Mild Hypothermia Preserves Contractile Function and Inhibits Prostaglandin E_2 Release from Metabolically Stressed Skeletal Muscle

Valerie A. Cwik, Ramanath Majumdar and Michael H. Brooke

Abstract: An *in vitro* model of muscle damage was used to investigate the protective effect of mild hypothermia in muscle injury. Rat epitrochlearis muscles were dissected in their entirety and suspended in Krebs-Ringer solution and DNP, a mitochondrial uncoupler, was added. PGE_2 and lactate release and the contractile response to stimulation were measured and compared to untreated controls. Experiments were done at 37, 35, 33 and 27°C. At 37°C, DNP stimulated muscle releases large amounts of PGE_2 and lactate and is unable to contract. As the temperature is reduced, there is progressive preservation of contractile force, although high lactate levels at the lowest temperatures indicate that the metabolic stress is still present. In contrast, DNP stimulated PGE_2 release is completely inhibited at or below 35°C and may be related to a similar protective phenomenon seen in experimental ischemic neuronal death.

Résumé: L'hypothermie légère protège la fonction contractile et inhibe la libération de prostaglandine E_2 par les muscles squelettiques soumis au stress. Nous avons utilisé un modèle *in vitro* de lésion musculaire pour investiguer l'effet protecteur d'une légère hypothermie dans le traumatisme musculaire. Des muscles épitrochléens de rat ont été disséqués et suspendus dans une solution de Krebs-Ringer à laquelle du DNP, un découpleur mitochondrial, a été ajouté. La libération de PGE_2 et de lactate ainsi que la réponse contractile à la stimulation ont été mesurées et comparées à celles de contrôles non traités. Les essais étaient faits à 37, 35, 33 et 27°C. A 37°C, le muscle stimulé par le DNP libère de grandes quantités de PGE_2 et de lactate et il est incapable de se contracter. A mesure que la température est abaissée, on observe une préservation progressive de la force contractile, bien que de fortes concentrations de lactate aux températures les plus basses indiquent que le stress métabolique est encore présent. Par contre, la stimulation de la libération de PGE_2 par le DNP est complètement inhibée à des températures de 35°C ou moins et peut être reliée à un phénomène protecteur semblable à celui observé dans la mort neuronale expérimentale par ischémie.

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Mechanisms which can prevent cell damage are a major interest in neurology because of the possible implications for therapy. Even if the primary neurological event cannot be prevented, the ability to decrease subsequent cell death may offer a viable treatment. This concept of "damage control" is being explored in stroke,[1-4] Parkinson's disease,[5] motor neuron disease[6,7] and muscular dystrophy.[8] Mild hypothermia exerts a profound protective effect against cerebral ischemia, both experimentally[9-14] and clinically.[15-18] An *in vitro* muscle preparation for investigating damage uses the rat epitrochlearis muscle and permits simultaneous monitoring of biochemical parameters and muscle function.[19-20] 2,4-dinitrophenol (DNP) which reversibly binds to mitochondria and uncouples oxidative phosphorylation is used as a metabolic stress to cause muscle damage. The release of prostaglandin E_2 (PGE_2) from the muscle cell is used as a measure of muscle damage. We have investigated the protective effect of mild hypothermia using this model.

Methods

Male Sprague-Dawley rats, weighing between 180 and 250 grams, were killed by a blow to the head quickly followed by cervical dislocation. Both epitrochlearis muscles were rapidly dissected, with as little trauma as possible. Muscles were discarded if there was obvious trauma or hemorrhage into the muscle. The intact muscle was then suspended in a small pyrex tube between two stainless steel clips in 3 ml Krebs-Ringer solution, pH = 7.4, which was oxygenated with a 95% O_2 /5% CO_2 mixture. This system was immersed in a water bath at a temperature of 37, 35, 33 or 27°C.

