Experimental Physiology

AT_1 receptors are necessary for eccentric training-induced hypertrophy and strength gains in rat skeletal muscle

Todd A. McBride

Department of Biology, California State University, Bakersfield, Bakersfield, CA 93311, USA

This study was undertaken to measure the response of skeletal muscle to eccentric contractions (EC) in the presence of the angiotensin type 1 (AT_1) receptor blocker, losartan. It was hypothesized that blocking AT_1 receptors prior to an initial bout of EC would prevent the muscle from developing the normal adaptation to EC as demonstrated by the repeated bout effect. It was also hypothesized that continuous AT_1 receptor blockade during EC training would significantly reduce muscle hypertrophy and strength gains that occur with repeated EC. Rats received losartan in their drinking water at either a low dose (20 mg (kg body weight)$^{-1}$ day^{-1}) or a high dose (40 mg (kg body weight)$^{-1}$ day^{-1}). Each bout of EC consisted of a total of 24 contractions. Rats were assigned to four groups: a single acute bout of EC ($n = 6$); two bouts of EC separated by 14 days ($n = 8$); and 4 weeks of training twice a week on the low dose ($n = 5$) or the high dose ($n = 9$). There was no effect of AT_1 receptor blockade on the initial loss of function following a single acute bout of EC, or on the repeated bout effect following a second exposure to EC. AT_1 receptor blockade did alter the results of EC training, in both the low and high dose groups. Losartan treatments prevented EC training-induced increases in muscle wet and dry weights compared to untreated rats. Finally, the low and high dose losartan treatments also prevented an increase in muscle contractile force following EC training compared to the untreated group. Functional AT_1 receptors are therefore not necessary for an acute adaptation to EC as demonstrated by the repeated bout effect, but are necessary for muscle hypertrophy and increased contractile force associated with EC training.

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Corresponding author T. A. McBride: California State University, Bakersfield, Bakersfield, CA 93311, USA.
Email: tmcbride@csub.edu

A novel bout of eccentric contractions (EC), during which the muscle lengthens while developing force, results in a well-characterized sequence of muscle damage and loss of contractile function. The damage and loss of function have been attributed to a number of mechanisms, including: disruptions in sarcolemmal integrity (Armstrong, 1990; McNeil & Khakee, 1992; McBride, 2003; Lovering & De Deyne, 2004); z-band streaming and sarcomere disruption (Friden *et al.* 1983; Morgan, 1990; Friden & Lieber, 1992; Morgan & Allen, 1999); cytoskeletal protein disruption (Lieber *et al.* 1994, 1996); and a failure of normal excitation–contraction-coupling mechanisms (Ingalls *et al.* 1998; Warren *et al.* 2001). The initial damage phase is followed by recovery of normal muscle function within days to weeks depending on the intensity of the EC protocol (Clarkson & Tremblay, 1988; Warren *et al.* 1993; Brooks *et al.* 1995; McBride *et al.* 1995, 2000; Ingalls *et al.* 2004). The recovery of skeletal muscle from EC injury includes an adaptive response, referred to as the repeated bout effect (Nosaka & Clarkson, 1995). The repeated bout effect results in a muscle that is resistant to damage and recovers normal function more rapidly following subsequent bouts of EC (Clarkson & Tremblay, 1988; Sacco & Jones, 1992; McBride *et al.* 1995; McHugh *et al.* 1999; McHugh, 2003; Ingalls *et al.* 2004). Although several mechanisms for the acute damage and loss of contractile function have been reported, the mechanisms for adaptation of skeletal muscle to EC are not well characterized. Examples of potential mechanisms for the repeated bout effect include neural, mechanical and cellular adaptations, which are outlined in two recent review articles (McHugh *et al.* 1999; McHugh, 2003).

When repeated over time in a training regime EC provide an effective stimulus for muscle hypertrophy and

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strength gains (Wong & Booth, 1988; Adams *et al.* 2002; McBride, 2003). A number of different markers used to identify intracellular pathways related to muscle cell growth and protein accumulation have been measured specifically following EC. These include the early response gene *c-fos* (McBride, 2003), mitogen-activated protein kinase (MAPK) (Nader & Esser, 2001), and 70-kDa S6 protein kinase ($p70^{S6k}$) (Barr & Esser, 1999). In most investigations, markers of cell growth have been measured following a single exposure to EC in conjunction with the initial damage response. It is not clear whether the intracellular pathways stimulated by the early events in the damage response to EC are the same as those responsible for the training effect accompanying repeated bouts of EC. Several intracellular pathways related to muscle cell growth and repair are stimulated by the mechanical stretch associated with EC, so overlap between acute and EC training responses is likely.

