ELSEVIER

European Journal of Pharmacology 274 (1995) 1–6

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[^{3}H]BQ-123, a highly specific and reversible radioligand for the endothelin ET_A receptor subtype

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Received 16 August 1994; revised MS received 17 October 1994; accepted 28 October 1994

Abstract

The mode of binding of [^{3}H]BQ-123 (*cyclo*(-D-Trp-D-Asp-[prolyl-3,4(*n*)-[^{3}H]]Pro-D-Val-Leu-)), an endothelin receptor antagonist radioligand, was evaluated in the human neuroblastoma cell line SK-N-MC at 37°C. Scatchard analysis indicated the presence of a single class of [^{3}H]BQ-123 binding sites with a high affinity of 3.2 nM. [^{3}H]BQ-123 binding achieved steady state within 7 min and dissociated with a half-time of 1.4 min, while [^{125}I]endothelin-1 binding barely reached a steady state even after 6 h and showed little dissociation. [^{3}H]BQ-123 binding was sensitive to endothelin-1 and endothelin-2 (K_i values = 0.058 and 0.10 nM, respectively) and the endothelin ET_A receptor-selective antagonist BQ-123 (K_i = 3.3 nM), while showing low affinity for endothelin-3 (K_i = 50 nM), the endothelin ET_B receptor-selective agonist BQ-3020 (K_i = 970 nM) and other bioactive peptides. Thus, [^{3}H]BQ-123 is a specific and reversible radioligand for endothelin ET_A receptors. The rapid reversibility of [^{3}H]BQ-123 binding should provide a tool for estimating the equilibrium inhibition constants (K_i values) of various compounds for endothelin ET_A receptors.

Keywords: Endothelin; Endothelin ET_A receptor; [^{3}H]BQ-123

1. Introduction

Endothelin-1 is a 21-amino acid peptide with potent and long-lasting vasoconstrictive activity, originally isolated from cultured porcine endothelial cells (Yanagisawa et al., 1988). Studies have revealed the existence of two additional related peptides termed endothelin-2 and endothelin-3 in mammalian species (Inoue et al., 1989). These endothelin family isopeptides and their receptors have been found in not only cardiovascular tissues but also several non-vascular tissues including those of the central nervous system (CNS) (Matsumoto et al., 1989; Koseki et al., 1989). Therefore, the presence of endothelin-1 immunoreactive peptides and their receptors within the CNS suggests an important role for these peptides there.

These endothelin family peptides produce multiple biological responses via at least two distinct endothelin receptors termed ET_A (selective for endothelin-1 and endothelin-2) and ET_B (nonselective for endothelin isopeptides) (Sakurai et al., 1992). Endothelin ET_B receptor-selective agonists such as sarafotoxin S6c, BQ-3020 and IRL-1620 are useful for analyzing the responses to and binding sites of endothelin ET_B receptors (Williams et al., 1991; Ihara et al., 1992a; Takai et al., 1992). In particular, the radioligands [^{125}I]BQ-3020 and [^{125}I]IRL-1620 are useful for characterizing the binding sites of endothelin ET_B receptors (Ihara et al., 1992a; Watakabe et al., 1992). The endothelin ET_A receptor-selective antagonists developed so far, such as BQ-123, BQ-153, BQ-485, BQ-610 and FR139317 (Ihara et al., 1992b; Ishikawa et al., 1992a,b; Itoh et al., 1993; Sogabe et al., 1993), have revealed that endothelin-1-induced vasoconstriction and hypertension are mediated mainly by endothelin ET_A receptors (Ihara et al., 1992b; Sogabe et al., 1993). In order to characterize the binding sites of endothelin ET_A receptors, [^{125}I]endothelin-1 is currently used (Nakamichi et al., 1992).