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One end of the muscle was suspended from a strain gauge connected to a strip chart recorder. The muscle was maintained under 400 mg of tension for the duration of the experiment. Maximal muscle twitch was evoked by a stimulus (50V for 10 msec) applied through one of the stainless steel clips. Maximal twitch was obtained immediately after suspension in the Krebs-Ringer solution. Fifteen minutes later the muscle was again maximally stimulated and the incubating medium was exchanged and discarded. Thirty minutes later (time point "0" on Figures 1-4), the muscle was again maximally stimulated, and the incubating medium was exchanged and saved for baseline PGE_2 and lactate levels. Muscles were discarded at this point if contractile force was less than 8 grams, greater than 8 grams but less than 50% of the opposite muscle from the same rat, or if there was a drop in contractile force of greater than 10 percent. For the remainder of the experiment a maximum contraction was obtained and incubating medium was exchanged and saved every 30 minutes for a total duration of 180 minutes. One portion of medium was immediately frozen in liquid nitrogen and stored at –70°C for PGE_2 analysis; another portion was stored at –20°C for lactate analysis. Control muscles were discarded if they failed to maintain 75% of their maximum contractile force at the 120 minute time point. At the end of the experiment muscles were removed from the apparatus and quickly blotted to remove excess water. The muscles were then immediately clamped between aluminum tongs, which had been precooled in liquid nitrogen, then immersed in liquid nitrogen. Muscles were stored at –70°C.

Treated muscles were handled identically to control muscles except that DNP (40 μM) was added to the incubating medium for thirty minutes after the baseline medium sample was obtained. At least 6 muscles were included in each treatment group at all temperatures. DNP (200 μM) was added to the incubating medium of 6 muscles at 27°C only.

Biochemical studies: The medium was assayed for lactate by standard spectrophotometric methods and the results are expressed as nmol/mg wet muscle weight/30 minutes.[21] PGE_2 analysis was performed by radioimmunoassay following the protocol supplied by ICN Biochemicals with the following slight modifications. Initial incubation of standards (12.5-300 pg) and test samples was carried out in the presence of antibodies at 4°C for 2 hours. $^{3H}PGE_2$ (8500 cpm/tube) was added to the mixture and further incubation was carried out overnight at 4°C. Results are expressed as pg PGE_2/mg wet muscle weight/30 minutes.

Statistical analyses: Results are expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using the Mann-Whitney nonparametric two sample test (Number Cruncher Statistical System software program, Kaysville, Utah, 1990).

Results

Control Muscle Over the 3 hour duration of the experiment at 37°C there was a linear decline in maximum twitch tension from 99% to 77% of maximum (Figure 1). Lactate release remained unchanged over the same time period (Figure 2). There was a small increment in the release of PGE_2 into the medium during the first 120 minutes of the experiment, which levelled off during the last 60 minutes (Figure 3). When the

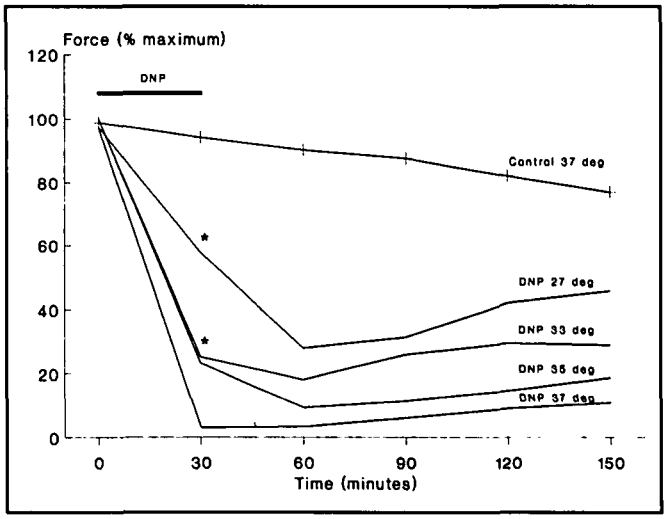

Figure 1: *Contractile force in DNP (40μM) treated muscles at 37, 35, 33 and 27°C. Control muscles at 37°C are included for comparison. Values are means for DNP treated muscles, and means ± SEM for control muscles. At the end of the DNP treatment period (30 minutes time point), there is a significant preservation of force in DNP treated muscles at 35, 33 and 27°C when compared to DNP stimulated muscles at 37°C (*p ≤ 0.0003, Mann-Whitney two sample test).*