The renin–angiotensin system (RAS) represents a hormonal pathway for muscle cell growth and hypertrophy that can be activated directly by mechanical stretch in cardiomyocytes (Sadoshima *et al.* 1993; Miyata *et al.* 1996; Malhorta *et al.* 1999). Two separate sources of angiotensin II (Ang II) may contribute to the hypertrophy response in both cardiac and skeletal muscle. The first source is systemic Ang II from the RAS, whereby circulating angiotensinogen produced by hepatocytes is converted to Ang I by renin released from the kidney and then to Ang II by angiotensin converting enzyme (ACE) released from the capillary endothelium of various tissues. The second source of Ang II is a separate local RAS that has been identified in many tissues, which is stimulated by local signals such as cell stretch (Sadoshima *et al.* 1993), resulting in a local production and release of Ang II in an autocrine/paracrine manner (Sadoshima *et al.* 1993; Jones & Woods, 2003). Both sources of Ang II then affect cells via the stimulation of angiotensin type 1 (AT_1) receptors on the cell surface.

Stimulation of the RAS is necessary for stretch- or overload-induced muscle cell hypertrophy in cultured neonatal rat myocytes (Sadoshima *et al.* 1993) and in adult rat skeletal muscle *in vivo* (Gordon *et al.* 2001). The dependence on a local RAS for hypertrophy of cardiomyocytes in an autocrine/paracrine fashion has been demonstrated *in vitro*, independent of the systemic RAS (Sadoshima *et al.* 1993). Skeletal muscle release of Ang II resulting from a tissue-specific stimulus such as stretching is most likely to depend on both the uptake and the storage of components from the systemic RAS as well as local production of Ang II by skeletal muscle cells (Jones & Woods, 2003). The addition of Ang II to chick heart cells *in vitro* (Baker & Aceto, 1990) and the infusion of Ang II into rats *in vivo* with the absence of a pressor response (Dostal & Baker, 1992; Susic *et al.* 1996) can also directly increase protein synthesis and cell growth in cardiac muscle, independent of a loading phenomenon. Collectively these observations make Ang II and the RAS an interesting candidate for a role in skeletal muscle repair, adaptation and hypertrophy in response to EC.

The goal of the present study was to determine whether adaptation to EC and skeletal muscle hypertrophy in response to an EC training regime are dependent on functional AT_1 receptors. It was hypothesized that blockade of AT_1 receptors prior to an initial bout of EC would prevent the muscle from developing the normal adaptive response as demonstrated by the repeated bout effect. This would result in a loss of function following a second bout of EC similar to that measured following an initial acute bout of EC. It was also hypothesized that continuous treatment to block AT_1 receptors during EC training would inhibit the normal training response and significantly reduce the strength and hypertrophy gains previously measured after EC training.

Methods

Animals

Female Sprague–Dawley rats, 3 months old, with body weights ranging from 240 to 270 g, were used. All animal care and use protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of California State University, Bakersfield, and were consistent with NIH guidelines. Animals were housed in a temperature-controlled room (19–21°C) with a 12 h–12 h light–dark cycle. Rats were provided with unlimited access to standard rat chow and water.

Blockade of AT_1 receptors

AT_1 receptors were blocked by administration of losartan in the drinking water at either a low dose (0.3 mg ml^{-1}) or a high dose (0.6 mg ml^{-1}). The losartan was a generous gift from Merck Research Laboratories, Rahway, NJ, USA. Daily water intake was monitored, and resulted in an average dose of 20 mg (kg body weight)$^{-1}$ day^{-1} in the low dose group and 40 mg (kg body weight)$^{-1}$ day^{-1} in the high dose group (Thienelt *et al.* 1997). Treatment started 3 days before the first bout of EC and continued until muscle contractile function was completed.

Confirmation of AT_1 blockade

A separate group of rats from those performing EC was used for evaluation of the losartan treatment. The effectiveness of losartan treatment via the drinking water was confirmed by measurement of the blood pressure response to a bolus injection of Ang II (1 μg (kg body weight)$^{-1}$ I.A.; Symons *et al.* 1999). All measurements were performed following 3 days of losartan treatment at either the low dose ($n = 3$) or the high dose ($n = 6$),

and compared to untreated controls ($n = 3$). Blood pressure was measured with a pressure transducer (Biopac, Santa Barbara, CA, USA) connected to a catheter placed in the carotid artery of the anaesthetized rats. Blood pressure values were continuously monitored during the procedure using a Biopac MP30 data acquisition system. The pressor response was measured as the difference between preinfusion systolic blood pressure (in mmHg) and the peak systolic blood pressure following Ang II infusion. Data acquisition and analysis were performed using Biopac BSL Pro software.

