptake were performed on the brush border membrane preparations in the presence of increasing osmolality induced by the addition of sucrose to the incubation medium (Murer and Gmaj, 1986). Increasing the osmolality from 300 to 600 mosM/kg had no effect on [^{125}I]endothelin-1 binding (5.4 ± 2.1% increase, NS) but, as expected, significantly reduced the uptake of ^{32}P measured at 60 min by 45 ± 2.6% ($p < 0.05$, $n = 3$). Therefore, the association of [^{125}I]endothelin-1 with the brush border membrane was due to binding rather than uptake of the radioligand.

4. Discussion

In the present study, we have demonstrated that [^{125}I]endothelin-1 binds to a single site in brush border membranes of rat renal cortex. The affinity of the brush border membrane for endothelin-1 and the number of binding sites were approximately twofold lower than those observed in the basolateral membrane. Based on the competition binding experiments with subtype selective ligands, the brush border membrane contains a high proportion of the ET_B receptor subtype (80%) while the basolateral membrane contains approximately 60% ET_B and 40% ET_A subtype receptor. The high proportion of endothelin ET_B receptors in both membranes is consistent with previous binding studies in isolated rat tubules (Takemoto et al., 1993).

While the K_d values obtained for the brush border and basolateral membranes are well within values reported for rat renal cortical membranes (Nambi et al., 1992; Gellai et al., 1994), it is interesting to note the different affinities of endothelin-1 for the two membrane preparations as well as the different proportions of the endothelin receptor subtypes. The reasons for this difference are not readily apparent since both membranes were assayed under identical conditions. However, it should be noted that the brush border membrane preparation originates predominately from the proximal tubule, while the basolateral membrane preparation likely represents a mixture of membranes from all cell types in the cortex (Murer and Gmaj, 1986) including the collecting tubule, glomerulus and vasculature, all of which have a high density of endothelin receptors (Dean et al., 1994; Nambi and Brooks, 1997). Thus, the heterogeneity of the basolateral membrane preparation with respect to cellular origin may account for the observed difference in K_d values and receptor subtype distribution obtained in the two membrane preparations.

The presence of high affinity endothelin-1 receptors on the basolateral membrane was predictable based on the previously reported ability of basolaterally applied endothelin-1 to stimulate proximal tubule fluid absorption (Garcia and Garvin, 1994). However, the role of the brush border membrane binding site described in the present study is unclear. We are aware of only one study which provides evidence for a functional role of luminal endothelin-1 in the proximal tubule. Eiam-Ong et al. (1992) demonstrated that endothelin-1 at concentrations as low as 100 pM stimulated Na^+/H^+ exchange when added directly to brush border membrane vesicles prepared from rabbit renal cortex. Although subtype selective antagonists were not used, endothelin-1 antiserum blocked this effect of endothelin-1. The potential signaling pathway activated by endothelin-1 to produce such an effect on Na^+/H^+ exchange is not known. However, angiotensin II (Morduchowicz et al., 1991) and dopamine (Sheikh-Hamad et al., 1993) have been shown to directly activate phospholipase A_2 and phospholipase C, respectively, in the brush border membrane, suggesting the presence of a local signal transduction mechanism in this membrane. Endothelin-1 has been shown to stimulate a number of phospholipases in various tissues including the kidney (Nambi and Brooks,

1997), and further studies are required to elucidate the signaling pathways activated by endothelin-1 in the brush border membrane.

In summary, we have demonstrated the existence of an endothelin-1 binding site in the brush border membrane from rat kidney which may be involved in the modulation of Na^+ transport. Although we utilized a number of peptidase inhibitors in the binding assay and could find no evidence for endothelin-1 uptake into brush border membrane vesicles, we cannot totally exclude the possibility that the brush border membrane binding site is involved in the degradation and/or internalization of endothelin-1 in a manner similar to other peptide hormones (Berg et al., 1988).

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References

Berg, J.A., Hayashi, M., Fujii, Y., Katz, A.I., 1988. Renal metabolism of atrial natriuretic peptide in the rat. Am. J. Physiol. 255, F466–F473.

