# Proceedings of the Anatomical Society of Great Britain and Ireland

A one day symposium of the Anatomical Society of Great Britain and Ireland was held at the Royal Free Hospital in London on the 17th September 1999, on the topic of 'Mechanisms of ageing and longevity'. The following are abstracts of communications and posters presented at the meeting.

## TALKS

**1 Extending lifespan by mutation, transgenesis and pharmacological intervention.** By G. J. LITHGOW, D. BARSYTE, M. S. GILL, N. JENKINS, G. McCOLL, S. MALIK, J. M. SAMPAYO, D. W. WALKER and G. A. WALKER. *School of Biological Sciences, University of Manchester, UK.*

The rate of ageing of the nematode *C. elegans* is partially governed by an insulin signalling like pathway. Mutations in genes encoding components of this pathway extend both mean and maximal lifespan anything up to 300% (Age mutants) and consequently are invaluable for identifying causal mechanisms of ageing. We have undertaken 3 approaches to understanding how this pathway affects ageing and in each used extended lifespan as the hallmark of altering ageing processes: (1) isolation of novel Age mutants; (2) transgenic modification of genes regulated by Age genes; (3) pharmacological intervention in ageing processes by targeting signalling pathways. Each approach has strengthened the view that genetically determined lifespan is mechanistically tightly linked to the expression of stress response genes. In addition we are interested in the evolutionary origins of ageing. Quantitative genetic theories of the evolutionary origins of ageing predict that alleles that confer extended lifespan may reduce fitness as a consequence of effects on early-life processes. We have undertaken laboratory natural section experiments to determine the fitness costs of extended lifespan and have obtained findings consistent with the pleiotropy theory of ageing.

**2 An integrated theory of ageing in the nematode *Caenorhabditis elegans*.** By D. GEMS. *University College London, UK.*

Numerous theories of ageing have been proposed, and many have been tested experimentally, particularly using nematode models such as *Caenorhabditis elegans*. By combining those theories of ageing that remain plausible with recent findings from studies of *C. elegans* lifespan mutants, an integrated theory of ageing has been devised. This is formed from 3 interconnected elements: the evolutionary theory of ageing, the oxidative damage theory of ageing, and a nonadaptive programmed ageing theory. This tripartite theory of ageing gives rise to a number of predictions that may be tested experimentally.

**3 Regulation of the long-term effects of sublethal oxidative stresses on the biomarkers of replicative senescence.** By J. REMACLE, P. DUMONT, C. FRIPPIAT, T. PASCAL[1], J.-F. DIERICK[1], F. CHAINIAUX[1], M. BURTON[1], E. S. GONOS[2], Q. M. CHEN[3], P. M. LARSEN[4], P. ROEPSTORF[4] and O. TOUSSAINT[1]. [1]*University of Namur, Belgium;* [2]*National Hellenic Research Foundation, Greece;* [3]*University of Arizona, USA; and* [4]*University of Odense, Denmark.*

Human diploid fibroblasts exposed to oxidative stresses with tert-butylhydroperoxide (t-BHP) applied at sublethal doses display stable morphological changes typical of senescent cells: irreversible growth arrest in G1/S phase of the cell cycle, long-term $p21^{waf\text{-}1}$ protein overexpression and pRb protein hypophosphorylation, senescence-associated $\beta$-galactosidase activity at pH 6.0 and the common 4977 kb mitochondrial DNA deletion 3 d after the stresses. At day 3 after sublethal single $H_2O_2$ and successive t-BHP stresses, increases are observed in the mRNA level of several genes (fibronectin, osteonectin, SM22, apolipoprotein J) overexpressed in replicative senescence. The level of mRNA of several cyclin-dependent kinase inhibitors ($p21^{waf\text{-}1}$, $p16^{ink\text{-}4}$, p14/15) is found to be increased using RNA protection assays.

The expression of some of the genes which are overexpressed in normal senescent cells and in stress induced senescent cells have been found to be controlled by p53 and/or retinoblastoma proteins. By proteome analysis and RT-PCR differential display analysis, many proteins/mRNAs are found for which expression is shared by senescent cells and cells exposed to sublethal stresses at least 3 d before. However proteins/mRNAs have also been found for which expression is specific either to senescent cells or to cells exposed to sublethal stresses of different natures, such as t-BHP or ethanol.