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SSDI 0014-2999(94)00670-9

However, [^{125}I]endothelin-1 binds to both endothelin ET_A and ET_B receptors nonselectively (Saeki et al., 1991) and the binding is very slow and shows little dissociation (Waggoner et al., 1992). These slow binding kinetics may be misleading with respect to the dissociation constants (K_d values) (Waggoner et al., 1992) and inhibition constants (K_i values) (Cheng and Prusoff, 1973), because these values are calculated on the assumption of an equilibrium steady state. Thus, an endothelin ET_A receptor-selective and reversible radioligand has been sought to characterize the binding kinetics of endothelin ET_A receptors. Using human neuroblastoma SK-N-MC cells, we report here the binding characteristics of [^{3}H]BQ-123 as a novel and reversible radioligand for human endothelin ET_A receptors.

2. Materials and methods

2.1. Materials

[^{3}H]BQ-123 (*cyclo*(-D-Trp-D-Asp-[prolyl-3,4(*n*)-[^{3}H]]Pro-D-Val-Leu-)) was synthesized at Amersham International, UK (41 Ci/mmol). It was dissolved in 10 mM Tris/HCl buffer, pH 7.4 and stored at 4°C for 3 months. [^{125}I]Endothelin-1 was purchased from Amersham International, UK (2000 Ci/mmol). BQ-123 (*cyclo*(-D-Trp-D-Asp-Pro-D-Val-Leu-)), BQ-153 (*cyclo*(-D-Trp-D-Cys(O_3H)-Pro-D-Val-Leu-)), BQ-485 (*N*-(1-perhydroazepinyl)carbonyl-Leu-D-Trp-D-Trp), BQ-610 (*N*-(1-perhydroazepinyl)carbonyl-Leu-D-Trp(N^{in}-CHO)-D-Trp), FR139317 (*N*-(1-perhydroazepinyl) carbonyl-Leu-D-Trp(N^{in}-Me)-D-2-pyridylalanine) and BQ-3020 (*N*-acetyl-Leu-Met-Asp-Lys-Glu-Ala-Val-Tyr-Phe-Ala-His-Leu-Asp-Ile-Ile-Trp) were synthesized at the Tsukuba Research Institute of Banyu Pharmaceutical Co., Japan. Endothelin-1, endothelin-2 and endothelin-3 were purchased from the Peptide Institute (Osaka, Japan). SK-N-MC cells derived from a human neuroblastoma were obtained from the American Type Culture Collection (Rockville, MD, USA). Girardi heart cells were obtained from Dainippon Seiyaku Co. (Osaka, Japan).

2.2. Binding experiments

SK-N-MC cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS), 100 units/ml of penicillin and 100 μg/ml of streptomycin at 37°C under an atmosphere of 95% air/5% CO_2, while Girardi heart cells were grown in minimum essential medium with Earle's salt (MEM) containing 10% FCS and the above-mentioned antibiotics under the same conditions of temperature and atmosphere. After washes with Hanks' balanced salt solution containing 0.1% glucose and 0.3% bovine serum albumin (buffer A), the SK-N-MC or Girardi heart cells were incubated at 37°C with [^{3}H]BQ-123 or [^{125}I]endothelin-1 in the presence of test compounds in a 95% air-5% CO_2 humidified atmosphere for 1 or 4 h, respectively. The cells were then washed 3 times with ice-cold buffer A and the cell-bound radioactivity was determined by a liquid scintillation counter (Packard: 2500TR) or a gamma counter (Packard: Cobra 5002). Nonspecific binding was defined by the addition of 1 μM BQ-123 or 0.2 μM endothelin-1 to each assay, respectively.

