The $\alpha_1$-adrenoceptor profile in human skeletal muscle resistance arteries in critical limb ischaemia

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Received 25 June 2002; accepted 12 September 2002

Abstract

Objective: We recently reported the hyper-responsiveness of human skeletal muscle resistance arteries (SkMRAs) to noradrenaline in critical limb ischaemia (CLI). In this study we investigated the characteristics of $\alpha_1$-adrenoceptor subtypes and evaluated the agonist affinity and adrenoceptor reserve in the ischaemic arteries. Methods: Human SkMRAs were isolated from non-ischaemic and ischaemic areas of limbs amputated for CLI. Subcutaneous resistance arteries were isolated from inguinal biopsies from healthy subjects. Arterial segments were mounted on a small vessel wire myograph. Results: Contractile responses to agonists, adrenaline and A-61603 ($\alpha_1$-selective) were significantly increased in ischaemic arteries compared to those in non-ischaemic arteries. Receptor inactivation studies indicated an increase in the $\alpha_1$-adrenoceptor reserve in the ischaemic arteries but the affinity of noradrenaline was unaffected. Healthy subcutaneous arteries had a similar noradrenaline affinity but a higher receptor reserve than skeletal muscle arteries. In the ischaemic arteries, the antagonists prazosin ($\alpha_1$-selective), 5-methyl-urapidil ($\alpha_1$-selective) and BMY 7378 ($\alpha_1$-selective) produced rightward shifts in the concentration response curves (CRCs) of noradrenaline giving $pK_{as}$ of 9.6±0.3, 8.4±0.2 and 7.1±0.4, respectively. Pretreatment with 10 $\mu$M chloroethylclonidine decreased the contractile responses to noradrenaline and A-61603 to 57±7 and 72±4% of their respective controls. Conclusions: These results demonstrate that the ischaemic SkMRAs have an increased $\alpha_1$-adrenoceptor reserve with no change in the predominant $\alpha_1$-adrenoceptor profile.

Keywords: Adrenergic (ant)agonists; Arteries; Ischemia; Receptors; Vasoconstriction/dilation

1. Introduction

Critical limb ischaemia (CLI) is a chronic ischaemic condition produced by occlusion of the large conducting arteries. A reduced perfusion pressure in the distal circulation is manifested in the early stages as a reduction in blood flow to the skeletal muscle vascular bed during mild exercise, termed intermittent claudication [1–3].

There is presently no perfect animal model to simulate this complex condition, caused by a variety of factors. The ischaemic limb amputated for CLI offers a good pharmacological model to study vascular dysfunction in the resistance arteries since both ischaemic and non-ischaemic (from the incision level selected for optimum wound healing) arteries can be isolated from the same amputated limb. There have been few studies that have focussed on the functional and structural alterations in the resistance vasculature of the skin and skeletal muscle in CLI. The haemodynamic environment in the resistance vasculature during CLI is characterised by a low-flow, low-pressure environment [1], which may directly or indirectly regulate the structure and function of the resistance vasculature [4,5].

We recently showed that the skeletal muscle resistance

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arteries (SkMRAS) from the distal part of the ischaemic limb produced exaggerated responses to noradrenaline [6,7] and that both $\alpha_1$- and $\alpha_3$-adrenoceptors contribute to this hyper-reactivity [8]. We sought the mechanisms underlying this effect on $\alpha_1$-adrenoceptors: selective antagonists and receptor inactivation experiments were carried out to identify possible changes in receptor subtypes and in the affinity of noradrenaline or in the $\alpha$-adrenoceptor reserve. Since this is the first analysis of receptor reserve in human resistance arteries it would have been ideal to employ equivalent biopsies from healthy subjects. Since it is impractical to obtain skeletal muscle biopsies from healthy subjects, we obtained and analysed subcutaneous resistance arteries (SCRAs) from healthy subjects. We have previously reported that they have a similar $\alpha_1$-adrenoceptor pharmacology to SkMRAS [9,10] and now report an analysis of their receptor reserve in receptor inactivation experiments for comparison with skeletal muscle arteries.