These results suggest that sublethal stresses can lead human fibroblasts to a state related to senescence. The work is now aimed at identifying the proteins/genes whose expression is similarly changed in normal senescent cells and in stress induced senescent cells, since these proteins are thought to regulate senescence.

Acknowledgements: FNRS & FRIA, Belgium; European Biomed & Health Programme; NATO & Fulbright Scholarships.

**4 Exercise, oxidative stress and muscle ageing.** By M. J. JACKSON and A. McARDLE. *Department of Medicine, University of Liverpool, UK.*

In recent years there has been increasing interest in the possibility that exercising skeletal muscle generates an increased amount of free radicals and other reactive oxygen species. There has also been considerable interest in whether this rise in free radical activity leads to oxidative damage to skeletal muscle and other tissues. Initial suggestions indicated that free radical mediated processes, such as lipid peroxidation were elevated during whole body exercise in humans and rats although whether this has any functional significance has become the subject of considerable controversy. An explanation for the lack of evidence for a chronic increase in oxidative damage in subjects who habitually take extensive exercise regimens may come from recent evidence indicating that muscle cells adapt to increased free radical activity during contraction to reduce the risk of free radical damage to the tissue. Thus exercise training has been shown to increase the activity of several antioxidant enzymes such as superoxide dismutase and catalase in muscle, and recent data indicates an increase in muscle heat shock protein (HSP) content following exercise. It is now recognised that these adaptations can protect skeletal muscle against further bouts of (normally) damaging contractile activity.

It has also become clear that the ability of aged organisms to adapt to stresses, such as oxidative stress, is diminished in comparison with young organisms. Our data indicate that this age related attenuation of the stress response also applies to responses to exercise induced oxidative stress. These data suggest that the increased susceptibility of aged muscle to exercise induced damage, and reduced recovery from damage, may be related to the inability to respond appropriately to the oxidative stress induced by contractile activity.

This work is supported by Research into Ageing, the Biotechnology and Biological Sciences Research Council and the Wellcome Trust.

**5 Cytokines, neurotrophins and neurodegeneration in the ageing CNS.** By N. J. MacDONALD[1], F. DECORTI[1], T. C. PAPPAS[2] and G. TAGLIALATELA[1]. [1]*Department of Anatomy & Neuroscience and Marine Biomedical Institute and* [2]*Division of Infectious Diseases, the University of Texas Medical Branch at Galveston, USA.*

Age associated neurodegenerative diseases such as Alzheimer's disease are characterised by neuronal impairments that lead to cognitive deficits. As many of the affected neurons depend on trophic factors such as neurotrophins (NTs), impairments in NT functions have been suggested to play a role in the onset of neuronal damage associated with Alzheimer's disease. Age related neurodegenerative diseases are also characterised by high CNS levels of pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF$\alpha$). Because TNF$\alpha$ receptors and certain NT receptors share a high degree of homology and are capable of activating similar signalling pathways, one possibility is that altered cytokine levels may affect NT function in the aged or diseased CNS. Here we wish to briefly review the evidence suggesting a role for cytokine and NT in the onset of age-associated neurodegenerative diseases. We propose that cytokine/NT interactions may alter neuronal homeostasis, thus possibly contributing to the neuronal degeneration occurring during such age associated CNS diseases as Alzheimer's disease.

**6 Glucocorticoids, stress responses and CNS ageing.** By J. SECKL. *Molecular Endocrinology Unit, Molecular Medicine Centre, Western General Hospital, University of Edinburgh, UK.*

With ageing, approximately one third of rats develop a syndrome comprising a triad of (1) excess levels of glucocorticoids, (2) loss of hippocampal cognitive function and (3) deleterious changes in the structure of hippocampal neurons and sometimes neuronal death. The causative role of glucocorticoids in this process was first illustrated more than 20 y ago by experiments in which glucocorticoid levels were maintained low throughout life by removing the adrenal glands. Such treatment largely prevented the emergence of memory defects and loss of neurons in the hippocampus with age. Similarly, manipulations in early life (neonatal handling, maternal grooming) which keep hormone levels low throughout life are also associated with the prevention of memory defects with age. Important recent data now show that in elderly humans, those individuals with rising cortisol levels with age subsequently show loss of memory function and shrinkage of the hippocampus (assessed by MRI). In contrast individuals in whom cortisol levels are low or decline with age, cognitive abilities and hippocampal volume are maintained with ageing.

