# Regional heterogeneity of α-adrenoreceptor subtypes in arteriolar networks of mouse skeletal muscle

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**Activation of vascular adrenoreceptors (ARs) governs the magnitude and distribution of muscle blood flow in accord with the distribution of AR subtypes. Functional studies in the rat cremaster muscle indicate that $\alpha_1$ARs predominate in proximal arterioles (first-order, 1A) while $\alpha_2$ARs predominate in distal arterioles (third-order, 3A). However, little is known of AR subtype distribution in arteriolar networks of locomotor skeletal muscles, particularly in the mouse. We tested the hypotheses that functional AR subtypes exhibit heterogeneity among branches of arteriolar networks in a locomotor muscle and that the nature of this heterogeneity can vary between muscles having diverse functions. In anaesthetized male C57BL/6J mice (3 months old), concentration–response curves ($10^{-9}$ M to $10^{-5}$ M, 0.5 log increments) were evaluated in the gluteus maximus muscle superfused with physiological saline solution (35°C, pH 7.4; $n \geq 5$ per group). Noradrenaline (NA, non-selective αAR agonist) constricted 1A, 2A and 3A with similar potency and efficacy. Phenylephrine (PE; $\alpha_1$AR agonist) evoked greater ($P < 0.05$) constriction in 3A (inhibited by $10^{-8}$ M prazosin; $\alpha_1$AR antagonist) while UK 14304 (UK; $\alpha_2$AR agonist) evoked greater ($P < 0.05$) constriction in 1A (inhibited by $10^{-7}$ M rauwolscine; $\alpha_2$AR antagonist). Isoproterenol (isoprenaline; βAR agonist) dilated 1A, 2A and 3A near-maximally with similar potency and efficacy; these dilatations were inhibited by $10^{-7}$ M propranolol (βAR antagonist) which otherwise had no effect on responses to NA, PE, or UK. Complementary experiments in the mouse cremaster muscle revealed a pattern of αAR subtype distribution that, while distinct from the gluteus maximus muscle, was consistent with that reported for the rat cremaster muscle. We conclude that functional αAR subtype distribution in arteriolar networks of skeletal muscle varies with muscle function as well as vessel branch order.**

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**Abbreviations** ARs, adrenoreceptors; GM, gluteus maximus muscle; NA, noradrenaline; PE, phenylephrine; PSS, physiological saline solution; SNP, sodium nitroprusside; 1A, first-order arterioles; 2A, second-order arterioles; 3A, third-order arterioles.

## Introduction

Vascular adrenoreceptors (ARs) are integral to the regulation of arterial blood pressure through adjustments in peripheral resistance. Sympathetic nerve activity releases noradrenaline (NA) from perivascular sympathetic nerve terminals (Luff, 1996) to elicit vasoconstriction and thereby restrict blood flow. In the microcirculation, neurovascular coupling is mediated through activating post-junctional αARs on arteriolar vascular smooth muscle cells, with functional $\alpha_1$AR and $\alpha_2$AR subtypes shown in animals and humans (Medgett & Langer, 1984; Flavahan *et al.* 1987; Faber, 1988; Aaker & Laughlin, 2002; Wray *et al.* 2004; Lambert & Thomas, 2005; Jackson *et al.* 2008). The magnitude of vasoconstriction in response to sympathetic nerve stimulation varies among arterioles differing in size and branch order. For resistance networks of skeletal muscle, sympathetic nerve stimulation evokes greater constriction in smaller arterioles located downstream when compared to larger arterioles and feed arteries located upstream (Folkow *et al.* 1971; Rosell, 1980; Marshall, 1982; Boegehold & Johnson, 1988; Ohyanagi *et al.* 1991; VanTeeffelen & Segal, 2003). Further,


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distal arteriolar branches exhibit greater 'escape' from constriction in resting muscle and more readily dilate (i.e. exhibit functional sympatholysis) during skeletal muscle contraction (Folkow *et al.* 1971; Marshall, 1982; Boegehold & Johnson, 1988; Anderson & Faber, 1991; VanTeeffelen & Segal, 2003). Thus, dilatation of distal arterioles effectively maximizes capillary perfusion and the functional surface area for exchange between microvessels and parenchymal cells while proximal arterioles and feed arteries remain constricted (and restrict muscle blood flow) to maintain systemic perfusion pressure (Folkow *et al.* 1971; Segal, 2000; Thomas & Segal, 2004).

The differential distribution of $\alpha_1$AR and $\alpha_2$AR subtypes has been used to explain why distal arterioles more readily undergo functional sympatholysis during muscle contraction when compared to proximal arterioles. Comprehensive studies of the functional distribution of $\alpha_1$ARs and $\alpha_2$ARs in arteriolar networks (e.g. in mediating the actions of NA and its analogues *in vivo*) have focused on the cremaster muscle of male rats (Faber, 1988; McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Ohyanagi *et al.* 1991). In response to pharmacological manipulations and to sympathetic nerve stimulation, $\alpha_1$AR activation produced relatively greater constriction of the larger proximal (first-order, 1A) arterioles, than did $\alpha_2$AR activation. In contrast, $\alpha_2$AR activation caused greater constriction of the smaller distal (third-order, 3A) arterioles than did $\alpha_1$AR activation (Faber, 1988; Ohyanagi *et al.* 1991). In turn, differential responses between proximal and distal arteriolar branch orders to acidosis or to muscle contraction have been attributed to corresponding differences in the susceptibility of respective $\alpha$AR subtypes to the actions of metabolic factors (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Wray *et al.* 2004). Thus relative to $\alpha_1$AR-induced vasoconstriction, $\alpha_2$AR-induced vasoconstriction has been proposed to be more susceptible to functional inhibition. However, not all studies have shown greater selectivity for inhibiting $\alpha_2$AR- *vs.* $\alpha_1$AR-mediated vasoconstriction. For example, vasoconstrictor responses to agonists that were selective for respective $\alpha$AR subtypes were attenuated to a similar extent during rhythmic handgrip exercise (Dinenno & Joyner, 2003; Rosenmeier *et al.* 2003).

A key functional difference between skeletal muscles and the cremaster muscle is that the former serve to stabilize and move the body while the latter has no skeletal attachments. Derived from musculature of the lower abdominal wall, the cremaster muscle supports the testes while regulating its temperature to maintain spermatogenesis. Thus, it is not clear whether the functional properties of $\alpha$AR subtype distribution determined in the rat cremaster muscle can be accurately extrapolated to skeletal muscles that are integral to locomotion. In the gluteus maximus muscle (GM; a powerful hip extensor) of C57BL/6 mice, recent findings have shown that subtle activation of $\alpha$ARs (i.e. below that causing any change in diameter) can blunt arteriolar dilatation to brief tetanic contractions and restrict muscle blood flow at rest as well as during exercise (Jackson *et al.* 2010; Moore *et al.* 2010). However, as holds for other skeletal muscles, the functional distribution of $\alpha$AR subtypes in the GM is unknown.

For the present study, our primary goal was to determine whether functional $\alpha$AR subtypes vary with arteriolar branch order in a representative locomotor muscle of C57BL/6J mice. Using intravital microscopy to study arteriolar networks in the GM, we tested the hypothesis that the activation of $\alpha_1$ARs and $\alpha_2$ARs would produce similar effects across arteriolar branch orders. In contrast to blocking $\alpha_1$AR or $\alpha_2$AR subtypes during exposure to NA (Faber, 1988), our experimental design relied upon agonists and antagonists that we confirmed to be selective for each $\alpha$AR subtype. This approach also avoided the possibility of non-selective inhibition of complementary receptors that might otherwise respond to NA. To test whether the variation in $\alpha$AR subtype distribution within the microcirculation differed between tissues in the same species, additional experiments were performed to evaluate the functional distribution of $\alpha_1$ARs and $\alpha_2$ARs in arteriolar networks of the cremaster muscle.

In contrast to vasoconstriction evoked through $\alpha$ARs, the activation of vascular $\beta$ARs produces vasodilatation as shown by isoproterenol addition to isolated arterioles in vitro (Aaker & Laughlin, 2002), intravenous infusion in anesthetized cats (Fronek & Zwiefach, 1975) or intra-arterial delivery to hindlimbs of exercising dogs (Buckwalter *et al.* 1997*a*). Remarkably, the effect of local $\beta$AR activation on intact arteriolar networks has received relatively little attention. Instead, to avoid possible actions of $\beta$AR activation, the functional distribution of $\alpha$AR subtypes has typically been studied in the presence of propranolol (Faber, 1988; McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Ohyanagi *et al.* 1991) without ascertaining whether this inhibition of $\beta$ARs affected responses to the activation of either $\alpha$AR subtype. To provide new insight with respect to the functional consequences of $\beta$AR activation in intact arteriolar networks controlling blood flow to the mouse GM, we evaluated the responses of successive arteriolar branches to $\beta$AR activation and determined whether $\beta$AR inhibition affected arteriolar responses to the activation of $\alpha$AR subtypes alone and in combination.