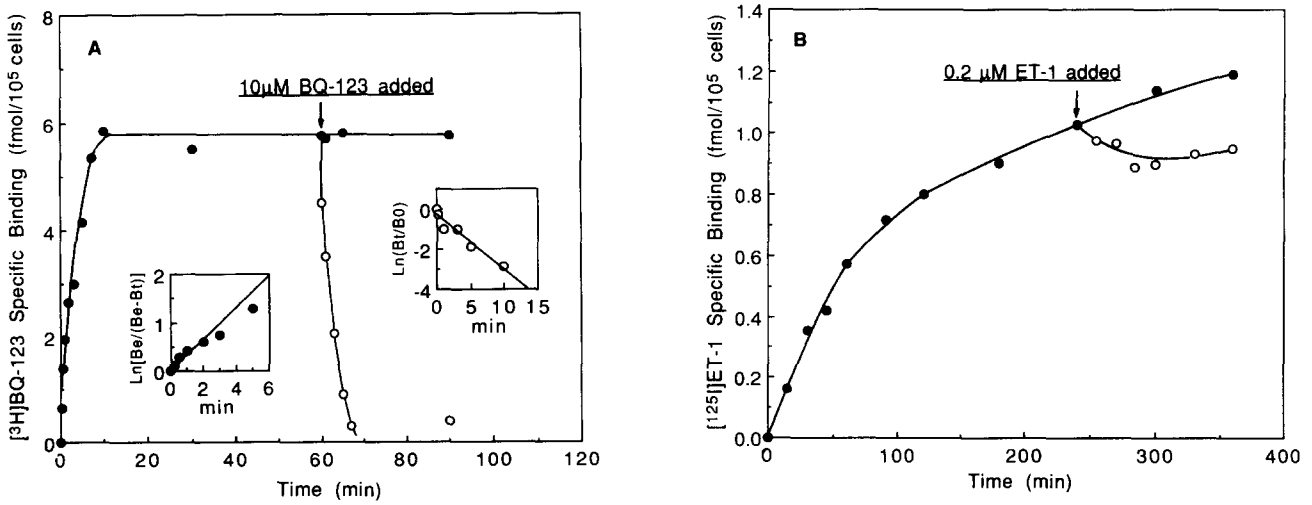

Fig. 1. Time course of (A) [^{3}H]BQ-123 and (B) [^{125}I]endothelin-1 ([^{125}I]ET-1) binding to SK-N-MC cells. After 1 or 4 h, (A) 10 μM BQ-123 or (B) 0.2 μM endothelin-1 (ET-1), respectively, was added to the assay tubes to evaluate the dissociation kinetics (open circles). Each point represents the mean value of triplicate determinations. Inserts (A), plots of the association and dissociation of the specific binding according to the first-order kinetic equation, as described in the data analysis section.

2.3. Data analysis

The IC_{50} values were determined by regression analysis of displacement curves. Saturation binding was analyzed by Scatchard plot to determine the equilibrium dissociation constant (K_d) and the concentration of binding sites (B_{max}). The k_{+1} and k_{-1} values were calculated from the data of the kinetic experiments. The k_{+1} values were determined from k_{obs}, the first order rate constant for association, according to the method of Bennet (1978). A plot of $\ln[B_e/(B_e - B_t)]$ vs. time, where B_e = the amount bound at equilibrium and B_t = the amount bound at various times before equilibrium, yields k_{obs}, the slope of the line. A plot of $\ln(B_t/B_0)$ vs. time, where B_t = the amount bound at various times after dissociation was initiated and B_0 = the amount bound at time = 0, yields the k_{-1} value, the slope of the line. The k_{+1} value was then calculated by the following formula: $k_{+1} = (k_{obs} - k_{-1})/F$, where F equals the free concentration of radioligands. The values of $T_{1/2}$, half-life, were calculated by the formula $T_{1/2} = 0.693/k_{-1}$. The Hill coefficients (n_H values) were determined by the method of Hill (1913). Student's t-tests (one-sample tests) were used to determine whether Hill slopes were significantly different from unity. The apparent inhibition constants (K_i values) were calculated from the equation $(K_i = IC_{50}/(1 + L/K_d))$ (Cheng and Prusoff, 1973). All values in this study are indicated as the mean ± S.E.M. values determined by triplicate determinations in more than three separate experiments.

