Electrically induced static exercise elicits a pressor response in the decerebrate rat

Scott A. Smith, Jere H. Mitchell and Mary G. Garry

Harry S. Moss Heart Center, Departments of Internal Medicine and Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9174, USA

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1. The purpose of this investigation was to determine if activation of the exercise pressor reflex in the decerebrate rat induced circulatory responses comparable to those reported in large mammalian species.

2. To activate both mechanically and metabolically sensitive afferent fibres, static hindlimb contractions were induced by stimulating the cut ends of L4 and L5 spinal ventral roots in Sprague-Dawley rats (300–400 g). To selectively stimulate mechanically sensitive receptors, hindlimb muscles were passively stretched.

3. In intact halothane-anaesthetized animals ($n = 10$), static contraction and passive stretch induced a *decrease* in mean arterial pressure (ΔMAP = -17 ± 3 and -8 ± 1 mmHg for contraction and stretch, respectively) and heart rate (HR). In contrast, MAP *increased* 23 ± 2 mmHg during contraction and 19 ± 3 mmHg during stretch in decerebrate rats ($n = 10$). These pressor responses were accompanied by a significant tachycardia. In decerebrate animals, the reintroduction of halothane attenuated the increase in MAP and HR caused by both contraction and stretch.

4. In both anaesthetized and decerebrate rats, sectioning the spinal dorsal roots innervating the activated skeletal muscle eliminated responses to contraction and stretch. This finding indicated that an intramuscular neural reflex mediated the response to each stimulus.

5. The results demonstrate that a decerebrate preparation in the rat is a reliable model for the study of the exercise pressor reflex. Development of the model would enable the study of this reflex in a variety of pathological conditions and allow investigation of the mechanisms controlling cardiovascular responses to exercise in health and disease.

Afferent signals from contracting skeletal muscle are an important source of neural input to the brain stem during exercise (Alam & Smirk, 1937; Coote *et al.* 1971; McCloskey & Mitchell, 1972; Kaufman & Forster, 1996). These exercise-induced signals are generated by activation of group III (predominantly mechanically sensitive A-δ fibres) and IV (predominantly metabolically sensitive C fibres) skeletal muscle afferents which reflexively increase arterial blood pressure (ABP) and heart rate (McCloskey & Mitchell, 1972; Kaufman *et al.* 1984). Haemodynamic regulation by this reflex loop, termed the exercise pressor reflex, is primarily mediated by increased efferent sympathetic nerve activity (Mitchell *et al.* 1983).

The rodent is an attractive candidate for the study of the exercise pressor reflex as disease models (e.g. heart failure, hypertension and diabetes) are readily available or easily produced. This is important as previous studies have suggested the exaggerated increases in ABP, sympathetic nerve activity and vascular resistance to exercise in patients with cardiovascular disease are due, in part, to an overactive exercise pressor reflex (Pickering, 1987; Magnusson *et al.* 1997; Piepoli *et al.* 1999). Furthermore, more genomic information is currently known about rodents than about larger mammals, presenting the opportunity to study this reflex at the level of cellular and molecular physiology.

Unfortunately, induction of static muscle contraction in the rat has been reported to elicit either no change (Vissing *et al.* 1991), an increase (Freda *et al.* 1999) or a decrease in arterial blood pressure (Overton & Stremel, 1992; Toney & Mifflin, 1996). The most obvious difference between these investigations was the method of anaesthesia. It is known that anaesthetic agents influence central haemodynamics as well as the cardiovascular responses to stresses such as haemorrhage and exercise (Vatner, 1978; Longnecker *et al.* 1982). However, experiments comparing the haemodynamic effects of pharmacological anaesthesia and decerebration in rats have shown that the circulatory responses to haemorrhage in the decerebrate animal closely resemble those elicited in the conscious state (Seyde *et al.* 1985).

Therefore, decerebration, which renders the animal insentient and so obviates the need for anaesthesia, may provide a superior model for the study of cardiovascular control in rodents.

The purpose of the present investigation was to compare the effects of static muscle contraction and passive stretch on cardiovascular control in anaesthetized and decerebrate rats. Intramuscular afferent fibres were stimulated by (i) involuntary muscle contractions induced by either electrical stimulation of sectioned spinal ventral roots or direct electrical stimulation of the sciatic nerve and (ii) passive muscle stretch. We hypothesized that the use of a decerebrate model would elicit circulatory responses to exercise similar to those previously observed in humans and large animals (McCloskey & Mitchell, 1972; Fisher & Nutter, 1974; Rowell *et al.* 1981). A preliminary report of these findings has been published (Smith *et al.* 2000).

METHODS

All procedures outlined in this investigation complied with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care Committee of the University of Texas Southwestern Medical Center. Experiments were performed on 37 Sprague-Dawley male rats (Harlan, body weight 300–400 g). Animals were housed in standard rodent cages and regulated on a 12 h light–dark schedule. Food and water were made available *ad libitum*.

