Difference in the sensitivity to Ca ion among glycerinated skeletal, cardiac and smooth muscles

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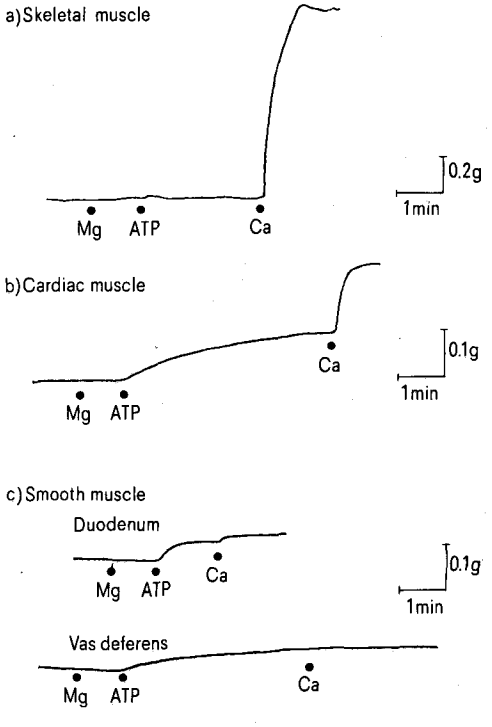
Summary. Glycerinated smooth muscle was contracted almost maximally with 15 mM Mg and 5 mM ATP, while extracted skeletal and cardiac muscles needed Ca ion with 15 mM Mg and 5 mM ATP for producing contraction.

Glycerol extracted skeletal muscles have been used as a model system for the study of contractile properties[2]. There are some investigations about glycerinated smooth[3] and cardiac muscles[4]. Filo et al.[5] reported that Mg ion dependence in the glycerinated smooth muscle is higher than that in the glycerinated skeletal muscle, inspite of similar dependence on Ca ion. Embry et al.[6] showed that the sensitivity to Ca ion in the cardiac muscle disappeared after glycerol extraction for more than 12 weeks. The Ca ion insensitive preparation was also reported in the natural actomyosin (myosin B) taken from arterial smooth muscle[7]. Bozler[8] reported that the Ca ion insensitive contraction, which was produced in glycerinated stomach muscle, could not be explained as contraction induced by a trace contamination of Ca ion in the solution. In the present study, the sensitivity to Ca ion was compared among glycerinated skeletal, cardiac and smooth muscles.

Materials and methods. Skeletal muscles (musculus adductor magnus) and cardiac muscles (ventricular myocardium) in both rats and dogs were split along fibres. Intestinal smooth muscles in dogs were separated to longitudinal and circular muscles. All muscles, which were tied to glass bars, were transferred into 2 mM $MgCl_2$ at 0 °C for 30 min, and immersed in 50% glycerol solution containing 60 mM KCl, 4 mM $MgCl_2$ and 15 mM Tris-HCl (pH 6.8) at 0 °C overnight. Then the glycerol solution was refreshed and the muscles were extracted under − 20 °C for 6 days. After 6 days, the muscles were washed thoroughly with the bath medium buffer solution and used in the experiments. $CaCl_2$ and $MgCl_2$ used in the present experiment were dissolved in the bath medium buffer solution. ATP was neutralized to pH 6.8 with KOH. The concentration of free Ca ions was obtained by using an EGTA-CaEGTA buffer system[9]. Double distilled water was used throughout the whole procedure. Tension development was recorded isometrically through a FD pick-up and a carrier amplifier (Nihon Kohden).

Results and discussion. Typical responses of glycerinated rat muscles to 15 mM $MgCl_2$, 5 mM ATP and 3×10^{-6} M Ca ion were shown in the figure. When Ca ion was added after Mg and ATP, skeletal muscles contracted rapidly. ATP after Mg produced a slow contraction in the cardiac muscles. The addition of Ca produced a rapid contraction



Typical responses of glycerinated rat muscles to Mg ion, ATP and Ca ion. Bath medium: 80 mM KCl, 4 mM EGTA and 20 mM Tris-maleate (pH 6.8). Bath volume: adjusted to 5 ml. Bath stirred using magnetic stirrer, and kept at 27 °C. ATP and $MgCl_2$ in final concentration of 5 mM and 15 mM respectively added to bath medium. Ca^{++} concentration 3×10^{-6} M, adjusted using EGTA-CaEGTA buffer system. Experiments performed with adding 100 µl of these solutions to bath medium.

Ca ion dependence of glycerinated muscles

	Ca	Tension (g/cm²)	% of $\frac{\text{response in Ca (−)}}{\text{response in Ca (+)}}$
A Rat muscle			
Skeletal muscle	−	5.4 ± 1.89 (7)	2.2 ± 0.68
	+	285.9 ± 56.82 (7)	
Cardiac muscle	−	38.3 ± 6.16 (7)	37.2 ± 4.20*
	+	103.8 ± 13.33 (7)	
Smooth muscle			
Duodenum	−	6.0 ± 1.30 (9)	75.1 ± 5.47*,**
	+	7.8 ± 1.40 (9)	
Colon	−	4.5 ± 1.02 (9)	68.2 ± 8.53*,**
	+	6.5 ± 1.32 (9)	
Vas deferens	−	4.9 ± 2.82 (5)	89.3 ± 5.15*,**
	+	5.2 ± 2.76 (5)	
B Dog muscle			
Skeletal muscle	−	5.9 ± 2.11 (6)	1.3 ± 0.45
	+	416.8 ± 44.13 (6)	
Cardiac muscle	−	33.7 ± 7.58 (6)	52.4 ± 6.61*
	+	62.9 ± 8.38 (6)	
Intestinal smooth muscle			
Longitudinal	−	62.4 ± 14.35 (5)	92.6 ± 2.95*,**
	+	66.5 ± 14.14 (5)	
Circular	−	30.6 ± 13.66 (5)	89.6 ± 5.04*,**
	+	34.0 ± 7.39 (5)	

