EXPERIENTIA

Vol. 34 - Fasc. 12 Pag. 1531-1672 15.12.1978

GENERALIA

Role of intracellular calcium in promoting muscle damage: a strategy for controlling the dystrophic condition

C.J. Duncan[1]

Department of Zoology, University of Liverpool, P.O. Box 147, Liverpool L69 3BX (Great Britain)

Summary. It is suggested that various muscle diseases and examples of experimentally-induced muscle damage arise because of a high calcium level in the myoplasm. When $[Ca^{2+}]_i$ is raised experimentally in amphibian or mammalian muscle by treatment with A23187 or caffeine, myofilament degradation follows quickly. Such a rapid action suggests the involvement of a sequence of proteolytic activity that is stimulated by a rise in $[Ca^{2+}]_i$. Ca^{2+} might either trigger protease activity directly or indirectly, or promote the release of lysosomal enzymes. A high $[Ca^{2+}]_i$ in dystrophic muscle is believed to be the resultant of a sequence of events that is summarized in the figure. Suggestions are presented for different ways in which the steady-state position of $[Ca^{2+}]_i$ might ultimately be controlled for the clinical amelioration of some dystrophic conditions.

The role of Ca^{2+} in the experimental induction of muscle damage

There is now a substantial body of evidence that shows that a marked rise in free intracellular Ca^{2+} ($[Ca^{2+}]_i$) in vertebrate skeletal muscle rapidly promotes damage to the myofilaments. Application of the divalent cation ionophore A23187 to frog skeletal muscle causes major ultrastructural damage and myofilament degradation. These effects are produced when extracellular Ca^{2+} ($[Ca^{2+}]_o$) is buffered at 5×10^{-7} M or when Ca^{2+} is omitted from the saline. It is believed that the ionophore acts primarily by releasing Ca^{2+} from the sarcoplasmic reticulum (SR) and the initial response of the muscle is the development of tension. However, degradative action is rapid, and widespread damage and dissolution of the myofilaments are evident 20 min after application of the ionophore[2]. A23187 produces comparable damage in mammalian muscle[3]; electron micrographs show initially a marked swelling of the mitochondria and it is believed that the mitochondria take up the excess Ca^{2+} released by the SR. After 40 min, however, the mitochondria are shrunken and it is believed that they have then released the accumulated Ca^{2+} because of overloading[4], and myofilament degradation quickly follows[3].

Similarly, caffeine at concentrations above 5 mM was shown to cause extensive ultrastructural damage to the myofilaments of frog skeletal muscle. This action occurred within 8 min. The effect was exacerbated at lower temperatures, whereas the myofilaments were protected by prior exposure to procaine[5]. It is suggested that caffeine promotes a Ca^{2+}-induced release of Ca^{2+} (the CROC) from the SR. Mammalian diaphragm muscle fibres are unaffected by exposure to caffeine, but if the muscle is stimulated in the presence of this drug, the characteristic myofilament dissolution follows rapidly and we conclude that in these fibres the synergistic effect of the depolarized release of Ca^{2+} from the SR, together with the action of caffeine in promoting a CROC, causes a marked rise in $[Ca^{2+}]_i$ in the muscle fibres[6]. Preliminary experiments also suggest that lindane is able to promote extensive damage in vertebrate skeletal muscle, the effect being accompanied by a marked rise in $[Ca^{2+}]_i$ (Publicover et al.[7]).

The conclusion from these studies is that myofilament degradation can be rapidly produced experimentally by agents that serve to raise $[Ca^{2+}]_i$.

