Diet restriction induced autophagy: A lysosomal protective system against oxidative- and pollutant-stress and cell injury

Michael N. Moore

Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK

Abstract

Nutrient deprivation or dietary restriction (DR) confers protection against ageing and stress in many animals and induced lysosomal autophagy is part of this mechanism. The effects of dietary restriction on the toxicity of copper and the polycyclic aromatic hydrocarbon phenanthrene have been investigated in the common marine mussel *Mytilus edulis*. The findings show that DR-induced autophagy facilitates the recovery of the digestive gland (i.e., molluscan liver analogue) from cell injury caused by both copper and phenanthrene. It is inferred that DR-induced autophagy and lysosomal proteolysis results in improved cellular “housekeeping” through the more efficient removal of oxidatively and pollutant damaged proteins (e.g., protein carbonyls, protein adducts, etc.) and that this contributes to stress resistance.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Diet restriction; Autophagy; Lysosomes; Lysosomal proteolysis; Pollutants; Oxidative-stress; Cell injury; Stress resistance; Mussels

Dietary restriction (DR) reduces protein oxidation and the effects of ageing; and also enhances stress resistance in mammals (Aksenova, Aksenov, Carney, & Butterfield, 1998; Cavallini, Donati, Gori, Pollera, & Bergamini, 2001; Ramaiah, Apte, & Mehendale, 2000). A major effect of DR is the induction of autophagic proteolysis in the cells of most eukaryotic organisms via the TOR signal transduction system (Cutler, Pan, Heitman, & Cardenas, 2001). It has been proposed that improved autophagy and lysosomal degradation might be part of the anti-ageing mechanisms.
of DR (Cavallini et al., 2001). Dysfunction of cellular lysosomes is a recognised mechanism of cell injury induced by many stressors including pollutants (Moore, 2002). There is currently little data available on whether nutritional status interacts with the harmful effects of pollutants. Consequently, since DR induces autophagy, but does not reduce lysosomal stability (even after 57 days at ambient temperature), in the hepatopancreatic or digestive gland epithelial (digestive) cells of molluscs (Bayne, Holland, Moore, Lowe, & Widdows, 1978), experiments were designed to test the hypothesis that autophagy facilitates protective resistance against pollutant-stress in marine mussels.

Mussels (Mytilus edulis, 50–65 mm shell length) were collected from the Lynher estuary near Plymouth and acclimated for 3 weeks at 15 ± 1 °C and 34 psu salinity and fed ad libitum on Phaeodactylum tricornutum. Mussels were not fed for 4 days prior to the experiment in order to trigger autophagy. Experimental treatments were set up in static 7 l polypropylene tanks, aerated continuously. Seawater was obtained from the Eddystone area southwest of Plymouth Sound and filtered through a 0.7 μm glass fibre filter. The experimental temperature and salinity was 15 ± 1 °C and 34 psu respectively. Mussels (2 animals/l) were provided with particulate food in the form of powdered kelp (Lusty’s Natural Products Ltd, Gloucester, UK) and soya flour at the rate of 17 mg/mussel/day of each. The food was administered continuously by peristaltic pump; and diet restricted animals received no food.

There were eight experimental treatments:
1. Fed.
2. Diet restricted.
3. Fed + copper (20 μg l⁻¹).
4. Diet restricted + copper (20 μg l⁻¹).
5. Fed.
6. Diet restricted.
7. Fed + phenanthrene (100 μg l⁻¹).
8. Diet restricted + phenanthrene (100 μg l⁻¹).

The toxicants were added to give the nominal concentration every 24 h and the water was also renewed every 24 h. Phenanthrene was administered in acetone solution (10 mg ml⁻¹) directly into the seawater to give 100 μg l⁻¹ seawater; the controls (fed and diet restricted) received 10 μl l⁻¹ acetone. Mussels (5 animals/sample) were sampled at the start of the experiment and after 3 days for each of the eight treatments. Copper and phenanthrene dosing stopped after 3 days and further samples were taken after 6 and 15 days.

Lysosomal stability (based on latency of β-N-acetylhexosaminidase) was determined in frozen sections of the digestive glands as described by Moore et al. (1984). Copper and phenanthrene analysis was performed as described by Moore et al. (1984) from pooled groups of five digestive glands.

