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Bone Fracture Induces Reflex Muscle Atrophy Which Is Sex-Dependent

H. URBANCOVÁ, P. HNÍK, R. VEJSADA

Institute of Physiology, Czech Academy of Sciences, Prague

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Summary

Longlasting nociceptive stimulation is known to cause atrophy of adjacent muscles. The aim of this study was to determine further the possible mechanisms of this pathological phenomenon. Unilateral fracture of the paw was performed under pentobarbital anaesthesia in several experimental groups (n = 8–11) of female and male rats. Dry muscle weights of the soleus (SOL), extensor digitorum longus (EDL), gastrocnemius (GA) and tibialis anterior (TA) were determined 7 days following the bone fracture and compared to the weight of contralateral control muscles. To demonstrate the reflex origin of this atrophy, deafferentation of the paw by dorsal root section (L_{4-6}) was performed before or after unilateral fracture of hindlimb metatarsal bones. In female rats, the fracture resulted in a significant loss of muscle weight in all the four muscles examined. When the hindlimb was deafferented prior to the fracture, no muscle atrophy developed, and neither did deafferentation itself cause any appreciable change in muscle weight except in male rats. This supports the concept that this type of atrophy is reflex in origin. Deafferentation, when performed after the fracture, did not prevent the weight loss in extensor muscles (SOL, GA), while the flexors (EDL, TA) did not in general lose any weight. The results in male rats had a similar trend as in female rats, although the weight loss was significantly smaller. Our results showed that the mechanism of reflex muscle atrophy following metatarsal bone fracture involves a component which is dependent on afferent information from the injured paw. Differences in the degree of affection of different muscle types (extensors vs flexors, slow vs fast muscles) and of female and male rats suggest that the muscle atrophy is the result of a complex process that probably also involves hormonal mechanisms.

Key words
Bone fracture – Muscle atrophy – Deafferentation

Introduction

Rapid wasting of muscles around painful joints was repeatedly described by neurologists, surgeons and physiologists (Raymond 1889, Harding 1929, Jirásek 1945, Thomsen *et al.* 1946 and others). Since the clinical and experimental observations were made on painful afflictions of joints, this type of atrophy was termed arthrogenic or arthritic muscle atrophy. It was subsequently denoted reflex muscle atrophy by Gutmann and Vodička (1953) and Vodička (1957) on the basis of their experimental results on rats involving crushing of the paw, or an injection of a small quantity of turpentine oil into the planta of one paw. In spite of extensive experimentation by Hník *et al.* (1974, 1977a, 1977b), when paw fracture was combined with an injection of turpentine oil into the paw, the actual mechanism which leads to this type of atrophy still remains poorly understood.

In the present set of experiments we studied three aspects of this problem in female rats and subsequently in male animals. Firstly, we wished to confirm that muscle atrophy induced by bone fracture is really of reflex origin by sectioning corresponding dorsal roots prior to fracture. Secondly, we attempted to ascertain for how long the painful stimulus must act for reflex atrophy to develop by deafferentating the hind limb subjected to the nociceptive stimulus at different times after bone fracture. And thirdly, some selected experimental procedures were repeated on male rats, in an attempt to ascertain whether there are not sexual differences in the susceptibility to muscle

atrophy induced by nociceptive stimulation. Some of these results have been published in preliminary form elsewhere (Urbancová *et al.* 1992).

Methods

Experiments were performed on adult female and male rats of the Wistar strain (180 g and 215 g mean body weight respectively). Each group comprised 9 - 11 animals. Fracture of metatarsal bones (F) was performed with two pairs of artery forceps on the right side under Sagatal /sodium oentobarbital) anaesthesia (60 mg/kg i.p.). Unilateral intradural section of L_4-L_6 dorsal roots was also performed under Sagatal anaesthesia. This extent of deafferentation eliminates the sensory inflow from the lower leg (see Vejsada and Hník 1980), including the planta, calf and shank muscles (namely the soleus (SOL) and gastrocnemius (GA) – both extensor muscles, and the extensor digitorum longus (EDL) and tibialis anterior (TA) – both ankle flexors). Seven days after the bone fracture, SOL and EDL muscles, and in some experiments also GA and TA were excised under general anaesthesia and exsiccated over P_2O_5 at 60-70 °C. The weight of muscles on the experimental side was expressed in percentage of corresponding muscles of the control side (mean values ± S.D.) and the differences were evaluated by Student's t test.