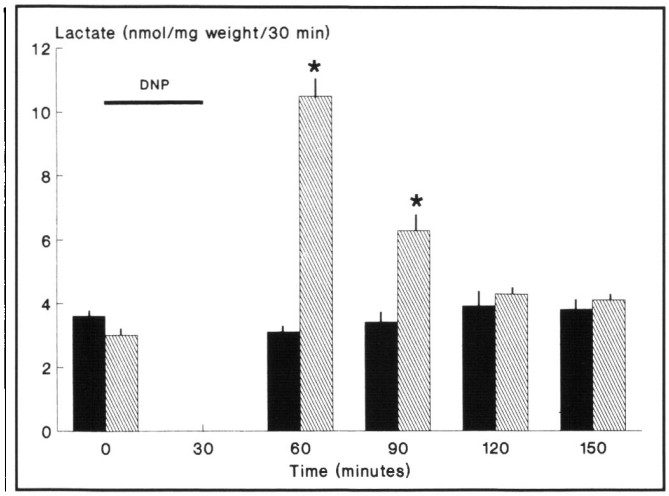

Figure 2: *Lactate release from control (solid bars) and DNP (40μM) treated (hatched bars) muscles at 37°C. Values are means ± SEM. Values at the 30 minute time point are not reported due to interference of lactate analysis by DNP in the medium. *Significant difference (p < 0.0001) between control and DNP treated muscles (Mann-Whitney two sample test).*

temperature of the experiment was decreased, the only difference was a slight progressive decrease in the amount of PGE_2 released (Figure 4).

DNP (40μM) stimulated muscle At 37°C, muscle exposed to 40μM DNP rapidly loses the ability to contract (twitch tension at 30 minutes is 3% of maximum value) (Figure 1). There is a very slight recovery by 150 minutes to 11% maximum value. Lactate release, as expected, is markedly accentuated by DNP stimulation (Figure 2). The concentration of lactate in the medium reaches 10.5 ± 0.6 nmol/mg muscle after 60 minutes

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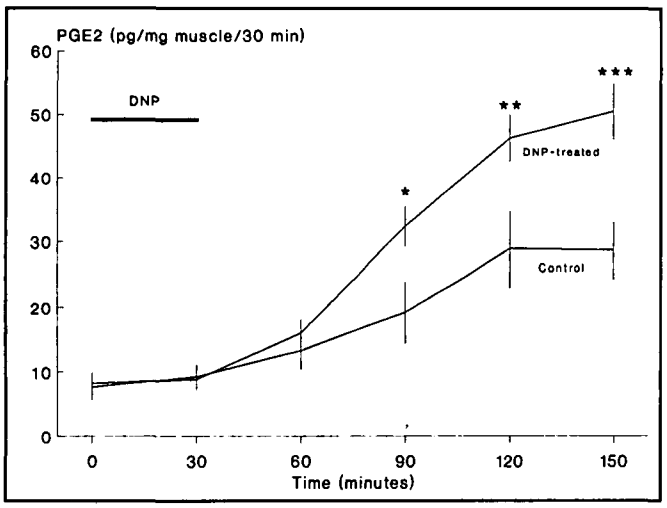

Figure 3: *PGE_2 release from control and DNP (40μM) treated muscles over 180 minutes at 37°C. Values are means ± SEM. Significant difference between control and DNP treated muscles: *p = 0.009, **p = 0.005, ***p = 0.004 (Mann-Whitney two sample test).*

