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Role of ET_B receptors in local clearance of endothelin-1 in rat heart: studies with the antagonists PD 155080 and BQ-788

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Abstract The effects of two endothelin (ET) receptor antagonists, PD 155080 (ET_A selective) and BQ-788 (ET_B selective), on cardiac function and ET-1 release were studied in isolated rat hearts. In normoxic hearts, infusion of PD 155080 (50 nM–5 μM) reduced coronary resistance, but had no effect on ET-1 release. Low concentrations of BQ-788 (2 and 20 nM) had no effect on coronary resistance; high concentrations (0.2 and 2 μM) increased it ~2-fold; all concentrations increased ET-1 release (up to 24-fold). Similar results were obtained in reperfused hearts. Although concentrations of ET-1 were higher in interstitial fluid than coronary effluent, levels never exceeded the low pg/ml range. These results indicate that (1) ET_A receptors mediate coronary constriction, whereas ET_B receptors bind and sequester ET-1, and (2) ET-1 displaced by ET_B antagonist accesses ET_A receptors resulting in coronary constriction.

Key words: Endothelin-1; Coronary resistance; Tissue clearance; ET_B subtype; Clearance receptor

1. Introduction

Endothelin-1 (ET-1), originally isolated from the conditioned medium of cultured vascular endothelial cells [1], is a polypeptide with numerous biological actions in vitro and in vivo. In vascular beds, ET-1 modulates coronary resistance by stimulating the release of endothelial relaxing factors or by constricting smooth muscle cells [2,3]. Whether endogenous ET-1 is a vasodilator or vasoconstrictor may depend on the concentration of ET-1 at vascular ET receptors, the density of receptor subtypes, the efficacy of receptor-effector coupling, and the turnover of receptors. One of us has previously determined interstitial ET-1 levels in rat hearts and found that interstitial ET-1 levels only reached the pg/ml, i.e. the vasodilator range and that cardiac ET-1 seemed to be involved in lowering vascular tone rather than increasing it [4]. In contrast, in the human forearm antagonism of ET_A receptors by BQ-123 caused vasodilatation [5], indicating that ET-1 is a net vasoconstrictor in forearm resistance vessels.

Infusion of dual ET_A/ET_B receptor antagonists into experimental animals increased plasma ET-1 levels manifold [6,7], possibly by displacing ET-1 bound to tissue ET_B receptors [8]. These in vivo data, together with preliminary ET-1 efflux measurements in rat hearts [9], raise the possibility of a local clearance mechanism mediated by ET_B receptors. We hypothesized that cardiac ET_A receptors mediate hemodynamic effects, and ET_B receptors clearance, or clearance and vascular, effects. Hearts were perfused with a colloid-free perfusion medium to generate interstitial fluid [4], and the effect of PD 155080, a ET_A receptor-selective antagonist [10], or BQ-788, a ET_B receptor antagonist [11], on myocardial, vascular, and electrical function as well as the rate of ET-1 secretion into coronary effluent (luminal release) and interstitial fluid (abluminal release) were monitored under normoxic, ischemic, and reperfusion conditions.

2. Materials and methods

2.1. Materials

PD 155080 (2-benzo[1,3]dioxol-5-yl-3-benzyl-4-(4-methoxy-phenyl)-4-oxobut-2-enoate×Na), BQ-788 (*N-cis*-2,6-dimethyl-piperidinocarbonyl-L-γ-methylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine×Na (=PD 160900-0015) and PD 142893 (Ac-D-3,3-diphenylalanine-L-Leu-L-Asp-L-Ile-L-Ile-L-Trp×2Na) were synthesized at Parke-Davis Pharmaceutical Research, Ann Arbor, MI, USA. All other materials were described previously [4,9].

2.2. Heart perfusions

Rat hearts were perfused in an upside-down position via the aorta at 9 ml/min per g heart wet weight. Interstitial fluid (transudate) produced by ventricles and appearing on their surface was collected under a latex cap using slight suction and was sampled in exchangeable vials [4]. Transudate flow slowly increased from an initial 50 ± 1 μl/min (45 min, end of equilibration period) to 128 ± 3 μl/min after 255 min. ET-1 was determined in timed collections of coronary effluent and transudate and expressed as concentration (pg/ml). Functional parameters were monitored throughout the experiment and included heart rate, left ventricular developed pressure (LVDevP; a measure of systolic function), left ventricular end-diastolic pressure (LVEDP; a measure of diastolic function), coronary perfusion pressure (CPP; a measure of coronary resistance), and the incidence of ventricular extrasystoles (VES) [4,12].