Experimental groups

Single bout of eccentric contractions. Animals in this group were subjected to a single acute bout of EC following the high dose losartan treatment (losartan single acute EC; $n = 6$) and compared to untreated animals performing the same acute bout of EC (untreated single acute EC; $n = 6$). Contractile function was measured in the right exercised and left contralateral control tibialis anterior (TA) muscles 2 days following the acute bout of EC. The TA muscles were then removed from the anaesthetized rats before they were killed by, and the muscles were immediately weighed to determine muscle wet weight values.

Two bouts of eccentric contractions. Animals in this group were subjected to two bouts of EC with 14 days recovery between each bout while receiving the high dose losartan treatment (losartan second EC; $n = 8$), and compared to untreated animals following the same EC protocol (untreated second EC; $n = 5$). Contractile function was measured in the right exercised and left control TA muscles 2 days following the second bout of EC. The TA muscles were then removed from the anaesthetized rats before they were killed by an overdose of anesthesia and a 1 ml bolus injection of KCl into the heart (SM), and the muscles were immediately weighed to determine muscle wet weight values.

Data from both the single bout and two bout eccentric contraction groups receiving losartan treatment were compared with data that were previously published in untreated rats (McBride *et al.* 2000). The same investigator used identical techniques and hardware to collect the data in both instances.

Eccentric training. Animals in this group received continuous losartan treatment in their drinking water at either a low dose ($n = 5$) or a high dose ($n = 9$), or no additional treatment (untreated; $n = 9$). Animals were trained with eight exposures to EC over a 4 week period, training on Tuesdays and Fridays each week. Each training bout was equivalent to the single bout and two bout EC protocols used in the other groups. Muscle contractile function in both the trained and contralateral control muscles was tested in all three groups 3 days following the final exposure to EC. The TA muscles were then removed from the anaesthetized rats before they were killed by an overdose of anesthesia and a 1 ml bolus injection of KCl into the heart (SM), and the muscles were immediately weighed to determine muscle wet weight values.

Muscle stimulation protocol for eccentric contractions

Animals were anaesthetized with ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, Iowa 60 mg kg^{-1}) and xylazine (Rompum, Bayer Corporation, Shawnee Mission, Kansas 12 mg kg^{-1}), and performed EC on a pulley device similar to the one described by Wong & Booth (1988). The rat was placed in the prone position on the supporting platform of a pulley apparatus designed to stabilize the leg and allow full ankle rotation. The hind foot was attached directly to a plate connected to the lever arm of the pulley system and the ankle stabilized with the foot at 90 deg with respect to the lower leg (neutral position). Two monopolar stainless-steel needle electrodes were inserted percutaneously near the sciatic notch to stimulate the sciatic nerve. Stimulation of the sciatic nerve above the branch point of the tibial and peroneal nerves caused the plantarflexors (triceps surae) to contract concentrically, stretching the dorsiflexors as they were also maximally activated. The dorsiflexors (tibialis anterior, TA) thus contracted eccentrically, lengthening, in opposition to the stronger ankle extensors. Stimulation consisted of 100 Hz stimulus trains with a 1 ms stimulus duration and a train duration of 2.5 s. The exercise protocol consisted of four sets of six repetitions with a 20 s rest between repetitions and a 5 min rest between sets. During each procedure only the right leg was stimulated to produce EC of the TA muscle. The left leg served as an unexercised contralateral control in all groups.

Muscle contractile function

TA contractile function was measured in-vivo at the designated time points following the final bout of EC. The animals were anaesthetized with ketamine 60 mg kg^{-1} and Rompum 12 mg kg^{-1}, and the TA exposed. The rat was placed on a warming pad to maintain body temperature and the animal and pad placed on a metal frame. The distal tendon of the TA was isolated and attached to a force transducer (Grass-FT-03) with silk suture (2–0). The TA was stimulated directly by a platinum plate electrode at supramaximal voltage with approximately 0.05 ms duration at optimal length (L_o). Maximum isometric twitch tension (P_t), rate of twitch relaxation (dR/dT), maximum isometric tetanic tension (P_o), and the maximum rate of force development during a tetanus at 330 Hz (dPo/dT) were recorded at 35C ± 0.5C. Output voltages from the force transducer were amplified

and recorded on an analog to digital acquisition system (Powerlab, ADInstruments, Colorado Springs, CO USA). Muscle temperature was monitored, and maintained at 35°C ± 0.5°C by radiant heat.

