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Sympathetic control of skeletal muscle function: possible co-operation between noradrenaline and neuropeptide Y in rabbit jaw muscles

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Abstract

Stimulation of the cervical sympathetic nerve at 10/s increases by 12.9 ± 0.7% peak tension of maximal twitches in the directly stimulated jaw muscles and markedly depresses (41.6 ± 1.3%) the tonic vibration reflex (TVR) elicited in the same muscles by vibration of the mandible. Both effects are not significantly influenced by administration of β-adrenoceptor antagonists. When both α- and β-adrenergic receptors are blocked, sympathetic stimulation induces a very small increase in twitch tension (3.8 ± 0.7%), while no detectable change in the TVR is observed. Close arterial injection of α_1-adrenoceptor agonist phenylephrine mimics the effects induced by sympathetic stimulation on twitch tension and TVR, dose-dependently. The noradrenaline co-transmitter neuropeptide Y also produces a long-lasting, dose-dependent increase in the twitch tension which is unaffected by blockade of adrenergic receptors as well as of the neuromuscular junctions. Contribution of neuropeptide Y to the sympathetically-induced reduction of the stretch reflex is not clearly demonstrated. These data suggest that co-operation between noradrenaline and neuropeptide Y may be effective in determining sympathetic modulation of skeletal muscle function.

Keywords: Jaw muscles; Sympathetic nervous system; Neuropeptide Y; Tonic vibration reflex; α_1-Adrenoceptors; Muscle contraction

Under different physiological conditions sympathetic nervous system can markedly affect skeletal muscle function by acting on the central nervous system pathways involved in the execution of motor tasks (Refs. in [5]). In addition, experimental evidence is available showing that catecholamines can act at muscular level by influencing afferent information as well as contraction of both fatigued and non-fatigued muscles (Refs. in [2]). Direct action of the adrenergic mediators on muscle spindles was first suggested by Hunt [10], but further studies either reconsidered the functional significance of such effect [11] or attributed the sympathetically-induced modulation of spindle afferent discharge to vasomotor changes [4]. More recently, we showed that the sympathetic influence on spindle afferent information from jaw muscles is relevant both under static conditions and during muscle length changes, and it is independent of vasomotor effects [16–18]. In particular, a decrease in spindle sensitivity has been considered responsible for the marked stretch reflex depression induced by sympathetic stimulation in rabbit jaw muscles [5,6,15]. In the same muscular district, activation of noradrenergic axons also affects muscle contraction by inducing a modest recovery in the tension depressed by prolonged stimulation [7,8] and a small increase in force developed by maximal twitches of non-fatigued muscle [5,7]. Postganglionic sympathetic neurones innervating jaw muscles potentially release both noradrenaline and its co-transmitter neuropeptide Y (NPY), since this peptide has been localised in a large percentage of superior cervical ganglion cells [12]. In the present paper the effects induced by sympathetic stimulation on muscle contraction and stretch reflex were further studied and the adrenergic receptors responsible for the sympathetic influence on motor function were investigated. In addition, the possible contribution of NPY to the above mentioned sympathetic effects was checked.

Two groups of experiments were performed on albino rabbits (weight 2.3–3.0 kg) in which either tension developed by directly stimulated jaw muscles or reflex responses to jaw muscle vibrations were recorded.

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In the first group of experiments, animals were anaesthetised with urethane (1.5 g/kg, i.v.) and blocked in a stereotaxic apparatus. Direct stimulation of the masseter muscle (MM) or the anterior digastric muscle (DM) was performed through Ag-AgCl electrodes sewn to the muscular surface. The stimulated muscle was immersed in a pool obtained with skin flaps, which was filled with warm mineral oil. Electrical stimuli (1–20 V, 0.5 ms pulse duration) were delivered at 0.1/s and the lowest stimulation parameters needed to obtain maximal contraction in each preparation were identified. Indirect stimulation of jaw muscles was not performed since stimulation of the intact muscle nerves is rather difficult because of the anatomical arrangement, and cutting the nerve would interrupt the sympathetic supply to the muscles. Maximal twitch tension of DM and MM was measured at the optimal muscle length by a force transducer respectively connected either to the cut central tendon of DM or to the mandibular symphysis. Lower jaw was blocked by cementing together upper and lower incisor teeth in the experiments in which DM tension was recorded.