3. Results

3.1. Binding kinetics of [^{3}H]BQ-123 and [^{125}I]endothelin-1

The time course of binding for [^{3}H]BQ-123 (3.0 nM) or [^{125}I]endothelin-1 (10 pM) was estimated at 37°C using SK-N-MC cells. As shown in Fig. 1A, specific binding for [^{3}H]BQ-123 increased rapidly with a half-time of about 3 min and achieved equilibrium within 7 min of reaction, with an apparent pseudo-first order rate constant k_{obs} of 0.48 ± 0.08 min^{-1}. The calculated association rate constant k_{+1} was 0.34 ± 0.06 min^{-1} nM^{-1}. Furthermore, after a steady state was reached, the addition of an excess of unlabeled BQ-123 (10 μM) caused a rapid release of bound radioactivity from the cells. The dissociation half-time of [^{3}H]BQ-123 binding was calculated from this study to be 1.4 ± 0.2 min. The off-rate constant k_{-1} was calculated as 0.51 ± 0.09 min^{-1}. The kinetically derived dissociation constant (k_{-1}/k_{+1}) was 1.6 ± 0.3 nM. This steady state was maintained for up to 90 min of incubation. Nonspecific binding of [^{3}H]BQ-123 determined by the addition of 1 μM BQ-123 was 3.4 ± 0.5% of the total binding. The nonspecific binding was not different from that determined with 10 nM endothelin-1 (4.1 ± 0.4%). Based on these data, a 1-h period of incubation was chosen for all subsequent experiments. In contrast, as shown in Fig. 1B, specific binding for [^{125}I]endothelin-1 increased slowly and did not reach steady state even after 6 h. The nonspecific binding of [^{125}I]endothelin-1 determined by the addition of 0.2 μM endothelin-1 was 2.3 ± 1.0% of the total binding. It was not different from that determined by 10 μM BQ-123 (2.0 ± 0.2%). After incubation for 4 h, 0.2 μM endothelin-1 was added to displace the radioligand. Most parts of the specific binding were resistant to displacement even after 2 h. The slow onset and offset binding profile of [^{125}I]endothelin-1 was remarkably distinct from the rapid and reversible kinetics of [^{3}H]BQ-123.

Fig. 2A shows the saturation binding of [^{3}H]BQ-123 to SK-N-MC cells. The amount of bound [^{3}H]BQ-123 increased in a concentration-dependent manner reaching a plateau. The binding of [^{3}H]BQ-123 to the cells, using concentrations between 1.5 and 95 nM, was saturable. Scatchard analysis of several binding isotherms

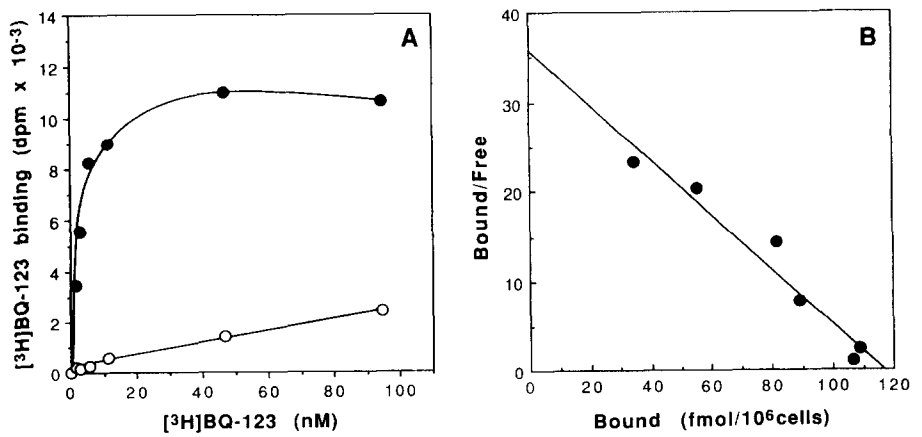

Fig. 2. (A) Saturation curve of [^{3}H]BQ-123 specific binding (●) to SK-N-MC cells. Nonspecific binding (○) was defined in the presence of 1 μM BQ-123. (B) Scatchard plot of the same data. Each point represents the mean value of triplicate determinations.