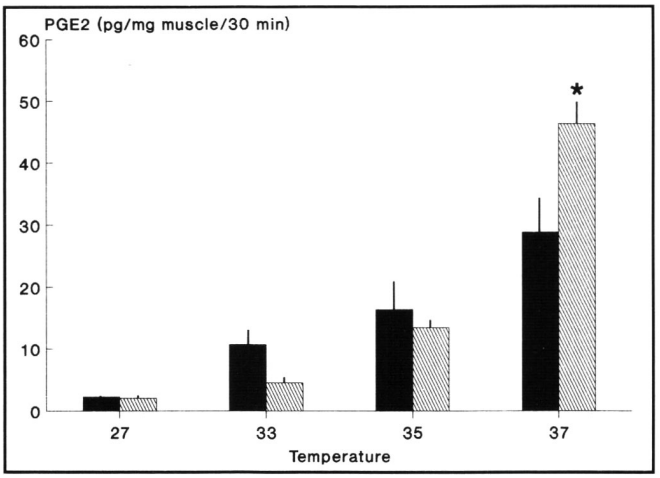

Figure 4: *PGE_2 release from control (solid bars) and DNP (40μM) treated (hatched bars) muscles at the 120 minute time point at 27, 33, 35 and 37°C. The late rise in PGE_2 release from DNP stimulated muscle is abolished at temperatures ≤ 35°C. Values are means ± SEM. *Significant difference (p = 0.005) between control and DNP treated muscles at 37°C (Mann-Whitney two sample test).*

(control = 3.1 ± 0.2 nmol/mg muscle) and then declines. PGE_2 release is identical to the control muscle for the first 60 minutes (Figure 3). Thereafter DNP stimulation results in an increase in PGE_2 release so that at 120 minutes the level of PGE_2 in the medium is 46.3 ± 3.6 pg/mg muscle, (controls = 28.7 ± 6.6 pg/mg muscle, p = 0.005).

DNP (40μM) stimulated muscle at 35, 33 and 27°C Reduction of the temperature is associated with a progressive preservation of the contractile ability of the DNP treated muscle (Figure 1). At 30 minutes, the force generated in response to supramaximal stimulation is 23% of maximum value at 35°C, 25% at 33°C and 58% at 27°C. Lactate release is only slightly reduced by decreasing the temperature: peak lactate release at 60 minutes is 10.5 ± 0.6 at 37°C, 8.6 ± 0.8 at 35°C, 6.9 ± 0.9 at 33°C, and 6.5 ± 0.5 at 27°C. However, the DNP stimulated PGE_2 release is abolished at temperatures of 35°C and below (Figure 4).

DNP (200μM) stimulated muscle at 27°C To determine whether the effect of hypothermia might be due to some impairment of the access of DNP to the tissues or to some lack of metabolic effect, the experiment was repeated using 200μM concentration of DNP at 27°C. Lactate rose to 13.7 ± 0.9, indicating a marked metabolic effect. PGE_2 levels were identical to untreated 27°C controls at all time points.

Discussion

Any manipulation having a protective effect, permitting a damaged cell to be rescued, may be important in trying to develop treatment for ischemic and degenerative neurological diseases. The use of hypothermia dates back several decades, but the temperatures used, which were as low as 27 to 30°C, were impractical as treatment in patients. Only recently has it been appreciated that a very minor reduction in temperature may provide a marked protective effect in experimental neuronal damage. The mechanism for this is unknown. The present report is the first demonstration that this effect of mild hypothermia is also present in muscle and may provide an impetus for further studies.

Clinically and experimentally, hypothermia is of proven benefit in protecting skeletal muscle against cell damage. It was recognized as early as 1939 that hypothermia significantly prolongs the amount of time a ligated limb can tolerate ischemia.[22] When reconstructive procedures of the hand or wrist are performed under bloodless conditions using a tourniquet, cooling of the limb before application of the tourniquet reduces myoglobin release from the ligated limb.[23] It also prolongs the time of safe ischemia beyond the commonly accepted 2 hour time limit without evidence of histological or electron microscopic damage.[24] Metabolic deterioration of traumatically amputated limbs can be minimized by transporting the limb on ice or in an ice bath, thereby preserving the viability of the limb for reattachment.[25] These observations have been supported by magnetic resonance spectroscopy which shows that cooling the limb preserves intracellular pH, slows depletion of muscle phosphocreatine and ATP and slows the accumulation of inorganic phosphate.[26]

In experimental crush injury of muscle the severity of the resulting edema is reduced by a moderate degree of hypothermia (27-30°C).[27] Hypothermia also reduces the infarct size[28] and creatine kinase, lactate dehydrogenase and potassium release in muscle subjected to ischemia under experimental conditions.[29] Even when hypothermia is instituted after prolonged ischemia, interstitial muscle pH is maintained and post-reperfusion edema is reduced, although there is little effect on infarct size.[30] Hypothermia reduces cellular metabolism, oxygen consumption and lactate production, and preserves pH, but the precise mechanism by which it protects against cell damage remains unknown.

