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Effects of selective ET_B-receptor stimulation on arterial, venous and capillary functions in cat skeletal muscle

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1 This paper describes, in quantitative terms, the *in vivo* effects of two selective ET_B-receptor agonists (IRL 1620 and BQ 3020) on vascular resistance (tone) in the following consecutive sections of the vascular bed of sympathectomized cat skeletal muscle: large-bore arterial resistance vessels (>25 μm), small arterioles (<25 μm) and the veins. The effects on capillary pressure and transcapillary fluid exchange were also recorded.

2 Both IRL 1620 and BQ 3020, infused i.a. to the muscle preparation, evoked an initial transient dilator response followed by a moderate dose-dependent constrictor response, both being preferentially confined to the small arterioles. The dilator response was associated with a transient increase, and the constrictor response with a sustained decrease, in capillary pressure, the latter causing net transcapillary fluid absorption. The capillary filtration coefficient decreased during the constrictor response, indicating constriction of terminal arterioles/precapillary sphincters.

3 The vascular responses to the ET_B-receptor agonists were unaffected by blockade of endothelium-derived nitric oxide (N^G-nitro-L-arginine methyl ester) and by selective ET_A-receptor blockade (FR139317). However, blockade of prostacyclin production with indomethacin decreased the amplitude of the dilator response, and decreased the time required to reach a steady-state vasoconstrictor response to the ET_B-receptor agonists.

4 The effect of ET_B-receptor stimulation on vascular tone was also evaluated *in vitro* on the cat femoral artery and vein. IRL 1620 had no effect on the femoral artery but caused a weak dose-dependent relaxation in the femoral vein. This large vein relaxation response seemed to be mediated by endothelium-derived nitric oxide and not by prostacyclin.

5 It may be concluded that ET_B-receptor stimulation is responsible for the dilator response, and can contribute to the constrictor response, elicited by endothelins in cat skeletal muscle *in vivo*.

Keywords: Arteries; capillaries; capillary pressure; endothelins; ET_A-receptor; ET_B-receptor; microcirculation; prostacyclin; veins

Introduction

Endothelin-1, -2 and -3 exert their actions in the cardiovascular system via at least two different receptor subtypes, the ET_A- and ET_B-receptors (Rubanyi & Parker Botelho, 1991; Sakurai *et al.*, 1992). Binding to ET_A-receptors on vascular smooth muscle cells elicits contraction, whereas binding to ET_B-receptors, considered to be located on the endothelial cells, causes relaxation, as evidenced by *in vitro* experiments (for references see Sakurai *et al.*, 1992). The latter effect has been attributed to a secondary release of endothelium-derived nitric oxide (EDNO) and/or prostacyclin (see Rubanyi & Parker Botelho, 1991; Sakurai *et al.*, 1992). *In vivo*, activation of the vascular ET_A-receptor elicits a vasoconstrictor response (see Sakurai *et al.*, 1992; Masaki *et al.*, 1992), whereas the contribution of the ET_B-receptor to the vascular effect evoked by the endothelins in the peripheral circulation is more uncertain. Some recent *in vivo* studies of the effects of ET_B-receptor agonists suggest that the ET_B-receptor can mediate not only a vasodilator, but also a vasoconstrictor response (Bigaud & Pelton, 1992; Gardiner *et al.*, 1994). These results were based on observations of ET_B-induced effects on blood flow velocity in some major arteries in the systemic circulation in the presence of ET_A-receptor blockade.

To elucidate further the possible role of the ET_B-receptor in vascular regulation *in vivo*, there is a need for more detailed information about the effects of selective ET_B-receptor agonists on volume blood flow in homogeneous tissues, instead of composite organs. Further, their control of different vascular functions, and their site(s) of action in the consecutive segments of the vascular bed, need to be defined.

In the present study an attempt was made to provide such information by studying the circulatory effects of two different newly described selective ET_B-receptor agonists, IRL 1620 (Takai *et al.*, 1992) and BQ 3020 (Ihara *et al.*, 1992) in a pure lower leg muscle preparation in the cat. The technique used permitted quantitative analyses of the resistance responses to these agonists in the whole vascular bed, and its consecutive segments: large-bore arterial resistance vessels (>25 μm), small arterioles (<25 μm) and the veins. The associated effects on hydrostatic capillary pressure and transcapillary fluid exchange were also recorded. To elucidate the mechanisms underlying the vascular responses to IRL 1620 and BQ 3020, comparative observations were made in the absence and presence of selective ET_A-receptor blockade (FR139317; Sogabe *et al.*, 1992; 1993), blockade of the production of EDNO (N^G-nitro-L-arginine methyl ester; L-NAME) or prostacyclin (indomethacin). As a complement to the *in vivo* study, effects of the ET_B-receptor agonists on vascular tone were analysed *in vitro* in large conduit vessels supplying the cat hindlimb, i.e. the femoral artery and vein.

Methods

In vivo *experiments on the skeletal muscle circulation*

Skeletal muscle preparation and recordings The study was performed on young adult male cats (mean body wt 4.2 kg), anaesthetized intravenously with α-chloralose (50 mg kg^{-1} body wt, if necessary later supplemented by 20 mg kg^{-1} body wt) applied via a venous catheter inserted under local anaes-

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thesia (lignocaine). After anaesthesia, a tracheal cannula was inserted to facilitate spontaneous respiration. Expiratory P_{CO_2} and body temperature were monitored continuously and stayed within normal limits during the experiments.