Surgical preparation

General procedures. Anaesthesia was induced with halothane (2–3 %) in pure oxygen. The trachea was exposed and an endotracheal tube inserted into the airway. Catheters (polyethylene tubing, PE-50) were inserted into the external jugular vein for the administration of drugs and into the common carotid artery for the measurement of ABP. Levels of inhalant gas were increased as indicated by a withdrawal reflex to pinching of the hindpaw, presence of a corneal reflex, and/or spontaneous increases in HR. A continuous infusion (2 ml 1 M $NaHCO_3$ and 10 ml 5 % dextrose in 38 ml Ringer solution) was established via the jugular vein (3–5 ml h^{-1} kg^{-1}) to stabilize fluid balance and maintain basal ABP (Quintin *et al.* 1989). In addition, dexamethasone (0.2 mg) was given intramuscularly to minimize oedema (Tian & Duffin, 1996). Animals were artificially ventilated using a mechanical respirator (Model 680, Harvard Apparatus) throughout the experiment. Arterial blood gases and pH were assessed periodically by an automated blood gas analyser (Model ABL 5, Radiometer) and maintained within normal ranges (arterial P_{O_2} > 80 mmHg, arterial P_{CO_2} 35–45 mmHg, pH 7.3–7.4). Body temperature was monitored using a rectal thermometer (YSI series 400) and maintained between 36.5 and 38.0 °C by a temperature-controlled water-perfused heating pad. All animals were held in a stereotaxic head unit (Kopf Instruments) and customized spinal frame. At the conclusion of the experiment, animals were humanely killed by intravenous administration of sodium pentobarbital (120 mg kg^{-1}).

Decerebration procedures. Decerebration was carried out using a method similar to that described by Sapru & Krieger (1978). This technique was used in 15 animals in which the ventral roots were exposed and in all animals in which the sciatic nerve was isolated ($n = 12$). To minimize cerebral haemorrhage, the remaining intact common carotid artery was isolated and ligated. A bilateral craniotomy was performed by drilling burr holes into the parietal skull. Subsequently, the portion of bone superior to the central sagittal sinus was removed. The dura mater was breached and reflected. The cerebral cortex was gently aspirated to visualize the superior and inferior colliculi. Using a blunt instrument, the brain was sectioned pre-collicularly and the transected forebrain aspirated. Small pieces of oxidized regenerated cellulose (Ethicon, Johnson & Johnson) were placed on the exposed surfaces of the brain and cotton balls were used to pack the cranial cavity. A minimum recovery period of 1.25 h was employed post-decerebration before data collection began. This allowed sufficient time for the effects of halothane anaesthesia to be eliminated from the preparation (Kohn, 1997).

Procedures for exposure of the sciatic nerve and lumbar ventral roots. In order to activate both mechanically and metabolically sensitive skeletal muscle afferent fibres, static hindlimb contractions were induced using two distinct methods. In one group of animals ($n = 25$), a laminectomy exposing the lower lumbar portions of the spinal cord (L2–L6) was performed. The dura of the cord was cut and reflected allowing visual identification of the L4–L6 spinal roots. The dorsal and ventral roots of L4 and L5 were carefully separated. The ventral roots were sectioned and the cut peripheral ends were positioned on insulated bipolar platinum electrodes. In a second set of rats ($n = 12$), the sciatic nerve was exposed using a dorsal approach. Connective tissue was carefully removed and the nerve placed on an insulated bipolar platinum electrode. In each method of preparation, the exposed neural tissue was covered in a pool of warm mineral oil (37 °C). The animals were secured within the spinal frame by clamps placed on rostral lumbar vertebrae. Further, the pelvis was stabilized with steel posts within the frame and the exercising limb was fixed in one position using clamps attached to the tibial bone. The calcaneal bone was sectioned and the Achilles' tendon connected to a force transducer (Grass Instruments, FT10) for the measurement of muscle tension. Electrical stimulations were performed using a Grass Instruments S88 stimulator. In animals in which ventral root stimulation was performed, preferential activation of mechanically sensitive intramuscular afferents was achieved by passively stretching the triceps surae muscles using a calibrated 9.5 mm rack and pinion system (Harvard Apparatus, Inc.). Care was taken to match the peak tension developed in response to electrical stimulation during the passive stretch experiments.

Data acquisition. ABP was recorded by connecting the carotid artery catheter to a pressure transducer (Model DTX plus-DT-NN12, Ohmeda). MAP was obtained by integrating the arterial signal with a time constant of 1–4 s. Heart rate was derived from the ABP pulse using a biotachometer (Gould Instruments). All data for these experiments were recorded directly by an eight channel thermal recorder (Astro-Med, Inc.) and subjected to A/D conversion (CED micro 1401, Cambridge Electronic Design Ltd) using commercially available data acquisition software (Spike 2, version 3, Cambridge Electronic Design Ltd) on a personal computer (Pentium III, 550 MHz, Dell Computer Corp.). Computer-acquired data were used in *post hoc* analyses.

Experimental protocols

Halothane-anaesthetized animals. Rats were divided into two groups based upon their method of surgical preparation. (1) Ventral root stimulation: electrically induced static muscle contraction of the triceps surae was performed by stimulating the L4 and L5 ventral roots for 30 s. Constant-current stimulation was used at 3 times motor threshold (defined as the minimum current required to produce a muscle twitch) with a pulse duration

of 0.1 ms at 40 Hz. These stimulation parameters have been shown to elicit consistent force generation during electrically induced contraction (McCloskey & Mitchell, 1972). Subsequently, passive stretch of the muscle was performed while attempting to generate the same magnitude and pattern of muscle tension developed during electrical stimulation. (2) Sciatic nerve stimulation: static contraction of hindlimb muscle was generated by depolarizing the sciatic nerve for 30 s at twice motor threshold (0.025 ms duration at 40 Hz). These parameters have been reported to minimize direct activation of skeletal muscle afferent fibres within the sciatic nerve (Rybicki & Kaufman, 1985). In all testing protocols each contraction or stretch was separated by a minimum of 10–15 min. In addition, the triceps surae muscles were pre-loaded with 70–100 g of tension prior to any manipulation.