The following experiments were performed on female as well as male rats:

1. Fracture of one paw (F)
2. Unilateral section of dorsal roots L_4-L_6 (DEA)
3. Fracture of the paw immediately after ipsilateral deafferentation (DEA + F)
4. Fracture of the paw 2 h, 1 h or 2 min before ipsilateral deafferentation (F + DEA).

The results of muscle weight (expressed as percentage of contralateral control muscles) were also recalculated after eliminating the maximum and minimum value in each subgroup. Since the mean values thus reduced did not differ appreciably from the non-reduced values, only the former results are presented in the Tables.

Table 1

The dry weight of four muscles (SOL – soleus, GA – gastrocnemius, EDL – extensor digitorum longus, TA – tibialis anterior) in female rats seven days after unilateral paw fracture compared with the dry weight of contralateral controls.

		SOL	GA	EDL	TA
F	R	17.8 ± 2.5***	204.4 ± 28.4***	22.0 ± 2.7*	68.7 ± 8.8***
	L	23.4 ± 2.9	265.3 ± 32.9	24.2 ± 2.2	87.3 ± 5.5
	R/L (%)	75.0 ± 7.1	77.5 ± 9.7	88.0 ± 10.3	80.6 ± 9.3
DEA	R	20.6 ± 3.9	-	21.9 ± 3.6	-
	L	20.4 ± 2.9	-	21.0 ± 3.5	-
	R/L (%)	100.2 ± 10.0	-	103.2 ± 9.8	-
DEA + F	R	19.3 ± 3.8	-	21.1 ± 2.3	-
	L	20.2 ± 3.6	-	20.9 ± 4.3	-
	R/L (%)	95.0 ± 16.3	-	107.4 ± 18.5	-
2MF + DEA	R	18.2 ± 3.6**	233.8 ± 38.6**	21.5 ± 3.4	75.1 ± 11.0**
	L	21.0 ± 3.3	248.3 ± 33.1	21.8 ± 3.1	82.3 ± 8.9
	R/L (%)	88.5 ± 12.6	93.9 ± 6.5	97.8 ± 9.2	91.2 ± 7.4
1HF + DEA	R	14.3 ± 5.8*	209.5 ± 44.9*	19.2 ± 4.4	68.0 ± 13.4
	L	19.0 ± 4.0	220.3 ± 46.1	19.3 ± 4.2	72.2 ± 15.4
	R/L (%)	87.1 ± 12.6	95.2 ± 5.3	98.3 ± 6.7	95.3 ± 12.1
2HF + DEA	R	14.8 ± 2.1**	-	17.9 ± 2.2	-
	L	19.4 ± 3.4	-	17.6 ± 2.2	-
	R/L (%)	79.3 ± 16.7	-	102.5 ± 12.8	-

Paw fracture (F), deafferentation (DEA), deafferentation preceding fracture (DEA + F) and fracture following dorsal root section by 2 min (2MF + DEA), 1 h (1HF + DEA) or 2 h (2HF + DEA) respectively. All values are means ± S.D. of dry muscle weight in milligrams and as percentage of the controls ± S.D. Statistical significance against control left side: * $p < 0.05$, ** < 0.01, *** < 0.001.

Table 2
Statistical significance of differences between particular groups of female rats according to the experimental procedure.

Experimental procedure	SOL	GA	EDL	TA
F vs				
DEA	$p<0.001$	-	$p<0.01$	-
DEA+F	$p<0.01$	-	$p<0.05$	-
2MF+DEA	$p<0.01$	$p<0.001$	$p<0.05$	$p<0.05$
1HF+DEA	$p<0.05$	$p<0.001$	$p<0.05$	$p<0.01$
2HF+DEA	ns	-	$p<0.05$	-
DEA vs				
DEA+F	ns	-	ns	-
2MF+DEA	$p<0.05$	-	ns	-
2MF+DEA	ns	-	ns	-

F – paw fracture, DEA – deafferentation, 2MF + DEA – fracture performed 2 min prior to deafferentation, 1HF and 2HF + DEA – fracture preceded dorsal root section by 1 h and 2 h, respectively.

1. Loss of muscle weight after fracture of the paw in female rats

Seven days after unilateral metatarsal bone fracture, all the muscles studied exhibited significant atrophy as compared with their contralateral controls (Table 1). The loss of muscle weight was most marked in the two extensor muscles (SOL and GA) and smallest in the flexor muscle EDL.