Observations were made on the acutely sympathectomized lower leg muscles of the right hindlimb, a preparation described in detail elsewhere (Mellander *et al.*, 1987). In brief, the muscle region was auto-perfused *in situ* via an arterial shunt placed between the femoral and popliteal artery. The muscle preparation, with intact arterial and venous supply, was enclosed in a Ringer fluid-filled plethysmograph, using special cannulation and instrumentation techniques described in detail elsewhere (Mellander *et al.*, 1987; Björnberg *et al.*, 1988). With this method, it is possible to obtain reliable continuous recordings of the following circulatory variables: arterial, arteriolar, capillary and venous pressures, regional blood flow, net transcapillary fluid flux, and resistances in the whole muscle vascular bed (R_T) and in the following consecutive vascular sections: large-bore arterial resistance vessels (> 25 μm; $R_{a,prox}$), small arterioles (< 25 μm; $R_{a,micro}$), and the veins (R_V). All parameters were recorded on a 10-channel Grass Polygraph.

After completion of surgery and instrumentation, the animals were left to equilibrate so as to permit the recovery of intrinsic myogenic vascular tone to a normal and stable level (Maspers *et al.*, 1990b) before the start of the experimental interventions.

Calculations and experimental protocols Total and segmental vascular resistances in the muscle preparation were derived as follows from the recorded regional blood flow (Q) and the relevant driving pressures obtained from the four pressure signals, arterial inflow pressure (P_A), pressure in arterioles of a size of about 25 μm ($P_{arteriole}$), capillary pressure towards the venous end of the capillaries ($P_{c,v}$) and the venous outflow pressure (P_V): $R_T = (P_A - P_V)/Q$; $R_{a,prox} = (P_A - P_{arteriole})/Q$; $R_{a,micro} = (P_{arteriole} - P_{c,v})/Q$; $R_v = (P_{c,v} - P_V)/Q$. Implicit in this methodological approach is that the sum of the three segmental resistances always equals the simultaneously recorded R_T value. These vascular resistances were continuously recorded with the aid of electronic divider circuits (for technical details see Björnberg *et al.*, 1988). All resistances are expressed as PRU values (peripheral resistance units; mmHg ml^{-1} min 100 g tissue).

The arterial versus venous resistance responses, i.e. changes in the pre- to postcapillary resistance ratio ($(R_{a,prox} + R_{a,micro})/R_v$) evoked by the ET_B-receptor agonists led to changes in hydrostatic capillary pressure and net transcapillary fluid exchange. Net transcapillary fluid movement during ET_B agonist infusion was assessed from the observed net change in tissue volume, after correction for the tissue volume change caused by the initial vascular capacitance response.

The capillary filtration coefficient (CFC) was used as an index of overall hydraulic conductivity across the exchange vessels in the muscle preparation, in turn reflecting the size of the functional capillary surface area available for fluid exchange (Folkow & Mellander, 1970). CFC (ml min^{-1} 100 g^{-1} mmHg^{-1}) was determined by raising venous outflow pressure by 5 mmHg and was calculated from the rate of the resulting net transcapillary fluid filtration divided by the directly observed increase in capillary pressure ($P_{c,v}$).

In vitro *experiments on femoral vessels*

Vessel preparation When the *in vivo* experiments were completed, the cat femoral artery and vein were rapidly removed, and placed in a 4°C aerated standard Na^+-Krebs buffer solution until use. The buffer solution contained (in mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.5 and glucose 11. The vessels were cut into small ring segments (1 mm long) under a dissecting microscope. For examination, each segment was carefully mounted on two L-shaped metal prongs (0.2 mm in diameter), one of which was connected to a Grass FT-03 transducer for continuous recordings of isometric tension. A basal tension of 5 mN was applied to the arterial segments, and of 1.5 mN to the venous segments, after which they were allowed to equilibrate for 2 h.

Experimental protocol The contractile capacity of the vessel segments was examined before the experimental interventions by a brief exposure to a high potassium (6×10^{-2} M) buffer solution (Högestätt *et al.*, 1983). In most cases precontraction of the vascular segments was produced with noradrenaline (3×10^{-5} M). To ascertain the specificity of the ET_B-induced vascular effects, paired experiments were always performed, one of the segments receiving the agonist, the other serving as a control. In some cases the response to ET-1 (10^{-11}–10^{-6} M) was tested at the end of the experiments.

The functional integrity of the endothelium was confirmed by the presence of a pronounced relaxation induced by acetylcholine (10^{-6} M), administered during stable precontraction with noradrenaline.

Endothelium denudation was performed by injection of 0.01% Triton X-100 through the vessel lumen for 10 s, followed by rinsing with buffer solution.

E_{max} was calculated as the maximum contractile effect compared to potassium-induced contraction and pD_2 as the negative logarithm of the molar concentration at half maximum of the response. The relaxant effect of the ET_B agonist was expressed as a percentage of the precontraction tension level. *n* refers to the number of segments tested.

Drugs

In the *in vivo* experiments, drugs were administered close-arterially to the muscle region via slow (0.01–0.2 ml min^{-1}) infusions (Harvard Apparatus, model 11) in the shunt between the femoral and the popliteal artery. The tip of the infusion needle was placed in the retrograde direction to facilitate the mixture of the drugs in the bloodstream. Control experiments in which isotonic saline was infused at the same rates demonstrated that infusion artifacts were negligible.

The following drugs were used: acetylcholine (Sigma, St Louis, MO, U.S.A.), BQ 3020 ([$Ala^{11,15}$]Ac-ET-1(6–21), Auspep, Parkville, Australia); endothelin-1 (Auspep); indomethacin (Dumex, Denmark); IRL 1620 (Suc-[Glu^9,$Ala^{11,15}$]-ET-1(8–21), Saxon Biochemicals, Hannover, Germany, and a generous gift from Ciba-Geigy, Japan); N^G-nitro-L-arginine methyl ester (L-NAME, Sigma); prazosin hydrochloride (Sigma); noradrenaline hydrochloride (Sigma); propranolol hydrochloride (ICI, Macclesfield, UK) and FR139317 ((R)2-[(R)-2-[(S)-2-[[1-(hexahydro-1H-azepinyl)] carbonyl]amino-4-methyl-pentanoyl]amino-3- [3- (1-methyl-1H-indolyl)] propionyl]amino-3-(2-pyridyl) propionic acid, Fujisawa Pharmaceutical Co., Osaka, Japan).