Decerebrate animals. In decerebrate rats the same techniques and protocols as described for halothane-anaesthetized animals were used. Additionally, in a subset of rats in which the ventral roots had been exposed ($n = 5$), a wide range of stimulus intensities (1–5 times motor threshold) were executed randomly to assess the relationship between tension development and cardiovascular responsiveness. Further, four different magnitudes of tension (250–1000 g) were used to assess the stimulus–response relationship to passive stretch. In a subgroup of rats in which the sciatic nerve was exposed ($n = 5$), variable intensities (1–3 times motor threshold) were developed randomly to assess the stimulus–response relationship to static contraction.

Experimental controls. Following the contraction and stretch protocols, several control experiments were completed. Firstly, the neuromuscular blocking agent pancuronium bromide (200 μg kg^{-1}) was administered I.V. Electrical activation of either the ventral roots or sciatic nerve was repeated using the stimulus parameters described previously. This manoeuvre was instituted to eliminate the possibility that cardiovascular responses were mediated by direct activation of sensory afferent fibres during stimulation protocols. In halothane-anaesthetized and decerebrate animals HR and ABP were stable between pressure-altering manoeuvres, providing evidence of the efficacy of each anaesthetic method during this period. Secondly, the L4, L5 and L6 dorsal roots were sectioned. These dorsal roots innervate the hindlimb and are carried via the sciatic nerve (Greene, 1963). Following dorsal rhizotomy, contraction and stretch protocols were repeated. A lack of responsiveness to manipulation after dorsal rhizotomy would confirm that contraction or stretch-induced haemodynamic alterations were of neural reflex origin. Thirdly, halothane anaesthesia was reintroduced to decerebrate animals for a minimum of 1 h. The level of halothane anaesthesia implemented (2% in pure oxygen) was identical to that used during the testing of brain-intact animals. Subsequently, the contraction and stretch protocols were repeated. These trials were completed to assess the effect of decerebration on the cardiovascular responses elicited by static muscle contraction and stretch. Finally, the viability of the preparation after neuromuscular blockade or dorsal root transection was confirmed by obtaining a response to a brief (30 s) hypoxic stimulus after discontinuing the use of the mechanical respirator.

Statistical analysis. Baseline values were determined by analysing 30 s of data immediately prior to a given manoeuvre. The peak response of each variable was defined as the greatest change from baseline elicited during the 30 s execution of the contraction or stretch. Analyses conducted within and between halothane-anaesthetized or decerebrate animal populations were made using one- or two-way ANOVA, as appropriate. When significance was indicated, a Student-Newman-Keuls *post hoc* test was executed. Linear regression analyses were used to characterize the stimulus–response relationships between the changes in MAP and tension produced in response to contraction or stretch. The α level was set at $P < 0.05$. Results are presented as means ± S.E.M.

RESULTS

Cardiovascular responses elicited by ventral root stimulation and passive stretch

Muscle contraction induced by ventral root stimulation and passive muscle stretch resulted in significant alterations in MAP and HR from baseline as illustrated in Fig. 1. Both static contraction and stretch produced depressor and bradycardic responses in the halothane-anaesthetized animal (Fig. 1*A*). In contrast, increases in ABP and HR were elicited during both manoeuvres in the decerebrate rat under conditions of equal tension development (Fig. 1*B*). Figure 2 is a summary of the population studies illustrated in the representative tracings shown in Fig. 1. Static muscle contraction significantly depressed MAP (−17 ± 3 mmHg) and HR (−7 ± 2 beats min^{-1}) in animals anaesthetized by halothane (Fig. 2*A*) while both variables increased (23 ± 2 mmHg and 12 ± 2 beats min^{-1}, respectively) in decerebrate rats (Fig. 2*B*). Likewise, in halothane-anaesthetized rats, passive muscle stretch significantly decreased MAP and HR by −8 ± 1 mmHg and −7 ± 1 beats min^{-1}, respectively, whereas in decerebrate animals MAP (19 ± 3 mmHg) and HR (12 ± 2 beats min^{-1}) were elevated in response to this manoeuvre. The re-administration of halothane in decerebrate animals ($n = 5$) attenuated the increase in MAP and HR by 48 ± 11% and 57 ± 8%, respectively, during static contraction and 67 ± 2% and 69 ± 6%, respectively, during passive stretch (Fig. 2*B*). In all cases, cardiovascular responses to either contraction or stretch were eliminated by sectioning the dorsal roots innervating the activated skeletal muscle (Fig. 2). Further, stimulation of the ventral roots after neuromuscular blockade did not elicit cardiovascular responses in either halothane-anaesthetized or decerebrate rats (Fig. 2). Basal and peak HR, MAP and tension, expressed as raw values for each study population, are presented in Table 1. Baseline and peak values for HR and MAP were significantly augmented in decerebrate animals compared to those of their halothane-anaesthetized counterparts.

In a subset of decerebrate animals, graded contraction and stretch elicited pressor responses that were linearly related ($r = 0.844$ and 0.836, respectively) to tension development (Fig. 3*A* and *C*). A significant main effect for each variable in response to graded electrical stimulation and passive stretch was calculated (MAP and tension, $P < 0.001$; Fig. 3*B* and *D*). In order to control for inter-subject variability in tension development, regression analyses were conducted by normalizing this variable to the maximal value obtained for each animal during contraction (1.001 ± 0.160 kg) and stretch (1.021 ± 0.027 kg). Cardiovascular responses were then matched to the corresponding percentage of maximal tension developed.