The causative agent in experimentally-induced muscle damage

The way in which a marked rise in $[Ca^{2+}]_i$ can promote muscle damage is unknown at present, but there are a number of ways in which this effect might be produced, for example: a) Ca^{2+}-activated proteases have been isolated from mammalian muscles; they are stimulated by Ca^{2+} concentrations in the

range 1–10 mM, are free in the myoplasm and they may have a role in the disassembly of intact myofibrils during the normal metabolic turnover in myofibrillar proteins[8–10]. Other intracellular proteases have been described[11–13] and it is possible that the marked rise in $[Ca^{2+}]_i$ acts, directly or indirectly, to stimulate these proteases of the myoplasm, so causing myofilament degradation. b) The evidence suggests that skeletal muscle contains few typical lysosomes and that the lysosomes are part of the sarcotubular system[14]. Thus, a marked rise in $[Ca^{2+}]_i$ may cause the release, by an unidentified mechanism, of acid hydrolases known to be present in the lysosomes system[15] which effect myofilament dissolution. Such an hypothesis is supported by studies in which inhibitors of catheptic enzymes can reduce the rate of protein degradation in normal and dystrophic muscle[16–18].

Once $[Ca^{2+}]_i$ has risen to a sufficiently high level, myofilament degradation follows more rapidly (within 8 min)[5] than would be expected in normal proteolysis, suggesting that degradation may be a multi-step process (cf. the cascade action of complement). Alternatively, degradation might be the result of the disassembly of the polymers of the myofilaments, rather than their proteolytic digestion.

$[Ca^{2+}]_i$ and proteases in myopathies

There is now a substantial body of evidence that implicates a rise in $[Ca^{2+}]_i$ in mammalian skeletal and cardiac muscle diseases[19]. In particular, Ca^{2+} levels are raised in the nuclei in Duchenne muscular dystrophy (DMD) and the mitochondria undergo ultrastructural changes, probably associated with Ca^{2+}-uptake, in a variety of myopathies[20,21]. Abnormality of mitochondrial function is reported in muscle from dystrophic chicken[22]. These organelles, therefore, apparently attempt to assist in the regulation of the high $[Ca^{2+}]_i$. DMD patients exhibit hypercontracted fibres[23], indicative of a permanent rise in $[Ca^{2+}]_i$. The Ca^{2+}-antagonist verapamil reduces cardiac necrosis in dogs[24]. Thus, there are clear parallels with events during the experimental induction of muscle damage[2–5].

Evidence has also accumulated recently concerning the involvement of protease activity in dystrophic conditions. A significant increase in the level of a muscle Ca^{2+}-activated, neutral protease is reported in DMD[25]; protein breakdown[26] and an increase in the specific activity of serine protease[27] are reported in the skeletal muscle of dystrophic mice; serine protease levels are also increased in DMD and Becker-type MD[28]. Finally, protease inhibitors such as leupeptin, pepstatin and antipain have variously been reported to reduce protein degradation in normal and dystrophic muscle[17], in cultured muscle cells[16] and in chickens with genetic dystrophy[18].

In summary, therefore, the studies on the experimental induction of muscle degradation may well constitute a useful model for a variety of myopathic conditions.

The biochemical lesion in dystrophic muscle

It is evident that DMD is associated with a generalized defect of the membranes not only of muscle[29–32] but also of other tissues. Both erythrocytes and the sarcolemma are altered in phospholipid composition[32,33] and erythrocytes have an altered molecular organization, as shown by electron spin resonance studies[34,35] and saturation transfer electron paramagnetic resonance spectroscopy[36]. Concomitantly, there are changes in the erythrocytes in the properties of the acetylcholinesterase[37], Na^+-K^+-ATPase[38–41], glycolytic enzymes[42] and protein kinase[43], surface deformations[44], Na^+ efflux[41], K^+ efflux[45], increased $[Na^+]_i$[46], survival[47], increased microviscosity[48] and reduced deformability[49]. Of particular significance is the elevation of Ca^{2+}-ATPase activity in DMD[50], suggesting that the cells are attempting to correct an elevated $[Ca^{2+}]_i$; the situation may be comparable to the elevated Na^+-K^+-ATPase activity in erythrocytes in hereditary spherocytosis where there is a rise in $[Na^+]_i$ in response to an increase in Na^+ permeability[51]. Increasing Ca^{2+} entry, by treatment of control erythrocytes with A23187, duplicates many of the changes in membrane properties seen in DMD, an observation consistent with the hypothesis that it is the change in Ca^{2+}-permeability that is of particular importance in this myopathy[52].