## Methods

### Animal care and use

All procedures were approved by the Animal Care and Use Committee of the University of Missouri and

performed in accord with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The present experiments comply with the policies and regulations of *The Journal of Physiology* (Drummond, 2009). Male C57BL/6J mice (3–5 months; 25–30 g) were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed at ∼22°C on a 12 h–12 h light–dark cycle with food and water provided *ad libitum* for at least 1 week before being studied. At the end of the experimental procedures each day, the anaesthetized mouse was killed with an overdose of pentobarbital (intraperitoneal injection) followed by cervical dislocation.

### Surgical preparation of GM and cremaster muscles

A mouse was anaesthetized with pentobarbital sodium (intraperitoneal injection, 60 mg $kg^{-1}$) supplemented as needed (intraperitoneal injection, 20 mg $kg^{-1}$) to prevent withdrawal from toe pinch. Hair was shaved from the hindquarters. Oesophageal temperature was maintained at 37–38°C by placing the mouse on an aluminum warming plate positioned on an acrylic platform. One muscle was studied per animal. Exposed muscles were superfused continuously (∼3 ml $min^{-1}$) with a bicarbonate-buffered physiological salt solution (PSS; 34–35°C, pH 7.4) of the following composition (in mM): 131.9 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.17 $MgSO_4$ and 18 $NaHCO_3$ equilibrated with 5% $CO_2$–95% $N_2$.

**Gluteus maximus muscle.** The left GM was prepared as recently described (Jackson *et al.* 2010; Moore *et al.* 2010) by removing overlying skin and connective tissue, separating the muscle from the spine along its origin, and reflecting it away from the body while maintaining its insertion on the femur. The muscle was spread over a transparent pedestal (Sylgard 184; Dow Corning, Midland, MI, USA) and pinned at the edges to approximate *in situ* dimensions.

**Cremaster muscle.** The left cremaster muscle was prepared by making a midline incision on the ventral surface of the scrotal sac, carefully separating the muscle from surrounding connective tissue, and then opening it along the ventral axis. An orchiectomy was performed and the cremaster muscle was spread over a transparent pedestal (Sylgard 184) and pinned at the edges to approximate *in situ* dimensions (Hungerford *et al.* 2000).

### Intravital microscopy

The completed experimental preparation was transferred to the fixed stage (Burleigh Gibraltar; Mississauga, Ontario, Canada) of an intravital microscope (Nikon E600FN; Melville, NY, USA) and equilibrated for at least 30 min prior to beginning a protocol. The microscope was mounted on an *X*–*Y* translational platform (Burleigh Gibraltar) enabling it to be moved to different observation sites without disturbing the preparation. Images were acquired using a Nikon ×20 SLWD objective (numerical aperture, 0.35) using Köhler illumination from a long-working-distance condenser (numerical aperture, 0.52). Images were digitized with a charge-coupled device video camera (KP-D50; Hitachi Denshi; Japan) and observed on a digital monitor (ViewEra V191HV; Walnut, CA, USA) at a total magnification of ×1300. A video calliper calibrated to a stage micrometer ($100 \times 0.01 = 1$ mm: Graticules Ltd, Tonbridge, Kent, UK) measured internal vessel diameter (spatial resolution $\leq 1\ \mu$m) as the widest distance between luminal edges. Output from the calliper was sampled at 40 Hz using a Powerlab/400 system (AD Instruments; Colorado Springs, CO, USA) coupled to a personal computer.

### Vessel classification

To the extent possible, arterioles selected for study were standardized across preparations. Arteriolar networks bifurcating into successive branch orders were typically located in the Inferior region of the GM (see Fig. 1 of Moore *et al.* 2010). During the equilibration period, networks were scanned by eye and branch orders were classified as follows. First-order (1A): the most proximal branch embedded within striated muscle fibres, with the 1A origin defined as the site where its feed artery entered the muscle. Second-order (2A): originating as the first major bifurcation from the 1A. Third-order (3A): originating as the first major bifurcation from the 2A. For each branch order, the observation site was at least 150 $\mu$m downstream of its origin. Respective sites were observed individually within each network by shifting the field of view with reference to anatomical landmarks. One arteriolar network was studied per mouse.

### Criteria for inclusion

After the initial 30 min equilibration, arterioles were evaluated for sensitivity to oxygen as an index of the preparation's integrity by equilibrating the superfusion solution with 21% $O_2$–5% $CO_2$ (balance $N_2$) for 5 min. Arterioles (2A and 3A) typically constricted by 5–10 $\mu$m during exposure to elevated $O_2$. The superfusion solution was then re-equilibrated with 5% $CO_2$–95% $N_2$ for the remainder of the experimental protocol. At the end of experiments, the maximum diameter of each observation site was determined during superfusion with $10^{-4}$ M sodium nitroprusside (SNP). Only preparations in which arterioles exhibited spontaneous vasomotor tone,

constricted to elevated oxygen, and dilated vigorously to SNP were included in the study. More than 90% of muscle preparations studied during the present experiments satisfied these criteria.

### Administration of agonists and antagonists

Adrenoreceptor agonists and antagonists were prepared fresh on the morning of an experiment, administered topically via addition to the superfusion solution and expressed as final concentrations over the tissue. The superfusion solution was delivered by gravity feed onto the preparation from a 60 ml chamber consisting of a vertically mounted syringe secured within an aluminum heater block (SW-60, Warner Instruments; Hamden, CT, USA). Chamber volume was maintained by gravity feed from a 500 ml reservoir. For cumulative concentration–response experiments to AR agonists, inflow from the reservoir was shut to fix the volume of solution within the chamber. The appropriate volume of stock concentration of agonist or antagonist was added to achieve the desired final concentration; continuous bubbling of gas within the chamber ensured rapid mixing. Stock solutions were prepared at concentrations ensuring that the volume added was always less than 1% of the chamber volume. Arteriolar diameters at each observation site were recorded for a given condition and then inflow from the reservoir was restored. Once the original volume in the chamber was attained, inflow from the reservoir was again shut and the next concentration of the agonist was administered. This procedure was repeated throughout concentration–response determinations. For treatment with antagonists, the desired concentration was prepared in the 500 ml reservoir supplying the 60 ml superfusion chamber to which agonists were added. When different antagonists were used for a given experiment, each was prepared in its own 500 ml reservoir. Drugs and chemicals and were purchased from Sigma-Aldrich (St Louis, MO, USA).

### Experimental protocols

The primary goal of these experiments was to determine the functional distribution of $\alpha_1$ARs, $\alpha_2$ARs and $\beta$ARs in 1A, 2A and 3A of the mouse GM.

**Protocol 1: functional distribution of arteriolar αARs in the GM.** We first evaluated the stability of AR activation in GM arterioles over the time course of a typical experiment (~3 h). One group of mice was used to evaluate the non-selective $\alpha$AR agonist NA. Another group of mice was used to evaluate the $\alpha_1$AR-selective agonist phenylephrine (PE) and a third group to evaluate the $\alpha_2$AR-selective agonist UK 14304. Each agonist was studied in half-log increments ($10^{-9}$ M to $10^{-5}$ M unless stated otherwise) by evaluating arteriolar diameters to cumulative increases in concentration. Each concentration of agonist was equilibrated for 3 min or until vessel diameters had reached stable values and the internal diameters of 1A, 2A and 3A were recorded (in random order). After the final agonist concentration was evaluated, superfusion with control PSS was restored for ~10 min while arterioles recovered to their initial (post 30 min equilibration) baseline diameters. This entire procedure was repeated 4 times for each agonist. One agonist was studied in each GM preparation.

**Protocol 2: functional distribution of arteriolar βARs in the GM.** While activating $\beta$ARs promotes vasodilatation, little is known of how $\beta$AR activation affects arteriolar diameters *in vivo* (Fronek & Zweifach, 1975) and few investigators have determined whether concomitant inhibition of $\beta$ARs affects arteriolar responses to $\alpha$AR activation (Marshall, 1982). Therefore, the functional distribution of $\beta$AR in arterioles of the GM was assessed by evaluating 1A, 2A and 3A diameters during cumulative addition of the $\beta$AR agonist isoproterenol ($10^{-11}$ M to $10^{-5}$ M). In one group of mice, responses to isoproterenol were re-evaluated after equilibrating with propranolol ($10^{-7}$ M) for at least 15 min. In three additional groups, we determined whether $\beta$AR inhibition with propranolol affected constrictions to NA, PE or UK 14304. During propranolol treatment, one $\alpha$AR agonist was evaluated in each GM preparation.