2. Deafferentation performed prior to fracture of the paw in female rats

Unilateral deafferentation by dorsal rhizotomy L_4-L_6 itself did not cause any appreciable muscle wasting of either the SOL or EDL 7 days after the operation (DEA in Table 1). When the fracture was performed immediately after dorsal root section, it did not cause any significant muscle atrophy in either of these two muscles (DEA + F in Table 1). It is thus evident that for muscle atrophy to develop after bone fracture, impulse activity from nociceptors must reach the spinal cord. The difference between muscles of group F and DEA + F was highly statistically significant ($p < 0.01$ for the SOL and $p < 0.05$ for the EDL). This finding corroborates the assumption that muscle wasting caused by bone fracture is "reflex" in origin.

Results

Table 3
The dry weight of four shank muscles (SOL – soleus, GA – gastrocnemius, EDL – extensor digitorum longus, TA – tibialis anterior) in male rats seven days after unilateral paw fracture compared with the dry weight of contralateral controls.

		SOL	GA	EDL	TA
F	R	22.9±3.6***	305.5±35.3***	28.8±3.9	107.3±8.3*
	L	27.2±4.1	345.2±29.9	30.6±2.6	112.2±8.0
	R/L (%)	84.2±4.4	88.4±5.0	94.4±11.1	95.7±4.8
DEA	R	23.5±3.6**	293.0±39.7**	27.2±2.4	89.1±10.1*
	L	26.9±4.3	319.9±36.4	27.8±2.2	100.7±13.2
	R/L (%)	88.1±12.1	91.7±7.4	97.9±5.1	89.4±12.4
DEA+F	R	28.9±6.0	378.3±32.0**	35.7±3.4	118.8±6.8**
	L	32.7±4.2	428.7±34.2	37.8±2.5	130.3±10.5
	R/L (%)	85.4±22.0	88.7±8.9	95.5±8.7	92.3±5.3
2MF+DEA	R	27.4±4.2**	355.9±62.3***	34.3±6.3	115.2±20.7*
	L	31.4±6.0	418.6±84.3	35.6±7.3	126.9±24.4
	R/L (%)	86.8±5.8	85.8±7.5	95.4±8.4	91.4±7.8

Paw fracture (F), deafferentation (DEA), deafferentation preceding fracture (DEA+F) and fracture following dorsal root section by 2 min (2MF + DEA). All values are means ± S.D. of dry muscle weight in milligrams and as percentage of the controls ± S.D. Statistical significance against control left side: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Since deafferentation annuls the effect of the nociceptive stimulus, the question arose as to how long must this stimulus act in order to induce muscle atrophy. We thus performed the following series of experiments in which we eliminated sensory information from the periphery by dorsal root section at different times after paw fracture.

3. Bone fracture performed at different times prior to deafferentation in female rats.

In these experiments, paw fracture was again performed under Sagatal anaesthesia either 2 h, 1 h or 2 min before dorsal root section. Muscles were excised 7 days later. It is evident from Table 1 that even when the nociceptive stimulus is only allowed to act 2 min before dorsal root section (2MF + DEA), significant wasting was noted in three of the four muscles followed (with the exception of EDL). When dorsal rhizotomy was performed one or two hours after paw fracture, loss of muscle weight gradually increased (1HF + DEA and 2HF + DEA) in the SOL (an extensor muscle) but not the EDL (a flexor muscle). In the GA and TA this progressing trend was not seen after 1HF + DEA (Table 1). It may thus be concluded that the nociceptive stimulus inducing muscle wasting does not need to act throughout the 7 days' period, but that the mechanism involved is triggered after a relatively short time. Statistical evaluation of the data described in Table 1 in female rats is presented in Table 2.

Table 4
Statistical comparison of muscle weight in female and male rats according to the experimental procedure. (See results presented in Tables 1 and 3)

Experimental procedure	SOL	GA	EDL	TA
F	$p<0.01$	$p<0.01$	ns	$p<0.001$
DEA	$p<0.05$	-	ns	-
DEA + F	ns	-	ns	-
2MF + DEA	ns	$p<0.05$	ns	ns

For further explanation see the text to Table 2.