showed a single class of binding sites characterized by the following parameters: $K_d = 3.2 \pm 0.6$ nM and $B_{max} = 68000 \pm 11000$ sites/cell ($n = 3$, Fig. 2B). The K_d value from equilibrium binding kinetics was slightly higher than the dissociation constant determined by kinetic experiments. Although it was impossible to estimate the equilibrium binding parameters of [^{125}I]endothelin-1 due to the lack of an equilibrium state of binding, the apparent binding parameters were calculated from Scatchard analysis of the saturation binding data of [^{125}I]endothelin-1; a linear Scatchard plot indicated the presence of a single population of high-affinity sites, $K_d = 34 \pm 3$ pM with $B_{max} = 53000 \pm 4000$ sites/cell ($n = 3$, data not shown), which was not significantly different from the B_{max} value for [^{3}H]BQ-123. In human Girardi heart cells, where endothelin receptors are predominantly of the endothelin ET_B type (Mihara and Fujimoto, 1992), specific binding of [^{3}H]BQ-123 was not found in the concentration range of 0.42–42 nM of [^{3}H]BQ-123, while the saturation kinetics of [^{125}I]endothelin-1 binding indicated the existence of saturable binding sites of endothelin-1 ($K_d = 32 \pm 8$ pM, $B_{max} = 7400 \pm 700$ sites/cell ($n = 3$, data not shown)).

3.2. Competitive binding studies

Fig. 3 illustrates the selectivity of various compounds for the endothelin ET_A receptors on SK-N-MC cells labeled by [^{3}H]BQ-123. Endothelin family peptides inhibited the specific binding of [^{3}H]BQ-123 in a concentration-dependent, monophasic manner. The IC_{50} values of endothelin-1, endothelin-2, endothelin-3 and BQ-3020 were 0.10 ± 0.01, 0.19 ± 0.02, 70 ± 14 and 1800 ± 400 nM, respectively. The endothelin ET_A receptor antagonist BQ-123 inhibited binding with an IC_{50} value of 4.9 ± 0.6 nM, while other non-related peptides such as neuropeptide Y, vasopressin, oxytocin, substance P, bradykinin, angiotensin II and CGRP inhibited less than 10% of the specific [^{3}H]BQ-123 binding even at 1 μM (data not shown).

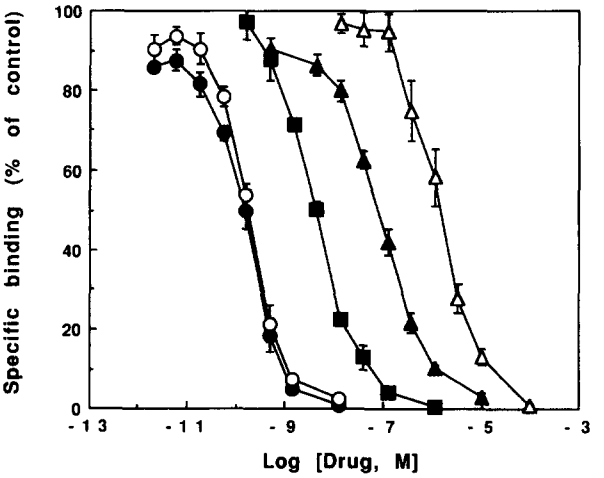

Fig. 3. Competitive inhibition of the specific binding of [^{3}H]BQ-123 to SK-N-MC cells by endothelin-1 (●), endothelin-2 (○), endothelin-3 (▲), BQ-3020 (△) and BQ-123 (■). Each point represents the mean ± S.E.M. value of more than three different experiments.

Table 1 provides calculated inhibition constants (K_i values) of various endothelin agonists and antagonists for the endothelin ET_A receptors labeled by either [^{3}H]BQ-123 or [^{125}I]endothelin-1. The inhibitory profiles for [^{3}H]BQ-123 binding were almost parallel to those for [^{125}I]endothelin-1 binding. The K_i values of endothelin-1 and endothelin-2 for [^{3}H]BQ-123 binding were close to those for [^{125}I]endothelin-1 binding, while K_i values of endothelin ET_A receptor antagonists such as BQ-123, BQ-153, BQ-485, BQ-610 and FR139317 and of endothelin ET_B receptor agonist such as endothelin-3 and BQ-3020 for [^{3}H]BQ-123 binding were significantly 3.2–12 times lower than those for [^{125}I]endothelin-1 binding. The Hill coefficients of these endothelin agonists and antagonists for [^{3}H]BQ-123 and [^{125}I]endothelin-1 binding inhibition had values close to unity except for that of endothelin-3 for [^{3}H]BQ-123 binding.