In previous studies (Ekelund *et al.*, 1993; Ekelund, 1994) on this muscle preparation *in vivo*, we have demonstrated that FR139317, administered i.a. at the dose of 1 mg kg^{-1} min^{-1}, entirely abolished the vasoconstrictor response to ET-1 (400 ng kg^{-1} min^{-1}, i.a.). This dose of FR139317 was therefore used in the present study. The dose of indomethacin used (10 mg kg^{-1}) can be considered to block effectively vascular cyclo-oxygenase and hence the production of prostacyclin in this muscle preparation (Ekelund, unpublished observations). Similarly, the dose of L-NAME (10 mg kg^{-1}, i.a.) used in this study is sufficient to inhibit EDNO formation (Ekelund *et al.*, 1992). Moreover, the doses of prazosin (1 mg kg^{-1}, i.a.) and propranolol (1.5 mg kg^{-1}, i.a.) used here are sufficient to block the α_1- and β-adrenoceptors (as evidenced by agonists in supramaximal doses) in this muscle preparation.

All drugs were dissolved in saline, and the solutions, when

necessary, were adjusted to isotonicity. Time data for the effects of the drugs given in Results are corrected for dead space delay in the tubings.

Statistics

Data are expressed as mean values ± s.e.mean. Group comparisons were performed by analysis of variance (ANOVA) followed by Bonferroni's test with a significance level of 5%, or by using paired (unless stated otherwise) Student's *t* test, differences being considered significant at P values <0.05.

Results

Vascular responses to ET_B-receptor agonists in skeletal muscle in vivo

General patterns of resistance response The two selective ET_B-receptor agonists, IRL 1620 and BQ 3020, were infused close-arterially in stepwise increasing doses (0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 $\mu g\,kg^{-1}$ muscle tissue min^{-1}) to determine the dose-response characteristics and the smooth muscle effector sensitivity in the consecutive sections of the muscle vascular bed. The threshold dose for vascular response was 0.8 $\mu g\,kg^{-1}\,min^{-1}$ for both IRL 1620 and BQ 3020, and this response was found to be a constriction, not a dilatation. For both agonists, the constrictor response was clearly dose-dependent in the dose range up to 6.4 $\mu g\,kg^{-1}\,min^{-1}$, higher doses eliciting no further vasoconstriction. The maximal constrictor responses to IRL 1620 and BQ 3020 seemed to be equally great, implying that the two agonists had similar constrictor efficacy. In these experiments with stepwise infusion up to 12.8 $\mu g\,kg^{-1}\,min^{-1}$, a vasodilator response to these agonists was never observed. In addition, close-arterial (local) infusion of IRL 1620 or BQ 3020 did not result in any change in blood pressure (systemic effect).

The pattern of overall and segmental vascular resistance responses in the muscle preparation to IRL 1620, as well as their time characteristics, are exemplified by the original tracings depicted in Figure 1, in which the ET_B agonist was administered at a dose of 3.2 $\mu g\,kg^{-1}\,min^{-1}$ in a single infusion experiment. It can be seen that, under these circumstances, IRL 1620 elicited after some delay a biphasic response, consisting of an initial short-lasting vasodilator component, followed by a gradually developing moderate vasoconstriction. The latter reached an approximate steady state after about 6 min. Upon cessation of the infusion, the resistance responses, with some segmental variation, disappeared gradually, complete recovery being attained not until some 10 min after the end of the infusion. The vascular response to IRL 1620, both with regard to the vasoconstrictor and vasodilator component, was present in all three consecutive segments of the muscle vascular bed, yet with a preferential site of action in the small arterioles (<25 μm; $R_{a,micro}$).

The corresponding pattern of vascular response to BQ 3020 (3.2 $\mu g\,kg^{-1}\,min^{-1}$), both with regard to amplitude and time course, was very similar, with an initial short-lasting vasodilatation and a subsequent more sustained vasoconstriction, both being preferentially confined to the small arterioles.

Compiled data for the resistance responses to ET_B-receptor agonists Compiled data for resistance responses to IRL 1620 ($n = 6$) and BQ 3020 ($n = 7$), infused close-arterially to the muscle region at the dose of 3.2 $\mu g\,kg^{-1}$ muscle tissue min^{-1}, are illustrated in Figures 2 and 3. This dose corresponds to about 1.8 $nmol\,kg^{-1}\,min^{-1}$ for IRL 1620 and 1.6 $nmol\,kg^{-1}\,min^{-1}$ for BQ 3020. The infusions were made when basal vascular tone, after recovery from the preparatory surgery, had reached a normal steady-state level. In the experiments with IRL 1620, vascular tone in the control state (Figure 2) corresponded to an average overall vascular resistance (R_T, total height of the bar) of 19.8 ± 2.0 PRU, of which 9.5 ± 1.0 PRU resided in the large-bore arterial resistance vessels ($R_{a,prox}$), 8.3 ± 1.0 PRU in the small arterioles ($R_{a,micro}$) and 2.0 ± 0.2 PRU in the veins (R_v). In the BQ 3020