Muscle wet and dry weights

Both the exercised and contralateral control TA muscles were removed immediately following the contractile measurements. The muscles were trimmed of connective tissue and weighed to determine muscle wet weight. Muscles were then dried in a desiccating oven at 70°C for 48 h and reweighed to determine muscle dry weight.

Statistics

Results are expressed as the mean ± S.E.M. of the indicated number of measurements for each group. Each treatment group is compared to its contralateral control by unpaired Student's *t* test, with comparisons between treatment groups performed by a one-way ANOVA. A Fisher PLSD and Scheffe *F* test were used to further analyse any differences indicated by the ANOVA. Differences were accepted as statistically significant at $P < 0.05$, but actual *P* values are indicated in the tables. Analyses were performed using the Statview software program (Abacus Concepts, Berkeley, CA, USA).

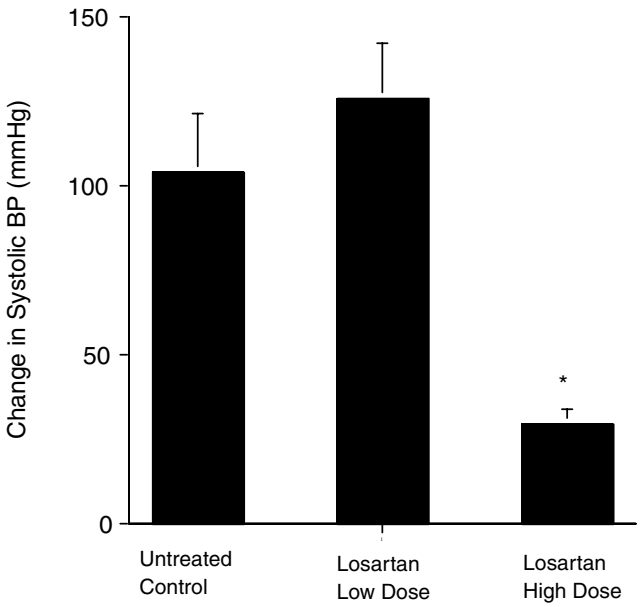

Figure 1. The change is systolic blood pressure following an I.A infusion of Ang II (1 μg (kg body weight)$^{-1}$) in control untreated rats ($n = 3$) and in low dose (20 mg (kg body weight)$^{-1}$ day^{-1}; $n = 3$) and high dose (40 mg (kg body weight)$^{-1}$ day^{-1}; $n = 6$) losartan-treated rats
Values are means + S.E.M. *$P < 0.05$ compared to untreated control.

Results

Blockade of AT_1 receptors

Results from a bolus infusion of Ang II on the pressor response in untreated and losartan-treated rats are presented in Fig. 1. Ang II infusion in untreated control animals produced a 103.9 ± 17.0 mmHg rise in systolic blood pressure. Losartan treatment in the low dose group did not prevent an Ang II pressor response (125.8 ± 15.9 mmHg). Losartan treatment in the high dose group produced a blockade of AT_1 receptors as confirmed by significantly blunting of the pressor response following the infusion of Ang II (29.5 ± 4.3 mmHg).

Muscle contractile function and wet weight following a single bout of EC

The values for TA muscle contractile function and muscle wet weight measured 2 days after a single acute bout of EC from both untreated and high dose losartan-treated rats are presented in Table 1. A single acute bout of EC resulted in a significant reduction in muscle contractile function compared to contralateral unexercised control muscles in both the untreated and losartan-treated groups (Table 1). Peak twitch tension (P_t) was reduced by 31 and 37.7% compared to contralateral unexercised control muscles in the untreated and losartan-treated groups, respectively. Maximum tetanic tension (P_o) was also significantly reduced by 19 and 24% in exercised muscles from untreated and losartan-treated muscles, respectively, compared to the unexercised contralateral control muscles. The rate of muscle tension development (dP_o/dt) and the rate of relaxation (dR/dt) were also significantly reduced by 19.5 and 45.7%, respectively, in untreated exercised muscles and by 31.3 and 52.9%, respectively, in losartan-treated exercised muscles compared to contralateral unexercised control muscles in each group (Table 1). The rate of tension development was also significantly reduced in exercised losartan-treated muscles compared to exercised muscles from the untreated group (Table 1). A single acute bout of EC also resulted in a significant increase in muscle wet weight by 10.7% in the untreated and 15.4% in the losartan-treated muscles compared to unexercised contralateral control muscles (Table 1). Both the untreated and losartan-treated groups therefore displayed similar deficits in contractile function and increases in muscle wet weight consistent with an acute injury response following an initial exposure to EC.

