Table 2. Action potential parameters and force of contraction as influenced by morphine (10 μmol/l) in rabbit papillary muscles and by ethylketocyclazocine (EKC) (10 μmol/l) in guinea pig papillary muscles (means ± SEM)

		APA (mV)	RP (mV)	OS (mV)	APD_{20} (ms)	APD_{90} (ms)	dV/dt_{max} (V/s)	Fc (mN)
Control	(n=5)	111 ± 3	−83 ± 1	26 ± 2	65 ± 5	165 ± 13	228 ± 33	0.68 ± 0.15
Morphine	(n=5)	111 ± 3	−83 ± 1	26 ± 3	66 ± 6	166 ± 12	235 ± 31	0.66 ± 0.15
Control	(n=6)	129 ± 1	−90 ± 0	39 ± 1	94 ± 5	175 ± 6	225 ± 28	0.1 ± 0.016
EKC	(n=6)	131 ± 2	−90 ± 0	41 ± 2	90 ± 6	174 ± 7	231 ± 30	0.09 ± 0.011

report[5] on the effects of morphine on rabbit papillary muscles has demonstrated a small negative inotropic effect which was eliminated upon the addition of naloxone 0.1 μmol/l. The morphine-induced negative inotropy was, however, not seen in the presence of atenolol 1 μmol/l, which led the authors to conclude that the effect of morphine on cardiac contractility was mediated by an indirect effect, located presynaptically at the adrenergic nerve terminals. This interpretation, however, is in conflict with the results of Starke et al.[9] and Fuder et al.[10], who have shown that the inhibition of noradrenaline release by opioids is mediated by kappa-receptors and not by μ-receptors. An alternative explanation for the results of Saxon et al.[5] would be an interaction between morphine and β-receptor activation affecting the adenylate cyclase. To test this possibility, we also investigated the interaction between morphine 10 μmol/l and isoprenaline 30 nmol/l. Under these conditions also, however, morphine failed to affect either the action potential or the force of contraction in human and guinea pig papillary muscles (not shown). We, therefore, favor the view that the action of the heart is not directly regulated by the release of endogenous opioids. This is unrelated to the possibility that endogenous opioids may significantly alter cardiac function by modulation of the release of noradrenaline[8-10] or acetylcholine[12-15].

Acknowledgments. We thank Dr H. Fuder (Pharmakologisches Institut der Universität Mainz) for valuable comments and suggestions. We also thank Prof. Satter (Abteilung für Herz-, Thorax- und Gefäßchirurgie der Chirurgischen Klinik der Johann Wolfgang Goethe-Universität Frankfurt) for supplying us with heart muscle preparations. H. N. and H. J. are supported by grants from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

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0014-4754/89/040337-03$1.50 + 0.20/0

Intramuscular comparison of myosin isozymes and light chains in rat extensor digitorum longus muscle

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Received 8 September 1988; accepted 12 January 1989

Summary. Complete muscle cross sections were obtained from the proximal and distal third regions of ten rat extensor digitorum longus muscles. Electrophoretic methods were then used to quantify the various myosin isozymes and light chains in each muscle specimen. The results demonstrated that the relative distribution of the various myosin isozyme and light chain variables do not vary significantly between the two sampling regions.
Key words. Myosin light chains; myosin isozymes; skeletal muscle.

In its native state, skeletal muscle myosin is a hexameric molecule consisting of two heavy chains and two pairs of light chains[1]. The polymorphic light and heavy chains are assembled in various combinations, producing a myosin molecule that exists in a large number of isozymic forms[2, 3].

Myosin isozymes are often regarded as markers representative of the physiological state of a muscle; the isozymes expressed by a particular muscle will depend upon the developmental stage[4, 5], the pathological or regenerative state[5–7], and the activity pattern[8] of the fibers that constitute the muscle.