In muscle, the membrane alterations in DMD are not confined to the sarcolemma; the Ca^{2+}-uptake mechanism of the SR is also impaired[53]. Similarly, biochemical alterations are reported in the fragmented SR from dystrophic mouse, chicken and human muscle[54–58], including alterations in Ca^{2+}-transport[57,59], ATP hydrolysis[57,59,60] and phosphoenzyme formation[57,59,60].

Although Ca^{2+}-fluxes have not been measured in dystrophic muscle, it appears therefore that there is a genetic generalized membrane defect in DMD which results in a rise in net Ca^{2+}-influx and an impaired Ca^{2+}-uptake by the SR causing, in turn, an elevated steady-state position of $[Ca^{2+}]_i$ in resting muscle.

Suggested sequence of events in Duchenne muscular dystrophy

A rise in $[Ca^{2+}]_i$ above normal physiological levels in frog skeletal muscle can cause a further Ca^{2+}-induced release of Ca^{2+} from the SR (the CROC)[61,62]. The effect is particularly evident at low $[Mg^{2+}]_i$, since active Ca^{2+} uptake by the SR is stimulated by Mg^{2+}[63]. The demonstration of the control by external Ca^{2+} of Ca^{2+}-efflux from rabbit SR vesicles[64,65] confirms that

the CROC is also a feature of mammalian muscle. $[Ca^{2+}]_i$ in normal frog muscle at rest $\simeq 2 \times 10^{-7}$ M; the threshold $[Ca^{2+}]_i$ for contraction at excitation $\simeq 6$–9×10^{-7} M; in a single twitch the myoplasmic concentration rises to approximately 10^{-5} M; the CROC operates when $[Ca^{2+}]_i \simeq 10^{-4}$ M at physiological levels of $[Mg^{2+}]_i$ (Endo[62]).

Caffeine promotes the CROC[5,62] and we have now shown that exposure of frog skeletal muscle to caffeine rapidly causes myofilament degradation and muscle damage[5]. This positive feedback system of the CROC, involving high levels of $[Ca^{2+}]_i$, is probably of little importance in normal muscle in vivo, but may well be the mechanism by which an elevated $[Ca^{2+}]_i$ in dystrophic muscle, caused by the Ca^{2+} released from the SR at excitation and associated with a raised steady-state level of $[Ca^{2+}]_i$, is converted to a dangerously high level that, either directly or indirectly, can cause the activation of the proteases. The CROC could therefore be one of the key steps in a dramatic rise in $[Ca^{2+}]_i$ and in the consequent induction of muscle necrosis (figure). Such events would occur but rarely in a muscle cell, and the rapid regeneration of muscle fibres caused by administration of bupivacaine is initially readily reversible[66]. However, rat muscle becomes abnormal after a number of cycles of degeneration and regeneration[67] and in DMD, the long-term repetition of this sequence in individual cells eventually causes the disappearance of the muscle fibres and the infiltration of connective tissue and fat.

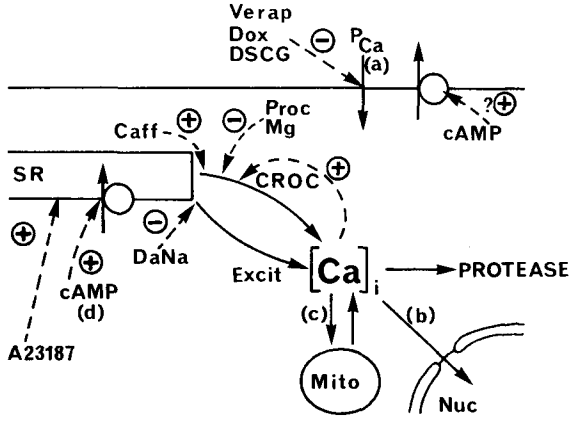
The specific biochemical lesion probably differs in various dystrophies (e.g. Na^+-K^+-ATPase is normal in dystrophic mouse erythrocytes[68]) but it is suggested that a common feature of many myopathies is an elevated muscle $[Ca^{2+}]_i$. It could be the result of an elevated Ca^{2+}-influx or reduced Ca^{2+}-efflux at the plasma membrane or impaired Ca^{2+}-uptake at the SR.

