cells was increased by adding HLS for 48 h in this study, which confirms their suggestion. The increase was not a consequence of an increased number of dead cells containing large amounts of calcium, because most of the dead cells, which were detached from the dish, could be removed by the washing procedure. Calcium overloading is thought to be a final common pathway of cell necrosis[9], and calcium antagonists are well known to reduce the calcium accumulation and cell necrosis in various disorders[10].

We used the concentration of 1 µg/ml of diltiazem in this study, because this dose was reported to bring about an 80% reduction in the sustained tension development of the thoracic aorta induced by ouabain but little change in the resting tension development and action potential duration of the papillary muscle[11,12]. This dose of diltiazem significantly reduced the calcium content of cultured aortic smooth muscle cells without HLS as well as the elevated calcium content of cultured aortic smooth muscle cells with HLS (fig. 2). On the basis of these data, we concluded that diltiazem, a calcium antagonist, suppressed the HLS-induced necrosis of cultured aortic smooth muscle cells by reducing the intracellular calcium content; and this might play an important role in the mechanisms of protection from atherosclerosis by calcium antagonists.

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Temperature dependence of neurotransmitter release in the antarctic fish *Pagothenia borchgrevinki*

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Summary. The quantal contents of endplate potentials from extraocular muscles of an antarctic fish *Pagothenia borchgrevinki* were measured over a range of temperatures. Quantal release was maximal at about 5 °C but showed little dependence on temperature between −2 °C and 10 °C. Above 10 °C quantal content declined until release ceased about 18 °C. In view of the fact that the ambient temperature at which these fish live is constant at −1.9 °C, the results suggest that *Pagothenia borchgrevinki* is only partially adapted to its environment despite 25 million years acclimatization.

Key words. Neuromuscular junction; quantal content; antarctic fish; temperature.

Antarctic fauna became isolated by the establishment of a circum-antarctic ocean circulation in the mid-Oligocene, about 25 million years ago[2]. Since that time, the evolution of antarctic fishes must have been strongly influenced by low temperatures and extensive areas of perennial sea ice. The sea temperature in deep coastal basins such as McMurdo Sound is constant throughout the year at −1.9 °C[3,4]. At this temperature temperate fish become comatose[5] so the very fact that antarctic species such as *Pagothenia borchgrevinki* live in McMurdo Sound must indicate that their nervous systems have become adapted to low temperature function. The temperature dependence of muscle contraction and of MEPP frequency and decay have been studied in *Pagothenia borchgrevinki*[6,7]. In the present experiments we set out to determine the temperature dependence of evoked transmitter release at the neuromuscular junction, to see how well it is tuned to the environment.

All experiments were carried out on the inferior oblique extraocular muscle of the nototheniid fish *Pagothenia borchgrevinki*[8], in a fish hut on the McMurdo Sound sea ice (77°51'S, 166°45'E). Specimens of *P. borchgrevinki* were caught on handlines and immediately decapitated. The inferior oblique muscle and its nerve were dissected free in an ice slurry and pinned in a bath filled with 5 °C physiological saline made up to match major ion concentrations in *P. borchgrevinki* serum[9] (258 mM NaCl, 7.47 mM KCl, 4.05 mM $CaCl_2$, 0.79 mM $MgCl_2$, buffered with 10 mM HEPES, pH 8.4). The nerve was taken up into a close fitting suction electrode and stimulated at 10 Hz with 0.1 ms voltage pulses. Intracellular recordings were made with 10–20 MΩ glass microelectrodes filled with 3 M KCl and using standard techniques, from the band of large white fibers on the midline side of the muscle. In normal saline, nerve stimulation evoked action potentials in the muscle fibers so transmitter release was lowered with Mg^{++} (25–50 mM) until only endplate potentials were recorded. Muscle fibers receiving focal, rather than multiple innervation were selected by stimulating the nerve with pulses of increasing voltage and selecting only muscle fibers where epp amplitude stayed constant rather than increasing in steps with voltage increase as axons were recruited. Usually the large white muscle fibers were focally innervated and the small red fibers multiply innervated by this criterion. Bath temperature was controlled by manual adjustment of a peltier unit under the recording chamber, with fluid temperature being measured by a thermister in the bath. Endplate potentials were stored as FM tape recordings and subsequently analyzed for quantal content by a modification of the method of variance, as described elsewhere[10–12].

