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ScienceDirect
Behavioural Brain Research 178 (2007) 1–9
BEHAVIOURAL BRAIN RESEARCH
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Research report

Effect of precocious locomotor activity on the development of motoneurones and motor units of slow and fast muscles in rat

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Received 12 October 2006; received in revised form 23 November 2006; accepted 24 November 2006
Available online 19 December 2006

Abstract

We have investigated the effect of precociously increasing locomotor activity during early postnatal development by daily treatment with the monoaminergic precursor L-DOPA on the survival of motoneurones supplying the slow soleus (SOL) muscle and the fast, tibialis anterior (TA) and extensor digitorum longus (EDL) muscles as well as the contractile and histochemical properties of these muscles. L-DOPA treatment resulted in a significant loss of motoneurones to the slow SOL muscle, but not to the fast TA and EDL muscles. Moreover, motoneurones to fast muscles also die as when exposed to increased activity in early life, if their axons are repeatedly injured. The loss of normal soleus motoneurones was accompanied by an increase in force of the remaining motor units and sprouting of the surviving axons suggesting a remodelling of motor unit organisation. The time to peak contraction of both SOL and EDL muscles from L-DOPA treated rats was prolonged at 8 weeks of age. At 4 weeks the soleus muscles of the L-DOPA treated animal developed more tension than the saline treated one. This difference between the two groups did not persist and by 8 weeks of age the muscle weight and tetanic tension from either group were not significantly different from control animals. The present study shows that early transient, precocious locomotor activity induced by L-DOPA is damaging to normal soleus but not to normal EDL/TA motoneurones.

Keywords: Neuromuscular activity; L-DOPA; Muscle; Motoneurone; Motor unit; Development

1. Introduction

Motoneurone activity patterns play an important role in the development and plasticity of the motor unit. In adults, the physiological and biochemical properties of muscle fibres belonging to the same motor unit are similar, and are adapted to the functional demands imposed upon them by the motoneurone that supplies them. During development, a dynamic matching takes place between the motoneurones and their associated muscle fibres that result in generation of motor unit anatomical and functional diversity (for review see Ref. [20]).

In neonatal rodents, motor units are large and have overlapping territories as each muscle fibre is innervated by several axons from different motoneurones. The establishment of motor unit architecture, where only one axonal branch innervates a single muscle fibre is achieved by elimination of polyneuronal innervation and is an activity-dependent process [1,21,24].

In the rat, the differentiation of the activity patterns of motoneurones supplying slow and fast hindlimb muscles takes place during the first 3 postnatal weeks associated with the development of postural and locomotor functions [1,19,32]. Neonatal rats are unable to produce coordinated locomotor activity and the emergence of quadrupedal locomotion during the second postnatal week depends on the functional and neurochemical maturation of descending pathways associated with postural functions [30]. There is considerable evidence based on studies in adult animals that descending catecholaminergic and serotonergic pathways are involved in controlling the spinal interneuronal networks which generate locomotion ([2,5,6], for review see Ref. [25]).

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0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.11.040

Similarly, studies using isolated in vitro spinal cord preparations in neonatal spinal cord have revealed that coordinated patterns of flexor and extensor muscle activity can be elicited by exogenous administration of neurotransmitter agonists including glutamate, dopamine, and serotonin [3,9,11,30]. These studies suggest that the spinal circuitry that generates locomotion is already established at birth. We have previously shown that exogenous administration of the monoaminergic precursor L-DOPA to early postnatal rats triggers sustained episodes of stereotyped overground locomotion characterized by rhythmic reciprocal bursts of electromyographic (EMG) activity in flexor and extensor muscles resembling to some extent the rhythmic locomotor pattern seen in mature animals [18].

Not only motor unit size and muscle development are influenced by activity, but the survival of developing motoneurones is also critically dependent on activity. Reducing activity to axotomized neonatal motoneurones protects motoneurones destined to die from their fate and allows their survival [16], while increasing their activity precociously causes them to die [26]. In view of the important role of activity on the survival of motoneurones as well as development and differentiation of slow and fast muscles we have studied here the effect of precociously increasing locomotor activity during early postnatal development using L-DOPA. Unlike other methods of increasing the activity of motoneurones such as electrical stimulation, L-DOPA, by activating the central pattern generators involved in locomotion, induces the orderly recruitment of motoneurones to hindlimb flexor and extensor muscles thus inducing a more natural increase in the synaptic drive to the motoneurones [8,18]. Following such daily transient increases in locomotor activity during early postnatal development we have investigated the survival of motoneurones supplying the slow soleus muscle (SOL) and the fast, tibialis anterior (TA) and extensor digitorum longus (EDL) muscles and the contractile and histochemical properties of their respective muscles.

2. Materials and methods

Our experiments were performed on Wistar rats of both sexes. All experimental procedures were carried out according to UK Home Office regulations and following EU guidelines of animal care.