Table 1. Cardiovascular responses to contraction via ventral root stimulation and passive stretch in halothane-anaesthetized and decerebrate rats

	Halothane		Decerebrate	
	Contraction	Stretch	Contraction	Stretch
MAP (mmHg)				
Baseline	79 ± 4	75 ± 6	91 ± 6†	92 ± 7†
Peak response	62 ± 2*	67 ± 5	114 ± 7*†	111 ± 8*†
HR (beats min^{-1})				
Baseline	270 ± 17	271 ± 14	398 ± 14†	404 ± 13†
Peak response	263 ± 15	264 ± 14	410 ± 13†	416 ± 12†
Tension (kg)				
Baseline	0.074 ± 0.010	0.081 ± 0.010	0.081 ± 0.003	0.088 ± 0.005
Peak response	0.953 ± 0.112*	1.095 ± 0.091*	0.990 ± 0.087*	1.101 ± 0.085*
n	10	10	10	10

Data are means ± S.E.M. MAP, mean arterial pressure; HR, heart rate. Stimulation parameters: 3 times motor threshold, 40 Hz, 0.1 ms. *Significantly different from baseline. †Significantly different from animals anaesthetized by halothane. $P < 0.05$.

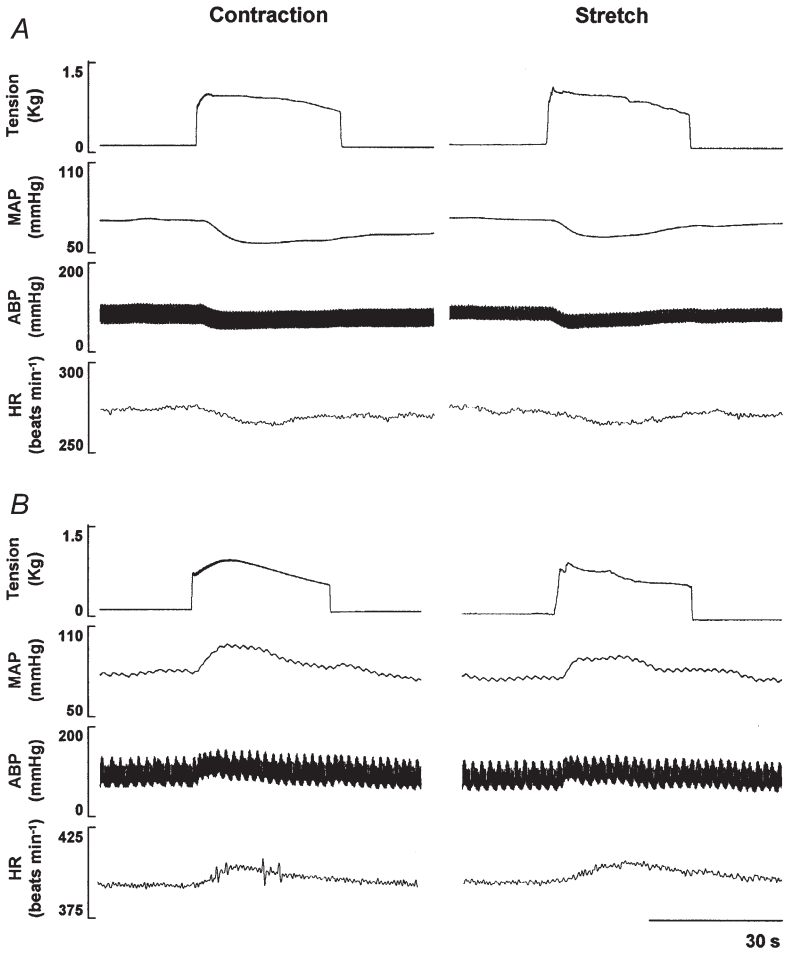

Figure 1. Cardiovascular responses elicited by spinal ventral root stimulation (contraction) and passive muscle stretch in halothane-anaesthetized (*A*) and decerebrate (*B*) rats

The contraction- and stretch-induced decreases in mean arterial pressure (MAP), arterial blood pressure (ABP) and heart rate (HR) in the pharmacologically anaesthetized animal were in direct contrast to the marked pressor and tachycardic responses elicited in the decerebrate model. The tracings depicted in *A* and *B* are from two different animals. Stimulation parameters: 3 × motor threshold, 40 Hz, 0.1 ms duration.

Cardiovascular responses elicited by sciatic nerve stimulation

The population data obtained in halothane-anaesthetized and decerebrate rats in response to static muscle contraction induced via electrical stimulation of the sciatic nerve are presented in Fig. 4. In halothane-anaesthetized rats, static muscle contraction increased MAP (ΔMAP = 10 $\pm$ 3 mmHg). This change in blood pressure was significantly less than the pressor response elicited by contraction in decerebrate animals (MAP = 49 $\pm$ 7 mmHg). There was no significant difference in the HR response to static contraction between halothane-anaesthetized or decerebrate animals (13 $\pm$ 3 and 15 $\pm$ 2 beats min^{-1}, respectively). After neuromuscular blockade in decerebrate rats, sciatic nerve stimulation induced increases in HR (3 $\pm$ 1 beats min^{-1}) and MAP (28 $\pm$ 3 mmHg) that were