In contrast to the previous work in muscle, studies of ischemic neuronal damage have demonstrated the beneficial effect of a very mild degree of hypothermia. Experimentally, carotid ligation in mammals at 37°C produces neuronal death in the hippocampus.[12,14] Intracellular pH drops rapidly and

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markedly,[31] ATP, phosphocreatine, glucose and glycogen levels fall, lactate rises and edema develops.[11,32] Sutherland and colleagues have reported that reducing the brain temperature from 38 to 35°C abolishes this type of neuronal injury.[9] This effect was demonstrated histopathologically and using magnetic resonance spectroscopy. Others have also documented this striking protective response clinically, biochemically and pathologically.[10-15,31,32]

The mechanisms mediating the protective effects of hypothermia in ischemic brain are not explained. In muscle, ischemia-reperfusion injury is associated with damage to the membrane by free radicals[33] and a similar mechanism may play a part in ischemic neuronal damage. A small but rapid rise in phospholipase A_2 activity occurs in the brain following cerebral ischemia[34] which is associated with a rise in the expected products of this enzyme, namely thromboxanes, leukotrienes and prostaglandins, at the site of injury. This occurs especially during the reperfusion period, and can be inhibited by pretreatment with indomethacin.[35-38] Moderate hypothermia (30-31°C) reduces leukotriene production at 10 minutes reperfusion, accompanied by reduced post-ischemic edema at 2 hours reperfusion.[39] The fact that a significant part of the change occurs during the reperfusion period is of interest. More importantly, it implies that there may be a window following the ischemic insult during which protective therapy may be instituted.

Another area which has gained recent prominence is that of the role of excitotoxins in ischemic neuronal death. Excitotoxic amino acids such as glutamate are released in large quantities following cerebral ischemia, resulting in excessive stimulation of neurons and eventual cell death. It is postulated that this is due to the increased entry of calcium into the cell via calcium channels regulated by NMDA receptors. NMDA receptor antagonists, NMDA receptor desensitisation and manoeuvres to reduce the release of glutamate, have been shown to be effective in reducing infarct size and protecting neurons against cell death in animal models of cerebral ischemia.[40-42] A possible link between these studies and those involving hypothermia is suggested by the finding that the effect of some NMDA blocking agents is due to their effect on lowering the animal's body temperature and not due to their action as NMDA receptor antagonists. When the brain temperature is maintained at 37°C NMDA blockade produces no protective effect.[43]

Experimental models of muscle damage are not as well standardized either *in vivo* or *in vitro*. One *in vitro* model which has been used in several experiments is an isolated muscle exposed to DNP or calcium ionophore.[19-20,44-47] The epitrochlearis muscle, a forelimb muscle, is particularly well-suited for these kinds of experiments as it is a thin ribbon of muscle that can be dissected end-to-end, and can be oxygenated by diffusion without the development of an hypoxic core.[19] This model permits simultaneous measure of contractile function and biochemical changes.

PGE_2 release from the muscle is used as an indirect indicator of the extent of muscle damage.[45] PGE_2 is one of the end products of a series of reactions initiated by the action of phospholipase A_2, a calcium-dependent, rate-limiting enzyme, that releases arachidonic acid from membrane phospholipids. Arachidonic acid is the precursor to the leukotrienes, via the lipoxygenase pathway, and to the prostacyclins, thromboxanes and prostaglandins, via the cyclooxygenase pathway. PGE_2 is not stored in the cell but under experimental conditions it is produced and released by skeletal muscle in response to a variety of stimuli including stretch, leukocytic pyrogen, 2,4-dinitrophenol (DNP), the calcium ionophore and arachidonic acid itself.[45,48-50] An increase in the efflux of PGE_2 as well as creatine kinase has been shown to occur in experimental models of muscle damage as well as in human muscle disease.[45,51]

