Alpha-1 adrenoceptor stimulation triggers axon-reflex vasodilatation in human skin

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Abstract

The aim of this study was to determine whether pre-treatment of human skin with the α_1-adrenoceptor antagonist terazosin would block vasoconstrictor responses and axon-reflex vasodilatation to the α_1-adrenoceptor agonist methoxamine. Drugs were administered by iontophoresis into the skin of the forearm of 15 healthy participants, and skin blood flow was monitored with a laser Doppler flow probe at the site of methoxamine iontophoresis (to monitor direct vasoconstrictor responses) or 5-10 mm from the site of methoxamine iontophoresis (to monitor axon-reflex vasodilatation). Experimental sites were pre-treated with terazosin (administered by iontophoresis for 10 minutes at 200 μA), and the same current intensity was passed through 0.9% saline to control for the nonspecific effects of iontophoresis. Pre-treatment with terazosin blocked vasoconstrictor responses to increasing doses of methoxamine, and also blocked vasodilatation several mm from the site of terazosin and methoxamine administration. These findings support the view that α_1-adrenoceptors play a role in generating axon-reflex vasodilatation, and thus might contribute to local vascular disturbances in acute and chronic inflammation.

Key words: axon-reflex vasodilatation; neurogenic inflammation; α_1-adrenoceptors; skin blood flow

Introduction

During noxious stimulation and tissue injury, inflammatory mediators such as hydrogen ions, bradykinin, prostaglandins and cytokines sensitize sensory afferents and initiate neurogenic inflammation (Richardson and Vasko, 2002). This response is coordinated by the sensory afferents that detect the noxious stimulus, and involves release of substance P and calcitonin gene-related peptide from the axonal network around the site of stimulation (Schmelz and Petersen, 2001). These neuropeptides increase vascular permeability and dilate blood vessels within the receptive field of the stimulated neuron (a process termed axon-reflex vasodilatation), thereby recruiting blood-borne immune cells and dispersing harmful substances.

Under certain conditions, the sympathetic neurotransmitter noradrenaline may enhance axon-reflex vasodilatation. The most convincing evidence of this has come from laboratory studies of rats. For example, sympathectomy was found to inhibit the flare generated by intradermal injection of capsaicin in the rat hindlimb, whereas intra-arterial injection of the α_1-adrenoceptor agonist phenylephrine restored the capsaicin-induced flare (Lin et al., 2003). In sympathetically-intact rats, blockade of peripheral α_1-adrenoceptors with the α_1-adrenceptor antagonist terazosin inhibited the flare to capsaicin. Similarly, sympathectomy and pre-treatment with terazosin inhibited sensitization of nociceptive C-fibres to intradermal injection of capsaicin in the rat hindpaw, whereas intra-arterial injection of noradrenaline restored the capsaicin-induced sensitization after sympathectomy (Ren et al., 2005). These findings are consistent with reports that sympathectomy inhibits behavioural signs of pain in certain animal models of inflammatory and neuropathic pain (Kinnman and Levine 1995; Kim et al., 1993; Shir and Seltzer, 1991; Xie et al., 2001). Conversely, α-adrenergic agonists evoke nociceptive discharge and pain in animals after nerve

injury and inflammation (Ali et al., 1999; Nam et al., 2000; Dogrul et al., 2006), and in experimental and clinical studies in humans (Torebjork et al., 1995; Fuchs et al., 2001; Mailis-Gagnon and Bennett, 2004; Jorum et al., 2007; Drummond, 2009).

Despite such findings, it has been assumed that sympathetic and nociceptive fibres do not interact directly under physiological conditions in human skin, as intradermal injection of noradrenaline or delivery through microdialysis fibres is painless and does not evoke axon-reflex vasodilatation (Zahn et al., 2004). Nevertheless, intradermal microdialysis of noradrenaline was found to decrease the temperature threshold of axon-reflex vasodilatation to local heat in healthy humans, and sympathetic blockade attenuated this response (Houghton et al., 2006; Hodges et al., 2008). Furthermore, pre-treatment of the skin with local anaesthetic agent blocked the flare produced by noradrenaline when it was introduced into the skin by iontophoresis (Drummond and Lipnicki, 1999). Together, these findings imply that noradrenaline facilitates axon-reflex vasodilatation initiated by heat or iontophoretic currents in humans, and thus might contribute to axon-reflex vasodilatation evoked by inflammatory mediators after nerve or tissue injury.