2. Methods

This study was performed in accordance with the Declaration of Helsinki [Cardiovascular Research 35 (1997) 2–3] of the World Medical Association and was approved by the local ethics committee. All the patients gave an informed consent. Table 1 gives the clinical information of the patients with CLI.

2.1. Preparation of skeletal muscle and subcutaneous resistance arteries

Skeletal muscle biopsies were obtained from the patients who fulfilled the criteria for CLI defined in the European Consensus Document [3], immediately after leg amputation. The level of amputation was always selected to be in a non-ischaemic area where the haemodynamics were physiologically normal as assessed by Doppler ultrasound and so the arterial segments obtained from this area represented an internal non-ischaemic control (proximal arteries). The distal portion of the limb appeared discoloured and so the arterial segments isolated from these tissues represent ischaemic ones (distal arteries). Non-ischaemic biopsies were obtained from either medialis (in the case of above-knee amputations) or gastrocnemius muscle (in the case of below-knee amputations). According to our preliminary experiments there was no significant difference in the potency and maximum responses of noradrenaline in the arterial segments from medialis (pEC$_{50}$: 6.0±0.2; maximum responses: 6.7±0.2 kPa, n = 7) and gastrocnemius (pEC$_{50}$: 6.1±0.1; maximum responses: 6.9±0.1 kPa, n = 10) muscles (see below for the definitions of pEC$_{50}$ and kPa). Ischaemic biopsies were always obtained from soleus muscle. Proximal and distal biopsies were obtained from the same patient when the contractile responses to $\alpha$-adrenoceptor agonists in proximal and distal arteries were compared. Subcutaneous arteries were obtained from the biopsies of inguinal subcutaneous fat from healthy subjects undergoing hernia operations. Biopsies were collected in physiological saline solution (PSS, see below for composition) and transported to the laboratory under ice-cold conditions. Resistance arteries (normalised diameter ($L_{0.9}$) of SkMRAS: 307±15 μm, n=70/23 (no. of arterial segments/no. of subjects); and of SCRA: 243±11 μm, n=5 (no. of subjects) were dissected out under a microscope (Zeiss) within 1–2 h.

2.2. Small vessel wire myography

Arterial segments of 2-mm length were mounted in a four-channel small vessel wire myograph (Danish Myotech, Aarhus, Denmark) for isometric tension measurements. The arterial segments were incubated in PSS of composition (mM): NaCl (119), KCl (4.5), NaHCO$_3$ (25), KH$_2$PO$_4$ (1), MgSO$_4$.7H$_2$O (1), (±)glucose (11) and CaCl$_2$ (2.5), at 37°C and gassed with carbogen. Then, 1 h after mounting, the resting tension–internal circumference relation was determined for each vessel segment [11]. The resting tension was then set to a normalised internal circumference of $L_{0.9}$ where $L_{0.9}$ = 0.9$L_{100}$ and $L_{100}$ is the internal circumference that the vessel would have under a transmural pressure of 100 mmHg (13.5 kPa). The software program MyoDAQ-Myodata (Danish Myotech, Aarhus, Denmark) was used for data acquisition. Subsequently, vessel viability was checked by exposure to high potassium solution (123 mM) twice and then to 10 μM noradrenaline in the presence of high potassium solution. Arterial segments were considered viable if they produced an effective resting transmural pressure (ERTP) of ≥100 mmHg (or 13 kilo pascals (kPa)) when stimulated with 123 mM KCl. ERTP was calculated from the Laplace equation:

$$ERTP =\text{wall tension} / (\text{internal circumference} / 2\pi)$$
which corrects for differences in length and diameter of arterial segments [11]. All the vessels were found to be viable according to this criterion.