Control of muscle $[Ca^{2+}]_i$ for the amelioration of Duchenne muscular dystrophy

If this hypothesis concerning the initial biochemical defect and the sequence of events leading eventually to the disappearance of the muscle cell is correct, the development of suitable protease inhibitors or of drugs promoting lysosomal stability might be fruitful lines of study. However, the study of the factors controlling $[Ca^{2+}]_i$ in the normal and diseased muscle cell, with particular reference to the possibility of maintaining $[Ca^{2+}]_i$ below the levels that would activate the proteases, may well be a profitable area of research for the amelioration of certain myopathies. There are a number of possible cellular sites that merit consideration, particularly since the rise in $[Ca^{2+}]_i$ to a critical level is probably a multi-step process (see summary in the figure).

a) *The plasma membrane.* Since the initial genetic lesion in some dystrophies probably causes an alteration in Ca^{2+}-fluxes across the plasma membrane, a specific reduction in Ca^{2+}-influx in the resting muscle could well prove to be beneficial. The anti-arrhythmic drug Verapamil, which reduces Ca^{2+}-influx, can prevent cardiac muscle necrosis in dystrophic hamsters[24]. Cromoglycate is a clinical antiallergic drug that is believed to act by interfering with Ca^{2+}-transport across the mast cell membrane. Similar drugs that are effective by mouth (e.g. Doxantrazole, AH 7725) are also available[69] and might be an alternative way of reducing Ca^{2+}-influx.

b) *The sarcoplasmic reticulum.* The CROC is specifically inhibited by procaine[70–72] and it is of interest that this agent also protects against caffeine-induced damage of the myofilaments[5]. The development of a clinical drug that can act in this way might constitute a major advance in the care of the dystrophic condition. The CROC is also sensitive to the level of $[Mg^{2+}]_i$; reducing free Mg^{2+} markedly lowers the minimum Ca^{2+} concentration that is effective in inducing Ca^{2+} release in skinned muscle fibres[62] and from SR vesicles[64]. Mg^{2+} accelerates the rate of phosphorylation of the Ca^{2+}-ATPase of the Ca^{2+}-uptake mechanism of the SR[73]. It may therefore be important that $[Mg^{2+}]_i$ in the skeletal muscle of dystrophic patients is not allowed to fall, so as to



To illustrate: 1. The interacting factors that determine the steady-state position of $[Ca^{2+}]_i$ in mammalian skeletal muscle[93], 2. The suggested sequence of events leading to myofilament dissolution and 3. The possible sites of action of drugs that may modify the system. Thus, at rest $[Ca^{2+}]_i$ is determined by Ca^{2+}-fluxes across the sarcolemma and by Ca^{2+}-uptake by the SR and mitochondria. Cyclic AMP probably modulates Ca^{2+}-uptake by the mammalian SR (d)[87,88]. It is suggested that in DMD the significant, genetic lesion is at the Ca^{2+}-channels (P_{Ca}) of the muscle plasma membrane (a), so that Ca^{2+}-influx is increased, but Ca^{2+}-uptake by the SR may also be impaired[53]. As a consequence $[Ca^{2+}]_i$ rises in DMD and the muscle attempts to correct the position by storage of Ca^{2+} in the nucleus (b)[20] and mitochondria (c)[4]. Ca^{2+} is released from the SR at excitation and, in DMD, $[Ca^{2+}]_i$ may then be raised to a level sufficient to promote a CROC[62], so increasing $[Ca^{2+}]_i$ to a concentration where myofilament dissolution quickly follows.
Caff, caffeine; DaNa, Dantrolene sodium; Dox, Doxantrazole; DSCG, Disodium cromoglycate; Nuc, nucleus; Proc, procaine; Verap, Verapamil.

afford protection against the development of a CROC. There are various reports of myocardial lesions in mammals depleted of Mg^{2+} in early life and it is noteworthy that the deposition of calcium salts on the myofibrillar matrix and in the mitochondria is a distinctive feature of such lesions[74]. Experimental Mg^{2+}-deficiency in the rat causes a rise in total Ca^{2+} in red and white skeletal muscle, although the effect is most significant in cardiac muscle[75].