In the present model of muscle damage we use DNP stimulation. DNP reduces ATP levels and uncouples mitochondrial phosphorylation.[52] In many ways it simulates the stress of intense exercise. The blood supply to a contracting limb muscle which is working at near maximum load is insufficient to supply the energy demands of the muscle. This results in a relative metabolic ischemia characterized by decreasing levels of high energy phosphates combined with inefficient mitochondrial respiration. Previous studies have shown that, at low concentrations, DNP stimulation mimics the biochemical effect of exercise in animals and in humans in health and disease.[53] Exposure to higher concentrations of DNP causes morphological changes in the muscle indicative of severe mitochondrial damage.[54]

At 37°C the use of DNP stimulation in the rat epitrochlearis muscle model results in an immediate fall in twitch tension followed 30-60 minutes later by an increased release of PGE_2. We have shown that a reduction of 2°C completely abolishes the DNP-provoked PGE_2 release, and preserves some contractile force in the immediate post-DNP treatment period. Greater reductions in temperature result in further preservation of contractile ability. While there is no evidence for a cause and effect relationship between loss of contractility and PGE_2 release, PGE_2 release may reflect irreversible muscle fiber damage. The delay between a significant fall in twitch tension (30 minutes, Figure 1), and a significant rise in PGE_2 release (90 minutes, Figure 3), suggests that there may be a window of time (60 minutes in our model) when muscle fibers are injured, but salvageable, and a time when therapeutic interventions may be of benefit. Of interest, therefore, is that reducing the temperature protects the contractile ability most during this "window".

In summary, reducing the temperature by 2°C has been shown by other workers to prevent ischemic neuronal death in brain. We now demonstrate that the same drop in temperature abolishes DNP-stimulated PGE_2 release and preserves contractile function in muscle. Although brain and muscle are quite dissimilar tissues, this may suggest a common protective cellular mechanism of hypothermia in muscle and brain. This *in vitro* model, which can be adapted to human muscle, may prove useful for the further investigation of the basic mechanisms of cellular ischemia in skeletal muscle and the development of therapeutic strategies to reduce the rate of irreversible cellular injury.

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References

1. Choi DW. Possible mechanisms limiting N-methyl-D-aspartate receptor overactivation and the therapeutic efficacy of N-methyl-D-aspartate antagonists. Stroke 1990; 21 (Suppl. 11): III20-2.
2. Collins RC, Dobkin BH, Choi DW. Selective vulnerability of the brain: new insights into the pathophysiology of stroke. Ann Intern Med 1989; 110: 992-1000.