The presence of functional endothelium was checked with 1 μM carbachol after pre-contracting with 1 μM noradrenaline. All the proximal arteries in the study produced >60% relaxation. In some experiments, the endothelium was mechanically removed as described earlier [8] by rubbing the luminal side of the arterial wall with hair that had been stored in ethanol and rinsed in PSS before use. Endothelial removal was confirmed by the lack of relaxation to carbachol when tested as described above.

After an equilibration period of 1 h, two to four concentration response curves (CRCs) were obtained to the adrenoceptor agonists in each arterial segment. No significant changes in EC₅₀ values or maximum responses of differences in the receptor density. The arterial segments pretreatment with phenoxybenzamine, an irreversible α-adrenoceptor antagonist. Concentrations of phenoxybenzamine required to decrease the maximum response to noradrenaline by between 20 and 80% were determined from preliminary experiments. These concentrations were 0.1 nM for SkMRAs and 100 nM for SCRAs, indicating differences in the receptor density. The arterial segments were incubated for 20 min and then washed for 45 min before a subsequent CRC to noradrenaline was obtained. The following relationship exists between the CRCs of the agonist before and after partial receptor inactivation [16]:

\[
\frac{1}{[A]} = \frac{1-q}{qK_A} + \frac{1}{q[A']}
\]

where [A] and [A'] are the corresponding equi-effective concentrations of the agonist before and after partial irreversible receptor inactivation, respectively, and q is the fraction of active (not alkylated) receptors remaining after irreversible inactivation. A plot of 1/[A] against 1/[A'] (double reciprocal plot) yields a straight line and the Kₐ for each individual experiment is calculated from the slope and the ordinate intercept using the following equation:

\[
K_A = \frac{\text{Slope} - 1}{\text{Intercept}}
\]

Mean negative log Kₐ (pKₐ) values were then calculated. The fraction of receptors occupied at a given concentration of noradrenaline and the receptor reserve in each individual experiment were calculated using the following equations [17]:

\[
\text{Receptor reserve} = \text{antilog}[-\log EC_{50} - pK_A]
\]

\[
\text{Fractional receptor occupancy} = \frac{[A]}{[K_A + [A]]}
\]

The receptor occupancies corresponding to 50% maximum responses were then obtained by interpolation, from plots of % maximum response against fractional receptor occupancy fitted to a hyperbolic curve.

2.5. Drugs

(-)-Noradrenaline (arterenol) bitartrate, (-)-adenaline (epinephrine) bitartrate, propranolol hydrochloride, corticosterone acetate and prazosin HCl were obtained from Sigma (Poole, Dorset, UK); cocaine HCl was obtained from Thornton and Ross (UK); RS 79948 ((8αR,12aS,13aS)-5,8,8α,9,10,11,12,12a,13a-decahydro-3-methoxy-12-(ethylsulphonyl)-6H-isoquinoline[2,1-g][1,6]-
naphthyridine) and A-61603 (N-[5-(4,5-dihydro-1H-imidazol-2-yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl]methanesulphonamide) were obtained from Tocris (Avonmouth, Bristol, UK); 5-methyl-urapidil, chloro-ethylclonidine HCl, phenoxycbenzamine HCl and BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4.5]decane-7,9-dione) were obtained from RBI (Natick, USA). The stock solution of 5-methyl-urapidil was prepared in 5% dimethyl sulfoxide and that of corticosterone acetate and phenoxycbenzamine HCl were prepared in 25% absolute ethanol. Stock solutions of all the other drugs were prepared in distilled water. PSS containing 123 mM KCl was prepared by replacing NaCl with an equimolar quantity of KCl.

2.6. Statistics

Maximum responses and pEC₅₀ values of the agonists were compared using Student’s t-test. A paired t-test was used to compare values from proximal and distal arteries from the same patient. For other comparisons an unpaired t-test was used.

