Muscle damage induced by the ionophore A23187 can be prevented by prostaglandin inhibitors and leupeptin

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Summary. Frog skeletal muscle incubated in vitro with the ionophore A23187 shows extensive morphological alterations. Myofilament disruption, presumably mediated by excess intracellular calcium, can be partially prevented by preincubating the muscle with inhibitors of prostaglandin synthesis and the lysosomal thiol protease inhibitor leupeptin.
Key words. Calcium; ionophore A23187; prostaglandins; skeletal muscle.

Exposure of mouse or frog skeletal muscle to the calcium ionophore A23187 causes extensive damage to the muscle fibers, and it has been suggested that A23187 releases Ca^{2+} from the sarcoplasmic reticulum which, in turn, activates muscle proteinases responsible for myofilament degradation[2,3]. A different possibility that must be considered is that A23187 promotes influx of Ca^{2+} into the muscle fibers from the extracellular space. Recently, Rodemann et al.[4] reported that in rat skeletal muscles incubated with A23187, protein degradation, as measured by the rate of tyrosine release, increases by 45–140%. This increase could be prevented by blocking the synthesis of prostaglandins. Since the rate limiting step in prostaglandin biosynthesis is calcium dependent, Rodemann et al.[4] concluded that Ca^{2+} promotes protein breakdown by stimulating synthesis of prostaglandin E_2 (PGE_2), which then activates lysosomal enzymes. I report here that the morphological damage produced by the ionophore A23187 in frog skeletal muscle can be partially prevented by pretreatment of the muscle with inhibitors of the synthesis of prostaglandins or the inhibitor of cathepsin B, leupeptin.

Materials and methods. Pairs of sartorius muscles were carefully dissected from small frogs (*Rana pipiens*). One muscle always served as control. The muscles were then stretched to normal length and pinned down through connective tissue in plexiglass chambers for continuous observation under a dissecting microscope. The incubating medium contained 1.8 mM $CaCl_2$, 2.7 mM KCl, 115 mM NaCl, 15 mM $NaHCO_3$ and was bubbled to pH 7.4 with a mixture of 95% air and 5% CO_2. Leupeptin, aspirin, indomethacin and corticosterone (all from Sigma) were dissolved in either ethanol or dimethylsulphoxide and added to the incubation bath 1 h prior to the addition of the ionophore. Proper controls were carried out with the solvents alone. Ibuprofen (Upjohn) was added to the bath as a benzyl alcohol solution. The ionophore A23187 (Sigma) was dissolved in ethanol and added to the bath to achieve the desired concentration (1–5 µM). Results are expressed as the time in minutes elapsed between adding the ionophore and the beginning of muscle fiber breakdown as observed through the dissecting microscope. Experiments were conducted at room temperature.

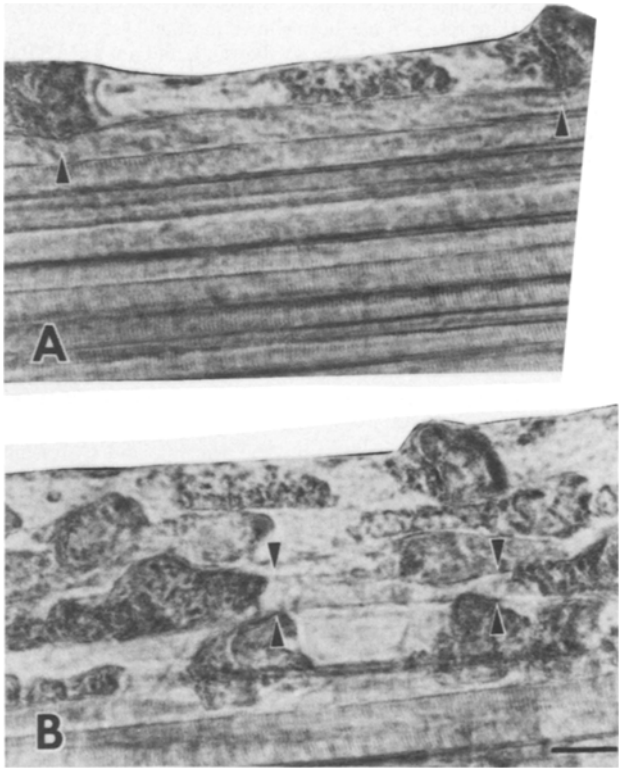
Effect of prostaglandin inhibitors and leupeptin on muscle preservation. Effects are expressed as the time (t) required to initiate fiber breakdown

	t (min)
A23187	9.6 ± 1.14*
A23187 + 25 µM leupeptin	24.6 ± 2.19
A23187	10.8 ± 1.48*
A23187 + 50 µg/ml ibuprofen	22.6 ± 2.30
A23187	12.4 ± 1.14*
A23187 + 3×10^{-6} M indomethacin	20.4 ± 2.30
A23187	10.2 ± 1.30*
A23187 + 5×10^{-4} M aspirin	23.4 ± 2.50
A23187	10.8 ± 1.30*
A23187 + 40 µg/ml corticosterone	18.0 ± 1.22

Muscles were incubated for 1 h prior to the addition of 2 µM A23187. Values expressed are mean ± SD. In all series n = 5. *$p < 0.001$ from Student's t-test.

Results and discussion. Upon adding the ionophore to the bath, one could see contraction of fibers in the surface of the muscle, some fibers forming so-called 'contraction knots'. Breakdown of individual muscle fibers could be observed approximately 10 min after adding the ionophore at a final concentration of 2 µM. A representative example is shown in the figure. Usually, as seen in the figure, edge fibers appeared to break down earlier than those in the medial section of the muscle. A possible explanation for this finding is that in edge fibers a greater area is exposed to the surrounding bathing solution. Results that are consistent with the ones presented here have been reported by others with regard to the effect of the ionophore on the fine structure of frog and mouse skeletal muscle[2,3]. Furthermore, it has been shown that the ionophore A204 also produces considerable damage to rat skeletal and cardiac muscles[5]. It is interesting to note in figure 1B that, although the myofibrillar system breaks down and there is swelling of the fibers, the sarcolemma appears intact.