Table 1
The inhibition constants (K_i) and Hill constants (n_H) of endothelin agonists and antagonists for [^{3}H]BQ-123 or [^{125}I]endothelin-1 binding in SK-N-MC cells

Compounds	[^{3}H]BQ-123		[^{125}I]Endothelin-1	
	K_i (nM)	n_H	K_i (nM)	n_H
Endothelin-1	0.058 ± 0.006	1.03 ± 0.07	0.057 ± 0.011	1.04 ± 0.10
Endothelin-2	0.10 ± 0.01	1.10 ± 0.03	0.17 ± 0.03	0.97 ± 0.04
Endothelin-3	50 ± 1	0.82 ± 0.01 [a]	180 ± 30	1.00 ± 0.02
BQ-3020	970 ± 90	1.04 ± 0.05	5000 ± 1000	0.97 ± 0.07
BQ-123	3.3 ± 0.5	1.05 ± 0.04	21 ± 1	1.06 ± 0.18
BQ-153	5.2 ± 1.2	0.98 ± 0.01	21 ± 5	0.89 ± 0.20
BQ-485	2.5 ± 0.5	1.03 ± 0.06	11 ± 4	1.04 ± 0.17
BQ-610	0.82 ± 0.32	1.09 ± 0.03	2.6 ± 1.0	0.90 ± 0.08
FR139317	0.89 ± 0.14	0.90 ± 0.05	11 ± 1	0.98 ± 0.14

The K_i values in this table represent mean ± S.E.M. values from more than three different experiments.
[a] Statistical significance from unity, $P < 0.05$.

4. Discussion

In this study, we characterized the binding sites of endothelin receptors in human neuroblastoma SK-N-MC cells using [^{125}I]endothelin-1 and a novel radioligand, [^{3}H]BQ-123. The saturation binding kinetics revealed a single class of BQ-123 binding sites with a high affinity of 3.2 nM, and the B_{max} value was not significantly different from that of [^{125}I]endothelin-1. [^{3}H]BQ-123 binding was sensitive to endothelin-1, endothelin-2 and endothelin ET_A receptor-selective antagonists such as BQ-123, BQ-153, BQ-485, BQ-610 and FR139317, while it showed low affinity for endothelin-3, the endothelin ET_B receptor-selective agonist BQ-3020 and various other bioactive peptides. [^{3}H]BQ-123 did not bind to human Girardi heart cells, on which endothelin ET_B receptors are predominant (Mihara and Fujimoto, 1992). These data indicate that [^{3}H]BQ-123 selectively labels endothelin ET_A receptors. Hill constants for the inhibition of [^{3}H]BQ-123 and [^{125}I]endothelin-1 binding were not significantly different from unity except for that of endothelin-3 with [^{3}H]BQ-123 binding. These data suggest that both [^{3}H]BQ-123 and [^{125}I]endothelin-1 share common binding sites on endothelin ET_A receptors. Furthermore, the significantly lower Hill constants of endothelin-3 for [^{3}H]BQ-123 binding may be due to interaction of endothelin-3 with the binding sites of BQ-123 on endothelin ET_A receptors in a negative cooperative manner.