In rats, the adrenergic component of axon-reflex vasodilatation to capsaicin is mediated by α_1-adrenoceptors (Lin et al., 2003) but whether this also applies in humans is uncertain because mechanisms of neurogenic inflammation differ between rat and human skin (Schmelz and Petersen, 2001; Sauerstein et al., 2000). Thus, the aim of the present study was to determine whether α_1-adrenoceptors also mediate axon-reflex vasodilatation in healthy human subjects. In particular, the aim was to establish whether prior administration of the α_1-antagonist terazosin would block axon-reflex vasodilatation to the α_1-agonist methoxamine. If so, this would support

the view that α_1-adrenoceptors play an important role in neurogenic inflammation in humans.

Method

Subjects

The sample consisted of 15 healthy volunteers (4 men and 11 women aged between 18 and 47 years) who were not taking prescription medication for any medical condition. They each provided informed consent for the procedures, which were approved by the Murdoch University Human Research Ethics Committee.

Procedures

The experiments were carried out in a temperature-controlled laboratory maintained at 22 $\pm$ 1^{o}C. The first step in each experiment was to introduce terazosin into the skin to block α_1-adrenoceptors. To determine whether the terazosin blockade was successful, vasoconstrictor responses to incremental doses of methoxamine were examined at terazosin-pretreated and control sites. Axon-reflex vasodilatation to methoxamine was examined adjacent to the site of terazosin pre-treatment and methoxamine administration in the final seven participants.

Pre-treatment with terazosin. Perspex iontophoresis capsules with an internal chamber diameter of 20 mm were attached with adhesive washers to the dorsal aspect of the left and right forearms, and 3 cm x 5 cm silver plates that acted as cathodes were attached to the wrists. Cotton swabs soaked with 0.9% saline were placed under the cathodes to enhance electrical contact with the skin. To ensure that the capsules adhered firmly and that good electrical contact was made, the forearm sites were shaved, if necessary, and cleaned with an isopropyl alcohol swab. Care was taken not to touch the skin with the razor while the hair was being removed. The perspex chambers were filled with a conducting medium (0.9% saline-control in one chamber

and 10 mM terazosin hydrochloride in the other; Sigma, Sydney, Australia) and anodal currents of 0.2 mA were passed through the solutions to repel positively changed ions into the skin for 10 minutes (Drummond, 2002).

Effect of the terazosin pre-treatment on vasoconstrictor responses to methoxamine (Figure 1A). Purpose-built iontophoresis capsules placed over the treated sites in the forearms were maintained at 40^{o}C to facilitate laser Doppler detection of cutaneous vasoconstriction (Lipnicki and Drummond, 2001). The capsules had an internal chamber diameter of 10 mm. The wide surface area probe of a Moor Instruments MBF3D laser Doppler flowmeter (Axminster, England) was inserted into the roof of the chamber several mm above the skin to monitor vasomotor responses at sites of drug entry. A solution of 10 mM methoxamine hydrochloride (Sigma, Sydney, Australia) was prepared on the day of the experiment with de-ionized water. The chamber beneath the flowmeter probe was filled with the mexthoxamine solution through a stainless steel inlet tube. After 10 minutes of skin heating, a 5 µA anodal current was directed through the drug solution for 60 seconds to introduce positively-charged methoxamine ions into the skin. Skin blood flow was monitored during the iontophoresis and for another four minutes afterwards at the terazosin-pretreated and control sites. The same procedure was repeated at currents of 10 µA through to 320 µA, doubling the current intensity after each step. As current duration was held constant at 60 seconds, the dose of methoxamine introduced into the skin was proportional to the current intensity. Cathodes on the wrists completed the electrical circuits.