Bath medium: 80 mM KCl, 20 mM Tris, 4 mM EGTA, pH 6.8 with maleate. ATP and $MgCl_2$ in final concentration of 5 mM and 15 mM respectively added to bath medium. Ca^{++} concentration adjusted using EGTA-CaEGTA buffer system. Ca(−): $<10^{-8}$ M, Ca(+): 3×10^{-6} M. Diameter of bundles of glycerinated fibres used: skeletal or cardiac muscles, 0.5 mm: duodenum, colon or vas deferens in rat, 1 mm: intestinal smooth muscles in dog, 0.8 mm. Crosssectional area of muscles was estimated by dividing weight of muscles by length, density taken as 1 g/cm³. Data shown as mean ± SE (number of experiments). Differences between skeletal and other muscles statistically significant (*$p<0.01$), and differences between cardiac and smooth muscles also statistically significant (**$p<0.01$).

in the cardiac muscles. In the smooth muscles, however, ATP after Mg produced almost maximum contraction and addition of Ca produced a little contraction, different from skeletal and cardiac muscles. No more contraction was observed by further addition of Ca in all preparations. Further addition of EGTA did not relax the smooth muscle, but gradually relaxed cardiac and skeletal muscles. These results are summarized in the table, A. Tension development of glycerinated smooth muscles was much weaker than that of glycerinated skeletal muscles. The ratio of the response in the absence of Ca (probably lower than 10^{-8} M of Ca ion) to the response in 3×10^{-6} M Ca ion were 2.2% and 37.2% for the skeletal and cardiac muscles respectively. The finding indicated that glycerinated cardiac muscles partially contracted without exogenous Ca ion. The values of glycerinated smooth muscles in rats were significantly higher than those of skeletal or cardiac muscles. The sensitivity to Ca ion among glycerinated skeletal, cardiac and smooth muscles in dogs (table, B) was similar to that in rats. The role of Ca ion in the contractile system of skeletal muscle was explained by Ebashi[10]. Binding of Ca ion to troponin removed the inhibition of troponin-tropomyosin to actin, so that actin filament interacted with myosin filament and a contraction produced. A similar mechnism have been assumed to exist in cardiac and smooth muscles[11]. Recently, it was reported that the regulation of the contraction via Ca ion was different in skeletal and in smooth muscles[12–15]. Under the conditions used in the present experiment, there are at least quantitative differences with respect to initiating contraction in the skeletal, cardiac and smooth muscles, respectively.

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Endolymphatic leakage in case of acute loss of cochlear microphonics[1]

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Summary. Rapid loss of cochlear microphonics in guinea-pigs previously exposed to high-energy impulse noise was shown to be related to the breakdown of the endolymphatic boundary. The cochlear duct was rendered leaky by deterioration of the reticular membrane, and damage of sensory and supporting cells.

Cochlear microphonics are considered indicative of the activity of cochlear sensory cells[2]. Accordingly, loss of cochlear microphonics (CM) following acoustic trauma was expected to parallel cellular damage at least in the basal turn of the cochlea. Light microscopic findings of less than 20% damaged hair cells are at variance with the almost 80% decrease of CM in guinea-pigs exposed to single impulses 2 h prior to sacrifice[3]. In the present paper we report on tracer studies providing evidence of the rapid breakdown of the endolymphatic barrier which is thought to impair hair cell function.

Material and methods. Anaesthetised young guinea-pigs subjected to resection of the tympanic bulla were individually exposed to 10 successive impulses of 164 dB SPL for 0.1 msec each produced by a spark-noise generator (built at the Zentralwerkstatt für Forschung und Entwicklung des Bereichs Medizin, FSU Jena). Previous to and immediately after impulse exposure CM were taken from the round window at frequencies from 500 to 10,000 Hz. About 40–50 min after exposure to impulse noise, perilymphatic perfusion with 6% horseradish peroxidase-Ringer pH 7.6 commenced for a period of 8 min. Following a 2-min rinse with Ringer-solution, the phosphate-buffered 2% glutaraldehyde-1.5% formaldehyde fixative was instilled for 30 min, and after removal the cochlea was fixed for another 180 min. Over-night the cochlea was incubated in phosphate buffer at 4 °C. Segments of the cochlear duct were micro-dissected free from the modiolus and, without removing the spiral ligament, the stria vascularis or Reissner's membrane, the specimens were subjected to the DAB-reaction for detection of peroxidase (for details, Geyer[4]). After completion of the histochemical procedure, the tissue was dehydrated and embedded in Durcupan.

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Mean amplitudes of cochlear microphonics registered from 11 guinea-pigs immediately following the exposure to 10 impulses of 164 dB SPL

Frequency (Hz)	500	1000	2000	3150	5000	8000	10,000
CM amplitude as % of the mean value previous to noise exposure ± SD	68 ± 2.7	70 ± 3.6	69 ± 4.0	67 ± 3.3	64 ± 2.5	62 ± 3.0	64 ± 4.1

According to the t-test, the amplitudes of CM before and after noise exposure differ from each other by a level of significance better than 1%.