2.3. Experimental protocols

(1) Normoxic perfusions. Hearts were equilibrated (45 min) and perfused for 210 min (total duration: 255 min) in the presence of vehicle, PD 155080 (50 nM), or BQ-788 (2 nM). For concentration-effect studies, the antagonists (PD 155080: 500 nM and 5 μM; BQ-788: 20, 200, 2000 nM) were tested cumulatively, starting at 45 min and allowing 15 min for each concentration, at the end of which CPP was determined (total duration: 75 min (PD 155080) or 90 min (BQ-788)). Because CPP was unchanged up to ~90 min after vehicle (Table 1), the CPP values so measured reflect the net effect of the antagonists.

(2) Ischemia/reperfusion. After equilibration (baseline), the protocol comprised a control (60 min), ischemic (60 min), and reperfusion period (30 min) (total duration: 195 min). Ischemia was generated by reducing coronary flow to 1 ml/min. PD 155080 (50, 500, and 5000 nM) and BQ-788 (2, 20, and 200 nM) were added to the perfusion medium during all three phases.

2.4. Determination of ET-1

The peptide was concentrated by solid-phase extraction followed by quantitative radioimmunoassay (RIA) as described previously [4].

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Abbreviations: ET-1, endothelin-1; CPP, coronary perfusion pressure; LVDevP, left-ventricular developed pressure; LVEDP, left-ventricular end-diastolic pressure; VES, ventricular extrasystole

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Briefly, coronary effluents and interstitial transudates were chromatographed on C2 cartridges, ET-1 was eluted with acetonitrile (70%), dried, and reconstituted for RIA using a commercial antibody (Peninsula Laboratories, Belmont, CA, USA). The cross-reactivity of other ET isomers and big ET-1 in this assay was $<5\%$ and $<37\%$, respectively, according to the supplier. No big ET-1 was detected in effluents or transudates in separate experiments using an antibody specific for big ET-1 [13].

2.5. Statistics

Group data are given as mean values ± S.E.M. (n, number of hearts). Data were subjected to two-way analysis of variance for repeated measurements to account for different treatments (control, ischemia, reperfusion) and factors (vehicle, antagonists), followed by the Scheffé test to compare single means. A P value <0.05 was considered significant.

3. Results

The basic hemodynamic parameters of the preparation using oxygenated perfusion medium and the effects of ET receptor antagonists are listed in Tables 1 and 2. As expected at constant flow perfusion, LVDevP was stable for several hours and CPP slowly increased with time ($P<0.05$ vs baseline). Heart rate and diastolic function (LVEDP) remained stable (not shown). Neither PD 155080 (50 nM) nor BQ-788 (2 nM) affected systolic function (LVDevP). The rise in coronary resistance (CPP) observed for vehicle was completely antagonized by PD 155080 (50 nM); a similar inhibition was observed at higher concentrations (0.5 and 5 μM, $n=4$; not shown). In the presence of BQ-788, CPP initially (2 and 20 nM) was unaffected, but increased above vehicle values at higher concentrations (0.2 and 2 μM; Tables 1 and 2). Basal rate of ET-1 secretion was 0.27 ± 0.01 pg/min (effluent) and 0.005 ± 0.001 pg/min (transudate), resulting in similar concentrations in effluents and transudates (less than ~0.1 pg/ml; Figs. 1 and 2). PD 155080 (50 nM) was without effect on ET-1 levels in effluent and transudate; higher concentrations (0.5 and 5 μM) were equally ineffective (n=4, not shown). BQ-788 (2 nM) caused a continuous increase in ET-1 concentration, whose dose dependence is shown in Table 2. The maximally active concentration (~2 μM) increased ET-1 ~8-fold (effluent) and ~24-fold (transudate).