Effect of the terazosin pre-treatment on axon reflex vasodilatation to methoxamine. In the final seven participants, additional sites on the forearms were pre-treated with terazosin and 0.9% saline (control). The pre-treatment procedures

were similar to those described above except that a central region of skin, 10 mm in diameter, was protected from the drug solution by a thick smear of petroleum jelly (Vaseline) (Figure 1B). Treated skin generally flushed after the iontophoresis of terazosin whereas the central region remained pale. After the pre-treatment, the petroleum jelly was removed and the subject rested quietly for 10 minutes to allow nonspecific vasomotor responses to the pre-treatment to subside. Iontophoresis capsules with a ring-shaped drug solution chamber (inner diameter 15 mm and outer diameter 19 mm) were then attached to the prepared sites and filled with 10 mM methoxamine solution. Adhesive tape was used to form a waterproof seal on each side of the drug solution chamber. The capsules were maintained at 32^{o}C (i.e., below the threshold for local heat-evoked vasodilatation) throughout the methoxamine iontophoreses that ranged in current strength from 5 µA to 320 µA, as described above. During each iontophoresis, positively-charged methoxamine ions were repelled away from the anode into the underlying skin for 60 seconds, followed by a 4-minute period of vascular monitoring. Skin blood flow was detected with a wide surface area laser Doppler flow probe positioned in the centre of the ring described by the iontophoresis chamber, 5-10 mm from the site of methoxamine administration over skin that had been covered with petroleum jelly during the pre-treatment (Figure 1B).

Data reduction and statistical approach

Skin blood flow was averaged for 1-2 minutes before the first iontophoresis to establish a baseline. As responses generally began during the 1-minute period of iontophoresis and peaked during the subsequent 4-minute period of monitoring, the mean response over the full 5-minute period was calculated. Blood flow was measured both in terms of mean flux (the low-frequency component of the signal

reflecting tissue perfusion to a depth of 1-2 mm) and the amplitude of the high frequency pulsatile waveform (representing the beat-to-beat difference between arterial inflow and venous outflow). As skin blood flow was recorded in arbitrary rather than absolute units, responses were expressed as the percent change from levels at baseline.

Differences in response to methoxamine at the terazosin-treated and control sites were investigated in Drug Treatment (terazosin, control) x Dose (the seven current intensities ranging from 5 μA to 320 μA) repeated measures analyses of variance. Where appropriate, the Greenhouse-Geisser epsilon was used to adjust the degrees of freedom to correct for violations of the sphericity assumption. Differences in blood flow between treated and control sites at baseline were investigated with Student's paired t-tests.

Results

At baseline, neither mean flux nor pulse amplitude differed between the terazosin-treated and control sites in skin heated to 40^{o}C, although values were substantially higher in heated than unheated skin (Figure 2). Similarly, blood flow did not differ significantly between control sites and sites that had been covered with petroleum jelly during the terazosin pre-treatment.

The terazosin pre-treatment blocked vasoconstriction to methoxamine (Figure 3). In particular, the Drug Treatment x Dose interaction was significant both for mean flux [$F(1.6,22.7) = 3.99$, $p<0.05$] (Figure 3A) and pulse amplitude [$F(2.2,31.1) = 3.50$, $p<0.05$] (Figure 3B), due to divergent responses between the treated and control sites at higher doses of methoxamine.

Similarly, the terazosin pre-treatment blocked signs of axon-reflex vasodilatation at higher doses of methoxamine (Figure 4). Again, the Drug Treatment

x Dose interaction was significant both for mean flux [$F(1.2,7.4) = 5.36$, $p<0.05$] (Figure 4A) and pulse amplitude [$F(1.7,10.3) = 8.90$, $p<0.01$] (Figure 4B).

Discussion

Pre-treatment with the α_1-antagonist terazosin blocked the vasoconstrictor response to the α_1-agonist methoxamine, indicating that the terazosin blockade was successful. The terazosin pre-treatment also blocked vasodilatation several mm from the site of methoxamine administration. Effects were detected not only for mean tissue perfusion (reflected by changes in flux) but also for pulsatile flow (reflected by the amplitude of beat-to-beat pulsations). These findings support the view that stimulation of α-adrenoceptors may play a role in generating the axon-reflex vasodilatation that accompanies acute and chronic inflammation not only in rats (Lin et al., 2003) but also in humans (Drummond and Lipnicki, 1999; Houghton et al., 2006; Hodges et al., 2008).