Muscle contractile function and wet weight following a second bout of EC

The values for TA muscle contractile function and muscle wet weight measured 2 days after a second bout of EC from

Table 1. Muscle weight and contractile properties from untreated and losartan-treated rats measured 2 days following a single acute bout of eccentric contractions

Treatment group	Muscle wet weight (mg)	P_t (g)	P_o (g)	dP_o/dt (g ms^{-1})	dR/dt (−g ms^{-1})
Untreated control‡ ($n = 6$)	508.5 ± 14.1	204.0 ± 8.6	977.2 ± 39.9	42.4 ± 2.1	12.7 ± 0.7
Untreated single acute EC‡ ($n = 6$)	562.7 ± 19.9*	155.8 ± 7.4**	791.7 ± 45.8**	34.1 ± 1.7**	6.9 ± 0.5**
Losartan control ($n = 6$)	591.8 ± 24.8	234.5 ± 7.1	1048.2 ± 28.7	40.0 ± 1.2	15.7 ± 1.2
Losartan single acute EC ($n = 6$)	683.0 ± 29.4*	146.0 ± 12.0**	796.5 ± 53.0**	27.5 ± 2.5**†	7.4 ± 0.6**

Values are reported as means ± S.E.M. *$P < 0.05$ and **$P < 0.01$ compared to contralateral control; †$P < 0.05$ compared to untreated exercised rats. P_t represents the peak isometric twitch tension. P_o represents the peak isometric tetanic tension. dP_o/dt represents the maximum rate of tension development during a tetanic contraction at 330 Hz. dR/dt represents the maximum rate of relaxation during an isometric twitch measurement. Losartan treatment consisted of the high dose, 40 mg (kg body weight)$^{-1}$ day^{-1}). ‡ Data from this group have been published previously (McBride *et al*. 2000), but are included in this paper to provide a non-losartan-treated group to allow comparison of the effects of losartan treatment.

Table 2. Muscle weight and contractile properties from untreated and losartan-treated rats measured 2 days following a second bout of eccentric contractions 14 days after the initial bout of eccentric contractions

Treatment group	Muscle wet weight (mg)	P_t (g)	P_o (g)	dP_o/dt (g ms^{-1})	dR/dt (−g ms^{-1})
Untreated control‡ ($n = 5$)	608.6 ± 18.7	211.0 ± 6.7	1147.4 ± 34.1	48.9 ± 1.7	10.6 ± 0.9
Untreated second EC‡ ($n = 5$)	637.8 ± 20.2	217.8 ± 13.9	1107.0 ± 33.6	44.4 ± 2.3	9.2 ± 0.9
Losartan control ($n = 8$)	545.4 ± 24.1	216.7 ± 8.1	981.9 ± 52.2	37.4 ± 2.4*	13.1 ± 0.9
Losartan second EC ($n = 8$)	580.9 ± 29.2	205.1 ± 8.0	990.9 ± 58.1	37.2 ± 2.4	11.2 ± 0.8

Values are reported as means ± S.E.M. *$P < 0.05$ compared to untreated control. P_t represents the peak isometric twitch tension. P_o represents the peak isometric tetanic tension. dP_o/dt represents the maximum rate of tension development during a tetanic contraction at 330 Hz. dR/dt represents the maximum rate of relaxation during an isometric twitch measurement. Losartan treatment consisted of the high dose, 40 mg (kg body weight)$^{-1}$ day^{-1}). ‡ Data from this group have been published previously (McBride *et al*. 2000), but are included in this paper to provide a non-losartan-treated group to allow comparison of the effects of losartan treatment.

untreated and high dose losartan-treated rats are presented in Table 2. A second bout of EC did not result in a deficit of contractile function as determined by P_t, P_o, dP/dt and dR/dt in either group when measured 2 days following the second bout of EC (Table 2). There was also no change in TA muscle wet weight compared to contralateral control muscles in either the untreated or losartan-treated groups following a second bout of EC (Table 2). The preservation of contractile function and the lack of a significant gain in wet weight in the exercised muscles from both the untreated and the losartan-treated groups represent an adaptation to EC, and are indicative of the repeated bout effect.

Muscle wet and dry weights and contractile function following EC training

The values for wet and dry weights in the TA muscles from the untreated trained, and both low and high dose losartan-treated rats following EC training are presented in Fig. 2 and Table 3. Four weeks of EC training resulted in significant increases in both muscle wet and dry weights in the trained TA muscles from untreated rats. The low and high dose losartan-treated rats experienced increases that were not statistically significant in both muscle wet and dry weights following 4 weeks of EC training.