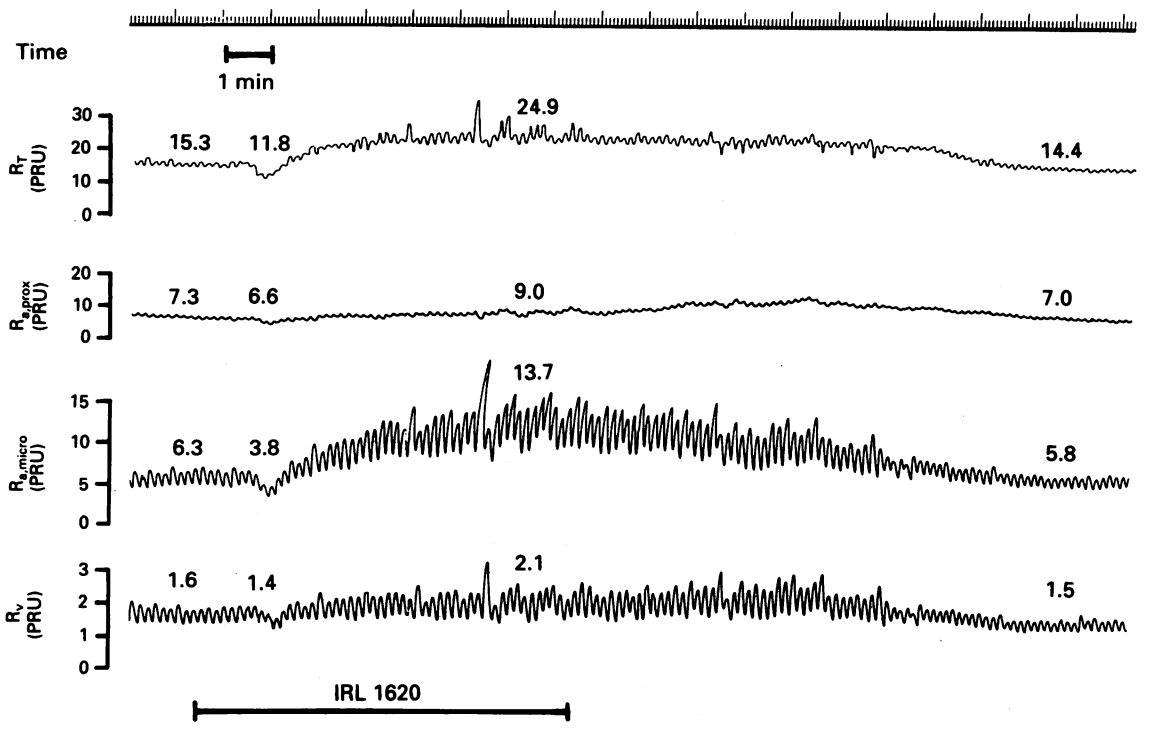

Figure 1 Pattern of vascular response in cat skeletal muscle to an i.a. infusion of IRL 1620 (3.2 $\mu g\,kg^{-1}\,min^{-1}$) on vascular resistance in the whole vascular bed (R_T) and its consecutive sections, large-bore arterial resistance vessels (>25 μm; $R_{a,prox}$), small arterioles (<25 μm; $R_{a,micro}$) and the veins (R_v). Note initial transient dilatation, followed by a slowly developing moderate vasoconstriction. Upon cessation of the infusion, the constrictor responses disappeared slowly.

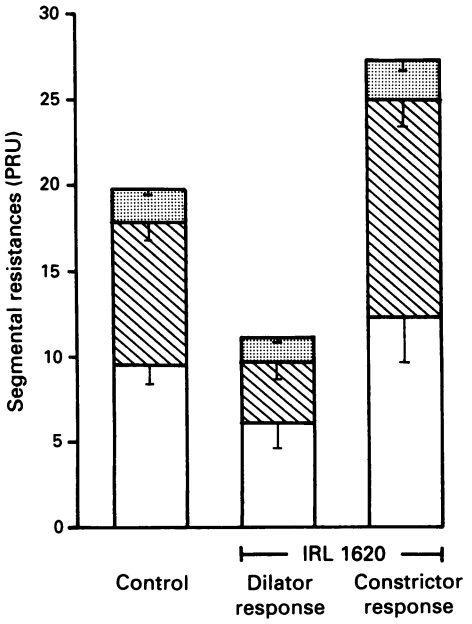

Figure 2 Compiled data ($n = 6$) for the initial transient peak dilator, and subsequent steady-state constrictor, response to IRL 1620 (3.2 $\mu g\ kg^{-1}\ min^{-1}$, i.a.) on total regional vascular resistance (total height of the columns), and its segmental distribution to the $R_{a,prox}$ (open area of column), $R_{a,micro}$ (hatched area of column) and R_v (stippled area of column) sections in cat skeletal muscle *in vivo*. Note that the vasodilator and subsequent vasoconstrictor responses were preferentially confined to the $R_{a,micro}$ section.

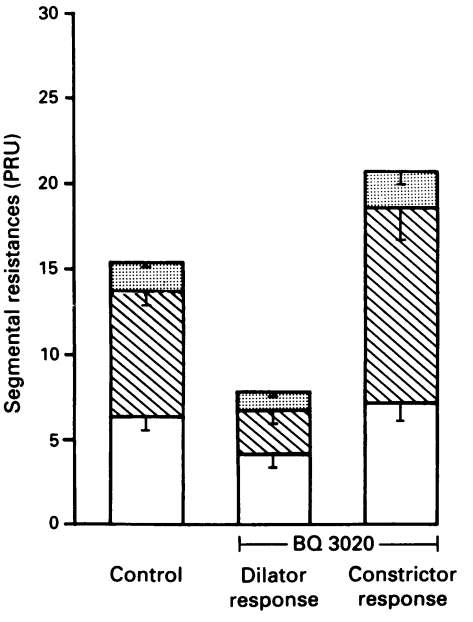

Figure 3 Compiled data ($n = 7$) for the initial transient peak dilator, and subsequent steady-state constrictor, response to BQ 3020 (3.2 $\mu g\ kg^{-1}\ min^{-1}$, i.a.) on total regional vascular resistance (total height of the columns), and its segmental distribution to the $R_{a,prox}$ (open area of column), $R_{a,micro}$ (hatched area of column) and R_v (stippled area of column) sections in cat skeletal muscle *in vivo*. Note that the vasodilator and subsequent vasoconstrictor responses were preferentially confined to the $R_{a,micro}$ section.

experiments, performed on another group of cats, R_T in the control state was 15.3 ± 0.7 PRU (Figure 3), of which 6.4 ± 0.7, 7.3 ± 0.7 and 1.7 ± 0.2 PRU resided in the $R_{a,prox}$, the $R_{a,micro}$ and the R_v sections, respectively.