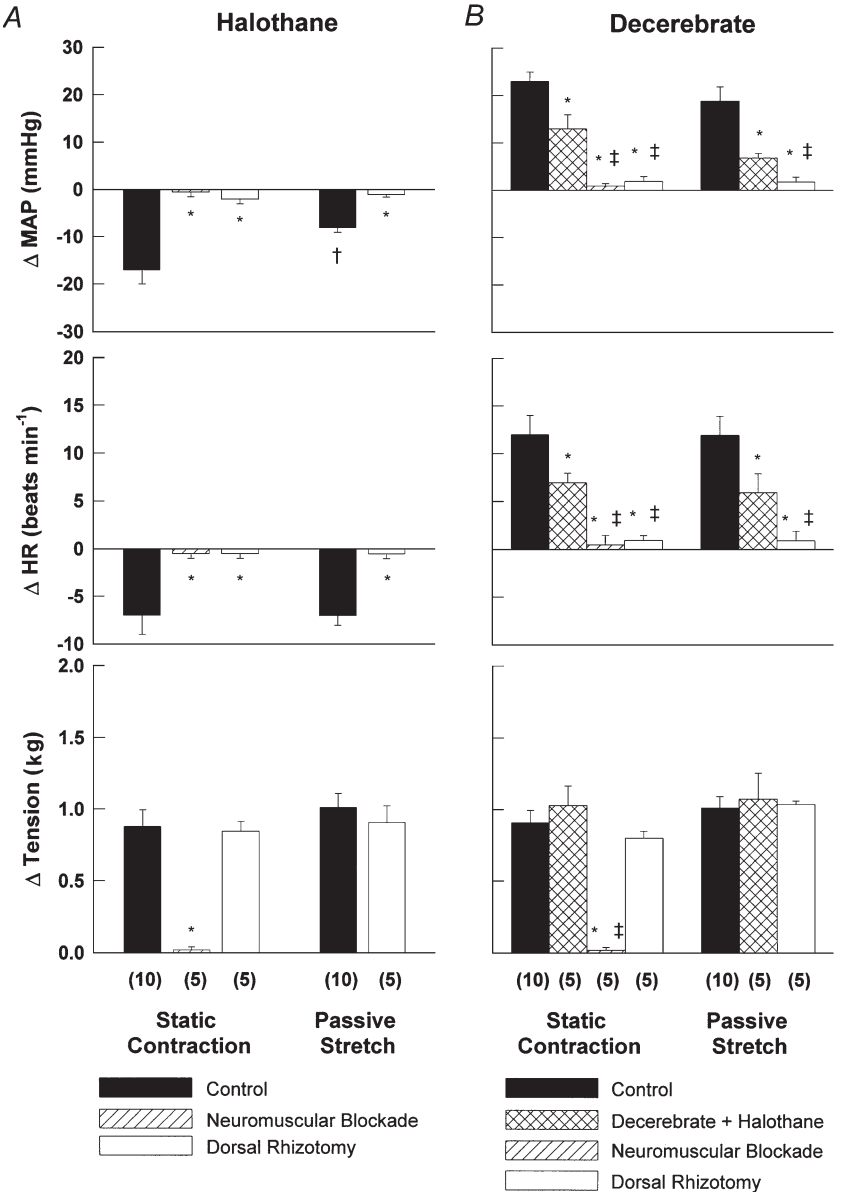

Figure 2. Effect of dorsal rhizotomy, neuromuscular blockade and halothane readministration (decerebrate animal only) on the cardiovascular responses evoked by ventral root stimulation and passive muscle stretch

The contraction- and stretch-induced decreases in mean arterial pressure (MAP) and heart rate (HR) in the halothane-anaesthetized animal (*A*) were reversed in the decerebrate rat (*B*). Denervation and/or neuromuscular blockade eliminated the responses to static muscle contraction and passive stretch in both animal preparations. *B*, the reintroduction of gas anaesthesia in decerebrate rats attenuated the increase in MAP and HR caused by both manoeuvres. (*n*), the number of rats tested in each protocol. Stimulation parameters, 3 $\times$ motor threshold, 40 Hz, 0.1 ms duration. * Significantly different from control perturbation. † Significantly different from control contraction. ‡ Significantly different from decerebrate + halothane condition. $P < 0.01$.