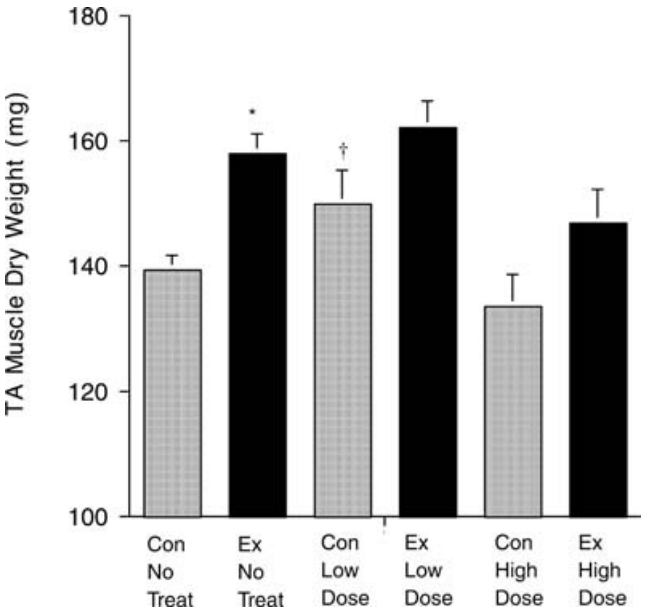

Figure 2. Comparison of tibialis anterior muscle dry weights between contralateral unexercised control muscles and eccentric exercised trained muscles from untreated ($n = 9$), low dose losartan-treated ($n = 5$) and high dose losartan-treated rats ($n = 9$)

Con, unexercised control; Ex, exercise trained; No Treat, no losartan treatment; Low Dose, low dose losartan treatment; High Dose, high dose losartan treatment. *$P < 0.05$ compared to contralateral control; †$P < 0.05$ compared to high dose control.

Table 3. Body and muscle weights measured in untreated and losartan-treated rats following eccentric training

Treatment group	Final body wt (g)	TA muscle wet wt (mg)	TA muscle dry weight (mg)
Untreated control‡ ($n = 9$)	253. 6 ± 3.5	564.5 ± 25.2	139.3 ± 6.9
Untreated trained‡ ($n = 9$)	253. 6 ± 12.5	605.6 ± 42.1*	157.8 ± 9.8*
Low dose control ($n = 5$)	268.4 ± 7.4†	567.8 ± 22.0	149.8 ± 5.3
Low dose trained ($n = 5$)	268.4 ± 7.4	630.2 ± 23.0	162.0 ± 4.3
High dose control ($n = 9$)	247.2 ± 5.3	528.4 ± 18.2	133.4 ± 5.2
High dose trained ($n = 9$)	247.2 ± 5.3	567.6 ± 19.6	146.9 ± 5.1

Values are reported as means ± S.E.M. *$P < 0.05$ compared to contralateral control; †$P < 0.05$ low dose control compared to high dose control. ‡ Data from this group have been published previously (McBride, 2003), but are included in this paper to provide a non-losartan-treated group to compare with the effects of losartan treatment.

Table 4. Muscle contractile force measured in untreated and losartan-treated rats following eccentric training

Treatment group	P_t (g)	P_t/MDW (g mg^{-1})	P_o (g)	P_o/MDW (g mg^{-1})
Untreated control‡ ($n = 9$)	208.4 ± 21.3	1.49 ± 0.04	938.4 ± 79.6	6.75 ± 0.23
Untreated trained‡ ($n = 9$)	240.1 ± 22.2*	1.52 ± 0.05	1068.5 ± 89.6*	6.77 ± 0.14
Low dose control ($n = 5$)	206.2 ± 6.0	1.38 ± 0.05	929.8 ± 45.4	6.22 ± 0.30
Low dose trained ($n = 5$)	210.8 ± 5.9	1.31 ± 0.07	998.2 ± 48.1	6.20 ± 0.42
High dose control ($n = 9$)	210.7 ± 3.8	1.58 ± 0.09	983.3 ± 38.8	7.38 ± 0.12
High dose trained ($n = 9$)	201.8 ± 4.3	1.39 ± 0.07	953.7 ± 46.9	6.53 ± 0.31

Values are reported as means ± S.E.M. *$P < 0.05$ compared to contralateral control; †$P < 0.05$ compared to high dose control. P_t represents the peak isometric twitch tension; P_t/MDW represents the peak isometric twitch tension normalized to muscle dry weight measured; P_o represents the peak isometric tetanic tension; P_o/MDW represents the peak isometric tetanic tension normalized to muscle dry weight. ‡ Data from this group have been published previously (McBride, 2003), but are included in this paper to provide a non-losartan-treated group to allow comparison of the effects of losartan treatment.