**Protocol 3: selectivity of αAR agonists and antagonists.** To investigate the selectivity of functional $\alpha$AR subtypes in arterioles of the mouse GM, we evaluated the effect of $\alpha$AR antagonists on responses to selective $\alpha$AR agonists. Antagonists were equilibrated for ~15 min before testing for their actions. One agonist was evaluated in the presence of respective antagonists in each GM preparation as follows.

*Protocol 3A: effect of αAR subtype antagonists on arteriolar responses to $\alpha_1$AR activation.* Responses to PE were first evaluated under control conditions. The agonist was removed and the preparation was superfused with control PSS for ~10 min. Responses to PE were next re-evaluated in the presence of rauwolscine ($10^{-7}$ M; $\alpha_2$AR antagonist), which was then removed and the preparation superfused with control PSS for ~10 min. Responses to PE were evaluated a final time in the presence of prazosin ($10^{-8}$ M, $\alpha_1$AR antagonist).

*Protocol 3B: effect of αAR subtype antagonists on arteriolar responses to $\alpha_2$AR activation.* Responses to UK 14304 were first evaluated under control conditions. The agonist was removed and the preparation was superfused with control PSS for ~10 min. Responses to UK 14304 were

then re-evaluated in the presence of prazosin ($10^{-8}$ M), which was subsequently removed and the preparation superfused with control PSS for ∼10 min. Responses to UK 14304 were evaluated a final time in the presence of rauwolscine ($10^{-7}$ M).

**Protocol 4: tissue specificity of αAR subtype distribution in arteriolar networks.** Experiments were performed in the cremaster muscle of additional mice to determine whether functional αAR subtype distribution of 1A, 2A and 3A varied between muscles. Diameter responses to cumulative increases in [NA] were evaluated first. Following washout and recovery, responses to cumulative increases in [PE] or [UK 14304] were evaluated (order randomized across experiments). Following further washout and recovery, responses to the third agonist were evaluated.

### Data presentation and statistical analysis

Data are presented as the diameter change (in μm) from resting baseline = $[(D_{rest} - D_{treatment})]$, as percentage constriction = $[(D_{rest} - D_{treatment})/D_{rest}]$ (where 100% corresponds to lumen closure), or as percentage maximal dilatation = $[(D_{treatment} - D_{rest})/(D_{max} - D_{rest})]$. The diameter following the initial 30 min equilibration was taken as $D_{rest}$. The diameter recorded at a given agonist concentration was designated $D_{treatment}$. The diameter recorded in the presence of $10^{-4}$ M SNP was designated $D_{max}$.

Data were analysed using one- and two-way repeated measures analysis of variance (Prism 5, GraphPad Software; La Jolla, CA, USA). When significant *F*-ratios were obtained, *post hoc* comparisons were made using Bonferroni or Tukey tests. Summary data are expressed as mean ± S.E. Differences were considered statistically significant at $P < 0.05$.

## Results

A total of 69 mice were used in the present experiments. Resting and maximal diameters of 1A, 2A and 3A for the GM and cremaster muscles studied are presented in Table 1.

**Protocol 1: functional distribution of αARs in the GM.** A comparison of relative responses to selective and non-selective αAR subtype activation is presented in Fig. 1. The concentration–response relationships to respective agonists were stable over time (see Figs. 1–3 in Supplemental material, available online only). Therefore, data from the four repeated determinations were averaged for each mouse. In response to NA (Fig. 1*A*), respective branch orders had similar percentage maximal peak constrictions (1A = 88 ± 7%, 2A = 97 ± 3%, 3A = 100 ± 0%). However, 3A exhibited greater ($P < 0.05$) responses than 1A or 2A between $3 \times 10^{-8}$ M and $10^{-6}$ M. Respective log $EC_{50}$ values were not significantly different (1A: −5.9 ± 0.1; 2A: −5.9 ± 0.1; 3A: –6.1 ± 0.3). In response to PE (Fig. 1*B*), peak constrictions of 3A (66 ± 5% of maximal) were greater ($P < 0.05$) than those of 1A (32 ± 3%) or 2A (47 ± 2%). However, the actual changes in diameter were similar across branch orders (see Fig. 3*B*). In response to UK 14304 (Fig. 1*C*), constrictions were greater ($P < 0.05$) for 1A (56 ± 3%) compared to 2A (31 ± 5%) or 3A (35 ± 6%). This pattern of response indicates greater functional activity of $\alpha_1$ARs in 3A and of $\alpha_2$ARs in 1A.

Because constrictions to PE or UK 14304 did not reach an apparent plateau, $EC_{50}$ values could not be determined for these agonists. In preliminary studies, exposure to PE at a concentration $>10^{-6}$ M was found to disrupt reproducible responses to consecutive concentration–response experiments, thereby establishing $10^{-6}$ M as the upper limit for PE concentration in these experiments.

**Table 1. Arteriolar diameters in mouse gluteus maximus and cremaster muscles**

| | Branch order | | |
|---|---|---|---|
| | 1A | 2A | 3A |
| Gluteus maximus | | | |
| Resting (μm) | 31 ± 1 | 20 ± 1 | 12 ± 1 |
| Maximal (μm) | 50 ± 1 | 39 ± 1 | 29 ± 1 |
| Cremaster | | | |
| Resting (μm) | 39 ± 1 | 22 ± 2 | 12 ± 1 |
| Maximal (μm) | 57 ± 4 | 39 ± 4 | 24 ± 3 |

Summary values (means ± S.E.) for internal diameters of arterioles *in vivo*. For each arteriolar branch order, resting values were recorded after the 30 min equilibration period following surgical preparation. Maximal values recorded at the end of experiments in the presence of $10^{-4}$ M SNP. Data for GM arterioles are from 60 mice. Data for cremaster muscle arterioles are from 9 mice.

**Protocol 2: functional distribution of βARs in the GM.** Each arteriolar branch order dilated in response to βAR activation with isoproterenol (Fig. 2). Peak absolute changes in diameter for 1A, 2A and 3A were not significantly different (18 ± 4 μm, 18 ± 3 μm and 17 ± 2 μm, respectively). Despite differences in resting and maximal diameters between arteriolar branch orders (Table 1), when responses to isoproterenol were expressed relative to respective maximal dilatations (i.e. diameter changes) obtained with SNP (Table 1), there were no significant differences in efficacy or sensitivity. This similarity in behaviour was reflected in respective log $EC_{50}$ values (1A: −8.7 ± 0.2; 2A: −8.8 ± 0.2; 3A: −9.2 ± 0.2). In the presence of propranolol ($10^{-7}$ M), responses to isoproterenol were shifted to the right by nearly 2 log-orders ($P < 0.01$),

confirming its effectiveness as an inhibitor of $\beta$ARs (Supplemental Fig. 4). The inhibition of $\beta$ARs had no effect on constrictions to NA, PE, or UK 14304 in 1A, 2A or 3A (Fig. 3).