In the second group of experiments, animals were anaesthetised with a mixture of urethane, ketamine and xylazine (300, 5 and 1.5 mg/kg, i.v., respectively), further doses of the last two drugs were supplemented when needed until pre-collicular decerebration was performed. In four animals only urethane and ketamine were used in order to check the possible influence of xylazine on the studied responses due to its α-adrenergic agonist action. Tonic vibration reflex (TVR) was elicited by applying to the lower jaw 4–5 s lasting vibrations at 150–180 Hz (15–50 μm peak-to-peak amplitude). Mandibular displacement signal was used for feedback control of the puller. Force developed by the jaw muscles was recorded by means of an isometric transducer put in series with the puller. Electromyographic (EMG) activity was bilaterally recorded from the MM through either gross 'belly-tendon' copper leads insulated except for the tip or bipolar coaxial electrodes inserted into the muscle (for further details, see [6]).

In both groups of experiments, the cervical sympathetic nerves (CSN) and thyroid artery were isolated. The peripheral cut end of the CSN was stimulated (5 min lasting trains of stimuli at 1–10/s, 0.5 ms pulse duration, 4–8 V) through platinum electrodes mounted inside a polyethylene cylinder filled with mineral oil. Thyroid artery was cannulated for administrating drugs as close as possible to the jaw muscles. Adrenergic agonists and neuropeptide Y were dissolved in saline and injected through a syringe pump (1 ml solution at a rate of 0.5 ml/min). In some experiments rabbits were paralysed (pancuronium bromide or tubocurarine, 0.3 and 0.25 mg/kg, respectively, i.v., repeated when needed) and artificially ventilated, the end-tidal CO_2 levels being maintained close to control values. Heart rate and arterial blood pressure were routinely monitored. Body temperature was kept at 38.0 ± 0.3°C by feedback-controlled heating blanket. All signals were recorded on a polygraph (ES1000, Gould Electronique, France) and stored on magnetic tape for further analysis. Force signals were analysed off-line by a PC equipped with AT-MIO-16L9 acquisition board (National Instruments) by using LabVIEW software (sampling frequency 1 KHz).

The following drugs were administered: phentolamine methanesulfonate (Sigma; 2.5–3.5 mg/kg), prazosin hydrochloride (Pfizer; 1.0–1.5 mg/kg), L-phenylephrine hydrochloride (Sigma; 3.5–28.0 μg/kg), propranolol hydrochloride (Sigma; 1.5–2.5 mg/kg), (±)isoproterenol hydrochloride (Sigma; 0.1–1.0 μg/kg), porcine neuropeptide Y (Peninsula Labs.; 25–500 pmol/kg). Data are expressed as means ± SEM; variance analysis and Student's t-test were carried out using SPSS program.

Unilateral stimulation of the CSN at 10/s enhanced peak tension of maximal twitches in the directly stimulated MM and DM of anaesthetised rabbits. The effects observed in the two muscles studied were similar, most of data were however collected in DM since, due to its anatomical arrangement, this muscle can be easily isolated thus avoiding spread of current to nearby tissues during electrical stimulation. Muscle twitch potentiation appeared with a latency of 10–20 s from CSN stimulation onset and reached a maximum increment ranging from 9 to 21% of controls (mean value 12.9 ± 0.7%; $n = 25$) within the following 1–2 min (Fig. 1A). At the end of CSN stimulation, peak amplitude returned to control values within 1–3 min. The enhancement of the developed force was associated with an increase in the duration of muscle twitch (Fig. 1A). CSN stimulation at frequencies as low as 1–2/s was sufficient to induce small but detectable increases in amplitude (3–4% of the control values) and duration of muscular responses. These effects were not influenced by blockade of neuromuscular junctions