The values for contractile force in the TA muscles from untreated and losartan-treated rats following a 4 week EC training protocol are presented in Table 4. EC training resulted in a significant increase in both P_t (15.2%) and P_o (13.9%) in the trained muscles from the untreated rats compared to untrained contralateral control muscles. EC training in losartan-treated rats did not result in a significant change in the force of muscle contraction as assessed by P_t and P_o in either the low dose or the high dose treatment groups compared to the untrained contralateral control muscles. The muscles from the high dose losartan-treated group actually demonstrated a slight non-significant decline in P_t (−4.2%) and P_o (−3.0%) following training compared to untrained contralateral control muscles. The low dose losartan-treated group demonstrated modest non-significant gains in both P_t (2.2%) and P_o (7.3%) following training compared to the contralateral control muscles. When the contractile values were normalized to muscle dry weight, none of the groups demonstrated a significant increase with training (Table 4). The contractile function normalized to muscle dry weight was significantly reduced in the trained muscles from the high dose losartan-treated group compared to unexercised contralateral control muscles (Table 4).

Discussion

The results show a difference between the acute and chronic effects of blockade of AT_1 receptors in skeletal muscles exposed to EC. Blockade of the AT_1 receptors did not prevent the loss in contractile function following the initial bout of EC or the repeated bout effect following a second bout of EC. It is an important point to demonstrate that the initial injury and loss of function occurs in the presence of AT_1 receptor blockade, as in untreated muscle (McBride *et al.* 1995, 2000). The normal contractile function observed 2 days following the second bout of EC is a true adaptive response, as opposed to the possibility that AT_1 receptor blockade prevents the injury and loss of function from occurring in the first place. To date, the early events in EC-induced injury and loss of function have been well characterized, as outlined previously (Morgan, 1990; Armstrong *et al.* 1991; Friden & Lieber, 1992; Brooks *et al.* 1995; Morgan & Allen, 1999), but the prevention of EC-induced injury in response to a naïve bout remains elusive. It is generally agreed upon that a muscle must undergo the initial damage and loss of function in order to become protected from the injury following repeated exposures to EC.

Several mechanisms have been proposed for the repeated bout effect. These include changes to the

sarcolemma (Clarkson & Tremblay, 1988), removal of weak fibres or sarcomeres that are more susceptible to injury (Armstrong *et al.* 1983), a remodelling of intermediate filaments and cytoskeletal proteins (Friden *et al.* 1983; Lieber *et al.* 1994), and the addition of new sarcomeres along the length of muscle fibres (Morgan, 1990; Lynn & Morgan, 1994). Ingalls *et al.* (2004) have provided the most detailed description of the repeated bout effect by measuring the recovery of the contractile response following five separate exposures to EC with 2 weeks between each bout. They continued to measure a loss of contractile force immediately following the fifth bout of EC, but there was a more rapid return to normal contractile force occurring by day 3 following the fifth bout (Ingalls *et al.* 2004). In the study of Ingalls *et al.* (2004), contractile function was not measured immediately after the second bout of EC. At this time point a deficit in function may still occur, followed by a more rapid return to normal function as measured 2 days after the second bout of EC in the present study. The adaptation to EC is not necessarily a total prevention of injury and loss of function, but rather a reduction in injury followed by a much more rapid recovery to normal contractile function. Either way, losartan treatment to block AT_1 receptors did not result in a deviation from the normal adaptive response reported previously utilizing the same EC protocol and assessment of contractile function (McBride *et al.* 1995, 2000).