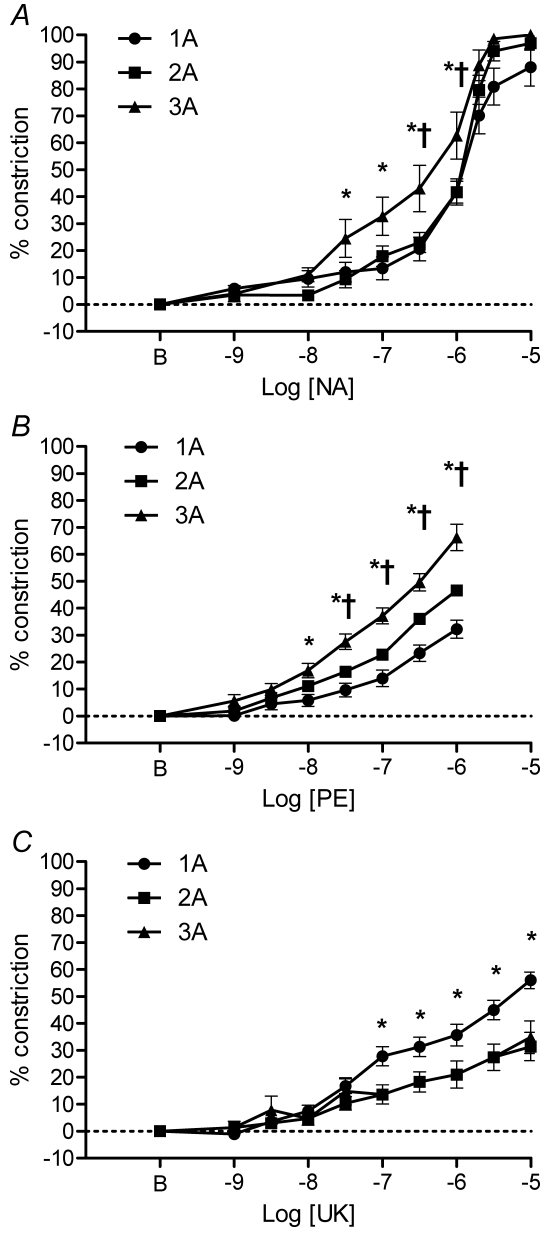


**Figure 1. Arteriolar constriction with $\alpha$AR activation in mouse GM**
*A*, non-selective activation of $\alpha$ARs with NA produced similar constrictions across arteriolar branch orders but 3A were more sensitive than 1A and 2A between $3 \times 10^{-8}$ and $10^{-6}$ M. $^{*}P < 0.05$, 3A different from 1A. $^{\dagger}P < 0.05$, 3A different from 2A ($n = 11$ for 1A and 2A, $n = 9$ for 3A). *B*, selective activation of $\alpha_1$ARs with PE constricted 3A relatively more than 1A or 2A. $^{*}P < 0.01$, 3A different from 1A. $^{\dagger}P < 0.01$, 3A different from 2A ($n = 21$ each). *C*, selective activation of $\alpha_2$ARs with UK 14304 (UK) constricted 1A relatively more than 2A or 3A. $^{*}P < 0.01$, 1A significantly different from 2A and 3A ($n = 20$ each). B indicates resting baseline.

**Protocol 3: selectivity of $\alpha$AR agonists and antagonists.** Results from these experiments are presented for 1A, 2A and 3A in Figs. 4–6, respectively, and confirm the selectivity of our pharmacological treatments. In response to PE, constriction of 1A was inhibited by $10^{-8}$ M prazosin (Fig. 4*A*) but was unaltered by $10^{-7}$ M rauwolscine (Fig. 4*B*). In response to UK 14304, 1A constriction was effectively maintained (except for an attenuated response at $10^{-5}$ M) in the presence of prazosin (Fig. 4*C*) but was inhibited by rauwolscine (Fig. 4*D*). For 2A, constriction in response to PE was inhibited by prazosin (Fig. 5*A*) but was unaffected by rauwolscine (Fig. 5*B*). In response to UK 14304, 2A constriction was maintained in the presence of prazosin (Fig. 5*C*) but was inhibited by rauwolscine (Fig. 5*D*). For 3A, constriction in response to PE was inhibited by prazosin (Fig. 6*A*) but maintained in the presence of rauwolscine (except for an attenuated response at $10^{-6}$ M) (Fig. 6*B*). In response to UK 14304, the modest level of 3A constriction was preserved in the presence of prazosin (Fig. 6*C*) but was inhibited by rauwolscine (Fig. 6*D*). Collectively, these data support the use of prazosin ($10^{-8}$ M) and rauwolscine ($10^{-7}$ M) as selective antagonists of $\alpha_1$ARs and $\alpha_2$ARs, respectively, in arterioles of the mouse GM.

**Protocol 4: tissue specificity of $\alpha$AR subtype distribution in arteriolar networks.** Because our results for the mouse GM indicated a different pattern of functional $\alpha$AR subtype distribution in respective arteriolar branch orders than reported for the rat cremaster muscle (Faber, 1988), we investigated responses to NA, PE and UK 14304 in arterioles of the cremaster muscle in mice identical to those in which the GM was studied (Fig. 7). For 1A, constrictions to NA were consistently less ($P < 0.05$) than those of 2A or 3A (Fig. 7*A*). Constrictions to PE appeared to be greatest in 3A, which were significantly different from

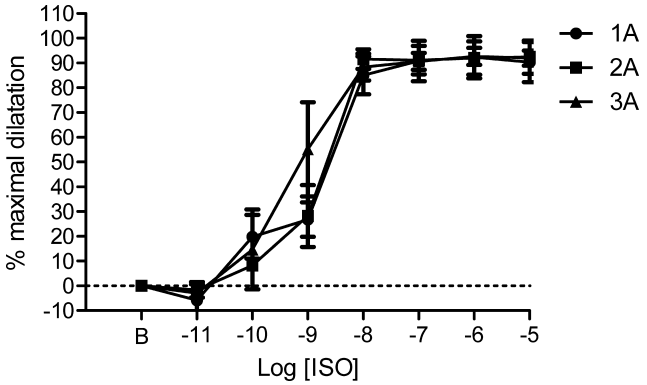


**Figure 2. Arteriolar dilatation with $\beta$AR activation in mouse GM**
Isoproterenol (ISO) dilated 1A, 2A and 3A to 90 ± 8%, 92 ± 7% and 92 ± 3% of the maximal dilatation (diameter change in $\mu$m) obtained with $10^{-4}$ M SNP (see Table 1). There were no significant differences between arteriolar branch orders ($n = 6$ arterioles for each branch order). B indicates resting baseline.

responses of 1A and 2A ($P < 0.05$) at $10^{-6}$ M (Fig. 7*B*). For UK 14304, 2A and 3A constricted progressively as agonist concentration increased with 3A exhibiting greater ($P < 0.05$) responses than 2A (Fig. 7*C*). In contrast, 1A showed little response to UK 14304. This pattern of arteriolar reactivity in the mouse cremaster muscle indicates greater functional activity of $\alpha_1$ARs in 1A and of $\alpha_2$ARs in 3A when compared to respective arteriolar branch orders in the GM. However this pattern is consistent with that observed for $\alpha_1$AR and $\alpha_2$AR subtype distribution first reported for the rat cremaster muscle (Faber, 1988).

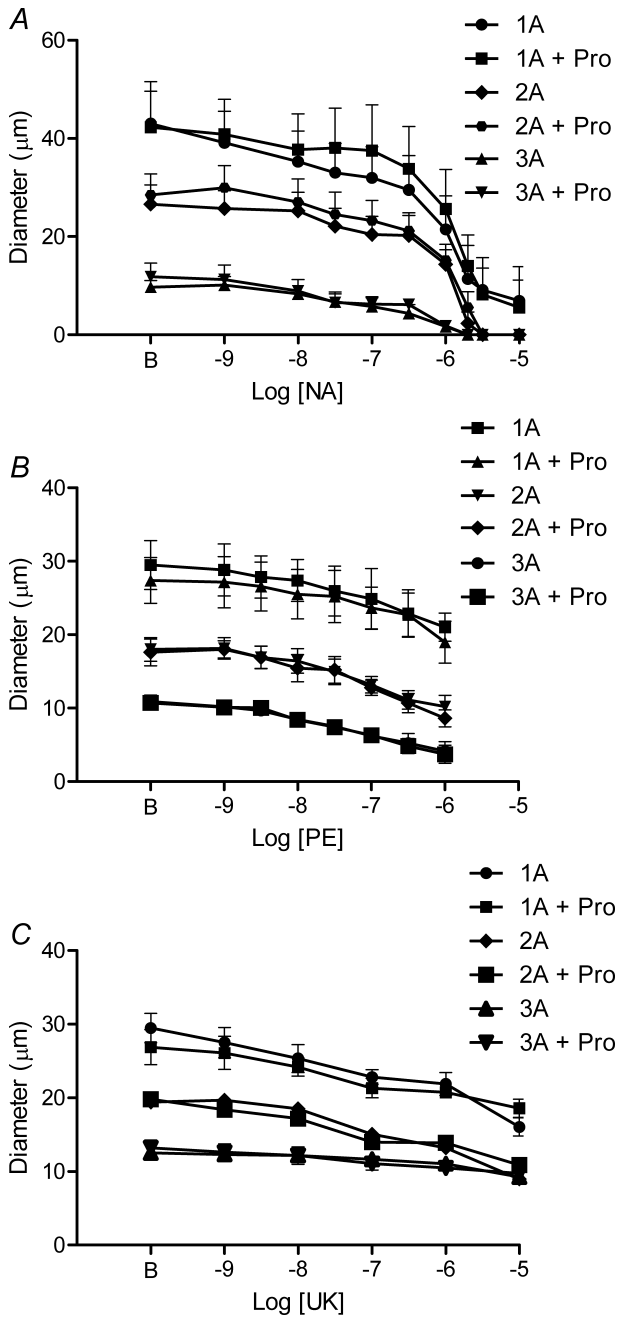


**Figure 3. Inhibition of βARs does not alter responses to αAR agonists**
Addition of propranolol (Pro, $10^{-7}$ M) had no significant effect on arteriolar constrictions to NA (*A*, $n = 4$), PE (*B*, $n = 5$), or UK 14304 (UK) (*C*, $n = 5$). B indicates resting baseline.