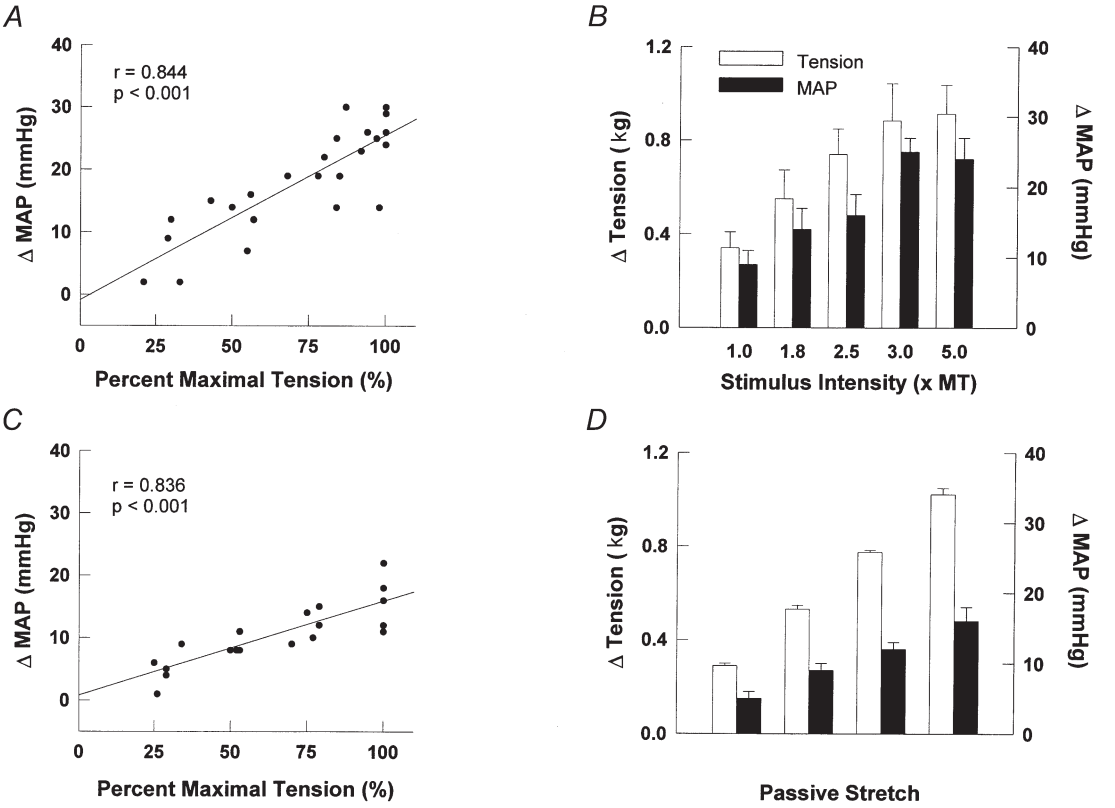

Figure 3. Changes in mean arterial pressure (MAP) and tension in response to graded electrical stimulation of spinal ventral roots (*A* and *B*, $n = 5$) and passive muscle stretch (*C* and *D*, $n = 5$)

Regression analyses were used to describe the line of best-fit depicted for both testing protocols. To control for inter-subject variability, tension was expressed as a percentage of maximum for each animal in *A* and *C*. MT, motor threshold. Stimulation parameters: 40 Hz, 0.1 ms duration.

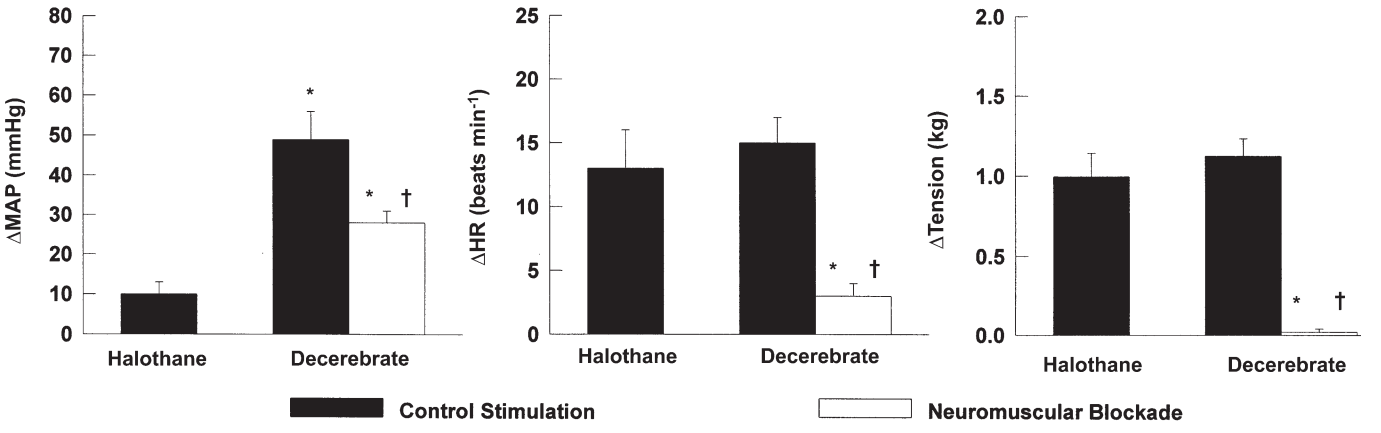

Figure 4. Cardiovascular responses elicited by sciatic nerve stimulation in halothane-anaesthetized and decerebrate rats ($n = 10$)

The large pressor response elicited by electrical stimulation in the decerebrate animal could not be abolished by neuromuscular blockade, indicating direct activation of somatosensory afferent fibres. This finding may also explain the increases in mean arterial pressure (MAP) and heart rate (HR) produced in the halothane-anaesthetized rat. Stimulation parameters: 2 × motor threshold, 40 Hz, 0.025 ms duration. * Significantly different from control stimulation in halothane-anaesthetized rats. † Significantly different from control stimulation in decerebrate animals. $P < 0.01$.

significantly attenuated from those elicited in the non-paralysed state. The increases in both HR and MAP post-paralysis were completely abolished by dorsal rhizotomy ($n = 2$).

Graded blood pressure responses were produced by stimulating the sciatic nerve over a range of one to three times motor threshold in a subgroup of decerebrate animals (Fig. 5*A*). The data indicate that the changes in arterial pressure were dependent upon contraction intensity, as we observed a significant main effect for both MAP and tension ($P < 0.001$). However, electrical stimulation of the sciatic nerve produced graded pressor responses that persisted after neuromuscular blockade. This persistent pressor response was evident at all levels of stimulation tested despite the lack of tension development (Fig. 5*B*).

Preparation viability

At any given stimulus intensity, both static contraction and passive stretch elicited reproducible cardiovascular responses during multiple trials (i.e. two to three manipulations). In addition, viability testing of animals 4–5 h post-decerebration confirmed that all preparations were able to elicit pronounced circulatory responses after neuromuscular blockade or following dorsal rhizotomy. Induction of hypoxic/hypercapnic stimuli produced increases in MAP of 40 ± 3 mmHg ($n = 20$). This finding suggests that the lack of responsiveness to electrical stimulation or stretch reported after administration of pancuronium bromide or deafferentation was not due to deterioration of the physiological preparation.