Adrenergic stimulation of sensory afferents

Although the means by which adrenergic agents affect axon reflex vasodilatation is uncertain, some findings suggest that direct stimulation of α_1-adrenoceptors on neural membranes contributes to antidromic neuronal discharge and axon-reflex vasodilatation. Messenger RNA (mRNA) for α_{1A}-, α_{1B}- and α_{2C}-adrenoceptors has been demonstrated within rat dorsal root ganglion neurons (Nicholson et al., 2005), and α_1-adrenoceptor mRNA increased in the dorsal root ganglia of rats after spinal nerve ligation and sciatic nerve transection (Xie et al., 2001; Maruo et al., 2006) and in rats with streptozotocin-induced diabetic neuropathy (Lee et al., 2000). At the physiological level, the α_1-agonist phenylephrine was found to mobilize intracellular calcium in cultured dorsal root ganglion cells, and to liberate

substance P from the distal terminals of nociceptive afferents in the urinary bladder (Trevisani et al., 2007).

Other data suggest that adrenergic agents stimulate the release of intermediary agents which excite nociceptive afferents. For example, phenylephrine was found to increase the secretion of nerve growth factor, a potent nociceptive agent, from cultured vascular smooth muscle (Tuttle et al., 1993), and to facilitate the nociceptive effects of adenosine triphosphate (Meisner et al., 2007). In the rat kidney, increases in renal sympathetic nerve activity were associated with increases in afferent renal nerve activity; in particular, noradrenaline acted via α_1-adrenoceptors to increase the synthesis and release of prostaglandin E_2 which, in turn, liberated substance P from nociceptive afferents (Kopp et al., 2007).

Although the studies reviewed above were carried out on rats rather than humans, it is tempting to speculate that direct stimulation of neural α_1-adrenoceptors or the release of intermediary agents such as prostaglandin E_2 evoked axon reflexes to methoxamine in the present study.

Role of inflammation

Iontophoretic currents induce an inflammatory response in human skin (Grossmann et al., 1995) which is mediated at the anode by prostaglandin synthesis (Tartas et al., 2005). However, current-evoked prostaglandin production does not account entirely for axon-reflex vasodilatation to methoxamine, because the response was profoundly inhibited by pre-treatment with the α_1-antagonist terazosin in the present study. The inflammatory mediators released by iontophoretic currents may disrupt the blood-nerve barrier, thereby increasing access to protected α_1-adrenoceptors in cutaneous nerve fascicles (Drummond, 1998). This could explain why the iontophoresis of adrenergic agonists evokes axon-reflex vasodilatation

(Drummond and Lipnicki, 1999) whereas administration via intradermal microdialysis fibres does not (Zahn et al., 2004).

Clinical implications

Although neurogenic inflammation is primarily protective, an abnormal excitability of this response could sensitize nociceptive afferents and promote chronic inflammation (Peters et al., 2006; Cunha et al., 2008). The present findings suggest that α_1-adrenoceptors contribute to this process by evoking axon-reflex vasodilatation. During chronic inflammation, increased access to neural α_1-adrenoceptors could establish a vicious circle between sympathetic neural discharge, neurogenic inflammation and pain. This might be important in genitourinary tract disorders (Trevisani et al., 2007) and in conditions such as complex regional pain syndrome, which is characterized by elevated levels of inflammatory mediators, enhanced axon-reflex vasodilatation and signs of adrenergic supersensitivity in the affected limb (Schinkel et al., 2006; Wesseldijk et al., 2008; Weber et al., 2001; Birklein and Schmelz, 2008; Drummond et al., 1996; Arnold et al., 1993; Chemali et al. 2001; Jorum et al., 2007; Gibbs et al., 2008).

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Figure legends

Figure 1. A. To investigate the effect of terazosin pre-treatment on vasoconstrictor responses to methoxamine, terazosin (or saline control) was introduced into a 20 mm diameter circle of skin, followed by methoxamine in a 10 mm diameter circle at the centre of the pre-treated site. The skin was heated to 40^{o}C, and skin blood flow was measured from the central methoxamine-treated site with a laser Doppler flow probe. B. To investigate the effect of terazosin pre-treatment on axon-reflex vasodilatation to methoxamine, terazosin (or saline control) was introduced into a ring of skin (outer diameter 20 mm and inner diameter 10 mm). The central region was covered with petroleum jelly to insulate against passage of the iontophoretic current. Methoxamine was then administered in a ring at the site of terazosin or saline pre-treatment (outer diameter 19 mm and inner diameter 15 mm). Skin blood flow was measured from the central untreated site with a laser Doppler flow probe.