One of the most attractive features of [^{3}H]BQ-123 is the rapid reversibility of its binding to endothelin ET_A receptors. Since binding of [^{3}H]BQ-123 to the cells was rapidly displaced by excess amounts of BQ-123 in a monophasic manner, [^{3}H]BQ-123 bound to SK-N-MC cells at least may not be internalized into the cells. The half-time of [^{3}H]BQ-123 for association and dissociation is of the order of a few minutes, while [^{125}I]endothelin-1 binds very slowly and shows little dissociation from the cells. The extremely slow binding kinetics of [^{125}I]endothelin-1 have also been described by others (Hemsen et al., 1990; Waggoner et al., 1992; Ihara et al., 1992a). Scatchard analysis is based on the equilibrium process of ligand binding with a receptor to form a dissociable receptor-ligand complex. Therefore, the K_d values derived from Scatchard analysis of [^{125}I]endothelin-1 binding data and the calculated K_i values may be misleading. However, [^{3}H]BQ-123 reached an equilibrium steady state within 7 min. Therefore, this compound is an ideal radioligand to evaluate the inhibition constants (K_i values) of various reversible compounds reflecting their equilibrium dissociation constants (K_d values). Although K_i values of endothelin agonists and antagonists for [^{3}H]BQ-123 binding are quite similar to the inhibition constants obtained by displacement of [^{125}I]endothelin-1 binding, the K_i values of endothelin ET_A receptor antagonists and endothelin ET_B receptor agonists for [^{3}H]BQ-123 binding are lower than those observed for [^{125}I]endothelin-1 binding. These differences in the calculated K_i values result from the lower reliability of K_i values calculated using the slow and almost irreversible binding kinetics of [^{125}I]endothelin-1, which does not reach an equilibrium steady state.

Recently, based on a chimera receptor technique, it was reported that the binding sites of BQ-123 on the endothelin ET_A receptor are distinct from the subdomains of endothelin receptors which determine the selectivity for endothelin isopeptides (Sakamoto et al., 1993). Therefore, it will be interesting to use [^{3}H]BQ-123 to determine whether recently developed nonpeptide endothelin antagonists (Fujimoto et al., 1992; Clozel et al., 1993) share a common binding site with BQ-123 on endothelin ET_A receptors. The development of a novel ET_A receptor-specific and reversible radioligand, [^{3}H]BQ-123, offers a specific tool to investigate the distribution of endothelin ET_A receptors, binding sites of endothelin antagonists and equilibrium inhibition constants for endothelin ET_A receptors.

Acknowledgement

We express our thanks to Ms. Donna LeBlanc, Merck & Co., for critical reading of the manuscript.

References

Bennet, J.P., 1978, Methods of binding studies, in: Neurotransmitter Receptor Binding, eds. H.I. Yamamura, S.J. Enna and M.J. Kuhar (Raven Press, New York) p. 57.

Cheng, Y.C. and H. Prusoff, 1973, Relationship between the inhibition constant (K_I) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction, Biochem. Pharmacol. 22, 3099.

Clozel, M., V. Breu, K. Burri, J.M. Cassal, W. Fischli, G.A. Gray, G. Hirth, B.M. Loffler, M. Muller, W. Neidhart and H. Ramuz, 1993, Pathophysiological role of endothelin revealed by the first orally active endothelin receptor antagonist, Nature 365, 759.

Fujimoto, M., S. Mihara, S. Nakajima, M. Ueda, M. Nakamura and K. Sakurai, 1992, A novel nonpeptide endothelin antagonist isolated from bayberry, *Myrica cerifera*, FEBS Lett. 305, 41.

Hemsen, A., A. FrancoCereceda, R. Matran, A. Rudehill and J.M. Lundberg, 1990, Occurrence, specific binding sites and functional effects of endothelin in human cardiopulmonary tissue, Eur. J. Pharmacol. 191, 319.

Hill, A.V., 1913, The combination of hemoglobin with oxygen and with carbon monooxide, Biochem. J. 7, 471.

Ihara, M., T. Saeki, T. Fukuroda, S. Kimura, S. Ozaki, A.C. Patel and M. Yano, 1992a, A novel radioligand [^{125}I]BQ-3020 selective for endothelin (ET_B) receptors, Life Sci. 51, PL47.

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

Inoue, A., M. Yanagisawa, S. Kimura, Y. Kasuya, T. Miyauchi, K. Goto and T. Masaki, 1989, The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes, Proc. Natl. Acad. Sci. USA 86, 2863.

Ishikawa, K., T. Fukami, T. Nagase, K. Fujita, T. Hayama, K. Niiyama, T. Mase, M. Ihara and M. Yano, 1992a, Cyclic pentapeptide endothelin antagonists with high ET_A selectivity. Potency- and solubility-enhancing modifications, J. Med. Chem. 35, 2139.