3. Results

3.1. Contractile responses to different α-adrenoceptor agonists in non-ischaemic (proximal) and ischaemic (distal) skeletal muscle resistance arteries

Table 2 gives the pEC₅₀ values and maximum contractile responses to the agonists and 123 mM KCl expressed as ERTP. Contractile responses to 123 mM KCl were not significantly different in proximal and distal SkMRAs. Significantly higher maximum responses were observed to the adrenoceptor agonists, adrenaline and A-61603, in distal arteries compared to proximal arteries (Table 2). No significant differences were observed in the pEC₅₀ values of the agonists in the proximal and distal arteries. Analysis of the data from the individual experiments showed that, with one exception where the maximum response to adrenaline was unchanged, distal arteries from each individual patient showed increased contractile responses to adrenaline and A-61603 compared to the paired proximal arteries (Fig. 1a,b).

3.2. Influence of endothelium on the contractile responses to noradrenaline in non-ischaemic (proximal) skeletal muscle arteries

Proximal arterial segments which were denuded of endothelium showed less than 20% relaxation to carbachol whereas those with intact endothelium showed 60–100% relaxation. The contractile responses to noradrenaline in arterial segments with and without endothelium showed no significant difference in either maximum responses (maximum ERTP of 8.3±1.3 kPa (n=6) and 8.3±1.6 kPa (n=6) with and without endothelium, respectively) or

![Fig. 1. Contractile responses to (a) adrenaline and (b) A-61603, expressed as effective resting transmural pressure (ERTP, kPa), in the individual arterial segments from proximal (non-ischaemic) and distal (ischaemic) skeletal muscle resistance arteries. Proximal and distal arteries obtained from the same patient are indicated by the same symbol.](image-url)

### Table 2

<table>
<thead>
<tr>
<th>Agonist</th>
<th>n</th>
<th>Maximum ERTP (kPa)</th>
<th>pEC₅₀</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>123 mM KCl</td>
<td>20</td>
<td>23.3±1.6</td>
<td>24.7±1.7</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>5</td>
<td>7.7±0.5</td>
<td>13.0±1.5*</td>
</tr>
<tr>
<td>A-61603</td>
<td>5</td>
<td>10.8±0.6</td>
<td>16.3±1.8*</td>
</tr>
</tbody>
</table>

*P<0.01, significantly higher than proximal.
pEC$_{50}$ values (6.0±0.2 ($n=6$) and 6.0±0.4 ($n=6$) with and without endothelium, respectively).

### 3.3. Receptor inactivation studies with phenoxybenzamine in non-ischaemic (proximal) and ischaemic (distal) skeletal muscle resistance arteries

Phenoxybenzamine (0.1 nM) decreased the contractile responses to noradrenaline to 41±6% ($n=4$) of the control in proximal arteries and to 61±3% ($n=4$) of the control in distal arteries with a rightward shift in the CRCs (Fig. 2a,b). The decrease observed in proximal arteries was significantly higher than that observed in distal arteries ($P<0.05$). In inguinal subcutaneous arteries phenoxybenzamine (100 nM) decreased the contractile responses to noradrenaline to 65±10% ($n=5$) of the control with a rightward shift in the CRCs (Fig. 2c). Table 3 gives the $pK_a$ values of noradrenaline and the $\alpha$-adrenoceptor reserve in the resistance arteries from different biopsies studied. No significant differences in the $pK_a$ values of noradrenaline were observed in the three groups of arteries studied. The receptor reserve obtained in the distal arteries was significantly higher than that of proximal arteries ($P<0.05$). The reserve observed in the subcutaneous arteries was significantly higher than that of skeletal muscle arteries ($P<0.01$). The fractional receptor occupancy–% response curves (Fig. 3) showed that receptor occupancy of 41 and 27% was required to produce 50% of the maximum response in proximal and distal arteries, respectively. In subcutaneous arteries 50% of the maximum response was obtained with receptor occupancy of 20%.