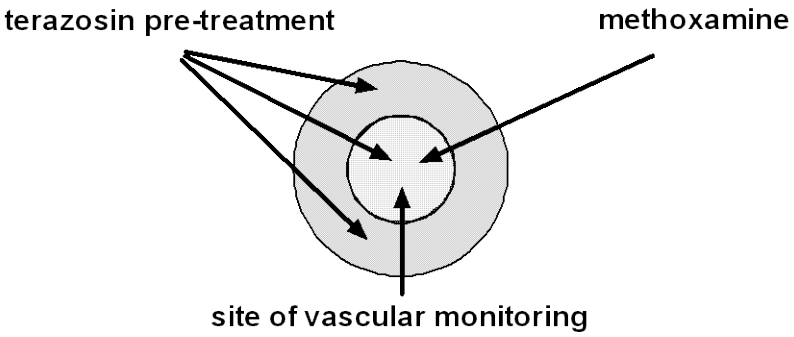
Figure 2. Skin blood flow (± S.E.) at baseline in skin maintained at 32^{o}C or 40^{o}C. Neither mean flux (A) nor pulse amplitude (B) differed significantly between the control site and the site of α_1-blockade in heated skin, or between control sites and sites that had been covered with petroleum jelly during the terazosin pre-treatment (unheated sites).

Figure 3. Change in skin blood flow (± S.E.) after the iontophoresis of methoxamine at sites pre-treated with the α_1-antagonist terazosin or 0.9% saline-control (see Figure 1A). The dose of methoxamine introduced into the skin was proportional to the iontophoresis current (measured in μA). Pre-treatment with terazosin blocked decreases in mean flux (A) and pulse amplitude (B) at higher doses of methoxamine (* difference between control and terazosin pre-treated site statistically significant, $p<0.05$). Skin blood flow increased above baseline at the terazosin-treated site after

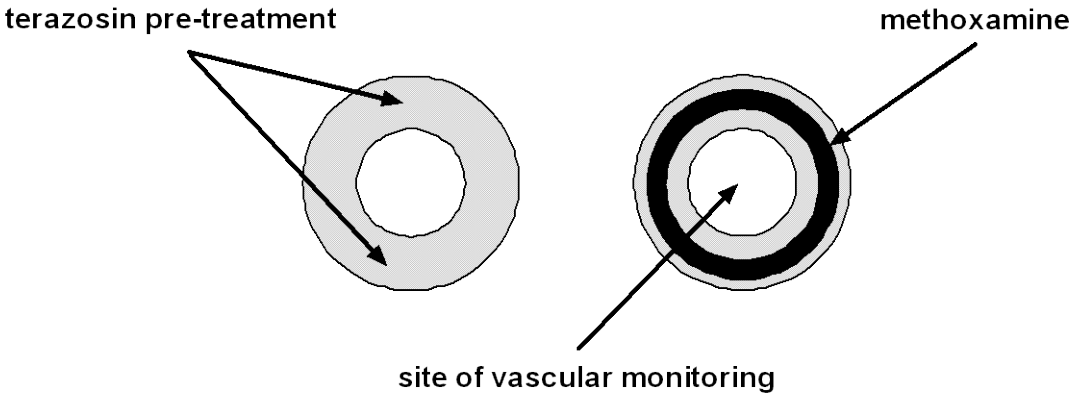
low doses of methoxamine, and decreased below baseline at the control site after high doses of methoxamine (# difference from baseline statistically significant, $p<0.05$).