Vasodilator component At the start of the infusions, both IRL 1620 and BQ 3020 elicited, after a delay of about 1 min, a clear-cut vasodilator response with its peak occurring about 90 s after the start of the infusion. For both agonists, the total duration of the dilator response was about 70 s. Compiled data for the total and segmental vascular resistance changes at the peak of these dilator responses to IRL 1620 and BQ 3020 are illustrated in Figures 2 and 3. The transient dilator response to IRL 1620 corresponded to an average decrease in R_T from the control value of 19.8 to 11.2 ± 2.0 PRU (-43%; $P<0.05$), in turn caused by a decrease in $R_{a,prox}$ of 36%, $R_{a,micro}$ of 57% and R_v of 25%. The initial dilator response to BQ 3020 was not significantly different, and corresponded to a decrease in R_T from 15.3 to 7.8 ± 0.8 PRU (-49%; $P<0.001$), in turn caused by a decrease in $R_{a,prox}$ by 36%, $R_{a,micro}$ by 64% and R_v by 35%. The vasodilator component to both ET_B agonists was thus present in all three consecutive sections, though preferentially confined to the small arterioles. From separate experiments in which single infusions of the ET_B agonists in doses other than $3.2\ \mu g\ kg^{-1}\ min^{-1}$ were made, the amplitude of this dilator response seemed to be dose-dependent.

In experiments ($n = 11$) in which a second infusion of either IRL 1620 or BQ 3020 was given after an interval with full recovery of vascular tone, the amplitude of the second dilator response was attenuated by about 55% compared to the first one, probably reflecting tachyphylaxis. The dilatation was further reduced during a third, and absent during a fourth, infusion. Moreover, cross-tachyphylaxis between the two ET_B-receptor agonists was observed. The data in Figures 2 and 3 refer to results from the first infusion in each animal.

Vasoconstrictor component After the initial dilator response to the ET_B agonists ($3.2\ \mu g\ kg^{-1}\ min^{-1}$), a gradually developing constrictor response always occurred, which, on average, reached a steady state after about 6.9 min for IRL 1620 and 5.6 min for BQ 3020. Compiled data from these experiments for the steady-state overall and segmental constrictor responses to IRL 1620 and BQ 3020 are shown in Figures 2 and 3. The constrictor response to IRL 1620 corresponded to a rise in R_T from the preinfusion control value of 19.8 to 27.3 ± 4.4 PRU ($+38\%$; $P<0.01$), explained by a resistance increase in the large-bore arterial resistance vessels to 12.3 PRU ($+29\%$), in the small arterioles to 12.7 ($+53\%$) and in the veins to 2.3 PRU ($+15\%$). The steady-state vasoconstriction elicited by BQ 3020 corresponded to an increase in R_T from 15.3 to 20.6 ± 2.3 PRU ($+35\%$; $P<0.05$), in turn caused by an increase in $R_{a,prox}$ to 7.2 ($+13\%$), $R_{a,micro}$ to 11.4 ($+56\%$) and R_v to 2.1 PRU ($+24\%$). These resistance changes were not significantly different from those evoked by IRL 1620. Thus, the vasoconstrictor response to both ET_B-receptor agonists was generalized, affecting all three consecutive vascular sections, yet with a preferential action, again, on the small arterioles.

Effects of ET_B-receptor agonists on capillary pressure and transcapillary fluid exchange Observations of ET_B-induced effects ($3.2\ \mu g\ kg^{-1}\ min^{-1}$) on hydrostatic capillary pressure ($P_{c,v}$) and transcapillary fluid movement were made on the same material as presented in Figures 2 and 3. $P_{c,v}$ in the control state averaged 19.5 ± 0.4 mmHg in the IRL 1620 experiments, and 19.5 ± 0.8 mmHg in the experiments with BQ 3020. An approximate transcapillary Starling fluid equilibrium (isovolumetric state) prevailed under these circumstances.

During the initial transient vasodilator response, IRL 1620 elicited an average peak increase in $P_{c,v}$ to 24.1 mmHg, due to its preferential dilator action on the precapillary vessels, in

turn decreasing the pre-/postcapillary resistance ratio (R_a/R_v) from 8.9 to 6.4. BQ 3020 infusion raised $P_{c,v}$ to 24.6 mmHg (decrease in R_a/R_v from 8.1 to 6.1).

During the steady-state constriction phase, IRL 1620 evoked a decrease in $P_{c,v}$ to 16.6 (− 2.9) mmHg ($P<0.01$), owing to an increase in R_a/R_v (from 8.9 to 10.9). BQ 3020 elicited a similar decrease in $P_{c,v}$ (to 16.7 mmHg; $P<0.01$). $P_{c,v}$ was maintained low as long as the vasoconstriction persisted, leading to a net transcapillary fluid absorption. The fluid absorption was, however, comparatively small, since there was a concomitant decrease in the capillary filtration coefficient (CFC). Thus, in separate experiments ($n = 11$), CFC was found to decrease from 0.010 ± 0.001 to 0.007 ± 0.001 ($P<0.05$, unpaired t test) during steady-state vasoconstriction evoked by IRL 1620.