## Discussion

This study is the first to examine the functional distribution of $\alpha_1$ARs and $\alpha_2$ARs in multiple arteriolar branch orders of a locomotor muscle in the mouse. We demonstrate that functional αAR subtype distribution in arterioles controlling blood flow to the GM varies between branch orders. Our data illustrate that $\alpha_2$AR reactivity is most prominent in proximal (1A) arterioles while $\alpha_1$AR reactivity is most prominent in distal (3A) arterioles. We therefore reject the hypothesis that functional αAR subtype distribution is uniform across arteriolar branch orders in the mouse GM. Moreover, the pattern of functional αAR subtype distribution shown here in the mouse GM contrasts with that reported for the rat cremaster muscle, where $\alpha_1$AR reactivity predominates in 1A and $\alpha_2$AR reactivity predominates in 3A. Remarkably, our findings in the mouse cremaster muscle are consistent with those in the rat cremaster muscle. In turn, we conclude that results from the cremaster muscle cannot be extrapolated to a locomotor muscle such as the GM or – by inference – to other locomotor skeletal muscles. The present findings illustrate the importance of determining the pattern of αAR subtype distribution in the muscle being studied for adrenergic reactivity to experimental treatments. Whereas responses to combined or selective αAR subtype activation were unaffected by βAR blockade, the functional distribution of βARs in GM arterioles appears uniform across branch orders. Indeed, signalling events initiated by βARs produced dilatations of respective arteriolar branch orders that were not different from dilatations obtained with the NO donor, SNP. We thereby demonstrate the efficacy of βAR-mediated dilatation throughout arteriolar networks controlling blood flow to skeletal muscle *in vivo*.

### Experimental activation of αARs

In the peripheral vasculature, sympathetic nerves release neurotransmitters which act on post-junctional αARs to initiate vasoconstriction (Vanhoutte *et al.* 1981). Experimentally, several methods have been employed to produce this response including electrical stimulation and the administration of αAR agonists. Perivascular sympathetic nerve activation can be accomplished by stimulating the paravertebral sympathetic chain (Hilton *et al.* 1970; Fleming *et al.* 1987; Boegehold & Johnson, 1988; Ohyanagi *et al.* 1991; Dodd & Johnson, 1993; Thomas *et al.* 2001) or through electrodes positioned

on the feed artery supplying the muscle (Marshall, 1982; VanTeeffelen & Segal, 2003; Haug & Segal, 2005). However, pre-junctional inhibition of neurotransmitter release or co-release of other substances (e.g. adenosine triphosphate and neuropeptide Y) may affect the pattern of post-junctional AR activation and vasoreactivity (Vanhoutte *et al.* 1981). In the present study, we were concerned with post-junctional AR distribution on arterioles and sought to avoid possible confounding mechanisms associated with nerve activation (Dodd & Johnson, 1993). Adrenergic agonists are effective whether administered through intravascular injection (Remensnyder *et al.* 1962; Hilton *et al.* 1970; Fronek & Zweifach, 1975; Dodd & Johnson, 1993; Buckwalter *et al.* 1997*a*) or applied topically in the superfusion solution (Faber, 1988; Haug & Segal, 2005; Jackson *et al.* 2010). We used the latter approach for the present experiments to minimize possible systemic effects of intravascular delivery (Dodd & Johnson, 1993). Our time controls based upon repeated concentration–response experiments confirmed that $\alpha$AR sensitivity and efficacy were well maintained over time (Supplemental Figs. 1–3). Furthermore, in contrast to earlier studies using nerve stimulation (Marshall, 1982; Boegehold & Johnson, 1988; Dodd & Johnson, 1993; VanTeeffelen & Segal, 2003), arterioles observed here did not undergo 'escape'. This difference may be attributable to constant exposure to the agonist in the superfusion solution instead of sympathetic nerve stimulation that can invoke prejunctional inhibition of neurotransmitter release (Vanhoutte *et al.* 1981; Dodd & Johnson, 1993).

### Effects of combined $\alpha$AR subtype activation

Because individual vessels within a resistance network may have differential sensitivities to NA (Marshall, 1982), it was of interest to ascertain the effects of combined $\alpha$AR subtype activation across arteriolar branch orders within the mouse GM. We found that cumulative addition of NA to the superfusion solution produced robust constriction of 1A, 2A and 3A (Figs 1*A* and 3*A*). Moreover, each branch

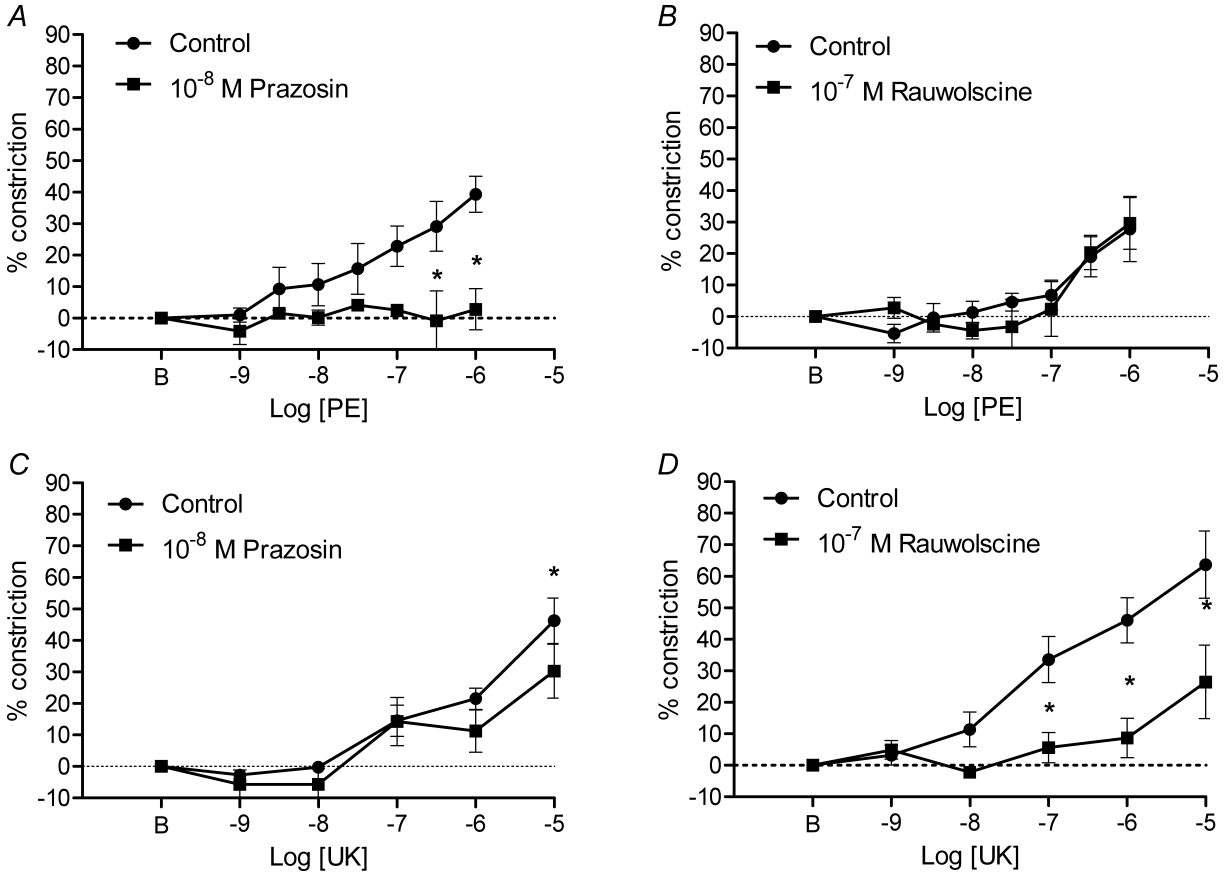


**Figure 4. Activation and inhibition of $\alpha_1$ARs and $\alpha_2$ARs in 1A of mouse GM**
*A*, the $\alpha_1$AR agonist PE progressively increased vasoconstriction that was inhibited by prazosin ($n = 6$). *B*, rauwolscine had no significant affect on constrictions to PE ($n = 5$). *C*, with the exception of an attenuated response at $10^{-5}$ M UK 14304 (UK), prazosin did not affect constrictions to $\alpha_2$AR activation ($n = 5$). *D*, constrictions to UK 14304 were inhibited by rauwolscine ($n = 6$). $^*P < 0.01$ in *A*; $P < 0.05$ in *C* and *D*. B indicates resting baseline.

order had similar maximum responses and log $EC_{50}$ values (Fig. 1). This behaviour contrasts with our findings in the mouse cremaster muscle (Fig. 7*A*) and with earlier studies of the rat spinotrapezius muscle (Marshall, 1982), where distal arterioles appeared to have greater sensitivity to NA. In addition, all branch orders in the GM underwent nearly complete closure at the highest concentrations of NA (Figs. 1*A* and 3*A*), which was not the case for 1A in the cremaster (Fig. 7*A*). The large and consistent arteriolar constrictions evoked by NA illustrated that respective branch orders within the GM are highly sensitive to $\alpha$AR activation and that NA produces similar relative responses across branch orders when $\alpha_1$ARs and $\alpha_2$ARs are stimulated simultaneously.