Intracellular penetrations were made on a total of 39 muscle fibers in muscles from 17 different fish. Six penetrations were each held for about 5 h, while the temperature was varied up and down between −3 °C and +20 °C and quantal content determinations were made at different temperatures. All of the resulting curves (e.g. fig. 1) had very similar shapes. Quantal content increased slightly from −2 °C to +6 °C but no marked temperature dependence was shown until about +9 °C, when quantal content started to decline. Release ceased at about +18 °C. Resting membrane potential also changed over the experimental temperature range (fig. 2), hyperpolarising as temperatures increased so that epp amplitude remained almost constant until

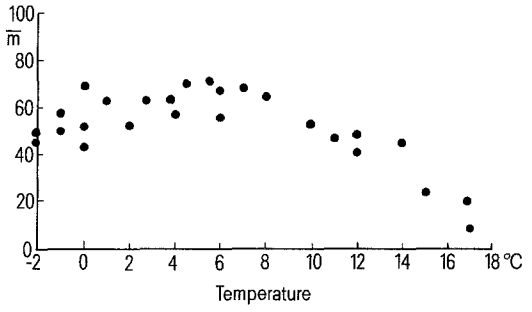

Figure 1. Effect of temperature on quantal release of transmitter in extraocular muscle of *Pagothenia borchgrevinki*. m̄ is average quantal content recorded at 10 Hz for 200 endplate potentials. Each point represents one determination of m. All points are from a single muscle fiber. Recording conditions and analysis of epps are described in the text.

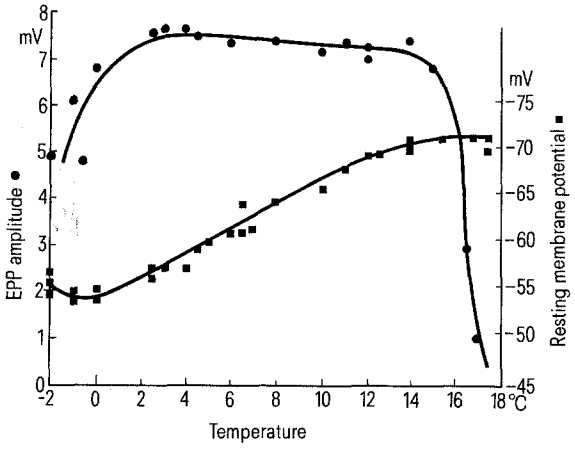

Figure 2. Effect of temperature on endplate potential amplitude and resting potential in extraocular muscle of *Pagothenia borchgrevinki*. Each circle represents average amplitude of 200 endplate potentials and each square one determination of resting membrane potential of the muscle fiber, the same as that in figure 1. Recording conditions are described in the text.

transmitter release failed at 16–17 °C (fig. 2). The progressive decrease in quantal content above 9 °C parallels a similar decrease in isotonic contraction for *P. borchgrevinki* extraocular muscle[7] and is probably the main factor responsible for high temperature failure of neuromuscular transmission in antarctic fishes.

The fact that transmitter release occurs at all at −2 °C indicates that *P. borchgrevinki* has made considerable adaptation to low temperature life. However, the fact that release is more effective at +5 °C than at the fish's ambient temperature of −1.9 °C (mean of 10 determinations of quantal content made between −3 °C and −1 °C on 6 muscle fibers each from a different fish was significantly different from mean of eight determinations made between +4 °C and +6 °C on the same muscle fibers: $0.005 < p < 0.01$, Student's t-test) shows that the adaptation is incomplete. Perhaps lack of competition in the antarctic environment has meant that there is no selective pressure for further fine-tuning of the cold adaptation, or perhaps the biochemical and biophysical constraints of membrane structure have not permitted adaptation to proceed further.

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Cholera toxin B-subunit incorporation into synaptic vesicles of the neuromuscular junction of the rat*

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Summary. The B-subunit of cholera toxin, a nontoxic macromolecule which binds specifically to GM1 ganglioside, was conjugated to colloidal gold and injected into skeletal muscle of the rat. It was taken up rapidly in vesicles in the terminal axons at the neuromuscular junctions. Injection of albumin-colloidal gold conjugates resulted in an insignificant uptake. The results indicate that uptake of extracellular macromolecules into the terminal axon of the neuromuscular junction may be greatly enhanced by binding to gangliosides at the presynaptic membrane, and that it may occur without association with vesicular recycling related to transmitter release.

Key words. Synaptic vesicles; endocytosis; cholera toxin; ganglioside; neuromuscular junction; rat.

Uptake of endo- and exogenous macromolecules occurs at the terminal axon of the neuromuscular junction[1] and has been considered an unspecific phenomenon related to recycling of synaptic vesicles during transmitter release[2]. Some of the incorporated macromolecules undergo retrograde axonal transport to the central nervous system[3]. Efficient transport of radio-