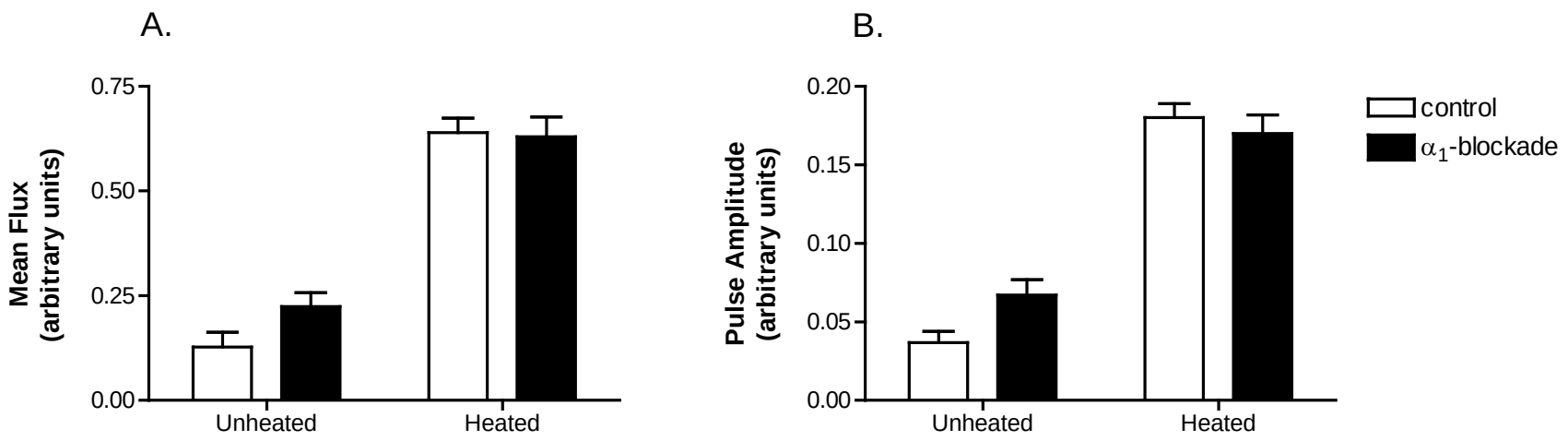
Figure 4. Change in skin blood flow (± S.E.) in the central untreated region after the iontophoresis of methoxamine in skin pre-treated with the α_1-antagonist terazosin or 0.9% saline-control (see Figure 1B). Skin blood flow increased at both sites after the iontophoresis of methoxamine (# difference from baseline statistically significant, $p<0.05$). However, pre-treatment with terazosin inhibited increases in mean flux (A) and pulse amplitude (B) at higher doses of methoxamine (* difference between control and terazosin pre-treated site statistically significant, $p<0.05$).