Dantrolene has been used clinically in the treatment of spasticity[76,77] and its major action is the inhibition of calcium release from the SR at excitation[78,79], as distinct from the CROC. Limited use of this drug in dystrophic muscle might allow contraction to proceed, whilst reducing the release of excess Ca^{2+}. Levels of $[Ca^{2+}]_i$ at rest are probably high in DMD, so that a reduction in the stimulated release of Ca^{2+} would be adequate for contraction, whilst reducing the risk of a Ca^{2+}-induced release of Ca^{2+} from the SR.

c) *Cyclic nucleotides.* It is now clear that the cyclic nucleotides have a major role in the control of $[Ca^{2+}]_i$ in many cells[80]. Cyclic AMP can act in a variety of ways. It can stimulate the mechanisms responsible for removing Ca^{2+}, or it can increase the influx of extracellular or the release of intracellular Ca^{2+}. Its action on Ca^{2+}-pumps has been described in cardiac muscle, where cAMP promotes Ca^{2+}-ATPase activity[81] and uptake of Ca^{2+} by the SR[82] by stimulating a protein kinase which phosphorylates a component of the SR[83]. Phosphorylation stimulates Ca^{2+}-uptake[84]. A similar mechanism may exist in smooth muscle where cAMP has been reported to stimulate the uptake of Ca^{2+} into various microsomal fractions[85]. Cyclic AMP is also believed to stimulate the Ca^{2+}-pump of the plasma membrane of both smooth and cardiac muscle[80]. In both tissues, stimulation of the β-receptors is also believed to modify cAMP levels[80] and it is significant that the action of the sympathomimetic amine isoproterenol in causing myocardial necrosis is believed to involve rises in $[Ca^{2+}]_i$ (Nirdlinger[86]). The ways in which cyclic nucleotides might interact in regulating Ca^{2+}-fluxes in skeletal muscle is less well known, but cAMP stimulates Ca^{2+}-uptake into the SR from cat and rabbit muscle[87,88]. Since intracellular levels of cyclic nucleotides can be raised by the use of phosphodiesterase inhibitors, this aspect of the regulation of $[Ca^{2+}]_i$ might be of importance and, even if cyclic nucleotides prove to have only a limited role in regulating $[Ca^{2+}]_i$ in skeletal muscle, these experiments suggest that they could probably be used in the protection of cardiac muscle from necrosis.

d) *Changes in extracellular Ca^{2+}.* Passive entry of Ca^{2+} is related to $[Ca^{2+}]_o$, and dystrophic hamsters placed on a Ca^{2+}-deficient diet have a reduced serum Ca^{2+} and show a reduction in the frequency and severity of necrosis in heart and skeletal muscle[89]. Furthermore, hereditary chicken muscular dystrophy (which has been considered as a model for human dystrophic diseases) responds favourably to penicillamine, the earlier the administration, the more favourable the response[90]. The chelating properties of this drug may be acting to produce an effect similar to that of a Ca^{2+}-deficient diet[19]. Clearly, the effects of maintaining clinically the serum free-Ca^{2+} at levels towards the lowest permissible would repay study. This suggestion may be important since serum Ca^{2+} has been shown to be elevated in children suffering from muscular dystrophy ($p < 0.001$) whereas serum Na^+ and K^+ were normal[91].