2.1. L-DOPA induced locomotion

In one group of animals where the effect of premature locomotor activity on the development of normal uninjured motoneurones and muscles was studied, activity was induced daily from birth until the animals were 12 days old by intraperitoneal (i.p.) injection of L-DOPA (100 mg/kg body weight). In another group of animals the effect of activity on injured motoneurones was investigated after unilateral sciatic nerve injury at 5 and 10 days of age. Unlike nerve injury at birth, where the majority of motoneurones die, the injury carried out at this age (5 days and later) leads to only a moderate loss of motoneurones [15] and therefore the effect of excess activity on injured motoneurones could be studied. In this group, the treatment started on postnatal day 5 (P5) and lasted until postnatal day 17 (P17). After the age of 9–10 days, the decarboxylase inhibitor, Carbidopa (10 mg/kg body weight) was added to the L-DOPA solution to enhance the action of L-DOPA. Control littermates were treated daily by i.p. injections with sterile saline for 12 days. They were kept away from their mother for the same period of time as the L-DOPA treated pups.

2.2. EMG recording

In 11 Wistar rat pups aged 7–9 days a pair of bipolar EMG recording electrodes was implanted into the SOL and either TA or EDL of one hindlimb as previously reported [18]. The EMG signals were amplified using a Neurolog NL 104 differential pre-amplifier (Digitimer, UK), filtered (bandpass 50 Hz–50 kHz) and recorded on a Racal 4DS FM tape recorder (Racal, UK). For quantitative analysis, the EMG recordings were played back from the tape recorder into a computer via an analogue to digital interface (CED 1401; Cambridge Electronic Design, UK).

Spontaneous EMG activity was recorded for 30 min from the unrestrained animal. Following recording of spontaneous EMG activity, the animals were injected i.p. with a single dose (150 mg/kg) of L-DOPA (Sigma, UK) dissolved in 0.1 ml of saline and the EMG activity was monitored for about 90–120 min after injection. The aggregate EMG activity from each muscle was determined by counting all the muscle action potentials above the noise level (about 50 μV) using a spike trigger (Neurolog NL 200; Digitimer) and displayed on a computer using a software package (MRATE, Cambridge Electronic Design). In each recording session, aggregate EMG activity was measured and the results were expressed as spikes per minute [18].

2.3. Muscle tension recordings

At 4 and 8–10 weeks of age the animals were anaesthetised with chloral hydrate (i.p. 4.5%; 1 ml/100 g body weight) and the distal tendons of the SOL, EDL, and TA muscles of both legs were dissected free of their surrounding tissue, and attached to strain gauges (Dynamometer UFI, Devices). The sciatic nerve was exposed and cut. The nerve to soleus and the deep peroneal nerve were dissected and prepared for stimulation. Isometric contractions were elicited by electrical stimulation of the motor nerve, via bipolar silver electrodes using a pulse width of 0.02 ms. The length of the muscle was adjusted at the beginning of the contraction experiment so that it developed maximal twitch tension. During a single twitch, the time to peak (TTP), i.e. time taken by the muscle to produce peak tension and the half relaxation time (1/2RT), i.e. time taken for the peak tension to drop to half its value were measured. To estimate the number of motor units in each muscle the stimulus strength was gradually increased to obtain stepwise increments of twitch tension, as individual motor axons were recruited. The number of stepwise increments was counted to give an estimate of the number of motor units present in the muscle. The mean motor unit force was obtained by dividing the maximum tetanic tension of each muscle by the number of increments of twitch tension in response to stimulation of the motor nerve. To determine the maximum tetanic tension, the motor nerve was stimulated repetitively at frequencies ranging from 10 to 100 Hz, for 600 ms and the maximum tetanic tension was determined at the optimal frequency of repetitive stimulation. Statistical differences in the muscle contractile properties were tested by a Mann–Whitney *U*-test. This non-parametric test was used because of relatively small sample size and due to the fact that data was not normally distributed.

2.4. Histology and histochemistry

After the tension experiments had been completed, the investigated muscles were dissected out from the rats, weighed and frozen in cooled isopentane. The corresponding muscles from either L-DOPA or saline treated animals were mounted next to each other, so that the pair of muscles underwent the same processing, i.e. sectioning (10 μm) and staining. The muscles were stained for succinate dehydrogenase (SDH), which reflects the oxidative capacity of the muscle fibres or processed for immunocytochemistry using a specific antibody against slow myosin. To visualize the endplates and the axons a modified, combined cholinesterase-silver stain [17,21] was used.

2.5. Retrograde labelling of motoneurones

The number of motoneurones in animals treated either by L-DOPA or saline was assessed using the retrograde tracer horseradish peroxidase (HRP) method as previously described in more detail [8,20]. Briefly, under chloral hydrate anaesthesia and using sterile precautions 15% solution of HRP (type VI; Sigma)