The hemodynamic parameters measured for the ischemia/reperfusion model are listed in Tables 3 and 4. Expectedly, low-flow perfusion resulted in global ischemia and greatly impaired cardiac function (not shown). After restoration of normal coronary flow, cardiac function recovered incompletely as expected. PD 155080 (50 nM) improved reperfusion systolic (LVDevP) and diastolic (LVEDP) function and completely prevented the ischemia-induced increase in CPP. Higher concentrations (0.5 and 5 μM) were similarly effective ($n=4$, not shown). The lowest concentration of BQ-788 (2 nM) did not affect cardiac function during the control, ischemic, and reperfusion periods (Table 3), a 10 times higher concentration appeared to reduce CPP ($P=0.055$), and the highest concentration tested (200 nM) considerably increased CPP (Table 4). The effects on ET-1 secretion into coronary effluent in the ischemia/reperfusion model are shown in Fig. 3. Antagonism of ET_A receptors with PD 155080 (50 nM) had no effect ($P>0.05$ vs vehicle), but antagonism of ET_B receptors with BQ-788 (2–200 nM) increased ET-1 secretion in all three phases (for clarity, data for ischemia not shown).

The effects of ET receptor antagonists on reperfusion VES, a common phenomenon in experimental ischemia/reperfusion and in ischemic syndromes in humans, are shown in Fig. 4. PD 155080 (50 nM) and the mixed ET_A/ET_B receptor antagonist PD 142893 were similarly effective (74 and 81% reduction, $P>0.05$), whereas BQ-788 was ineffective at 2 nM, protective at 20 nM (56% reduction), and deleterious at 200 nM (1.5-fold increase). The drugs had similar effects in early and late reperfusion. The protective effect of 20 nM BQ-788 was abol-

Table 1
Effect of PD 155080 (ET_A receptor antagonist, 50 nM) and BQ-788 (ET_B receptor antagonist, 2 nM) on left ventricular and coronary function under normoxic conditions

Time (min post mounting)	Vehicle		PD 155080		BQ-788	
	LVDevP (mm Hg)	CPP (mm Hg)	LVDevP (mm Hg)	CPP (mm Hg)	LVDevP (mm Hg)	CPP (mm Hg)
45	87 ± 4	53 ± 2	84 ± 1	53 ± 2	87 ± 2	51 ± 2
75	82 ± 4	57 ± 2	83 ± 2	54 ± 3	87 ± 2	55 ± 3
105	80 ± 3	59 ± 1	84 ± 2	55 ± 3	84 ± 2	58 ± 2
135	83 ± 2	63 ± 3	84 ± 2	55 ± 3	84 ± 2	62 ± 1
165	87 ± 3	68 ± 3	84 ± 2	55 ± 2*	82 ± 2	68 ± 1
195	85 ± 1	73 ± 3	82 ± 2	55 ± 2*	79 ± 2	74 ± 2
225	82 ± 2	78 ± 8	82 ± 2	57 ± 3*	79 ± 2	79 ± 1
255	77 ± 2	83 ± 8	81 ± 2	58 ± 3*	78 ± 1	82 ± 1

Data are mean values ± S.E.M., $n=6$. Heart rate was 300 ± 17 beats/min (baseline) and was not affected by antagonists ($P>0.05$). *Significantly different from vehicle. The differences vs. baseline (45 min) are not indicated separately.

Table 2
Concentration dependence of BQ-788 effects on coronary perfusion pressure and ET-1 release in coronary effluent and interstitial transudate

BQ-788 concentration (nM)	CPP (mm Hg)	ET-1 level in	
		Effluent (pg/ml)	Transudate (pg/ml)
Vehicle	50 ± 1	0.03 ± 0.001	0.10 ± 0.013
2	51 ± 1	0.04 ± 0.001*	0.15 ± 0.006*
20	55 ± 2	0.04 ± 0.001*	0.16 ± 0.015*
200	96 ± 1*	0.23 ± 0.004*	2.4 ± 0.17*
2000	100 ± 2*	0.25 ± 0.001*	2.3 ± 0.18*

The antagonist was added cumulatively, starting after equilibration (45 min), allowing 15 min for each concentration. Data are mean values ± S.E.M., $n=5$. *Significantly different from vehicle.