4. Reflex atrophy in male rats and the effects of deafferentation performed before or after paw fracture

The experiments in male rats were performed by the same procedure as in the females. The results are summarized in Table 3. When muscle atrophy evoked by the nociceptive stimulus is compared, it may be seen that muscle wasting in male rats is significant but smaller than in the females (Table 4). This applies to the SOL, GA and TA. However, similarly as in female rats, the EDL was more resistant to paw fracture than the other three muscles studied (Tables 2, 3 and 4). There was no muscle wasting of the SOL and EDL after 7 days' unilateral deafferentation in female rats without nociceptive stimulation (Table 1). However, significant atrophy was found in the SOL, GA and TA after dorsal root section in the males (Table 3), with the exception of the EDL. This intersexual difference was statistically significant for $p < 0.05$. However, when bone fracture was performed after deafferentation, no further loss of muscle weight occurred in the males (Tables 2, 3 and 4). It can thus be concluded that muscle atrophy induced by a nociceptive stimulus is less marked in male than in female rats. This fact is documented in Table 4.

Discussion

The present results have shown that paw fracture by itself causes significant muscle atrophy. This is hardly surprising in view of the fact that "arthrogenic" (Harding 1929, Thomsen *et al.* 1946 and others) and the so-called "reflex" muscle atrophy (Gutmann and Vodička 1953, Hník *et al.* 1974) have already been studied. However, several new aspects of this phenomenon have been supplemented in the present paper.

Firstly, fracture of the paw induces muscle atrophy after 7 days even when this nociceptive stimulus is applied under Sagatal anaesthesia, the effect of which lasts for several hours. As was reported previously, reflex muscle atrophy is already present 3 days after application of the nociceptive stimulus (Hník *et al.* 1974).

Secondly, deafferentation by dorsal root section eliminates muscle wasting in female rats, if performed prior to paw fracture. This corroborates the findings of Harding's experiments. Harding (1929) studied "arthrogenic" muscle atrophy in rabbits and, for this type of atrophy to develop, intact sensory inflow into the spinal cord is essential. Both types of atrophy thus deserve the attribute "reflex".

Thirdly, a novel aspect to the problem of reflex atrophy has been the finding that male rats are less sensitive to the nociceptive stimulus than the females. This result certainly requires further experimental analysis, since it indicates that intersexual differences may play an important role in the susceptibility of male rats to a longlasting nociceptive stimulus, such as bone fracture. It may be argued that hormonal influences would not be expected to affect selectively muscles of the afflicted extremity. However, the experiments of Bajusz (1958), for example, showed that, although hypoxia by arterial ligature does not by itself cause atrophy of lower leg muscles in rats, exposure of the animals to stressful situations leads to selective wasting of muscles in the restricted vascular territory.

Fourthly, considerable effort was made previously in our laboratory to ascertain the probable mechanisms leading to reflex muscle atrophy. Disuse itself does not seem to be the primary cause, since EMG recordings from the SOL (the muscle most affected by reflex atrophy) returned to normal within 1 - 2 hours after paw fracture and injection of turpentine oil into the planta (Hník *et al.* 1977b). It was also shown that neither restriction of blood flow by ligature of the iliac artery nor adrenalectomy (nor the administration of corticosteroid hormones) affected the development of reflex muscle atrophy (Hník *et al.* 1977a). An attempt was also made to ascertain whether nociceptive stimulation leads to changes in the incorporation of labelled lysine into spinal motoneurones innervating the lower leg muscles in rats. Even these results gave negative results. This led to the conclusion that neuronal protein metabolism is not altered during reflex muscle atrophy (Jakoubek *et al.* 1980). Of course, these authors may not have chosen a sufficiently representative amino acid to test their hypothesis that the protein metabolism of spinal motoneurones is altered under the influence of the nociceptive stimulus.

In the present experiments we tried to substantiate the hypothesis that the muscle atrophy after bone fracture, which develops in most shank muscles, is due to increased afferent activity evoked by the fracture. The injury discharges from the injured paw can lead to excessive release of excitatory amino acids (see Palečková *et al.* 1992) and neuromodulators, such as substance P (see Brodin *et al.* 1987) from primary afferents that is thought to cause sensitization of dorsal horn neurones, activation of second messenger systems and prooncogenes such as C-fos (see also Paleček *et al.* 1992). It is thus obvious that the mechanism of reflex muscle atrophy is more complex than was originally anticipated. Besides a purely spinal mechanism, supraspinal centres also appear to be involved (Hník *et al.* 1977b). The greater susceptibility of female rats to chronic nociceptive stimulation are a further aspect contributing to the complexity which leads to reflex muscle atrophy.

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Reprint Requests
Dr. P. Hník, Institute of Physiology, Czech Academy of Sciences, 142 20 Prague 4, Czech Republic.