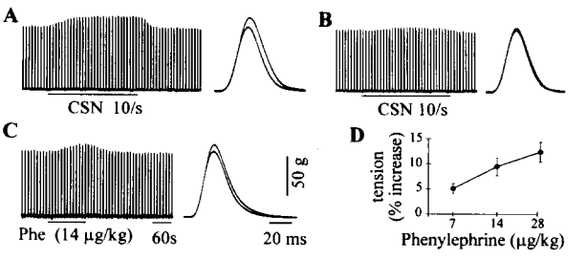

Fig. 1. Increase in amplitude and duration of maximal twitches induced by CSN stimulation and α_1-adrenoceptor activation in the digastric muscle. (A) Left, slow speed recording of muscle twitches shows the time course of the sympathetic effect; right, superimposed traces of control and maximum sympathetic effect displayed at a larger time scale. (B) A small enhancement is induced by CSN stimulation after administration of both α- and β-adrenoceptor antagonists (phentolamine, 3.5 mg/kg and propranolol, 1.5 mg/kg, i.v., respectively). (C) Effect of CSN stimulation is mimicked by i.a. injection of α_1-adrenoceptor agonist phenylephrine. (D) Dose-response relationship of muscle twitch potentiation induced by phenylephrine.

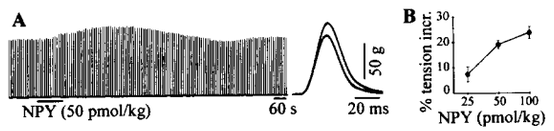

Fig. 2. Increase in amplitude and duration of DM maximal twitches induced by NPY administration. (A) Time course of the effect and single traces superimposed, as in Fig. 1. (B) Dose-effect relationship.

obtained by administration of curarizing agents (tubocurarine or pancuronium bromide, 0.25 and 0.3 mg/kg, respectively). Administration of the β-adrenoceptor antagonist propranolol (1.5–2.5 mg/kg, i.v.; $n = 6$) also failed to elicit any significant change in the sympathetically-induced increase of twitch tension, and injection of the β-adrenoceptor agonist isoproterenol (0.1–1.0 μg/kg, i.a.) did not usually produce any increase in either amplitude or duration of maximal twitches. Small effects were occasionally observed but they did not exhibit any dose-response relationship. After administration of both β- and α-adrenoceptor antagonists (propranolol and phentolamine, 1.5 and 2.5–3.5 mg/kg, i.v., respectively), the sympathetically-induced effect was markedly reduced. The residual increase in twitch tension was 3.8 ± 0.7% ($n = 13$) of the control values (Fig. 1B). Intra-arterial injection of the selective α_1-adrenoceptor agonist phenylephrine mimicked the increase in amplitude and duration of maximal twitches induced by CSN stimulation. The effect of phenylephrine was dose-dependent, the enhancement in peak tension being 5.1 ± 0.9% ($n = 13$) at the dose of 7 μg/kg, 9.4 ± 0.8% ($n = 14$) at the dose of 14 μg/kg, and 12.3 ± 1.1% ($n = 19$) at the dose of 28 μg/kg. The difference among the three groups is statistically significant (variance analysis, $P < 0.0001$; test of linearity, $P < 0.0001$).

Once studied the adrenergic action on skeletal muscle contraction, we investigated whether the noradrenaline co-transmitter neuropeptide Y might contribute to the sympathetically-induced muscle twitch potentiation. Injection of porcine NPY (25–100 pmol/kg, i.a.) induced a dose-dependent increase in amplitude and duration of maximal twitches which was very similar to the effects induced by CSN stimulation and phenylephrine administration (Fig. 2). The time course of NPY effects was however much slower. In particular, the increase in muscle twitch amplitude showed a latency of 1–2 min from the start of the injection, reached a maximum increment in the following 2.0–4.5 min and returned to the control values in the next 9–16 min (mean time from peak effect to control value was 11.7 ± 0.8 min). The increment of the developed tension was 7.5 ± 2.9% ($n = 6$) at 25 pmol/kg, 19.0 ± 1.6% ($n = 10$) at 50 pmol/kg and 23.7 ± 2.4% ($n = 10$) at 100 pmol/kg. The dose-dependent increase was statistically significant (variance analysis, $P < 0.0005$; test of linearity, $P < 0.0005$). The effects induced by NPY injection were not altered by blockade of α- and β-adrenoceptors (phentolamine 3.0 mg/kg, propranolol 1.5 mg/kg, i.v.) as well as of neuromuscular junctions (tubocurarine, 0.3 mg/kg, i.v.).