It is, therefore, suggested that one strategy for ameliorating the effects of some dystrophic conditions is dependent on a detailed understanding of the interacting factors controlling $[Ca^{2+}]_i$ in the normal skeletal muscle cell. In summary, Ca^{2+} is released from the SR at excitation and, in normal muscle, $[Ca^{2+}]_i$ rises from about 10^{-7} M to 10^{-5} M. In dystrophic muscle the higher steady-state position of $[Ca^{2+}]_i$ probably causes $[Ca^{2+}]_i$ to rise to an abnormally high level when stimulated, which occasionally (perhaps when $[Ca^{2+}]_i \simeq 10^{-4}$ M) may trigger a CROC by positive feedback, so that $[Ca^{2+}]_i$ briefly rises further to a concentration that is sufficient to stimulate proteolytic destruction. There have been suggestions that the ultimate disappearance of muscle fibres in DMD is due to the long-term repetition of the degenerative-regenerative cycle[92]. Thus, modest improvements in the control of $[Ca^{2+}]_i$, which might be achieved by a combination of some of the different treatments suggested above, may produce a substantial improvement in delaying the onset of permanent subcellular damage.

1 This work was supported in part by the Muscular Dystrophy Group of Great Britain. I am grateful to Miss S. Scott for assistance in the preparation of the manuscript.

2 H.E. Statham, C.J. Duncan and J.L. Smith, Cell Tiss. Res. *173*, 193 (1976).
3 S.J. Publicover, C.J. Duncan and J.L. Smith, J. Neuropath. exp. Neurol., in press.
4 S.J. Publicover, C.J. Duncan and J.L. Smith, Cell Tiss. Res. *185*, 373 (1977).
5 C.J. Duncan and J.L. Smith, Naunyn-Schmiedebergs Arch. Pharmac., in press.
6 C.J. Duncan and J.L. Smith, in preparation.
7 S.J. Publicover, C.J. Duncan and J.L. Smith, in preparation.
8 M.K. Reddy, J.D. Etlinger, M. Rabinowitz, D.A. Fischman and R. Zak, J. biol. Chem. *250*, 4278 (1975).
9 W.R. Dayton, D.E. Goll, M.G. Zeece, R.M. Robson and W.J. Reville, Biochemistry *15*, 2150 (1976).
10 W.J. Reville, D.E. Goll, M.H. Stromer, R.M. Robson and W.R. Dayton, J. Cell Biol. *70*, 1 (1976).
11 R.R. Kohn, Lab. Invest. *20*, 202 (1969).
12 T.R. Koszalka and L.L. Miller, J. biol. Chem. *235*, 665 (1960).
13 T.R. Koszalka and L.L. Miller, J. biol. Chem. *235*, 669 (1960).
14 D. Seiden, Z. Zellforsch. *144*, 467 (1973).
15 A.A. Iodice, V. Leong and I.M. Weinstock, Archs Biochem. Biophys. *117*, 447 (1966).
16 E.B. McGowan, S.A. Shafiq and A. Stracher, Expl Neurol. *50*, 649 (1976).
17 P. Libby and A.L. Goldberg, Science *199*, 534 (1978).