Effects of blockade of cyclo-oxygenase, ET_A-receptors, EDNO production and adrenoceptors on vascular responses to ET_B-receptor agonists In separate experiments, the vascular resistance responses to IRL 1620 or BQ 3020 (3.2 μg kg^{-1} min^{-1}) were analysed in the absence and presence of blockade of prostacyclin with indomethacin, of EDNO with L-NAME, of ET_A-receptors with FR139317, of α_1-adrenoceptors with prazosin and of β-adrenoceptors with propranolol.

Illustrated in Figure 4a are the compiled data for the amplitude of the initial dilator response to IRL 1620 in the absence and presence of indomethacin ($n = 7$). Blockade of prostacyclin production attenuated ($P<0.05$; unpaired t test), but did not abolish ($P<0.05$), the initial dilator response to IRL 1620. However, the duration of the dilatation was unaffected by indomethacin.

Blockade of EDNO formation ($n = 7$) had no effect on the dilator response to IRL 1620. The dilatation in the presence of L-NAME (R_T − 44%) was not significantly different ($P<0.05$; unpaired t test) from that in the absence of L-NAME (R_T − 58%). Further, blockade of ET_A-receptors ($n = 3$) or β-adrenoceptors ($n = 6$) did not affect the initial dilator response to IRL 1620. The amplitude of the vasoconstrictor response (R_T) to IRL 1620 was not significantly different (unpaired t tests) in the absence and presence of indomethacin ($n = 13$), L-NAME ($n = 9$), FR139317 ($n = 10$) or prazosin ($n = 2$). However, the time required to reach steady-state constriction was significantly shorter in the presence (4.4 ± 0.3 min), than in the absence (7.2 ± 0.3 min), of indomethacin. L-NAME, FR139317 or prazosin had no effect in this respect. In the described experiments, L-NAME *per se* increased basal vascular tone (R_T) in the control state before IRL 1620 administration, due to inhibition of the basal endogenous release of EDNO, in agreement with previous results (Ekelund & Mellander, 1990). Therefore, an additional statistical analysis was made using matched controls with a corresponding level of basal vascular tone. The results from this analysis were not different from those described above.

Analogous experiments were performed with BQ 3020 in the absence and presence of indomethacin ($n = 7$), FR139317 ($n = 3$), L-NAME ($n = 6$) and prazosin ($n = 2$). The results were principally the same as those obtained with IRL 1620. As can be seen in Figure 4b, indomethacin attenuated ($P<0.05$; unpaired t test), but did not abolish ($P<0.05$), the vasodilator response to BQ 3020.

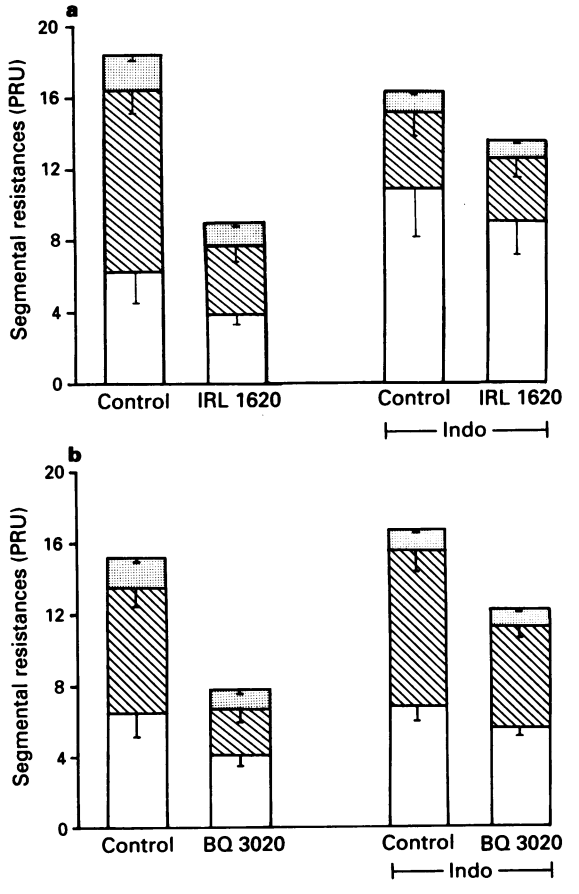

Figure 4 (a) Initial vasodilator response to IRL 1620 in the absence (left columns) and presence (Indo) of blockade of prostacyclin production with indomethacin ($n = 7$). (b) Initial vasodilator response to BQ 3020 in the absence (left columns) and presence (Indo) of blockade of prostacyclin production with indomethacin ($n = 7$). Prostacyclin blockade attenuated, but did not abolish, the dilator response to both ET_B-receptor agonists. Other details as in Figure 3.

In vitro *study of smooth muscle effects of ET_B-receptor agonists in femoral artery and vein*

In the absence of precontraction, IRL 1620 (10^{-10}–10^{-6} M) elicited no response in the femoral artery ($n = 12$) or vein ($n = 15$), nor did it induce a response in the noradrenaline-precontracted femoral artery ($n = 20$). These results were the same in the absence and presence of an intact endothelium.