### 3.4. Studies with different $\alpha$-adrenoceptor antagonists in the ischaemic (distal) skeletal muscle resistance arteries

Prazosin (0.1 μM), a selective $\alpha_1$-adrenoceptor antagonist over $\alpha_2$ [18], produced a parallel rightward-shift in the CRCs to noradrenaline in distal arteries with a 689-fold decrease in the potency without affecting the maximum response to noradrenaline ($n=4$) (Fig. 4). The observed shift gave a $pK_\beta$ of 9.6, indicating a high affinity of this antagonist as previously observed in proximal arteries [9] (Table 4a).

In distal arteries 5-methyl-urapidil (300 nM), a selective antagonist of the $\alpha_{1A}$-adrenoceptor subtype [19], decreased the potency of noradrenaline by 125-fold giving a $pK_\beta$ value of 8.4±0.2 ($n=8$) (Fig. 5) without affecting the maximum responses. This affinity estimate obtained in the distal arteries is similar to that reported for proximal arteries [9] (Table 4a). Incubation of arterial segments with 5-methyl-urapidil for 30 min did not increase the basal tension ruling out a possible agonist effect on 5HT$_{1A}$ receptors [20].

Table 4b shows the effect of chloroethylclonidine, which preferentially alkylates the $\alpha_{1B}$-adrenoceptor subtype [21], on the contractile responses produced by the two agonists, noradrenaline and A-61603, in proximal (taken

![Figure 2. Concentration–response curves to noradrenaline in skeletal muscle. (a) Proximal (non-ischaemic, $n=4$), (b) distal (ischaemic, $n=4$), and (c) subcutaneous ($n=5$) resistance arteries before (■) and after (▲) partial receptor inactivation with phenoxybenzamine.](image-url)
Table 3
The dissociation constant (pKₐ) of noradrenaline and adrenoceptor reserve in human skeletal muscle (n=4 for both proximal and distal) and subcutaneous resistance arteries (n=5)

<table>
<thead>
<tr>
<th></th>
<th>Skeletal muscle</th>
<th>Subcutaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>pKₐ</td>
<td>6.0±0.2</td>
<td>6.1±0.2</td>
</tr>
<tr>
<td>Adrenoceptor reserve</td>
<td>1.4±0.4</td>
<td>4.8±1.4*</td>
</tr>
</tbody>
</table>

*P<0.05, significantly higher than that of proximal.
**P<0.05, significantly higher than that observed in proximal and distal skeletal muscle arteries.

Table 4
Affinity estimates of different α₁-adrenoceptor antagonists (a) and the effect of chloroethylclonidine on noradrenaline- and A-61603-mediated contractile responses (b) in proximal (non-ischaemic) and distal (ischaemic) skeletal muscle resistance arteries

(a) Affinity estimates

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>pKᵦ/pA₂</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prazosin</td>
<td>9.2*</td>
<td>9.6±0.3</td>
<td></td>
</tr>
<tr>
<td>5-Methyl-urapidil</td>
<td>8.5*</td>
<td>8.4±0.2</td>
<td></td>
</tr>
<tr>
<td>BMY 7378</td>
<td>6.5±0.3*</td>
<td>7.1±0.4</td>
<td></td>
</tr>
</tbody>
</table>

(b) Effect of chloroethylclonidine

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline</td>
<td>69±8*</td>
<td>56±7*</td>
</tr>
<tr>
<td>A-61603</td>
<td>69±6*</td>
<td>72±4**</td>
</tr>
</tbody>
</table>

*P<0.05, significantly different from that of proximal.
**P<0.05, significantly higher than responses to noradrenaline in distal arteries; similar to that of A-61603 in proximal arteries (see text for details).

*Taken from Jarajapu et al. [9].

The decrease observed in noradrenaline-mediated responses was higher than that observed with A-61603 in these arteries (Table 4b). The decrease produced by chloroethylclonidine in noradrenaline-mediated responses in distal arteries was higher than that previously reported in proximal arteries whereas A-61603-mediated responses were reduced to a similar extent in both proximal and distal arteries (Table 4b). Incubation of arterial segments with chloroethylclonidine had no effect on baseline tension, ruling out any agonist action at α₁-adrenoceptors [22,23].