### Effects of selective $\alpha$AR subtype activation

Concentration–response experiments to NA established that $\alpha$AR were functionally expressed in 1A, 2A and 3A of the GM (Fig. 1*A*). However, our goal was to determine the profile of functional $\alpha$AR subtypes in respective arteriolar branch orders. Remarkably, selective activation of $\alpha_1$ARs and $\alpha_2$ARs resulted in different response characteristics in 1A, 2A and 3A (Fig. 1). The $\alpha_1$AR agonist PE evoked similar absolute diameter changes in respective branches (Fig. 3*B*). When these data were normalized to account for respective differences in arteriolar diameter, constrictions were significantly different with 3A > 2A > 1A (Fig. 1*B*). It therefore appears that functional $\alpha_1$AR are distributed unevenly between arteriolar branch orders, with 3A being the most responsive to PE. In turn, $\alpha_2$AR stimulation with UK 14304 evoked constrictions in 1A that were greater than those evoked in 2A or 3A (Figs. 1*C* and 3*C*). Observing that 3A constricted to closure in response to NA and nearly to closure with PE but not to UK 14304 (Fig. 3) supports the contention that $\alpha_2$ARs are distributed heterogeneously among arteriolar branch orders of the mouse GM. Furthermore, neither $\alpha_1$AR nor $\alpha_2$AR activation alone are as effective as combined $\alpha_1$AR + $\alpha_2$AR activation in constricting arterioles in this muscle. By inference, responses to respective selective agonists (Fig. 1*B* and *C*) provide an index of the contribution of respective $\alpha$AR subtypes during their combined response to NA (Fig. 1*A*).

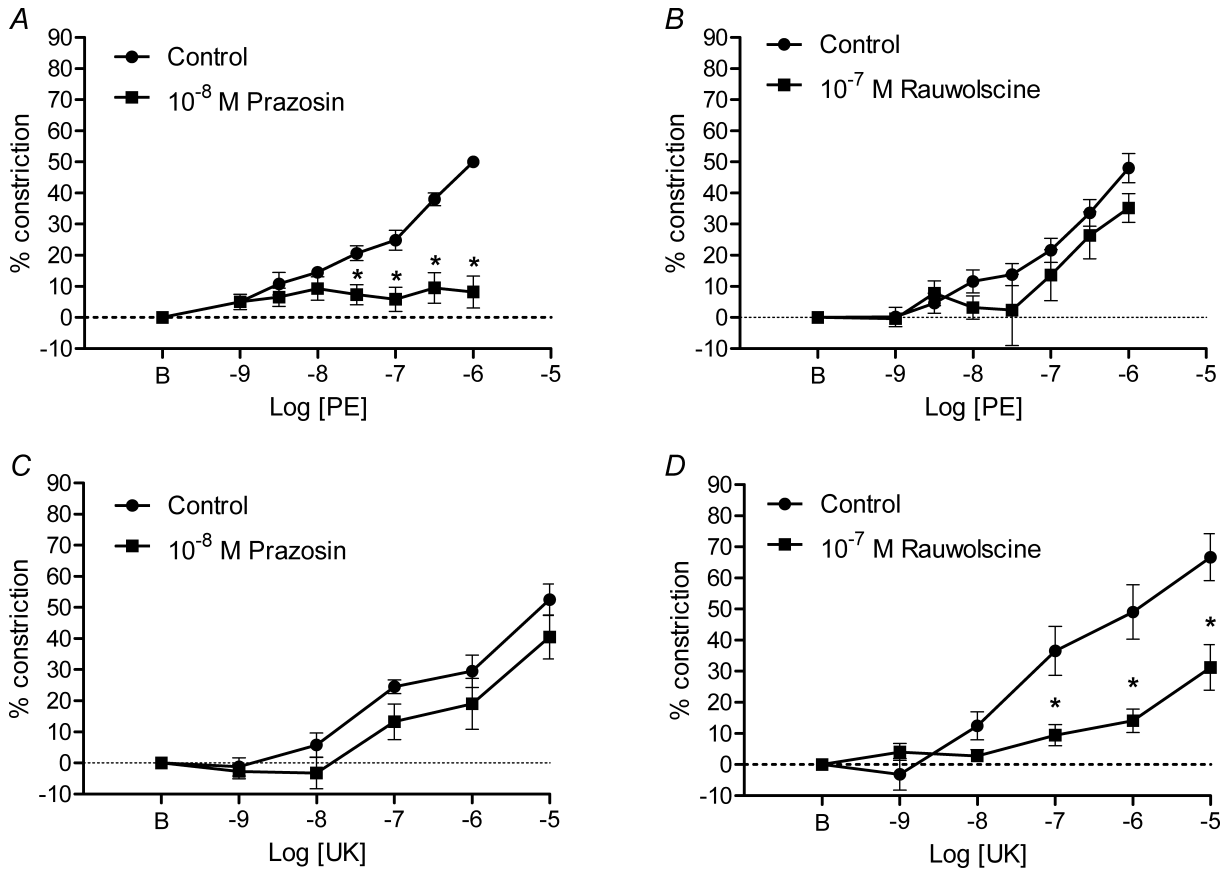


**Figure 5. Activation and inhibition of $\alpha_1$ARs and $\alpha_2$ARs in 2A of mouse GM**
*A*, the $\alpha_1$AR agonist PE progressively increased vasoconstriction that was inhibited by prazosin ($n = 6$). *B*, constrictions to PE were preserved in the presence of rauwolscine ($n = 5$). *C*, constrictions to the $\alpha_2$AR agonist UK 14304 (UK) were preserved in the presence of prazosin ($n = 5$). *D*, constrictions to UK 14304 were inhibited by rauwolscine ($n = 6$). *$P < 0.01$ in *A*; $P < 0.05$ in *D*. B indicates resting baseline.

### Effects of selective αAR subtype antagonists on selective αAR subtype activation

Effective use of αAR antagonists has been a powerful tool in resolving which AR subtype mediates specific physiological responses (Vanhoutte *et al.* 1981; Timmermans *et al.* 1987; Faber, 1988; Haug & Segal, 2005). In the mouse GM, it was unknown whether blockade of one αAR subtype would increase or decrease the sensitivity of arterioles to the remaining αAR subtype. To address this concern, we performed comprehensive control experiments to directly test the selectivity of αAR subtype antagonists as used in the present study. The $\alpha_1$AR antagonist prazosin ($10^{-8}$ M) inhibited responses to the $\alpha_1$AR agonist PE (Figs 4*A*, 5*A* and 6*A*) and this effect was most pronounced in 3A (Fig. 6*A*), where responses to AR stimulation were mediated primarily through $\alpha_1$ARs (Fig. 1). Although prazosin reduced maximal constriction to UK 14304 in 1A slightly (Fig. 4*C*), it had no other significant effects on responses to this $\alpha_2$AR agonist (Figs. 4*C*, 5*C* and 6*C*). The $\alpha_2$AR antagonist rauwolscine ($10^{-7}$ M) produced the largest inhibition to UK 14304 in 1A where the potency of this $\alpha_2$AR agonist was greatest (Figs. 1*C* and 4*D*). Rauwolscine also inhibited responses to UK 14304 in 2A and in 3A where responses to this $\alpha_2$AR agonist were depressed relative to 1A responses (Fig. 1*C*). We conclude that, as used in the present study, prazosin and rauwolscine were selective inhibitors of respective αAR subtypes of the mouse GM.