Small amplitude vibrations (15–50 μm at 150–180 Hz) applied to the mandible of decerebrate rabbits induced TVR consisting of tonic contraction of the jaw elevator muscles lasting as long as the vibratory stimuli. Unilateral stimulation of the CSN at 10/s induced a marked decrease in both developed tension and EMG activity in the jaw muscles ipsilateral to the stimulated side (Fig. 3). Tension reduction was 41.6 ± 1.3% of control values, as estimated in 45 trials which also include data collected in our previous study [6]. The depressant action induced by sympathetic stimulation was not significantly affected by propranolol administration (1.5–2.5 mg/kg, i.v.; $n = 7$) while it was abolished by blockade of α-adrenoceptors (phentolamine, 3.5 mg/kg, $n = 5$; prazosin 1.0–1.5 mg/kg, $n = 5$). In four more experiments in which both α- and β-adrenoceptor antagonists were administered, no significant change in TVR could be observed during CSN stimulation. However, it has to be considered that the variability of the reflex responses under control conditions in the decerebrate animals may not allow to detect and correctly estimate possible small modifications in the studied responses. The sympathetically-induced TVR decrease was mimicked by activation of α_1-adrenoceptors (phenylephrine, 3.5–28.0 μg/kg, i.a.) while isoproterenol (0.1–1.0 μg/kg, i.a.) did not produce any reduction of the reflex responses. Fig. 3D shows the dose-response relationship of the effects induced by phenylephrine injection. TVR amplitude was 95.0 ± 1.7% ($n = 11$) at 3.5 μg/kg, 88.3 ± 0.8% ($n = 13$) at 7 μg/kg, 78.8 ± 1.7% ($n = 10$)

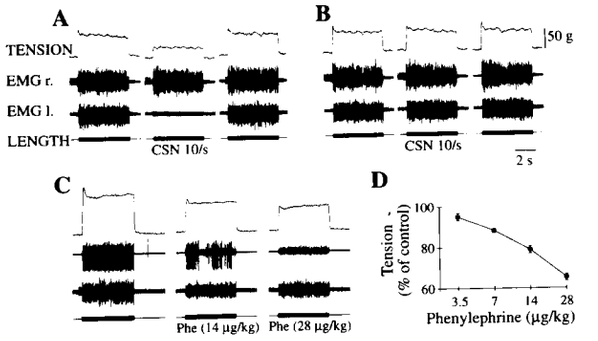

Fig. 3. Sympathetically-induced depression of the TVR is mediated by α-adrenoceptors. (A) From left to right, the three vertical strips correspond to single TVRs which were evoked respectively under control conditions, during CSN stimulation and 5 min after the end of stimulation. Unilateral stimulation of the CSN suppresses EMG activity in the MM ipsilateral to the stimulated nerve and markedly reduces the reflexly developed tension. (B) α-Adrenoceptor blockade (phentolamine, 3 mg/kg, i.v.) abolishes the sympathetically-induced TVR decrease. (C) Reduction in both EMG activity and muscle tension is induced by phenylephrine administration. (D) Dose-response relationship showing the TVR depression induced by phenylephrine.

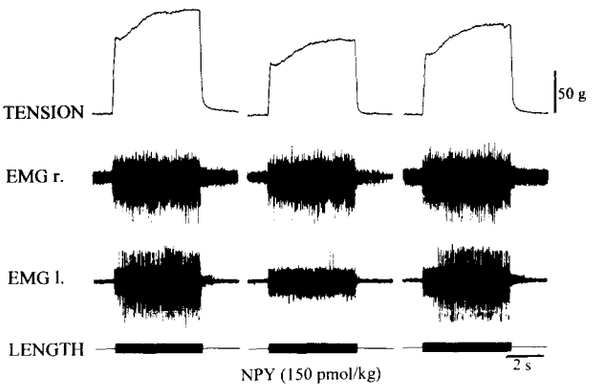

Fig. 4. TVR reduction induced by injection of NPY into the left thyroid artery. A decrease in both EMG activity and tension of jaw closing muscles can be observed 4 min after NPY administration. Traces on the right show EMG and force returned to control values 13 min after NPY injection.

at 14 μg/kg, and 65.1 ± 1.7% ($n = 13$) at 28 μg/kg. Difference among groups is statistically significant (variance analysis, $P < 0.0001$; test of linearity, $P < 0.0001$). No detectable differences were found between data collected in experiments in which anaesthesia included or not xylazine, thus showing that the α_2-adrenoceptor agonist action of this drug does not significantly interfere with the effects induced by either electrical stimulation of noradrenergic axons or phenylephrine administration.