18 A. Stracher, E.B. McGowan and S.A. Shafiq, Science *200*, 50 (1978).
19 K. Wrogemann and S.D.J. Pena, Lancet *1*, 672 (1976).
20 C.A. Maunder, R. Yarom and V. Dubowitz, J. neurol. Sci. *33*, 323 (1977).
21 M.A. Oberc and W.K. Engel, Lab. Invest. *36*, 566 (1977).
22 J.H. Thakar, Biochem. Med. *19*, 108 (1978).
23 M.J. Cullen and J.J. Fulthorpe, J. neurol. Sci. *24*, 179 (1975).
24 K.A. Reimer, J.E. Lowe and R.B. Jennings, Circulation *55*, 581 (1977).
25 N.C. Kar and C.M. Pearson, Clin. chim. Acta *73*, 293 (1976).
26 P.M. Daniel, O.E. Pratt and E. Spargo, J. Physiol. *275*, 41P (1978).
27 Y. Sanada, N. Yasogawa and N. Katunuma, J. Biochem. *83*, 27 (1978).
28 N. Katunuma, N. Yasogawa, K. Kito, Y. Sanada, H. Kawai and K. Miyoshi, J. Biochem. *83*, 625 (1978).
29 D.L. Schotland, E. Bonilla and M. Van Meter, Science *196*, 1005 (1977).
30 S. Mawatari, A. Takagi and L.P. Rowland, Archs Neurol. *30*, 96 (1974).
31 L.P. Rowland, Archs Neurol. *33*, 315 (1976).
32 T.A. De Kretser and B.G. Livett, Biochem. J. *168*, 229 (1977).
33 J.L. Howland and S.L. Iyer, Science *198*, 309 (1977).
34 D.A. Butterfield, Biochim. biophys. Acta *470*, 1 (1977).
35 B. Sato, K. Nishikida, L.T. Samuels and F.H. Tyler, J. clin. Invest. *61*, 251 (1978).
36 L.S. Wilkerson, R.C. Perkins, R. Roelofs, L. Swift, L.R. Dalton and J.H. Park, Proc. natl Acad. Sci. USA *75*, 838 (1978).
37 P.K. Das, D. Graesslin and H.W. Goedde, Biochem. Soc. Trans. *4*, 723 (1976).
38 H.D. Brown, S.K. Chattopadhyay and A.B. Patel, Science *157*, 1577 (1967).
39 P.Q.R. Siddiqui and R.J.T. Pennington, J. neurol. Sci. *34*, 365 (1977).
40 T.W. Pearson, Life Sci. *22*, 127 (1978).
41 G. Souweine, J.C. Bernard, Y. Lasne and J. Lachanat, J. Neurol. *217*, 287 (1978).
42 A. Bosia, G.P. Pescarmona and P. Arese, Eur. J. clin. Invest. *1*, 413 (1971).
43 S.L. Iyer, P.A. Hoenig, A.P. Sherblom and J.L. Howland, Biochem. Med. *18*, 384 (1977).
44 D.W. Matheson and J.L. Howland, Science *184*, 165 (1974).
45 J.L. Howland, Nature *251*, 724 (1974).
46 R.M. Dowben and K.R. Holley, J. Lab. clin. Med. *56*, 867 (1959).
47 B.T. Adornato, L. Corash and W.K. Engel, Neurology *27*, 1093 (1977).
48 R.I. Sha'afi, S.B. Rodan, R.L. Hintz, S.M. Fernandez and G.A. Rodan, Nature *254*, 525 (1975).
49 A.K. Percy and M.E. Miller, Nature *258*, 147 (1975).
50 A. Hodson and D. Pleasure, J. neurol. Sci. *32*, 361 (1977).
51 J.S. Wiley, Br. J. Haematol. *22*, 529 (1972).
52 C.A. Dise, D.B.P. Goodman, W.C. Lake, A. Hodson and H. Rasmussen, Biochem. biophys. Res. Commun *79*, 1286 (1977).
53 J.B. Peter, M. Worsfold and W. Fiehn, in: Exploratory Concepts in Muscular Dystrophy, vol. 2, p. 491. Ed. A.T. Milhorat. Excerpta Medica, Amsterdam 1975.
54 F.J. Samaha and J. Gergeley, New Engl. J. Med. *280*, 184 (1969).
55 A. Takagi, D.L. Schotland and L.P. Rowland, Archs Neurol. *28*, 380 (1973).
56 A. Martonosi, Proc. Soc. exp. Biol. Med. *127*, 824 (1968).