Neither prazosin nor rauwolscine had significant effects on resting arteriolar diameters (data not shown). Thus if there were constitutive activation of αAR for arterioles in the GM at rest, it was at a level below that required to enhance spontaneous vasomotor tone. This conclusion is consistent with recent findings in the GM, where the non-selective AR antagonist, phentolamine ($10^{-6}$ M), also had no effect on resting arteriolar diameter (Jackson *et al.* 2010; Moore *et al.* 2010). It should be recognized that as a barbiturate anaesthetic, pentobarbital inhibits sympathetic nerve activity. Such actions may explain the

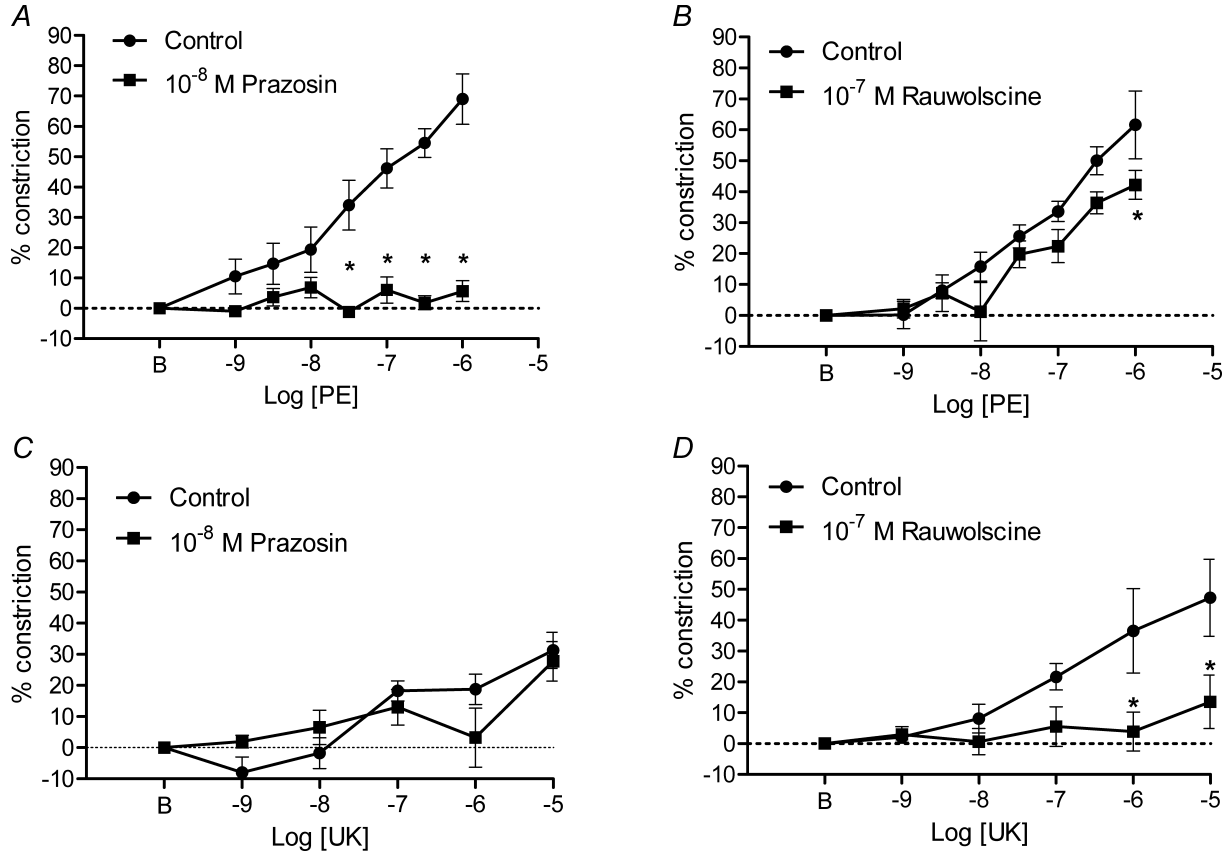


**Figure 6. Activation and inhibition of $\alpha_1$ARs and $\alpha_2$ARs in 3A of mouse GM**
*A*, the $\alpha_1$AR agonist PE progressively increased vasoconstriction that was inhibited by prazosin ($n = 6$). *B*, with the exception of an attenuated response at $10^{-6}$ M, responses to PE were preserved in the presence of rauwolscine ($n = 5$). *C*, constrictions to the $\alpha_2$AR agonist UK 14304 (UK) were preserved in the presence of prazosin ($n = 5$). *D*, constrictions to UK 14304 were inhibited by rauwolscine ($n = 6$). One 3A in this group constricted to closure which elevated the mean response. In all other mice, peak constriction of 3A was typically <30% in response to UK 14304 (see Fig. 1*C*). *$P < 0.01$ in *A*; $P < 0.05$ in *B* and *D*. B indicates resting baseline.

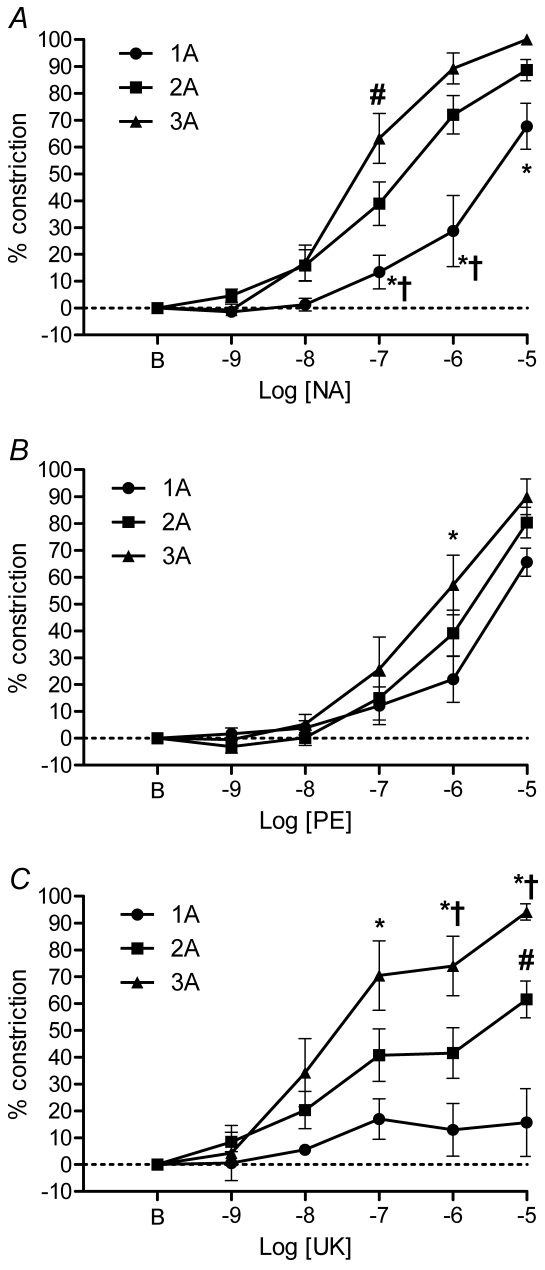


**Figure 7. Arteriolar constriction to αAR activation in mouse cremaster muscle**
*A*, non-selective activation of αARs with NA produced variable levels of percentage maximal constriction across arteriolar branch orders with 3A more sensitive than 2A at $10^{-7}$ M and 1A less sensitive than 2A or 3A between $10^{-7}$ and $10^{-5}$ M. $^{*}P < 0.05$, 1A different from 3A. $^{\#}P < 0.05$, 2A different from 3A. $^{\dagger}P < 0.05$, 2A different from 1A. *B*, selective activation of $\alpha_1$ARs with PE produced similar percentage maximal constriction across arteriolar branch orders but 3A were more sensitive than 1A at $10^{-6}$ M. $^{*}P < 0.05$, 3A different from 1A. *C*, selective activation of $\alpha_2$ARs with UK 14304 (UK) constricted 3A relatively more than 1A or 2A. $^{*}P < 0.05$, 3A different from 1A. $^{\#}P < 0.05$, 2A different than 1A. $^{\dagger}P < 0.05$, 3A different from 2A. $n = 9$ in all panels. B indicates resting baseline.

lack of effect of αAR antagonists on resting tone. In contrast, findings in conscious humans (Dinenno *et al.* 2001) and animals (Buckwalter *et al.* 1997*b*; O'Leary *et al.* 1997) have shown that the inhibition of αARs with phentolamine or prazosin enhance limb blood flow at rest and during exercise. Nevertheless, constitutive activity of αARs is present in the GM preparation of anaesthetized mice as recently illustrated: despite no effect on resting diameter, phentolamine ($10^{-6}$ M) enhanced the magnitude of rapid onset vasodilatation in 'Old' (20 month) male mice (Jackson *et al.* 2010) and promoted spreading dilatation along arterioles in 'Young' (3 month) male mice not different from those studied here (Moore *et al.* 2010). Through direct observations of the microcirculation, the GM preparation is affording new insight into such subtle physiological consequences of adrenergic signalling in the regulation of skeletal muscle blood flow and these actions may well be enhanced in the absence of anaesthesia.