DISCUSSION

The results of this investigation provide evidence that static muscle contraction induced by ventral root stimulation and passive muscle stretch were able to elicit increases in HR and MAP in the decerebrate rat. Furthermore, these changes were mediated by a neural reflex, since transection of the dorsal roots innervating the activated skeletal muscle abolished the response. Moreover, neuromuscular blockade eliminated the increase in HR and MAP caused by ventral root stimulation, indicating that the response elicited was due exclusively to muscle contraction. In addition, graded activation of intramuscular afferent fibres by both contraction and stretch elicited increases in MAP that were linearly related to the intensity of the stimulus in a manner similar to that reported in other mammalian models (Perez-Gonzalez, 1981; Mitchell *et al.* 1983). Given that models of cardiovascular pathology are readily accessible and easily produced in the rat, this investigation describes a novel preparation that can be used to study the exercise pressor reflex in both health and disease.

The use of the rat as a model for the exercise pressor reflex is controversial. In some studies, hindlimb muscle contractions have been shown to produce large depressor responses (Overton & Stremel, 1992; Toney & Mifflin, 1996). In this investigation, a marked decrease in MAP was produced in response to muscle contraction via ventral root stimulation in halothane-anaesthetized rats. In contrast, others have been able to elicit robust pressor responses to muscle contraction induced by electrical stimulation of the tibial nerve in chloral hydrate-anaesthetized rats (Caringi *et al.* 1997; Freda *et al.* 1999). In the current study, static contraction via sciatic nerve stimulation produced an increase in MAP and HR in both anaesthetized and decerebrate animals. However, this response was not abolished by induction of neuromuscular blockade in the decerebrate rat. Therefore, it is likely that the pressor responses induced by sciatic nerve stimulation in both study populations were due, in part, to direct electrical activation of afferent fibres, a conclusion supported by the complete elimination of the response after dorsal rhizotomy. This finding was surprising as the stimulation parameters used (i.e. twice motor threshold, 0.025 ms duration, 40 Hz) have been shown not to activate group III and IV fibres within the sciatic nerve of larger mammals (Rybicki & Kaufman, 1985). Based on these results, there is a need to

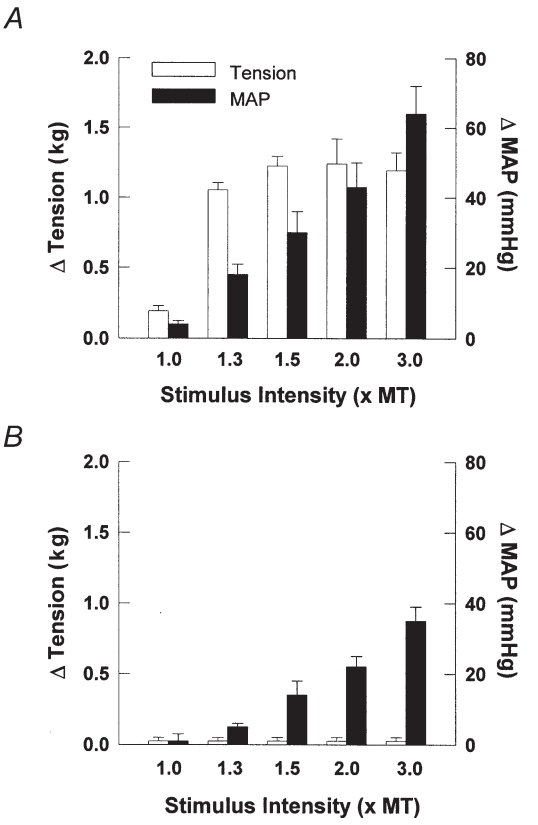

Figure 5. Changes in mean arterial pressure (MAP) and tension in response to graded electrical stimulation of the sciatic nerve before (*A*) and after neuromuscular blockade (*B*)

The increases in MAP persisted after induction of neuromuscular blockade in the absence of tension development. This finding suggests that electrical activation of muscle afferents must have occurred. MT, motor threshold. Stimulation parameters: 40 Hz, 0.025 ms duration. $n = 5$.

define the operating characteristics (e.g. the activation threshold) of afferent fibres within whole nerves innervating the hindlimb of the rat. Given these findings, the differences in the responses produced in this and previous studies (Overton & Stremel, 1992; Toney & Mifflin, 1996; Caringi *et al.* 1997; Freda *et al.* 1999) are probably due to the technique employed to induce muscle contraction and/or to the regimen used for anaesthesia.

Evidence exists which suggests that the neural circuitry of the rat possesses the ability to drive sympathoexcitatory responses mediated by intramuscular afferent input. For example, significant increases in adrenal sympathetic nerve activity have been described in anaesthetized rats in response to static hindlimb contraction (Vissing *et al.* 1991). Toney & Mifflin (2000) demonstrated that neurons in the rat nucleus tractus solitarii (NTS), a medullary autonomic nucleus intimately involved in cardiovascular regulatory control, receive excitatory input from skeletal muscle receptors in response to both muscle contraction and stretch. Although the effects of activating NTS neurons remains undetermined (i.e. sympatho-inhibition *vs.* excitation) in the rat, these findings do provide electrophysiological evidence that input from peripheral muscle afferents is projected to the NTS in this species. Furthermore, previous experimentation in decerebrate rats has elicited increases in blood pressure and HR in response to stimulation of the mesencephalic locomotor region (MLR) that were similar to those in dynamically exercising conscious animals (Bedford *et al.* 1992). More recently, Potts *et al.* (2000) have reported that activation of forelimb intramuscular receptors via electrically induced contraction produced a vasoconstrictor-mediated increase in pressure in an *in situ* decerebrate rat preparation. The data presented in this investigation extend the latter finding to the larger muscle groups of the hindlimb in an *in vivo* model. In addition, this study demonstrates that the responses can be driven by various somatic sensory stimuli (i.e. mechanical and metabolic).