Ishikawa, K., T. Fukami, T. Nagase, T. Mase, T. Hayama, K. Niiyama, K. Fujita, Y. Urakawa, U. Kumagai, T. Fukuroda, M. Ihara and M. Yano, 1992b, Endothelin antagonistic peptide derivatives with high selectivity for ET_A receptors, Eur. Peptide Symposium, 273 (abstract).

Itoh, S., T. Sasaki, K. Ide, K. Ishikawa, M. Nishikibe and M. Yano, 1993, A novel endothelin ET_A receptor antagonist, BQ-485, and its preventive effect on experimental cerebral vasospasm in dogs, Biochem. Biophys. Res. Commun. 195, 969.

Koseki, C., M. Imai, Y. Hirata, M. Yanagisawa and T. Masaki, 1989, Autoradiographic distribution in rat tissues of binding sites for endothelin: a neuropeptide?, Am. J. Physiol. 256, R858.

Matsumoto, H., N. Suzuki, H. Onda and M. Fujino, 1989, Abundance of endothelin-3 in rat intestine, pituitary gland and brain, Biochem. Biophys. Res. Commun. 164, 74.

Mihara, S. and M. Fujimoto, 1992, Non-isopeptide-selective endothelin receptors in human Girardi heart cells, Life Sci. 50, 219.

Nakamichi, K., M. Ihara, M. Kobayashi, T. Saeki, K. Ishikawa and M. Yano, 1992, Different distribution of endothelin receptor subtypes in pulmonary tissues revealed by the novel selective ligands BQ-123 and [$Ala^{1,3,11,15}$]ET-1, Biochem. Biophys. Res. Commun. 182, 144.

Saeki, T., M. Ihara, T. Fukuroda, M. Yamagiwa and M. Yano, 1991, [$Ala^{1,3,11,15}$]Endothelin-1 analogs with ET_B agonistic activity, Biochem. Biophys. Res. Commun. 179, 286.

Sakamoto, A., M. Yanagisawa, T. Sawamura, T. Enoki, T. Ohtani, T. Sakurai, K. Nakao, T. Toyooka and T. Masaki, 1993, Distinct subdomains of human endothelin receptors determine their selectivity to $endothelin_A$-selective antagonist and $endothelin_B$-selective agonists, J. Biol. Chem. 268, 8547.

Sakurai, T., M. Yanagisawa and T. Masaki, 1992, Molecular characterization of endothelin receptors, Trends Pharmacol. Sci. 13, 103.

Sogabe, K., H. Nirei, M. Shoubo, A. Nomoto, S. Ao, Y. Notsu and T. Ono, 1993, Pharmacological profile of FR139317, a novel, potent endothelin ET_A receptor antagonist, J. Pharmacol. Exp. Ther. 264, 1040.

Takai, M., I. Umemura, K. Yamasaki, T. Watakabe, Y. Fujitani, K. Oda, Y. Urade, T. Inui, T. Yamamura and T. Okada, 1992, A potent and specific agonist Suc-[Glu^9,$Ala^{11,15}$]-endothelin-1(8–21) IRL-1620 for the ET_B receptor, Biochem. Biophys. Res. Commun. 184, 953.

Waggoner, W.G., S.L. Genova and V.A. Rash, 1992, Kinetic analyses demonstrate that the equilibrium assumption does not apply to [^{125}I]endothelin-1 binding data, Life Sci. 51, 1869.

Watakabe, T., Y. Urade, M. Takai, I. Umemura and T. Okada, 1992, A reversible radioligand specific for the ET_B receptor: [^{125}I]Tyr^{13}-Suc-[Glu^9,$Ala^{11,15}$]-endothelin-1(8–21), [^{125}I]IRL-1620, Biochem. Biophys. Res. Commun. 185, 867.

Williams, D.L., K.L. Jones, D.J. Pettibone, E.V. Lis and B.V. Clineschmidt, 1991, Sarafotoxin S6c: an agonist which distinguishes between endothelin receptor subtypes, Biochem. Biophys. Res. Commun. 175, 556.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto and T. Masaki, 1988, A novel potent vasoconstrictor peptide produced by vascular endothelial cells, Nature 332, 411.