Illustrated in Figure 5 is the effect of IRL 1620 (10^{-11}–10^{-6} M; $n = 16$) in the precontracted femoral vein. With

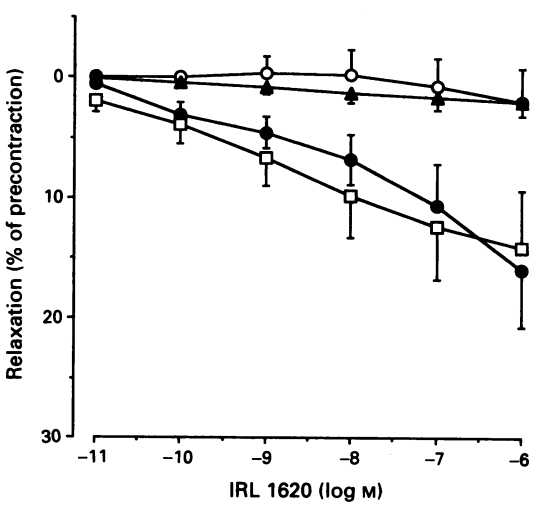

Figure 5 Concentration-response curve to IRL 1620 in cat isolated femoral vein precontracted with noradrenaline (3×10^{-5} M). Depicted are mean (± s.e.mean) responses in the control state (●, $n = 16$), in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 10^{-5} M; ▲, $n = 7$) or indomethacin (10^{-5} M; □, $n = 7$), and of the response to IRL 1620 in the absence of the endothelium (○, $n = 5$). The relaxation is expressed as a percentage of the precontraction tension level.

intact indothelium, the ET_B agonist elicited a dose-dependent relaxation, at a dose of 10^{-6} M reducing the precontraction tension by 17% ($P<0.01$). The relaxation to IRL 1620 was absent in endothelium-denuded vein segments. Segments with intact endothelium were also examined during blockade of EDNO and prostacyclin production (Figure 5). The relaxation response to IRL 1620 was abolished ($P<0.05$) by L-NAME at a dose of 10^{-5} M ($n=7$), but not at 10^{-6} M. The relaxation response was unaltered ($P>0.05$) in the presence of indomethacin (10^{-5} M; $n=7$).

In the femoral vein with intact endothelium, BQ 3020 ($10^{-11}-10^{-6}$; $n=3$) elicited a relaxation response which was similar to that evoked by IRL 1620, decreasing precontraction tension by an average of 22% at 10^{-6} M.

In both the femoral artery and vein, ET-1 ($10^{-11}-10^{-6}$ M) elicited strong dose-dependent contractions. FR139317 (10^{-6} M) caused parallel shifts to the right of the concentration-response curves (pD_2 significantly different for both artery and vein) but did not affect the E_{max} values.

Discussion

In some recent studies (Ekelund *et al.*, 1993; Ekelund, 1994), the vascular effects *in vivo* of ET-1, ET-2 and ET-3 were analysed in cat skeletal muscle with the same technical approach as was used in the present investigation. The results showed that all three endothelins elicited a transient dilatation followed by a slowly developing pronounced vasoconstrictor response. The constrictor response was preferentially confined to the small arterioles, and was mediated via the ET_A-receptor only, as indicated by abolition of the response by selective ET_A-receptor blockade (FR139317), and the observed rank order of constrictor activity, ET-1 > ET-2 > ET-3. The initial transient dilator response, however, was not mediated via the ET_A-receptor, but more likely via the ET_B-receptor. The present *in vivo* investigation aimed at defining, in quantitative terms, the site(s) of action of selective ET_B-receptor agonists on vascular resistances in the consecutive sections of the muscle vascular bed, and the effects on capillary pressure and transcapillary fluid exchange. The following results might warrant some comments.

The results demonstrated that both IRL 1620 and BQ 3020, infused i.a. to the muscle preparation, evoked an initial short-lasting dilator response and a subsequent dose-dependent vasoconstrictor response. The ET_B-receptor in the vascular bed of skeletal muscle thus mediates not only a vasodilator, but also a constrictor response. Although vascular smooth muscle adjustments in all three consecutive sections contributed to these responses, the effector reactivity in functional terms was greatest in the small arterioles ($<25\,\mu m$). Larger vessels were less responsive to ET_B-receptor stimulation, which was further supported by our *in vitro* observations on the femoral artery and vein. The pattern of segmental resistance response was thus similar to that evoked by the endothelins (Ekelund, 1994). These results, taken together, might suggest that both the ET_A- and the ET_B-receptors are especially abundant in the small arterioles.

There was no significant difference in the amplitude (peak resistance change) and time characteristics of the vascular responses between the two ET_B-receptor agonists. Moreover, the responses were unaffected by ET_A-receptor blockade. The effects of the two different ET_B agonists were thus strikingly similar, and the present comparative results indicate that, from a functional point of view, IRL 1620 and BQ 3020 can be considered equally effective and highly selective ET_B-receptor agonists in the vascular bed of skeletal muscle *in vivo*. Hence, these compounds might become useful as specific pharmacological tools for future functional investigations of the role of the ET_B-receptor in circulatory regulation in physiological and/or pathophysiological situations.

The initial transient vasodilator responses to IRL 1620 and BQ 3020 were not significantly different from those elicited by the endothelins (Ekelund *et al.*, 1993; Ekelund, 1994) with regard to amplitude, duration and time to peak dilatation. This finding seems to support our previous tentative conclusion (Ekelund, 1994) that the dilator responses to the endothelins are mediated via the ET_B-receptor.

The dilator response to ET_B-receptor stimulation by both agonists was attenuated, but not abolished, in the presence of indomethacin. On the assumption that the dose of indomethacin used completely blocked vascular cyclo-oxygenase, this observation indicates that the transient dilator response to ET_B-receptor stimulation in skeletal muscle is partly, but not entirely, mediated by secondary release of vasodilator cyclo-oxygenase products, most probably endothelium-derived prostacyclin. Thus, the findings in cat skeletal muscle seem to be compatible with the previous postulate (e.g. Sakurai *et al.*, 1992) that ET_B-receptor stimulation is responsible for endothelin-induced prostacyclin production. However, both the ET_A- and ET_B-receptor subtypes have been claimed to be capable of mediating endothelin-induced metabolism of arachidonic acid (Aramori & Nakanishi, 1992), and prostaglandin production. Hence the specific receptor subtype responsible for prostacyclin release may vary in different vessels and/or species. For instance, in the rat lung and rabbit kidney, the release of prostacyclin by ET-1 seems to be mediated by the ET_A-receptor (D'Orléans-Juste *et al.*, 1992; Télémaque *et al.*, 1992), whereas in the rat kidney both receptor subtypes (Warner *et al.*, 1993) may mediate the release of prostanoids.