The distribution of myosin isozymes in single muscle fibers is not known. Also unknown is whether the distribution of myosin isozymes and light chains are uniform between regions of muscle that share the same muscle fibers (i.e. regions homogenous in their histochemical fiber type composition). If the distribution between histochemically similar regions does vary then the relevance of studies examining muscle myosin would be somewhat questionable since the results of these analyses would be typical of a particular region of muscle only.

The purpose of the present study, therefore, was to determine whether the relative distribution of myosin isozymes and light chains is uniform among areas of muscle that share the same fibers. In order to investigate this problem, electrophoretic techniques were used to determine the relative isozyme and light chain distribution in two distinct regions of the rat fast-twitch extensor digitorum longus (EDL) muscle.

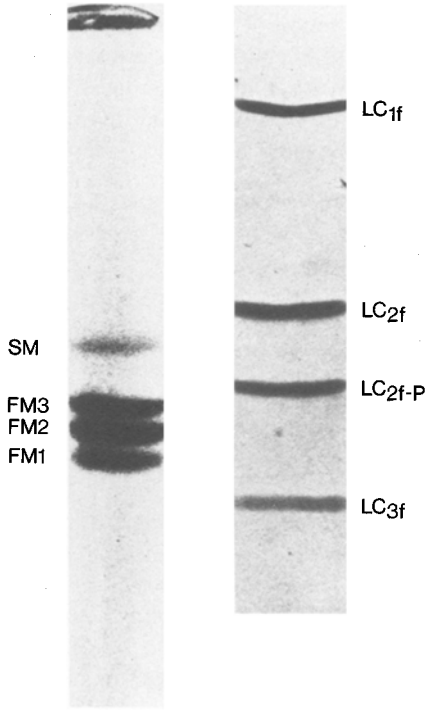
Materials and methods. Ten adult male Wistar rats weighing 127 ± 10 g were housed in an animal facility under temperature and light controlled conditions and fed rat chow and water ad libitum.

Under pentobarbitone sodium anaesthesia (0.44 ml/kg i.p.), the right extensor digitorum longus (EDL) muscle from each animal was excised by a cut through the tendons of origin and insertion. The EDL muscle is a fast contracting, pinnate, fusiform muscle[9]. The fibers originate from a tendonous attachment to the lateral epicondyle of the distal femur, divide into four distinct muscle groups near the ankle, and insert via four separate tendons on the distal phalanx of digits 2, 3, 4 and 5 of the foot. Thus, to make valid comparisons between different regions of the EDL muscle, two whole muscle cross sections (approximately 5 mg each) were taken from distinct levels of the muscle belly, proximal to its division into the four separate muscle groups, and where the population of muscle fibers was common to both areas. One cross section was taken from the proximal third of the muscle belly and the other cross section from the distal third portion. The muscle specimens were trimmed of connective tissue, quick-frozen in liquid nitrogen, and stored at − 80 °C until further analysis.

Each cross section of EDL muscle was homogenized and diluted with homogenizing buffer[10]. Native myosin was isolated from pyrophosphate polyacrylamide gels following electrophoresis at 90 V for 3.5 h at 1 °C[1]. Light chains were subsequently separated by isoelectric focusing (pH gradient 4.5–6.0) for 3.5 h on 3.4% polyacrylamide gels[10]. After fixing in 20% trichloracetic acid, gels were silver stained[11] to reveal the different light chain bands contained within the sample (fig.). Each band from each sample was scanned using an LKB 2202 Ultroscan laser densitometer. The relative percentages of the three fast light chains (LC_{1f}, LC_{2f}, and LC_{3f}) and the two slow light chains (LC_{1s} and LC_{2s}) were determined with an LKB 2200 integrator. The present method also identified the phosphorylated forms of the myosin light chain (designated LC_{2f-P} and LC_{2s-P}); therefore, LC_{2f} and LC_{2s} were computed as the sum of $LC_{2f} + LC_{2f-P}$ and $LC_{2s} + LC2_{s-P}$, respectively.