In contrast to the repeated bout effect, the ability of skeletal muscle to respond to EC training was significantly reduced by blockade of AT_1 receptors. This result is consistent with data reported by Gordon *et al.* (2001), who were the first to describe the necessity of Ang II and functional AT_1 receptors for overload-induced hypertrophy in rat skeletal muscle. The results of the present study provide additional information by demonstrating a similar dependence on functional AT_1 receptors for hypertrophy in response to brief intermittent periods of stretching and loading by EC. This is in contrast to a relatively continuous stretch and load in the synergist ablation model used by Gordon *et al.* (2001). It is also interesting to note that the effect of losartan on EC training was dose dependent. Both the low and high dose losartan treatments had similar non-significant changes in muscle mass compared to contralateral control muscles. The muscles from the low dose treatment groups, however, demonstrated a non-significant increase in force, compared to a decline in force in the muscles from high dose treatment groups following training. The low dose losartan treatment was also able to affect the EC training response of muscle at a dose that did not block the Ang II pressor response. The mechanism for this is unclear, but Gordon *et al.* (2001) have commented that adaptations occurring in response to skeletal muscle overload may include an upregulation of AT_1 gene expression resulting in an increase in the density of AT_1 receptors. This speculation is based on the observations of Zhang *et al.* (1995), who measured an increase in mRNA specific for AT_1 receptors in overloaded cardiomyocytes. Further speculation at this point might also include a change in the sensitivity of AT_1 receptors following stretching or loading. More research in this area is clearly needed to provide a more complete understanding of the role that AT_1 receptors play in load-induced skeletal muscle hypertrophy. The present study is also novel in that it provides a measurement of muscle contractile function following EC training in conjunction with blockade of AT_1 receptors.

An argument could be made that the lack of a hypertrophy response and the absence of an increase in contractile force in this study is more a function of the relatively short training duration rather than of AT_1 receptor blockade. The training protocol did, however, produce a significant increase in both muscle mass and contractile force in muscles from untreated rats compared to unexercised controls (Tables 3 and 4). Using a similar EC training protocol, Adams *et al.* (2002) also measured a significant increase in muscle mass in addition to higher DNA and RNA content, but did not measure muscle contractile function. Ingalls *et al.* (2004) did not measure a significant change in muscle mass or protein content, but did measure a significant increase in muscle contractile force with as few as five exposures to EC separated by 2 weeks recovery between each EC event. Based on previous reports, the EC training protocol used here provides sufficient stimulus for a significant increase in both muscle hypertrophy and contractile force. The absence of a training response can therefore be attributed to the losartan treatment and AT_1 receptor blockade. Losartan treatment and AT_1 receptor blockade did not have a negative affect on the contractile properties of unexercised control muscle.

It therefore appears that the intracellular mechanisms responsible for the repeated bout effect and EC training-induced hypertrophy rely on separate pathways, with the adaptive response independent and the training response dependent on functional AT_1 receptors. Multiple pathways for the initiation of cardiac and skeletal muscle hypertrophy have been described. A functional muscle hypertrophy, as defined here by an increase in muscle protein and contractile force, is the end result of a complex combination of neural, chemical, hormonal and physical stimuli. Some chemical mediators, such as clenbuterol and insulin-like growth factor-I (IGF-1) *in vitro* (Maclennan & Edwards, 1989; Vandenburgh *et al.* 1991), can stimulate muscle hypertrophy independent of mechanical load, while others, such as anabolic steroids, rely on the addition of a mechanical stimulus to be effective (Tingus & Carlsen, 1993). The biochemical stimuli also differ in their origins, with some released and acting on a systemic level, while others occur as a local phenomenon in an autocrine/paracrine manner.

Ang II is clearly one such chemical/hormonal stimulus important for cardiac (Morgan & Baker, 1991; Dostal & Baker, 1992) and skeletal muscle hypertrophy during a functional overload (Gordon *et al.* 2001). Ang II is released by cardiac cells in response to stretch (Sadoshima *et al.* 1993; Malhorta *et al.* 1999) and contributes to the overload-induced hypertrophy response. Stretch or overload of cardiac muscle, however, does not appear to be necessary for Ang II to stimulate hypertrophy (Morgan & Baker, 1991; Dostal & Baker, 1992; Black *et al.* 1995; Susic *et al.* 1996). This same effect of Ang II independent of overload does not appear to be true for skeletal muscle hypertrophy. The addition of an exogenous source of Ang II was able to restore the majority of an overload hypertrophy response in animals treated with an ACE inhibitor to block endogenous sources of Ang II, but it did not have an effect on non-overloaded contralateral muscles (Gordon *et al.* 2001). In skeletal muscle, load appears to be necessary for Ang II stimulation of hypertrophy. Perhaps Ang II plays more of a permissive role in skeletal muscle hypertrophy in response to load, compared to a more direct role in cardiac muscle hypertrophy. Losartan treatment did not affect mass and function of unexercised contralateral control muscles in this study.

In conclusion, functional AT_1 receptors are not necessary for skeletal muscle to recover from EC-induced muscle injury and loss of function, or to undergo adaptation necessary for the repeated bout effect. Functional AT_1 receptors are necessary for the training effect of EC that results in muscle hypertrophy and increases in muscle contractile force. Based on these observations, it appears that muscle recovery and adaptation to EC rely at least in part on unique intracellular signalling pathways compared to EC training-induced muscle hypertrophy.

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