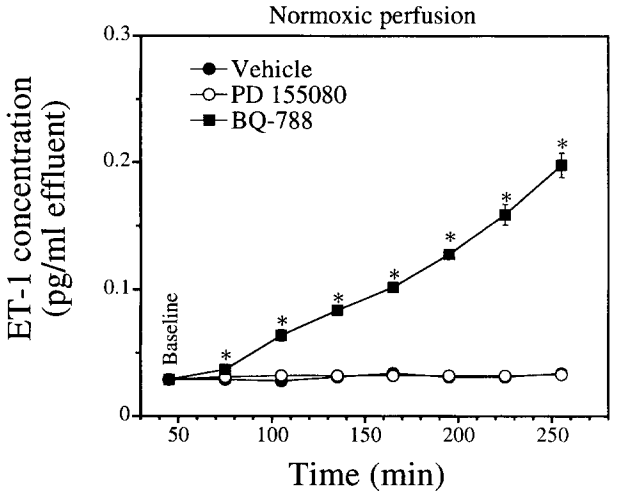

Fig. 1. Effects of PD 155080 (50 nM) and BQ-788 (2 nM) on basal ET-1 secretion into coronary effluent of isolated rat hearts at normoxic conditions. The antagonists were added after collection of the baseline sample (45 min). Mean values ± S.E.M., $n = 6$. *Significantly different from vehicle ($P < 0.05$).

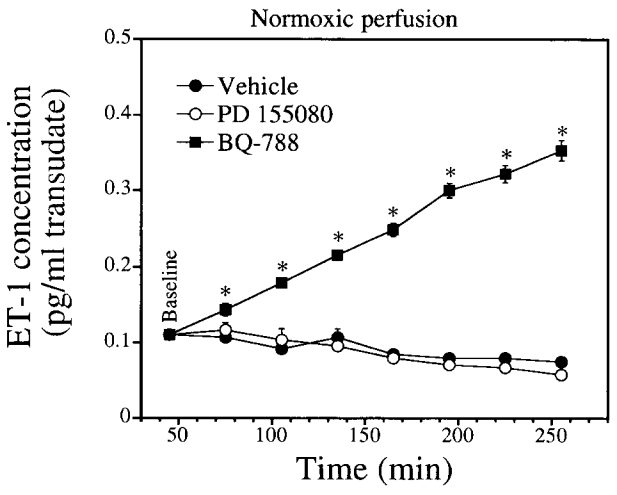

Fig. 2. Effects of PD 155080 (50 nM) and BQ-788 (2 nM) on basal ET-1 secretion into interstitial transudate of isolated rat hearts at normoxic conditions. The antagonists were added after collection of the baseline sample (45 min). Mean values ± S.E.M., $n = 6$. *Significantly different from vehicle ($P < 0.05$).

ished by concomitant infusion of indomethacin (10 μM) so that both the incidence of VES (167 ± 15) and CPP (100 ± 5 mm Hg) were restored to vehicle level ($n = 3$; data not shown).

4. Discussion

This study shows that the ET_A receptor subtype mediates myocardial and coronary effects of endogenous ET-1, whereas the ET_B subtype appears to bind and sequester ET-1 without mediating hemodynamic effects in a rat heart model. Because BQ-788 increased ET-1 release from the heart in a time- and concentration-dependent manner without affecting CPP or myocardial function (at 2 and 20 nM; Tables 1 and 2), this strongly indicates that cardiac ET_B receptors are involved in the local sequestration and clearance of ET-1, both in normoxia and after ischemia/reperfusion. After 0.2 or 2 μM BQ-788, considerably more ET-1 was displaced than in the presence of less antagonist, resulting in measurable vasoconstrictor effects (increase in CPP), presumably consequent to binding and activation of ET_A receptors. To avoid any binding to ET_B receptors, a low concentration of PD 155080 (50 nM) was initially chosen and later supplemented with higher concentrations (500 and 5000 nM), all of which consistently inhibited the time-dependent rise in CPP, both in normoxia and after ischemia, but were entirely ineffective at mobilizing ET-1 into effluent or transudate. The much higher efficacy of PD 155080 in this preparation than in rabbit femoral and pulmonary artery ($K_B \sim 500$–1500 nM) may be due to differences in tissues (microvascular bed vs. conduit arteries) and species, but is supported by binding data ($IC_{50} < 10$ nM at the ET_A receptor [10]).