Dean, R., Zhuo, J., Alcorn, D., Casley, D., Mendelsohn, F.A.O., 1994. Cellular distribution of ^{125}I-endothelin binding in rat kidney following in vivo labeling. Am. J. Physiol. 267, F845–F852.

Edwards, R.M., Stack, E., Trizna, W., 1997. α-Ketoglutarate transport in rat renal brush-border and basolateral membrane vesicles. J. Pharmacol. Exp. Ther. 281, 1059–1064.

Eiam-Ong, S., Hilden, S.A., King, A.J., Johns, C.A., Madias, N.E., 1992. Endothelin-1 stimulates Na^+/H^+ and Na^+/HCO_3^- transporters in rabbit renal cortex. Kidney Int. 42, 18–24.

Felder, C.C., McKelvey, A.M., Gitler, M.S., Eisner, G.M., Jose, P.A., 1989. Dopamine receptor subtypes in renal brush border and basolateral membranes. Kidney Int. 36, 183–193.

Garcia, N.H., Garvin, J.L., 1994. Endothelin's biphasic effect on fluid absorption in the proximal straight tubule and its inhibitory cascade. J. Clin. Invest. 93, 2572–2577.

Gellai, M., DeWolf, R., Pullen, M., Nambi, P., 1994. Distribution and functional role of renal ET receptor subtypes in normotensive and hypertensive rats. Kidney Int. 46, 1287–1294.

Ihara, M., Noguchi, K., Saeki, T., Fukuroda, T., Tsuchida, S., Kimura, S., Fukami, T., Ishikawa, K., Nishikibe, M., Masaki, T., 1992. Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. Life Sci. 50, 247–255.

Kaufmann, M., Muff, R., Stieger, B., Biber, J., Murer, H., Fischer, J.A., 1994. Apical and basolateral parathyroid hormone receptors in rat renal cortical membranes. Endocrinology 134, 1173–1178.

Kniaz, D., Pahlavan, P., Valaitis, D., Arruda, J.A.L., 1991. High affinity binding sites for VIP in renal cortical membranes; possible role of VIP in renal transport. Kidney Int. 39, 266–272.

Morduchowicz, G.A., Scheikh-Hamad, D., Dwyer, B.E., Stern, N., Jo, O.D., Yanagawa, N., 1991. Angiotensin II directly increases rabbit renal brush-border membrane sodium transport: presence of local signal transduction system. J. Membr. Biol. 122, 43–53.

Murer, H., Gmaj, P., 1986. Transport studies in plasma membrane vesicles isolated from renal cortex. Kidney Int. 30, 171–186.

Nambi, P., Brooks, D.P., 1997. Effects of endothelin in the kidney. In: Huggins, J.P., Pelton, J.T., (Eds.), Endothelins in Biology and Medicine. CRC Press, Boca Raton, FL, pp. 171–190.

Nambi, P., Wu, H.L., Pullen, M., Aiyar, N., Bryan, H., Elliott, J., 1992. Identification of endothelin receptor subtypes in rat kidney cortex using subtype-selective ligands. Mol. Pharmacol. 42, 336–339.

Ohlstein, E.H., Beck, G., Douglas, S.A., Nambi, P., Gleason, J., Ruffolo, R.R. Jr., Feuerstein, G., Elliott, J.D., 1994. Non-peptide endothelin receptor antagonists: II. Pharmacological characterization of SB 209670. J. Pharmacol. Exp. Ther. 271, 762–768.

Sheikh-Hamad, D., Wang, Y-P., Jo, O.J., Yanagawa, N., 1993. Dopamine antagonizes the actions of angiotensin II in renal brush-border membranes. Am. J. Physiol. 264, F737–F743.

Takemoto, F., Uchida, S., Ogata, E., Kurokawa, K., 1993. Endothelin-1 and endothelin-3 binding to rat nephrons. Am. J. Physiol. 264, F827–F832.

Williams, D.L., Jones, K.L., Pettibone, D.J., Lis, E.V., Clinschmidt, B.V., 1991. Sarafotoxin 6c, an agonist which distinguishes between endothelin receptor subtypes. Biochem. Biophys. Res. Commun. 175, 556–561.