### Regional differences in the functional distribution of αAR subtypes

The most comprehensive examination of functional αAR distribution in the microcirculation of skeletal muscle has come from studies in the rat cremaster muscle (Faber, 1988; McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Ohyanagi *et al.* 1991). Findings from these experiments indicated that functional $\alpha_1$AR predominate in 1A while functional $\alpha_2$AR predominate in 3A. Because our observations in the mouse GM indicated a reciprocal pattern of αAR subtype expression, we tested whether such properties were inherent to the GM, or whether they were manifest in other vascular beds of the mouse. As shown in the mouse cremaster muscle (Fig. 7), PE produced robust constriction of each arteriolar branch order. In contrast to the GM (Fig. 1*C*), UK 14304 evoked the greatest constrictions of cremasteric 3A, with little effect on the diameter of 1A (Fig. 7*C*). Our data from arterioles of the mouse cremaster muscle are therefore consistent with the αAR subtype distribution described for arterioles of the rat cremaster muscle (Faber, 1988; McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Ohyanagi *et al.* 1991).

The present findings collectively indicate that αAR subtype distribution in arteriolar networks varies between the GM and the cremaster muscle of the mouse. Studies of the rat cremaster muscle have concluded that the preponderance of $\alpha_2$ARs on 3A contributes to the ability of these vessels to escape from sympathetic vasoconstriction (McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991). However, our finding that functional $\alpha_1$ARs predominate over $\alpha_2$ARs in 3A of the GM (Fig. 1*B* and *C*) contrasts with such inferences. In turn we suggest

that the greater ability of distal arterioles to dilate during sympathetic nerve activity in other preparations of skeletal muscle (Folkow *et al.* 1971; Marshall, 1982; VanTeeffelen & Segal, 2003) may occur irrespective of $\alpha$AR subtype distribution. Although adrenergic sensitivity has been shown to vary between resistance vessels of different skeletal muscles (Hilton *et al.* 1970; Gray, 1971; Laughlin & Armstrong, 1987), it does not appear related to corresponding differences in muscle fibre type (Aaker & Laughlin, 2002; Lambert & Thomas, 2005). As shown for cutaneous arterioles in the guinea-pig ear, regional variability in sensitivity to $\alpha$AR subtype-selective agonists may be associated with the proportion of sympathetic axons containing the co-transmitter neuropeptide Y (Morris, 1994). In turn, differences between muscles in vascular sensitivity to NA or to sympathetic nerve activity may reflect corresponding differences in the density of terminal sympathetic innervation (Hilton *et al.* 1970). Nevertheless, we conclude that findings based upon the pattern of functional $\alpha$AR subtype distribution in arterioles of the cremaster muscle need not apply to skeletal muscles involved in locomotion. Nor should results shown here for the GM be generalized to other skeletal muscles without appropriate controls.

### Activation and inhibition of $\beta$ARs

Vasodilatation in response to $\beta$AR activation with isoproterenol has been demonstrated in arterioles isolated from rat skeletal muscles (Aaker & Laughlin, 2002), arterioles controlling blood flow in the tenuissimus muscle of anesthetized cats (Fronek & Zweifach, 1975) and to hindlimbs of exercising dogs (Buckwalter *et al.* 1997*a*). In previous studies, propranolol was used proactively to block $\beta$ARs without evaluating their role in responses to adrenergic agonists (Medgett & Langer, 1984; Flavahan *et al.* 1987; Faber, 1988; McGillivray-Anderson & Faber, 1990; Anderson & Faber, 1991; Ohyanagi *et al.* 1991). Here we illustrate that isoproterenol is capable of eliciting near-maximal dilatation across arteriolar branch orders of the mouse GM with similar efficacy and potency (Fig. 2). Given such capacity to relax vascular smooth muscle, we questioned whether constitutive activation of $\beta$ARs may influence constrictor responses to $\alpha$AR activation. Propranolol was found to have no effect on resting diameters (data not shown) thus constitutive $\beta$AR activity was not apparent during our experiments. Selective inhibition of $\beta$ARs with propranolol was demonstrated by the shift in responses to isoproterenol by $\sim$2 log orders in concentration (Supplemental Fig. 4) while having no effect on constrictions to NA, PE, or UK 14304 (Fig. 3). Our findings thereby confirm earlier studies illustrating that $\beta$ARs were not stimulated during activation of $\alpha$ARs (Marshall, 1982; Morris, 1994). Given such pronounced arteriolar dilatations in response to pharmacological activation of $\beta$ARs (Fig. 2), the contribution of these receptors in regulating peripheral resistance remains to be ascertained. In contrast, the role of $\beta$ARs on skeletal muscle fibres in promoting glycogenolysis has been well defined (Dietz *et al.* 1980). As shown in rats (Martin *et al.* 1989*b*) and humans (Martin *et al.* 1989*a*) the density of $\beta$ARs was several-fold greater for slow-twitch oxidative (Type I) muscle fibres compared to fast-twitch glycolytic (Type II) muscle fibres. However, $\beta$ARs had higher affinity in the Type II fibres (Martin *et al.* 1989*b*). While the density of $\beta$ARs on arterioles was similar between muscles, arterioles were more numerous in the soleus (Type I) compared to the gastrocnemius (Type II) muscle (Martin *et al.* 1989*b*), consistent with greater oxidative capacity of the soleus muscle.

### Summary and conclusions

The location of AR subtypes and the control they exert over the resistance vasculature is integral to sympathetic control of muscle blood flow concomitant with the regulation of blood pressure, particularly during muscular exercise. The present study provides evidence that $\alpha$AR subtypes are not uniformly distributed in arteriolar networks of the mouse GM. Third-order arterioles exhibited relatively greater responses to PE than UK 14304, indicating that functional $\alpha_1$AR activation is able to evoke relatively greater constriction in distal arterioles when compared to that evoked by $\alpha_2$ARs. In contrast, 1A in the GM exhibited relatively greater responses to UK 14304 than to PE, indicating that $\alpha_2$AR activation is able to evoke relatively greater constriction in proximal arterioles of this muscle. Prazosin ($10^{-8}$ M) effectively inhibited $\alpha_1$ARs while preserving $\alpha_2$AR responses. Rauwolscine ($10^{-7}$ M) effectively inhibited $\alpha_2$ARs while preserving $\alpha_1$AR responses. This pattern of functional $\alpha$AR subtype distribution in arterioles of the GM differed from that found in the mouse cremaster muscle (or first reported in the rat cremaster muscle; Faber, 1988), where $\alpha_1$ARs predominate in 1A while $\alpha_2$ARs predominate in 3A. The functional distribution of $\beta$ARs appears to be uniform across arteriolar branch orders of the GM as 1A, 2A and 3A responded similarly to isoproterenol with near-maximal dilatations. Vasoconstriction to $\alpha$AR activation was not affected by $\beta$AR inhibition as propranolol had no effect on arteriolar responses to NA, PE or UK 14304, while clearly inhibiting responses to isoproterenol. The consistency observed among our results obtained with agonists and antagonists targeted to AR subtypes confirms the selectivity of our pharmacological interventions.

Distal branches of the resistance network have long been recognized to more readily 'escape' from sympathetic vasoconstriction (Folkow *et al.* 1971; Mellander, 1971; Marshall, 1982). Comprehensive studies in the rat cremaster muscle have associated such behaviour with the

prevalence of $\alpha_2$ARs on distal arterioles, in contrast to the prevalence of $\alpha_1$ARs on proximal arterioles. Our present findings in the mouse cremaster muscle support these original studies. Nevertheless, our present findings in the mouse GM illustrate that this reasoning need not apply to other skeletal muscles. Implicit to this interpretation is that properties other than $\alpha$AR subtype contribute to the ability of distal arterioles to oppose sympathetic vasoconstriction. The interaction between sympathetic neuroeffector signalling and metabolic demand in skeletal muscle should account for the functional distribution of $\alpha$AR subtypes in the muscle of interest. Insight gained from the present experiments provides a foundation for the mouse GM as a model for investigating signalling events mediated through $\alpha_1$ARs *vs.* $\alpha_2$ARs which contribute to the adrenergic restriction of muscle blood flow with ageing (Proctor & Joyner, 1997; Dinenno *et al.* 2001; Jackson *et al.* 2010).

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## Author contributions

A.W.M., W.F.J. and S.S.S. contributed to the conception, experimental design, analysis and interpretation of the experiments contained in this study. A.W.M. performed all of the experiments in the laboratory of S.S.S. at the University of Missouri and prepared the first draft of this article which was edited by W.F.J. and S.S.S. All co-authors have approved the version submitted to be considered for publication.

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