It is unclear why decerebration was necessary to reverse the depressor response to contraction and stretch elicited in anaesthetized animals. Examining locations within the central nervous system where halothane is known to alter neuronal function may provide a viable explanation. Using expression of the immediate-early proto-oncogene c-*fos* as a marker, several investigators have identified the medulla, pons, midbrain, hypothalamus, thalamus and cerebrum as sites of action for halothane in the rat (Takayama *et al.* 1994; Clement *et al.* 1996). More importantly, halothane-induced c-fos protein expression in these areas is essentially equal (Takayama *et al.* 1994). It is possible, therefore, that the depressor response elicited in this study could have been driven by halothane-induced changes in neuronal activity in *each* of these brain structures. The re-administration of halothane after removal of the supra-mesencephalic brain elicited an attenuated increase in arterial pressure to contraction and stretch (as compared to decerebrate conditions), not a depressor response. Thus, it would appear that halothane, although still effective on the brain stem in the decerebrate preparation, cannot drive a depressor response unless all sites of its action remain intact.

Given the known depressive cardiovascular effects and varied sites of action of halothane within the central nervous system, we contend that the pressor response elicited within this investigation was attributable to the discontinuation of pharmacological anaesthesia rather than secondary to the decerebration procedure. In support of this finding, it has been shown in other models that a larger pressor reflex is generated in decerebrate animals (in the absence of anaesthesia) in response to exercise than in anaesthetized animals (Iwamoto *et al.* 1985). Even more compelling is that the increases in HR and MAP produced in the present study complement previous reports of pronounced pressor responses to both static (Tipton *et al.* 1988) and dynamic (Baum & Shropshire, 1975; Sturek *et al.* 1984) exercise in conscious rats. Therefore, the decerebrate model developed in this investigation mimics the physiological response to exercise in conscious animals.

An alternative explanation is that brain centres critical to the muscle reflex were removed by decerebration (Eldridge *et al.* 1981; Vertes & Crane, 1996). For example, a significant proportion (i.e. approximately 50 %) of the contraction-evoked depressor response described in anaesthetized rats has been attributed to the action of adrenal catecholamines on β-adrenoreceptors (Toney & Mifflin, 1996). Since adrenal catecholamine release, a sympathetically mediated response, can be elicited by stimulation of hypothalamic nuclei (Vissing *et al.* 1989), it is possible that the decerebration procedure used in the present study eliminated a potentially important component of the muscle reflex. However, neuroanatomical and electrophysiological studies have demonstrated that skeletal muscle afferent fibres project to several medullary nuclei known to control sympathetic nerve activity (Kalia *et al.* 1981; Iwamoto *et al.* 1989; Bauer *et al.* 1992). Since the medulla was fully intact in this preparation, it is unlikely that activation of this region of the brain stem was disrupted, preserving its ability to drive sympatho-excitatory responses. In support of this contention, it has been shown in other animal models that the cardiovascular response to muscle contraction is essentially complete at the level of the medulla, being only slightly diminished when supra-mesencephalic input is eliminated (Iwamoto *et al.* 1985).

Potential limitations in the design of this model are recognized. Firstly, baseline HR and MAP were significantly greater in decerebrate rats than in their halothane-anaesthetized counterparts. These increases were probably due to the discontinuation of pharmacological anaesthesia. Alternatively, it is possible that the decerebration procedure induced a hyperadrenergic condition. If the latter possibility occurred, the responses to static contraction and stretch may have been attenuated in decerebrate animals due to a diminished sympathetic reserve capacity. Finally,

although baroreceptor signals from aortic and cardiopulmonary regions remained intact, carotid baroreflex function was compromised by the surgical preparation. As has recently been described, inputs from skeletal muscle and baroreceptor afferents undergo significant interaction within the central nervous system (Potts *et al.* 1998). Therefore, elimination of the carotid baroreflex may have affected the responses to contraction and stretch.

In this investigation, a novel exercise model has been developed in a species in which cardiovascular disease is readily available or easily induced. Alterations in skeletal muscle morphology and metabolism associated with the genesis of cardiovascular pathology have led to the hypothesis that the exercise pressor reflex may become hyperactive after development of disease (Piepoli *et al.* 1999). However, the contribution of this neurally mediated peripheral reflex to the evolution of abnormal circulatory control is poorly understood. The utility of this model lies in its ability to assess both peripheral and central autonomic control mechanisms using much of the physiological technology currently used in larger animal preparations. For example, the techniques of microdialysis and electrophysiological neuronal recordings can now be used at the level of the spinal cord and brain stem to assess synaptic events and cellular behaviour during disease. Further, much of the genome of the rat has been described allowing the use of cellular and molecular techniques previously unavailable in large mammalian populations. Equally important, the exercise-induced circulatory adjustments elicited in this preparation are similar to those reported in other mammalian models such as the mouse (Kramer *et al.* 2001), cat (Coote *et al.* 1971; McCloskey & Mitchell, 1972), dog (Fisher & Nutter, 1974) and human (Rowell *et al.* 1981; Hansen *et al.* 1994). Given these findings, in conjunction with the functional utility of the preparation, use of this model provides the potential to further our understanding of circulatory regulation during exercise in both health and disease.

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Corresponding author

S. A. Smith: Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, USA.

Email: scott.smith@utsouthwestern.edu