NPY administration (25–500 pmol/kg, i.a.) induced a small decrease in the reflexly developed tension which might be associated with a reduction of the discharge in the recorded motor units ipsilateral to the injected side (Fig. 4). TVR depression reached maximum values ranging from 10 to 25% of the controls 4–7 min after NPY injection and slowly returned to controls within the next 5 to 15 min. However, NPY effects were not consistently found in all trials and they were not dose-dependent.

The results described above show that sympathetic stimulation, at frequencies within the physiological range, markedly influences motor function by affecting both the muscular contraction and the stretch reflex. Force developed by maximal twitches is enhanced by 12.9 ± 0.7% in the directly stimulated jaw muscles. The increase in tension is associated with a prolongation of the twitch duration which, under conditions of repetitive stimulations, can produce a larger tension development due to increased fusion of incomplete tetanic contractions. The enhancement in twitch amplitude and duration, induced in the jaw muscles by stimulation of the sympathetic axons at physiological frequencies, is comparable with that previously observed in cat hind limb muscles following i.v. injection of large doses of catecholamines ([3] and Refs. in [2]). Noradrenaline released by postganglionic sympathetic axons increases the developed force through activation of α_1-adrenergic receptors, since the sympathetically-induced effect is markedly reduced by α-adrenoceptor blockade and it is mimicked by α_1-adrenoceptor agonist dose-dependently. The noradrenaline co-transmitter NPY also induces a long-lasting, dose-dependent increase in peak tension and duration of maximal twitches.

The evidence that muscle twitch potentiation is mediated by α_1-adrenoceptors and is also induced by NPY administration raises the question on whether such effects may be secondary to vasoconstriction. This possibility can however be excluded since potentiating effects of catecholamines were also observed in in vitro preparations ([1] and Refs. in [2]). In addition, the decrease in muscle blood supply in in vivo experiments either reduced the developed tension or was ineffective, depending on the different sensitivity to hypoxia of the fibre types present in the studied muscle [9]. The presence of potentiating effects of noradrenaline and NPY in animals in which neuromuscular junctions had been blocked by curarizing agents also suggests that the increase in twitch tension is not related to an influence on the acetylcholine release from motor nerve terminals.

All the data discussed above allow us to conclude that the increase in twitch tension is likely due to direct action exerted by noradrenaline and by NPY on skeletal muscle fibres. Numerous experimental data are available in the literature showing that the effects induced by sympathetic nervous system activation on vascular and other smooth muscle preparations are due to the co-operation between noradrenaline and its co-transmitter NPY (Refs. in [13,19]). Also, many NPY-immunoreactive fibres have been found in the skeletal muscles and NPY has been suggested to significantly participate in the sympathetic regulation of muscle blood flow ([14] and Refs. in [13]). The above data make it reasonable to hypothesise that, in the muscular district, the adrenergic-peptidergic cooperation is not limited to vascular smooth muscle but it is also effective on skeletal muscle fibres.

When muscular contraction is reflexly induced by activation of muscle spindle afferents, the modulatory effect of sympathetic nervous system is the result of the potentiating action exerted on muscle fibre contraction and of the change induced on spindle afferent information. Under these experimental conditions, sympathetic stimulation produces a marked depression of the stretch reflex, due to the marked reduction in spindle sensitivity to muscle length changes which prevails on the modest increase in muscle tension [5,6,15]. As in the case of twitch potentiation, TVR decrease is mimicked by α_1-adrenoceptor activation. Contribution of NPY to the sympathetically-induced depression in the reflex responses is questionable since TVR depression was not consistently observed following NPY injection, it appeared with a latency quite longer than that of muscle contraction effects and no dose-response relationship could be clearly

evidenced. However, it can not be excluded that the peptide co-released with noradrenaline may give a minor contribution to the sympathetic modulation of the stretch reflex.

All the data discussed above point to a significant influence exerted by the sympathetic nervous system on the skeletal muscle function. Such action may be effective under numerous physiological conditions in which the sympathetic outflow is modified and can be attributed to the combined action of noradrenaline (activating α_1-adrenoceptors) and NPY, the contribution of this peptide being more relevant on skeletal muscle fibre contraction than on spindle afferent information.

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