Exposure of fed mussels to copper (20 μg l⁻¹ initial daily concentration) and phenanthrene (100 μg l⁻¹ initial daily concentration) resulted in severe damage to the lysosomal system in the digestive gland epithelial (digestive) cells (Fig. 1). There was evidence for partial recovery after 12 days in clean seawater, but only in the case of copper exposure (Fig. 1). Phenanthrene exposed mussels did not show evidence for
recovery (Fig. 1). Dietary restriction of mussels exposed to copper facilitated recovery with limited recovery after 3 days and full recovery of lysosomal integrity after 12 days in clean seawater (Fig. 1). With phenanthrene, DR reduced the severity of lysosomal damage and there was a full recovery after 12 days in clean seawater (Fig. 1). The concentration of copper was greater after 3 days in digestive glands of DR mussels; and phenanthrene concentration in the digestive gland tissues was essentially the same in both treatments after 3 days with and without food (Fig. 1).

These results show that DR-induced autophagy provides protection against the injurious effects of both copper and phenanthrene. Copper is known to facilitate the generation of oxyradicals, leading to oxidative damage to proteins and other cellular constituents, so it can be reasonably inferred that autophagy is protecting against this form of cellular damage, as well as the direct toxicity of phenanthrene (Kirchin, Moore, Dean, & Winston, 1992; Moore et al., 1984; Viarengo, Moore, Pertica, Mancinelli, & Accomando, 1992).

DR activates autophagic bulk degradation of proteins and cellular organelles, via autolysosomes, probably as a survival mechanism (Moore, 2002; Fig. 2). However, under ambient environmental conditions, starvation does not reduce the stability of the digestive cell lysosomes for up to 2 months, indicating that there is strong physiological regulation of the autophagic process (Bayne et al., 1978). Even though autolysosomal enlargement does occur, patho-physiological changes appear to be minimised as indicated by the maintenance of lysosomal integrity (Fig. 2(b)).
Recent investigations on mammalian cells indicate that prior induction of autophagy protects cells against oxidative injury that would otherwise lead to programmed cell death (Persson, Nilsson, & Brunk, 2001).

Physiological function emerges from the interactions between cellular proteins: pollutant-induced damage to cellular proteins will, therefore, impact on integrated physiological function. Consequently, increased removal of proteins damaged by reactive oxygen species (ROS) and adduct formation with reactive ligands, by means of improved autophagic proteolysis, will probably result in improved cellular “housekeeping” and help to maintain function during stress (Kirchin et al., 1992; Moore, 2002).

An age-related decline in lysosomal function occurs in mussels and similar changes have been described in mammals; however, DR is believed to improve autophagic/lysosomal capacity and this confers anti-ageing protection (Cavallini et al., 2001; Hole, Moore, & Bellamy, 1993). Autophagy and lysosomal function are highly

Fig. 2. Micrographs of mussel digestive tubules showing lysosomes reacted for the lysosomal marker enzyme $\beta$-N-acetylhexosaminidase in the digestive cells of fed controls (a) and enlarged autophagolysosomes in the DR treatment (ration – restricted for 19 days) (b) indicative of DR-induced autophagy. The frozen tissue sections (10 $\mu$m) were prepared as described by Moore et al. (1984). Scale bar = 20 $\mu$m.
conserved evolutionarily; and it is likely that this ancient system has remained largely unchanged because it conferred anti-ageing and stress resistant properties (Cutler et al., 2001). Furthermore, these relatively simple functional systems in well defined organisms, such as mussels, are particularly well suited to screen compounds for important, unwanted toxic effects including food additives and/or pharmaceutical agents that may be only incompletely broken down by conventional waste treatment.

Autophagy is triggered by DR, salinity increase and hypoxia in mussels (Bayne et al., 1978; Moore, Lowe, & Moore, 1979). Since these conditions often prevail in estuarine environments, the repeated triggering of autophagy may be a significant contributory factor to stress tolerance in mussels and other intertidal animals. The environmental significance of this lysosomal-autophagic protection system remains to be fully assessed, but we can speculate that it may play a protective role in the survival of animals chronically exposed to stress and pollution.

Acknowledgements

The work was partly funded by the NERC (UK) core science research programme of the Plymouth Marine Laboratory and partly by the DEFRA (UK) PREDICT 2 contract (AE1136/CSA 6132). The author gratefully acknowledges the expert assistance of Sue Farrar and John Cleary for the chemical analyses.

References