BMY 7378 (1 μM), a selective antagonist of the α₁-adrenoceptor subtype [24], produced a 14-fold decrease in
The potency of noradrenaline without affecting the maximum response in the distal arteries (Fig. 6). The observed shift gave a $pK_A$ of $7.1 \pm 0.4$ ($n=4$) not significantly different from that reported in proximal arteries [9] (Table 4a).

4. Discussion

This study showed that the increased contractile responses to $\alpha$-adrenoceptor agonists in resistance arteries from ischaemic skeletal muscle were accompanied by an increase in the $\alpha$-adrenoceptor reserve without any change in the agonist affinity and in the characteristics of $\alpha_1$-adrenoceptor subtypes. This is the first study to indicate a pathophysiological increase in the receptor reserve in a human disease.

The present study with human ischaemic arteries is in agreement with animal studies that showed increased arterial sensitivity or responsiveness to $\alpha$-adrenoceptor agonists following either reduced blood flow, ischaemia or ischaemia–reperfusion [25–27]. However, the present study points to a pathophysiological increase in the receptor reserve associated with the arterial hyper-reactivity in ischaemia, independent of changes in contractility, endothelial function, receptor subtype or receptor affinity.

4.1. Hyper-responsiveness to adrenoceptor agonists in ischaemic skeletal muscle resistance arteries

The adrenoceptor agonists used in the present study were the endogenous activators of adrenoceptors (the catecholamines noradrenaline and adrenaline) and a selective $\alpha_{1A}$-adrenoceptor agonist, A-61603 [28].

The larger contractile response to all three agonists observed in the distal ischaemic arteries was not via a non-specific increase in ability to contract since KCl produced similar contractions in non-ischaemic and ischaemic arteries. It has been reported that endothelial dysfunction associated with ischaemia may enhance the contractile responses to adrenoceptor agonists [29]. However, denudation of endothelium did not affect the contractile responses of non-ischaemic skeletal muscle arteries, ruling out endothelial dysfunction as a potential contributor to hyper-responsiveness in ischaemia. Although the patient group is heterogeneous with regard to sex, underlying complications, drug treatment and life style, these factors do not account for the greater responses in ischaemic arteries as non-ischaemic vessels from each patient served as an internal control and the data were consistent across the patients.

Although there is a possibility that distal vessels may have increased responsiveness to $\alpha$-adrenoceptor agonists compared to proximal arteries regardless of the presence of CLI, it is likely that CLI is a causative factor. Direct comparison in healthy limbs is difficult to achieve since control tissue is not easily available, particularly distal tissue. To address this we carried out a comparison of responsiveness to noradrenaline in non-ischaemic arteries from medialis muscle (above-knee amputations) with gastrocnemius muscle (below-knee amputations). This analysis shows no differences between the proximal and distal arteries (Section 2.1), thus suggesting that any differences seen in CLI between proximal and distal arteries are indeed due to the presence of ischaemia.

4.2. Effect of ischaemia on the $\alpha$-adrenoceptor reserve in the skeletal muscle resistance arteries

This is the first study to determine agonist (noradrenaline) affinity and $\alpha$-adrenoceptor reserve in human resistance arteries. The $pK_A$ observed for noradrenaline in human resistance arteries from skeletal muscle or subcutaneous fat (6.0) was low compared with that obtained from previous functional studies, e.g. rat aorta (6.7) [31], rabbit aorta (6.4) [32] and rat pulmonary artery (6.6) [31]. The affinity of the agonist was not affected by chronic ischaemia. According to the occupancy–response curves, the agonist noradrenaline could evoke contractile responses in the ischaemic arteries with a receptor occupancy lower than that needed in the non-ischaemic arteries (27% compared with 41% for a response that is 50% of maximum response). In other words a significant increase in the $\alpha$-adrenoceptor reserve in the ischaemic arteries makes the occupancy–response relationship for the agonist more favourable in the ischaemic arteries than in the non-ischaemic arteries.