The myosin isozymes were isolated on 4.5% pyrophosphate polyacrylamide tube gels following electrophoresis at 50 V for 48 h at 1 °C (fig.). The fast and slow isozyme bands were stained with Coomassie brilliant blue, and the relative percentage of each of the three fast isozymes (FM1, FM2, and FM3) and slow isozyme (SM) were determined by laser densitometry and integration.

Specimens obtained from the same muscle were always electrophoresed together as a single batch to eliminate potential variance between different runs of electrophoresis. For the light chain determination, a total of two separate runs of electrophoresis were necessary to analyze all the samples. For the isozyme analysis, all the samples were assayed in a single run of electrophoresis.



Electrophoretic determination of myosin isozyme (left) and light chain (right) bands from rat EDL muscle. (Since the percentage of slow myosin light chains (LC_{1s} and LC_{2s}) in EDL muscle was low, the corresponding bands could not be photographically reproduced.)

Table 1. Relative proportion (%) of myosin light chains from cross sections obtained from the distal third and proximal third regions of the rat extensor digitorum longus muscle. Values are average of 10 animals ± SD

Light chain	Region of EDL muscle	
	Distal third	Proximal third
LC_{1f}	23.9 ± 4.2	22.8 ± 3.0
LC_{2f}	58.9 ± 6.4	62.8 ± 4.3
LC_{3f}	12.9 ± 2.8	11.8 ± 4.7
LC_{1s}	1.2 ± 3.3	0.2 ± 0.2
LC_{2s}	3.1 ± 5.0	2.4 ± 2.5

Table 2. Relative proportion (%) of myosin isozymes from cross sections obtained from the distal third and proximal third regions of the rat extensor digitorum longus muscle. Values are average of 10 animals ± SD

Isozyme	Region of EDL muscle	
	Distal third	Proximal third
FM1	17.5 ± 5.8	15.1 ± 3.1
FM2	36.2 ± 7.2	36.2 ± 2.5
FM3	42.1 ± 2.7	45.8 ± 5.1
SM	4.2 ± 11.2	2.9 ± 1.1

A Student's t-test was performed on paired comparisons between specimens from the proximal third and distal third regions of the EDL for each of the myosin isozyme and light chain variables quantified. A 5% level of significance was used for all statistical analyses.

Results. The relative distribution of the various myosin isozyme and light chain bands is given in tables 1 and 2, respectively. The Student's t-test revealed no significant differences between the proximal and distal portions of EDL muscle for any of the variables measured.

Discussion. The present investigation has demonstrated that myosin isozymes and light chains are uniform between different regions of the fast-twitch EDL muscle. It is important to note that the specimens obtained from the two regions shared the same muscle fibers. It has been previously demonstrated that muscles characterized by compartments of histochemically distinct fiber types exhibit a corresponding distribution of myosin isozymes [12, 13]. Single fiber analysis suggests that this regional distribution can be ascribed to the unique composition of light and heavy chains that characterize each muscle fiber of a given type [2, 3]. Significant variation between specimens from the two regions sampled in the present study would thus have been likely had they been composed of contrasting fiber types.

Our findings suggest that the relative distribution of myosin isozymes and light chains is homogeneous along single muscle fibers. They do not reveal anything about the distribution of myosin within a myofibril. Miller et al.[14] found in nematode muscle that thick filaments were composed of two different myosins. This finding would be compatible with the results of the present investigation if the distribution of different myosins were homogeneous both within a single sarcomere and between the different sarcomeres making up to myofibril. Further work will be necessary to determine the precise distribution of myosin within a single thick filament, sarcomere, and myofibril, and how these various distributions are related to the overall distribution of myosin isozymes in single muscle fibers and whole muscles.

Acknowledgments. The authors wish to thank Dr R. I. Woods (Cambridge) and Linda Seto (Waterloo) for their invaluable technical assistance. This work was supported in part by the Natural Science Engineering and Research Council of Canada.

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0014-4754/89/040339-03$1.50 + 0.20/0