Previously, it was shown that the uptake of ET-1 into rat tissues in vivo results in its rapid clearance from the plasma by a ET_B receptor-specific mechanism [8]. The present data extend these observations and suggest that ET-1, in addition to its removal by lung tissue, is also cleared locally in the heart and that, by implication, the removal process may generally involve ET_B receptors. Hence, with respect to the increased plasma levels of ET-1 observed after infusion of subtype nonselective antagonists [6,7], the increase probably resulted from

Table 3
Effect of PD 155080 and BQ-788 on left ventricular and coronary function under normoxic (control) and reperfusion conditions

Treatment	Control (60 min)	Reperfusion	
		3 min	30 min
Vehicle			
LVDevP	88 ± 3	61 ± 2	65 ± 3
CPP	52 ± 3	78 ± 2	97 ± 6
LVEDP	0	21 ± 2	13 ± 2
PD 155080 (50 nM)			
LVDevP	84 ± 2	66 ± 3	78 ± 2*
CPP	54 ± 2	54 ± 4*	59 ± 2*
LVEDP	0	17 ± 2	7 ± 1*
BQ-788 (2 nM)			
LVDevP	84 ± 2	58 ± 2	65 ± 1
CPP	55 ± 2	80 ± 2	97 ± 3
LVEDP	0	23 ± 3	13 ± 1

All data are given in mm Hg. Data are mean values ± S.E.M., $n = 6$. Heart rate was 292 ± 11 beats/min (baseline) and was not affected by antagonists ($P > 0.05$). *Significantly different from vehicle. The differences between treatments (reperfusion vs. control) were analyzed by analysis of variance as described in Section 2, but symbols are omitted for clarity.

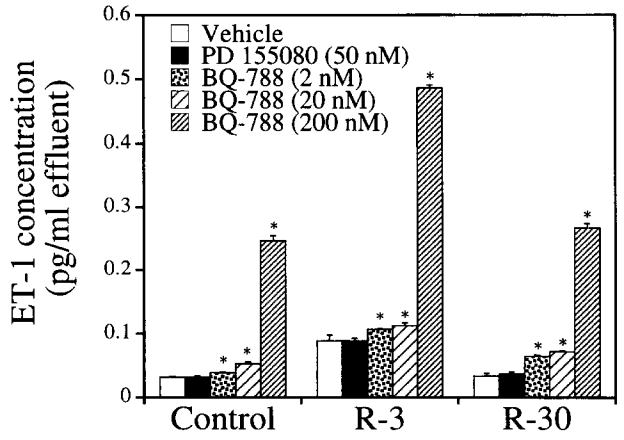

Fig. 3. Effect of PD 155080 and BQ-788 on ET-1 secretion into coronary effluent under control and reperfusion (R) conditions (R-3: 0–3 min; R-30: 20–30 min). The antagonists were added to perfusate during the control, ischemic (not shown) and reperfusion phases. Mean values ± S.E.M. of 6 hearts in each case. *Significantly different from vehicle ($P < 0.05$). The differences between treatments (reperfusion vs. control) were analyzed by analysis of variance as described in Section 2, but symbols are omitted for clarity.

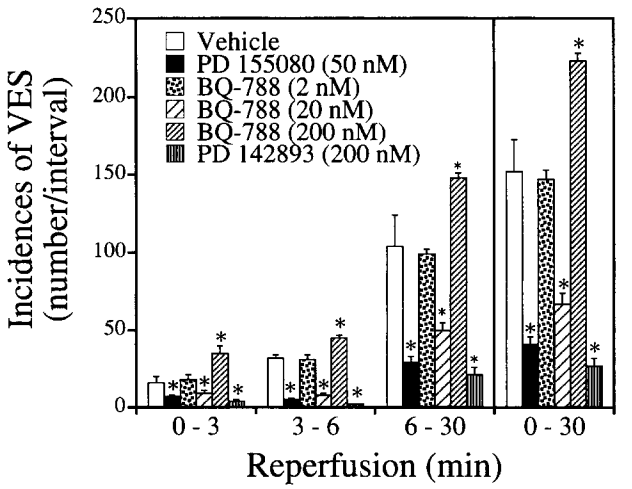

Fig. 4. Effect of PD 155080, BQ-788, and PD 142893 (mixed ET_A/ET_B receptor antagonist) on the incidence of ventricular extrasystoles (VES) during reperfusion. The antagonists were added to perfusate during the control, ischemic (not shown) and reperfusion phases. Mean values ± S.E.M. of 4 (vehicle) or 6 hearts. *Significantly different from vehicle ($P < 0.05$). The differences between treatments (reperfusion vs. control) were analyzed by analysis of variance as described in Section 2, but symbols are omitted for clarity.

the interaction of these antagonists with ET_B receptors in peripheral tissues, including the heart.