The mechanism underlying the remaining ET_B-mediated dilator response *in vivo* during prostacyclin blockade is unclear, but could involve a direct effect of ET_B-receptors situated on the vascular smooth muscle, or an ET_B-mediated release of vasodilator substances other than those synthesized by vascular cyclo-oxygenase. EDNO did not seem to be involved in this mechanism, since the vasodilator response to ET_B-receptor stimulation was not significantly modified by EDNO blockade with L-NAME. This finding is in agreement with previous *in vivo* results obtained with ET-1 during EDNO blockade (e.g. Gardiner *et al.*, 1989; Ekelund *et al.*, 1993), and provides further evidence against the hypothesis derived from *in vitro* experiments (see Rubanyi & Parker Botelho, 1991; Sakurai *et al.*, 1992), that the vasodilatation and the consequent hyperaemic response to the endothelins is caused by ET_B-mediated release of EDNO.

The amplitude of the vasoconstrictor response was found not to be modified by indomethacin, which indicates that the ET_B-induced vasoconstrictor response was not due to the release of vasoconstrictor cyclo-oxygenase products. It is possible that the vasoconstrictor response to IRL 1620 and BQ 3020 is elicited by ET_B-receptors situated on the smooth muscle cells *proper* (cf. Moreland *et al.*, 1992).

Endothelin-1, at least *in vitro*, shows an about equal affinity for ET_A- and ET_B-receptors (Saeki *et al.*, 1991; Takai *et al.*, 1992). The present result, demonstrating that ET_B-receptor stimulation leads to a constrictor response, could *a priori* suggest that the vasoconstrictor response to ET-1 can be mediated by both receptor subtypes in skeletal muscle *in vivo*. However, we have previously demonstrated that the ET-1-mediated vasoconstriction in muscle *in vivo* can be abolished by selective ET_A-receptor blockade alone (FR 139317; Ekelund *et al.*, 1993). Further, in the present *in vitro* study, FR139317 competitively inhibited the ET-1-induced contraction of the femoral artery and vein. Future experiments with selective and true antagonists to the ET_B-receptor might permit more definite conclusions about the receptor mechanisms underlying the endothelin-induced vasoconstrictor response.

In the feline isolated femoral vein, IRL 1620 elicited a weak dose-dependent relaxation. This finding is one of the first demonstrations of a sustained relaxation to selective ET_B-receptor activation in a vein. The results indicated that this relaxation response was endothelium-dependent and abolished by L-NAME, which suggests that an endothelial

ET_B-receptor relaxed the vessel via EDNO release. Similar results with IRL 1620 have been reported for other large vessels, e.g. the rat aorta (Fujitani *et al.*, 1993). Thus, the ET_B-receptor-mediated vasodilator response in skeletal muscle *in vivo* (Figures 2 and 3), and the relaxation of the femoral vein *in vitro* (Figure 5), seem to be elicited via different postreceptor mechanisms, which in fact have been reported to differ considerably in different vessels (for references see Masaki, 1993; Simonson, 1993). The functional importance of the ET_B-mediated relaxation response in the femoral vein for blood flow regulation *in vivo* is probably very small, since the contribution of large conduit vessels to the overall vascular resistance regulation is almost insignificant. If the *in vivo* constrictor response to ET_B-receptor stimulation is caused by receptors situated on the vascular smooth muscle cells proper (see above), the absence of a constrictor response in the femoral vein could suggest a lack of such receptors in this vessel. The cat femoral artery might lack both types of ET_B-receptors, since it was non-reactive to ET_B-receptor stimulation.

It appears that the haemodynamic significance *in vivo* of the described vasodilator response to ET_B-receptor stimulation in skeletal muscle must be rather trivial in view of its transient nature. The subsequent sustained constrictor response may be more important in this respect. This response was also associated with a decrease in capillary pressure which seemed to be more pronounced per unit resistance increase than that to most other vasoconstrictors, e.g. neurally released noradrenaline (Maspers *et al.*, 1990a). As indicated by the observed decrease in CFC, this response further involved a maintained constriction of the terminal arterioles/precapillary sphincters, and a consequent reduction of the size of the functional capillary surface area. The decrease in CFC implies that the net transcapillary fluid absorption evoked by the capillary pressure drop becomes attenuated. A maintained constriction of precapillary sphincters is usually not evoked by other constrictor agents (e.g. neurally released noradrenaline), due to secondary metabolic/myogenic counteraction as a result of the associated decrease in blood flow and vascular transmural pressure. The sustained precapillary sphincter constrictor response to ET_B-receptor stimulation may thus present further evidence in support of the view of an abundance of ET_B-receptors in the microcirculation.

Our previous investigations, demonstrating quite unusual haemodynamic and capillary effects of the endothelins in muscle tissue (Ekelund *et al.*, 1993; Ekelund, 1994), suggested that they might be of importance in long-term, rather than short-term, regulation of vascular tone *in vivo*, perhaps especially during pathophysiological conditions. From the present results, it may be concluded that ET_B-receptor stimulation is responsible for the dilator response, and can contribute to the constrictor response, elicited by the endothelins in skeletal muscle *in vivo*.

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