The occupancy–response relationship to noradrenaline was almost linear in the case of proximal skeletal muscle arteries and shows that ~40% of the receptors need to be occupied to produce a half-maximal response. In earlier animal studies, noradrenaline, considered to be a full
agonist, produced half-maximal responses with a lower receptor occupancy, e.g. rat aorta (6%) [31] and rat portal vein (7%) [33]. Our present study of subcutaneous resistance arteries provides the only available data for healthy human resistance arteries. In these arteries, the receptor reserve was still low (20% occupancy for 50% response). It seems therefore that human resistance arteries may, in general, have a low receptor reserve and that this may be modulated by chronic conditions, e.g. chronic ischaemia.

The basis for the increased α-agonist reserve in the distal ischaemic arteries could be either an increased expression of α1A-adrenoceptors, an increase in the proportion that is functionally coupled, an increase in the efficiency of coupling or modified signal transduction downstream from the receptor activation. An increase in the receptor number would not theoretically increase the maximum contractile responses to a full agonist unless accompanied by one or more of the other above mentioned mechanistic changes. In fact each of these other changes itself could result in an increase in receptor reserve without an increase in receptor number. In the rat tail artery, ischaemia–reperfusion increases arterial contractility to noradrenaline without a change in the expression of α1-adrenoceptors or their associated G-proteins. Rather it was associated with an increase in α1-adrenoceptor-Gαq/11 protein coupling and the resultant α1-adrenoceptor-mediated phosphoinositide signaling [27]. The exact mechanisms underlying the hyper-responsiveness of the human skeletal muscle resistance arteries in critical limb ischaemia to α-adrenoceptor agonists should be the focus of future analysis.

4.3. The α1-adrenoceptor subtypes in non-ischæmic and ischæmic skeletal muscle resistance arteries

Our earlier studies using antagonists and comparing A-61603 with noradrenaline showed that the α1A-adrenoceptor is the predominant subtype in human resistance arteries, viz. non-ischæmic skeletal muscle and subcutaneous resistance arteries [9,10]. A similar higher potency of A-61603 compared to that of noradrenaline in the ischæmic skeletal muscle resistance arteries and the similarity in the effects of the antagonists, prazosin, 5-methyl-urapidil and BMY 7378, all indicate that the major adrenoceptor subtype mediating contraction in SkMRAs is unchanged by ischaemia.

In distal arteries, the sensitivity of noradrenaline-mediated responses to chloroethylclonidine was significantly higher than that of A-61603. This is in contrast to the situation in non-ischæmic arteries, where the sensitivity of the responses mediated by both the agonists to chloroethylclonidine was similar. This may suggest a small contribution of α1B-adrenoceptors to noradrenaline-mediated responses in the ischæmic arteries. Although an increased expression of functional α1B-adrenoceptor subtype in hypoxic conditions was reported in rat aortic smooth muscle [30], the differences in the distribution of α1-adrenoceptor subtypes with the anatomical regions cannot be ruled out.

In conclusion, this study showed hyper-responsiveness of the skeletal muscle resistance arteries to the adrenoceptor agonists in ischaemic conditions. The α1A-adrenoceptor was found to be the predominant subtype that mediates the contractile responses to noradrenaline in the ischæmic arteries. The affinity of noradrenaline and different antagonists to α1-adrenoceptor subtypes was not affected by the ischaemic environment. The α1-adrenoceptor receptor reserve was increased but the α1-adrenoceptor profile was almost unaltered in ischaemic conditions in human skeletal muscle resistance arteries.

Acknowledgements

The authors are thankful to the surgeons and the theatre staff from the Department of Vascular Surgery at Glasgow Royal Infirmary and Gartnavel General Hospital, Glasgow. The authors are also thankful to Dr Colin Berry and Dr Andrew Renwick at Western Infirmary, Glasgow. Yagna P.R. Jarajapu was supported by the School of Biological and Biomedical Sciences, Glasgow Caledonian University during this study.

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