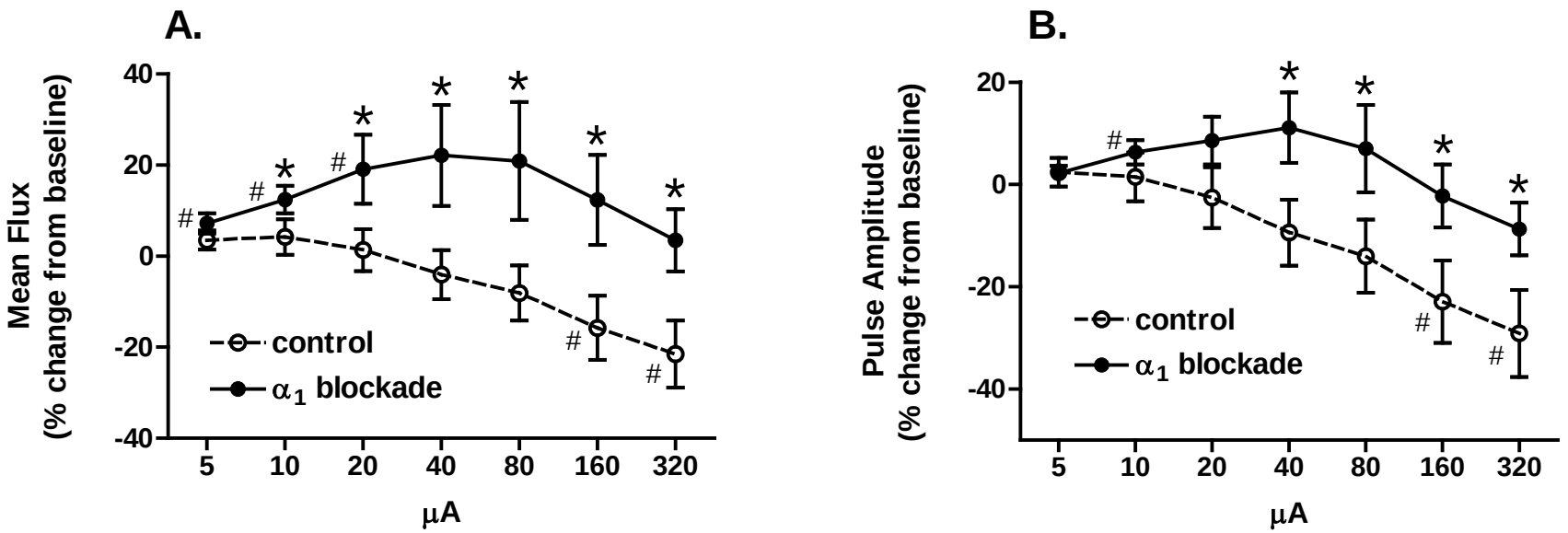
A. Vasoconstriction

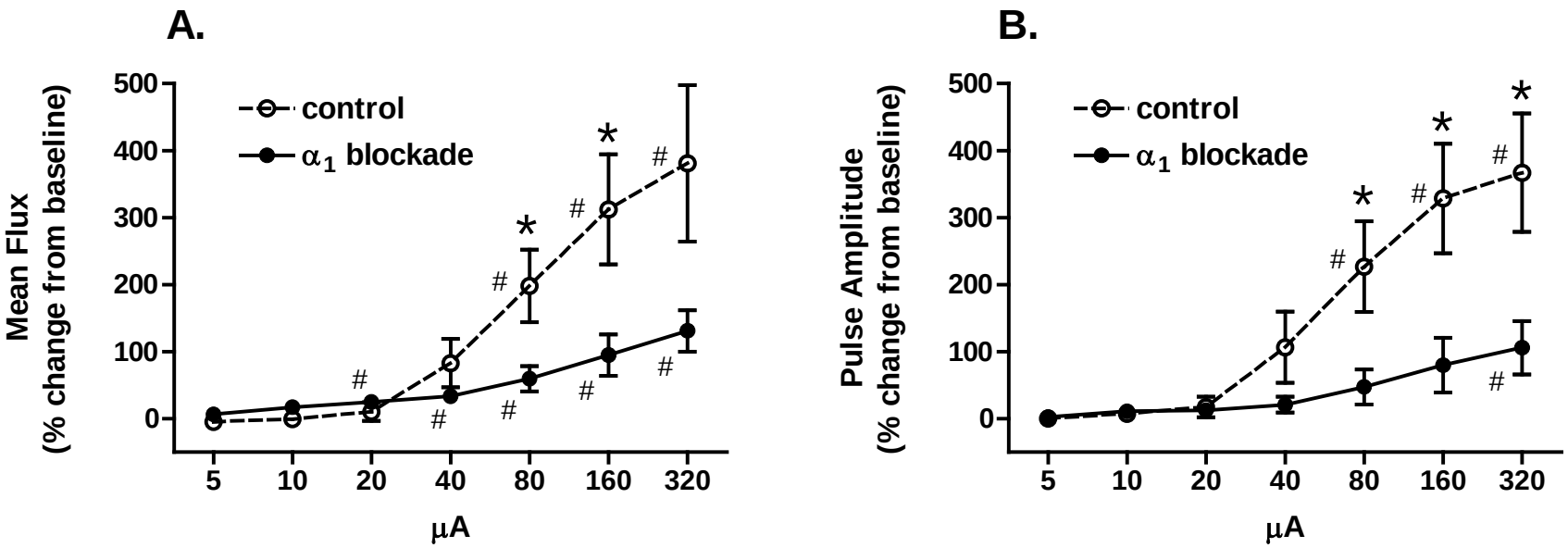


B. Axon-Reflex Vasodilatation




A.
B.
Mean Flux
(arbitrary units)
0.75
0.50
0.25
0.00
Unheated
Heated
Pulse Amplitude
(arbitrary units)
0.20
0.15
0.10
0.05
0.00
Unheated
Heated
control
α1-blockade


A.
Mean Flux
(% change from baseline)
40
20
0
-20
-40
5
10
20
40
80
160
320
μA
control
α1 blockade
#
*
B.
Pulse Amplitude
(% change from baseline)
20
0
-20
-40
5
10
20
40
80
160
320
μA
control
α1 blockade
#
*


A.
Mean Flux
(% change from baseline)
500
400
300
200
100
0
control
α1 blockade
5
10
20
40
80
160
320
μA
#
*
B.
Pulse Amplitude
(% change from baseline)
500
400
300
200
100
0
control
α1 blockade
5
10
20
40
80
160
320
μA
#
*