In their original work, Publicover et al.[2] and Statham et al.[3] proposed that A23187 disrupts the muscle fibers by increasing



A Light micrograph of a section of the muscle after 10 min of exposure to 2 µM A23187. Only the first fiber (edge fiber) is damaged. The myofibrillar apparatus is broken and accumulation of intracellular material is pressing (arrowheads) on the adjacent fiber. *B* Same preparation as in A (displaced only a few µm) after 25 min of incubation with the ionophore. At this time, the first 5 fibers show severe damage; however, the sarcolemma, indicated between arrowheads for the 4th fiber, remains uninterrupted. Calibration bar, 50 µm.

the intracellular Ca^{2+} concentration, which in turn would activate a Ca^{2+}-activated neutral proteinase (CANP). A CANP has been described in mammalian skeletal muscle[6,7]; also, a CANP with higher than normal activity has been reported in dystrophic hamsters and mice[9]. However, the role of a CANP in degradation of muscle proteins is still not clear. A likely role for CANP would be to initiate myofilament degradation which would then be followed by activation of lysosomal enzymes.

The involvement of lysosomal enzymes in the action of the ionophore A23187 has been inferred from experiments in which the lysosomal thiol protease inhibitor leupeptin has been used. Thus, Rodemann et al.[4] showed that the increase in protein degradation induced by A23187 in mammalian skeletal muscle can be prevented by pretreatment with leupeptin. Furthermore, the same authors reported that the increase in lysosomal enzymes, specifically cathepsin B, is mediated by prostaglandins. In fact, in rat skeletal muscle A23187 increased the release of prostaglandins E_2 and $F_{2\alpha}$ 3–4 fold. There is, however, no direct evidence indicating that the ionophore increases the activity of lysosomal enzymes.

The results reported here, and summarized in the table, indicate that the morphological damage produced by the ionophore A23187 in frog skeletal muscle is mediated by prostaglandins. Since prostaglandins are widely distributed in the animal kingdom[10,11], it should come as no surprise to find them in frog skeletal muscle. With all the inhibitors of prostaglandin used, and also leupeptin, there was a significant difference in the time necessary to initiate fiber breakdown. Furthermore, once breakdown had started it progressed much slower in preparations preincubated with the inhibitors than in control preparations. It is clear, however, that damage could not be totally prevented even when Ca^{2+} was removed from the incubation medium. It is quite possible that the ionophore is able to release Ca^{2+} from intracellular stores, as it has been shown in other systems[12–14].

Rodemann et al.[4] postulated that cathepsin B and not a CANP is responsible for the increase in protein degradation induced by A23187 in rat skeletal muscle. Their conclusion was based on the fact that the sulfhydryl inhibitor mersalyl, while it completely inhibited the CANP, did not prevent the increase in protein breakdown. In the present work, experiments conducted with mersalyl proved inconclusive. Addition of mersalyl (200 μM) to the incubating bath produced muscle twitching which sometimes led to fiber damage. This effect of mersalyl is mediated by excitation of the nerve terminals since it can be prevented by preincubation with d-tubocurarine. In fact, Binah et al.[15] have shown that mersalyl, as well as other mercurials, greatly increases the frequency of the spontaneous miniature endplate potentials in the frog neuromuscular preparation in vitro.

The present results provide evidence for a role of prostaglandins in skeletal muscle fiber destruction induced by Ca^{2+}. Nevertheless, a direct action of the ionophore on the muscle enzymatic systems, CANP or lysosomes, cannot be ruled out at this time. These results are important in the context of trying to find mechanisms that can minimize the morphological alterations that occur in skeletal muscle in different pathological conditions.

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Beta-adrenergic-stimulated adenylate cyclase activity in normal and EBV-transformed lymphocytes

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Summary. Beta-adrenergic-associated cyclic AMP accumulation was studied in intact lymphocytes before and after transformation with Epstein-Barr virus into immortal cell lines. Although a marked reduction in isoproterenol-stimulated cyclic AMP synthesis was observed in transformed cells, forskolin-stimulated cyclic AMP accumulation was preserved. A parallel loss of ^{125}-iodocyanopindolol binding sites suggests that the reduction in beta-adrenergic-stimulated AMP synthesis is due to receptor down regulation.
Key words. Epstein-Barr virus; beta-adrenergic receptors; cyclic AMP synthesis; forskolin; adenylate cyclase; lymphocytes.

Epstein-Barr virus (EBV)-transformed lymphocytes are a powerful tool in immunology and virology since EBV virus induces unlimited growth of human B cells with an extremely high efficiency[2], concomitantly with preservation of the differentiated state as evidenced by the prolonged in vitro secretion of specific monoclonal antibodies[3,4]. Although specific immunological characteristics are maintained by transformed or immortal cell lines[3,4], less is known about the fate of other lymphocyte functions after EBV transformation. Such non-immunological functions are of particular interest, however, in investigations that use transformed lymphoblasts as a model system for studying the interaction of environment and heredity in the etiology of various disease states[5–7]. Circulating lymphocytes, including both B and T cells, possess a beta-adrenergic receptor linked to adenylate cyclase, which has been the subject of extensive clinical and biochemical investigations[8–11]. The presence of a well-defined adenylate cyclase complex in circulating lymphocytes and the demonstrated importance of cyclic AMP in a variety of cell processes prompted us to follow changes in this system subsequent to EBV transformation and growth in culture. The