3. Albers GW, Goldberg MP, Choi DW. N-methyl-D-aspartate antagonists: ready for clinical trial in brain ischemia? Ann Neurol 1989; 25: 398-403.
4. Lipton SA. Calcium channel antagonists in the prevention of neurotoxicity. Adv Pharmacol 1991; 22: 271-297.
5. Lieberman A (ed.). Emerging perspectives in Parkinson's disease. Neurology 1992; 42 (Suppl): 41-48.
6. Oppenheim RW. Motor neuron diseases: high hopes for a trophic factor. Nature 1992; 358: 451-452.
7. Sendtner M, Schmalbruch K, Stockli KA, et al. Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. Nature 1992; 358: 502-505.
8. Jackson MJ, Edwards RH. Free radicals and trials of antioxidant therapy in muscle diseases. Adv Exp Med Biol 1990; 264: 485-491.
9. Sutherland GR, Lesiuk H, Hazendonk P, et al. Magnetic resonance imaging and ^{31}P magnetic resonance spectroscopy study of the effect of temperature on ischemic brain injury. Can J Neurol Sci 1992; 19: 317-325.
10. Clifton GL, Jiang JY, Lyeth BG, et al. Marked protection by moderate hypothermia after experimental traumatic brain injury. J Cereb Blood Flow Metab 1991; 11: 114-121.
11. Busto R, Dietrich WD, Globus MY-T, et al. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987; 7: 729-738.
12. Minamisawa H, Smith M-L, Siejso BK. The effect of mild hyperthermia and hypothermia on brain damage following 5, 10, and 15 minutes of forebrain ischemia. Ann Neurol 1990; 28: 26-33.
13. Young RSK, Olenginski TP, Yagel SK, Towfighi J. The effect of graded hypothermia on hypoxic-ischemic brain damage: a neuropathological study in the neonatal rat. Stroke 1983; 14: 929-934.
14. Welsh FA, Sims RE, Harris VA. Mild hypothermia prevents ischemic injury in gerbil hippocampus. J Cereb Blood Flow Metab 1990; 10: 557-563.
15. Connolly JE, Boyd RJ, Calvin JW. The protective effect of hypothermia in cerebral ischemia: experimental and clinical application by selective brain cooling in the human. Surgery 1962; 52: 15-24.
16. Uihlein A, MacCarty CS, Michenfelder JD, Terry HR, Daw EF. Deep hypothermia and surgical treatment of intracranial aneurysms. JAMA 1966; 195: 127-129.
17. Siebke H, Breivik H, Rod T, Lind B. Survival after 40 minutes' submersion without cerebral sequelae. Lancet 1975; 1: 1275-1277.
18. Sekar TS, MacDonnell KF, Namsirikul P, Herman RS. Survival after prolonged submersion in cold water without neurologic sequelae. Arch Intern Med 1980; 140: 775-779.
19. Nesher R, Karl IE, Kaiser KE, Kipnis DM. Epitrochlearis muscle. I. Mechanical performance, energetics, and fiber composition. Am J Physiol 1980; 239: E454-E460.
20. Brooke MH, Choski R, Kaiser KK. Inosine monophosphate production is proportional to muscle force *in vitro*. Neurology 1986; 36: 288-291.
21. Lowry OH, Passonneau JV. A flexible system of enzymatic analysis. Orlando, FL: Academic Press, 1972.
22. Allen FM. Surgical considerations of temperature in ligated limbs. Am J Surg 1939; 45: 459-464.
23. Ikemoto Y, Kobayashi H, Usui M, Ishii S. Changes in serum myoglobin levels caused by tourniquet ischemia under normothermic and hypothermic conditions. Clin Orthop 1988; 234: 296-302.
24. Swanson AB, Livengood LC, Sattel AB. Local hypothermia to prolong safe tourniquet time. Clin Orthop 1991; 264: 200-208.
25. Tamai S. Digit replantation: analysis of 163 replantations in an 11 year period. Clin Plast Surg 1978; 5: 195-209.
26. Sapega AA, Heppenstall RB, Sokolow DP, et al. The bioenergetics of preservation of limbs before replantation. J Bone Joint Surg 1988; 70-A: 1500-1513.
27. McMaster WC, Liddle S. Cryotherapy influence on posttraumatic limb edema. Clin Orthop 1980; 150: 283-287.
28. Wright JG, Kerr JC, Valeri CR, Hobson RW. Regional hypothermia protects against ischemia-reperfusion injury in isolated canine gracilis muscle. J Trauma 1988; 28: 1026-1031.
29. Presta M, Ragnotti G. Quantification of damage to striated muscle after normothermic or hypothermic ischemia. Clin Chem 1981; 27: 297-302.
30. Wright JG, Araki CT, Belkin M, Hobson RW. Postischemic hypothermia diminishes skeletal muscle reperfusion edema. J Surg Res 1989; 47: 389-396.