57 S.D. Hanna and R.J. Baskin, Biochem. Med. *17*, 300 (1977).
58 D. Scales, R. Sabbadini and G. Inesi, Biochim. biophys. Acta *465*, 535 (1977).
59 R.E. Mrak and R.J. Baskin, Biochem. Med. *19*, 47 (1978).
60 S.D. Hanna and R.J. Baskin, Biochim. biophys. Acta *540*, 144 (1978).
61 L.E. Ford and R.J. Podolsky, J. Physiol. (Lond.) *223*, 21 (1972).
62 M. Endo, Physiol. Rev. *57*, 71 (1977).
63 E.W. Stephenson and R.J. Podolsky, J. gen. Physiol. *69*, 1 (1977).
64 A.M. Katz, D.I. Repke, G. Fudyma and M. Shigekawa, J. biol. Chem. *252*, 4210 (1977).
65 M.M. Sorenson and L. De Meis, Biochim. biophys. Acta *465*, 210 (1977).
66 I. Jirmanova and S. Thesleff, Z. Zellforsch. *131*, 77 (1972).
67 W.J.K. Cumming, M.A. Johnson, J.B. Harris and W.G. Bradley, Muscular Dystrophy Group of Great Britain, unpublished abstract (1977).
68 G. Souweine, M.O. Rolland, I. Maire and M.C. Donas, J. Neurol. *217*, 183 (1978).
69 J.C. Foreman and L.G. Garland, Br. Med. J. *1*, 820 (1976).
70 M.B. Feinstein, J. gen. Physiol. *47*, 151 (1963).
71 H.C. Lüttgau and H. Oetliker, J. Physiol. (Lond.) *194*, 51 (1968).
72 L.E. Ford and R.J. Podolsky, J. Physiol. (Lond.) *223*, 1 (1972).
73 P.J. Garrahan, A.F. Rega and G.L. Alonso, Biochim. biophys. Acta *448*, 121 (1976).
74 H.A. Heggtveit, Ann. N.Y. Acad. Sci. *162*, 758 (1969).
75 M.P. Ryan and M.F. Ryan, Biochem. Soc. Trans. *5*, 1744 (1977).
76 S.B. Chyatte, J.H. Birdsong and B.A. Bergman, South Med. J. *64*, 180 (1971).
77 M. Chipman, S. Kaul and M. Lambi, Dis. Nerv. Syst. *35*, 427 (1974).
78 H.E. Statham and C.J. Duncan, Eur. J. Pharmac. *39*, 143 (1976).
79 K. Hainaut and J.E. Desmedt, Nature *252*, 728 (1974).
80 M.J. Berridge, in: Advances in Cyclic Nucleotide Research, vol. 6, p. 1. Ed. P. Greengard and G.A. Robison, Raven Press, New York 1975.
81 H.L. Wray and R.R. Gray, Biochim. biophys. Acta *461*, 441 (1977).
82 M.L. Entman, G.S. Levey and S.E. Epstein, Circulation Res. *25*, 429 (1969).
83 M.A. Kirchberger, M. Tada, D.I. Repke and A.M. Katz, J. molec. cell. Cardiol. *4*, 673 (1972).
84 M. Tada, M.A. Kirchberger and A.M. Katz, J. biol. Chem. *250*, 2640 (1975).
85 R.G.G. Andersson, Acta physiol. scand. suppl., *382*, 1 (1972).
86 E.L. Nirdlinger and P.O. Bramante, J. molec. cell. Cardiol. *6*, 49 (1974).
87 E.P. Bornet, M.L. Entman, W.B. Van Winkle, A. Schwartz, D.C. Lehotay and G.S. Levey, Biochim. biophys. Acta *468*, 188 (1977).
88 A. Fabiato and F. Fabiato, Biochim. biophys. Acta *539*, 253 (1978).
89 G. Jasmin and B. Solymoss, Proc. Soc. exp. Biol. Med. *149*, 193 (1975).
90 T.-H. Chou, E.J. Hill, E. Bartle, K. Wolley, V. LeQuire, W. Olson, R. Roelofs and J.H. Park, J. clin. Invest. *56*, 842 (1975).
91 T.S. Danowski, P.M. Wirth, M.H. Leinberg, L.A. Randall and J.H. Peters, Am. J. Dis. Child. *91*, 346 (1956).
92 B.A. Kakulas and R.D. Adams, Ann. N.Y. Acad. Sci. *138*, 90 (1966).
93 C.J. Duncan and H.E. Statham, J. Physiol. (Lond.) *268*, 319 (1977).