In view of the constant rate of synthesis observed over 255 min, the additional ET-1 appearing in transudate and effluent was in all likelihood peptide displaced from tissue receptors. A continuous increase in ET-1 appearance (Figs. 1 and 2) is expected as a direct result of the binding reaction kinetics involving a very potent endogenous ligand pre-bound to receptors (ET-1) and a much less avid antagonist. The relative concentrations of ET-1 in effluent and transudate also merit mention. Both were similar at baseline (Figs. 1 and 2), but transudate levels increased three times more after BQ-788 than effluent levels (Table 2), suggesting that the pharmacodynamic effects of ET_B receptor antagonism in this model appear to be determined mainly by the transudate (i.e. tissue) level of ET-1. Whether the same ET_B receptors also mediate the coronary relaxation observed in rat hearts after infusion of exogenous ET-1 [9] or else whether coronary ET_B receptors may mediate dual, i.e. indirect vasodilator as well as vasoconstrictor effects as suggested for dog heart [14], is unclear and requires further work.

Considerable evidence suggests that myocardial ischemic injury is modulated by ET-1 released during ischemia or reperfusion [15], but its pathophysiological effects are far from clear, in particular the effects of antagonists on post-ischemic function and infarct size are variable and appear to depend on the antagonist used. Thus, the mixed ET_A/ET_B receptor antagonist TAK-044 showed a limited inhibitory effect on the extension of infarct size in rats [16], whereas BQ-123 (ET_A receptor antagonist) was without effect in the same model [17]. Another ET_A receptor antagonist, FR 139317 was without effect in a rabbit model of ischemia/reperfusion [18], and led to an increase, rather than a decrease in infarct size in dogs [19]. Our results may be of relevance to these discrepancies. Thus, an antagonist may be expected either to antagonize endogenous ET-1 despite mobilization from inactive receptors (mixed ET_A/ET_B receptor antagonist), or to antagonize ET_A receptor-mediated effects without displacing inactive ET-1 (ET_A receptor antagonist), or to antagonize ET_B receptor-mediated effects and to displace ET-1 from inactive receptors, followed by possible action on ET_A receptors (ET_B receptor antagonist). Naturally, the net antagonist effect will also depend on the antagonist concentration attained at the respective subtypes.

Previous evidence has implicated ET_A receptors in the genesis of reperfusion arrhythmias in rats [20]. Here, the proarrhythmic role of this subtype was confirmed and a role for the ET_B receptor was excluded. Expectedly therefore, the subtype non-selective antagonist PD 142893 [21] showed a similar antiarrhythmic potency as the ET_A selective antagonist PD 155080 (Fig. 4). The receptor mediating the proarrhythmic effect of ET-1 is probably localized on the myocardial cell and may be activated by ET-1 generated locally by cardio-

Table 4
Effect of BQ-788 on coronary perfusion pressure under normoxic and reperfusion conditions

BQ-788 concentration	Baseline (30 min)	Control (60 min)	Reperfusion	
			3 min	30 min
Vehicle	52 ± 3	57 ± 2	78 ± 2	97 ± 6
2 nM	52 ± 3	55 ± 2	80 ± 2	97 ± 3
20 nM	51 ± 3	52 ± 3	75 ± 2	83 ± 3
200 nM	51 ± 3	104 ± 3*	115 ± 2*	136 ± 2*

The antagonist was added during all three phases, one concentration per heart. Data (mm Hg) are mean values ± S.E.M., $n = 4$–6. *Significantly different from vehicle.

myocytes as a consequence of ischemia [22]. The lower proarrhythmic potency of 20 nM than either 2 or 200 nM BQ-788 was unexpected; because it was abolished by indomethacin, it is likely due to increased release of prostacyclin [23,24] stimulated by ET-1. At 200 nM BQ-788, the beneficial effect of the prostanoid turns into an overall deleterious effect due to the severalfold higher ET-1 level (Fig. 3).

In conclusion, a ET_A receptor antagonist was cardioprotective whereas an antagonist of ET_B receptors caused increased coronary and interstitial levels of ET-1 resulting in heightened coronary constrictor effects. These results are relevant to the potential therapeutic role of ET receptor antagonists in diseases associated with acute vasoconstriction or vasospasm, including coronary vasospasm [25] and myocardial infarction [26]. A mixed ET_A/ET_B receptor antagonist was recently shown to increase the circulating plasma level of endogenous ET-1 in humans some 10-fold [27], indicating that displacement from ET_B receptors occurs in humans as well and that this phenomenon is likely to be of clinical importance.

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