31. Norwood WI, Norwood CR. Influence of hypothermia on intracellular pH during anoxia. Am J Physiol 1982; 243: C62-C65.
32. Berntman L, Welsh FA, Harp JR. Cerebral protective effect of low-grade hypothermia. Anesthesiology 1981; 55: 495-498.
33. Harris K, Walker PM, Mickle DAG, et al. Metabolic response of skeletal muscle to ischemia. Am J Physiol 1986; 250: H213-H220.
34. Edgar AD, Strosznajder J, Horrocks LA. Activation of ethanolamine phospholipase A_2 in brain during ischemia. J Neurochem 1982; 39: 1111-1116.
35. Dempsey RJ, Roy MW, Meyer K, Cowen DE, Tai H-H. Development of cyclooxygenase and lipoxygenase metabolites of arachidonic acid after transient cerebral ischemia. J Neurosurg 1986; 64: 118-124.
36. Moskowitz MA, Kiwak KJ, Hekimian K, Levine L. Synthesis of compounds with properties of leukotrienes C_4 and D_4 in gerbil brains after ischemia and reperfusion. Science 1984; 224: 886-889.
37. Yoshida S, Inoh S, Asano T, et al. Effect of transient ischemia on free fatty acids and phospholipids in the gerbil brain. J Neurosurg 1980; 53: 323-331.
38. Gaudet RJ, Alam I, Levine L. Accumulation of cyclooxygenase products of arachidonic acid metabolism in gerbil brain during reperfusion after bilateral common carotid artery occlusion. J Neurochem 1980; 35: 653-658.
39. Dempsey RJ, Combs DJ, Maley ME, et al. Moderate hypothermia reduces postischemic edema development and leukotriene production. Neurosurgery 1987; 21: 177-181.
40. Miller LP, Hsu C. Therapeutic potential for adenosine receptor activation in ischemic brain injury. J Neurotrauma 1992; 9 (Suppl 2): S563-S577.
41. Park CK, Nehls DG, Graham DI, Teasdale GM, McCulloch J. The glutamate antagonist MK-801 reduces focal ischemic brain damage in the rat. Ann Neurol 1988; 24: 543-551.
42. Simon R, Shiraishi K. N-methyl-D-aspartate antagonist reduces stroke size and regional glucose metabolism. Ann Neurol 1990; 27: 606-611.
43. Buchan A, Pulsinelli WA. Hypothermia but not the N-methyl-D-aspartate antagonist, MK-801, attenuates neuronal damage in gerbils subjected to transient global ischemia. J Neuroscience 1990; 10: 311-316.
44. Duncan CJ, Jackson MJ. Different mechanisms mediate structural changes and intracellular enzyme efflux following damage to skeletal muscle. J Cell Sci 1987; 87: 183-188.
45. Jackson MJ, Wagenmakers AJM, Edwards RHT. Effect of inhibitors of arachidonic acid metabolism on efflux of intracellular enzymes from skeletal muscle following experimental damage. Biochem J 1987; 241: 403-407.
46. Phoenix J, Edwards RHT, Jackson MJ. Inhibition of Ca^{2+} -induced cytosolic enzyme efflux from skeletal muscle by vitamin E and related compounds. Biochem J 1989; 257: 207-213.
47. Jones DA, Jackson MJ, McPhail G, Edwards RHT. Experimental mouse muscle damage: the importance of external calcium. Clin Sci 1984; 66: 317-322.
48. Vandenburgh HH, Hatfaludy S, Sohar I, Shansky J. Stretch-induced prostaglandins and protein turnover in cultured skeletal muscle. Am J Physiol 1990; 259: C232-C240.
49. Baracos V, Rodemann HP, Dinarello CA, Goldberg AL. Stimulation of muscle protein degradation and prostaglandin E_2 release by leukocytic pyrogen (interleukin-1). N Engl J Med 1983; 308: 553-558.
50. Rodemann HP, Goldberg AL. Arachidonic acid, prostaglandin E_2 and $F_2\alpha$ influence rates of protein turnover in skeletal and cardiac muscle. J Biol Chem 1982; 257: 1632-1638.
51. Jackson MJ, Brooke MH, Kaiser K, Edwards RHT. Creatine kinase release and prostaglandin E_2 release from isolated Duchenne muscle. Neurology 1991; 41: 101-104.
52. Kariman K, Chance B, Burkhart DS, Bolinger LA. Uncoupling effects of 2,4-dinitrophenol on electron transfer reactions and cell bioenergetics in rat brain *in situ*. Brain Res 1986; 366: 300-306.
53. Heller SL, Brooke MH, Kaiser KK, Choski R. 2,4-Dinitrophenol, muscle biopsy, and McArdle's disease. Neurology 1988; 38: 15-19.
54. Melemd C, Karpati G, Carpenter S. Experimental mitochondrial myopathy produced by *in vivo* uncoupling of oxidative phosphorylation. J Neurol Sci 1975; 26: 305-318.