The neonatal studies suggest that measures to increase glucocorticoid receptor density in the hippocampus sensitise the brain to negative feedback control, thus keeping glucocorticoid levels low. The major positive regulators of hippocampal receptor expression are neurotransmitters, particularly serotonin and noradrenaline. Such effects occur via specific transmitter receptors, second and third messenger cascades and activation of the glucocorticoid receptor gene promoter. Recent data suggest that antidepressant drugs which potentiate monoamine function increase glucocorticoid receptor expression selectively in the hippocampus, thus keeping glucocorticoid levels low. Indeed antidepressants improve cognitive performance in young rats and prevent the emergence of memory defects with ageing.

Another approach arises from the recent discovery that enzymes modulate glucocorticoid access to nuclear receptors. 11$\beta$-hydroxysteroid dehydrogenase catalyses the interconversion of active cortisol (corticosterone in rats and mice) with inert 11-keto forms (cortisone, 11-dehydrocorticosterone). The type 1 isozyme (11$\beta$-HSD1) is highly expressed in the brain, including the hippocampus where it acts as a reductase, regenerating active corticoids in cells. Enzyme inhibition attenuates the neurotoxicity of 11-keto steroids and mice with targeted disruption of the 11$\beta$-HSD1 gene appear protected from age-associated cognitive impairments. Unlike adrenalectomy or early life manipulations, treatments with antidepressant drugs and perhaps enzyme inhibitors might be appropriate for

humans in middle life, raising the possibility of therapies to prevent glucocorticoid-associated CNS ageing.

**7 Calcium homeostasis, synaptic function and neuronal ageing.** By E. C. TOESCU[1] and A. VERKHRATSKY[2]. [1]*Department of Physiology*, *Birmingham University*, *and* [2]*School of Biological Sciences*, *University of Manchester*, *UK*.

The last decade witnessed a significant turn in our understanding of the mechanisms responsible for the decline of cognitive functions in aged brain. As has been demonstrated by detailed morphological reassessments, the senescence related changes in cognition cannot be attributed to a simple decrease in the number of neurons. It is becoming clearer now that a major cause of age induced deterioration of brain capability involves much subtler changes at the level of synapses. These changes are either morphological (i.e. reduction in the number of effective synapses) and/or functional (i.e. changes in the efficacy of the synapses remaining) all leading to a literal rewiring of the CNS. Important questions are now raised regarding the mechanisms which mediate these synaptic changes. Clearly an important candidate is calcium, whose cytotoxic role is already firmly established. The wealth of evidence collected so far regarding the changes of $Ca^{2+}$ homeostasis in the aged neurons shows that the overall duration of cytoplasmic $Ca^{2+}$ signals gets longer. This is the most consistent result, demonstrated on different preparations and using different techniques. What is not yet clear is the underlying mechanism, as this result could be explained either through an increased $Ca^{2+}$ influx or because of a deficit in the $Ca^{2+}$ buffering/clearance systems. It is conceivable that these prolonged $Ca^{2+}$ signals may exert a local excitotoxic effect, removing preferentially the most active synapses. Uncovering of the role of $Ca^{2+}$ in the synaptic function of the aged brain presents an exciting challenge for all those involved in the neurobiology of senescent CNS.

**8 Control of the Forkhead transcription factor AFX by phosphatidyl inositol 3-kinase/protein kinase B and Ras/Ral-mediated signalling.** By G. J. P. L. KOPS[1], R. H. MEDEMA[2], N. D. DE RUITER[1], A. M. M. DE VRIES-SMITS[1], D. R. POWELL[3], J. L. BOS and B. M. Th. BURGERING[1]. [1]*Laboratory for Physiological Chemistry and Centre for Biomedical Genetics*, *University of Utrecht*, *The Netherlands*; [2]*Department of Haematology*, *University Hospital Utrecht*, *The Netherlands*; [3]*Department of Pediatrics*, *Baylor College of Medicine*, *Houston*, *Texas*, *USA*.

The phosphatidyl inositol 3-kinase (PI 3-kinase) effector protein kinase B (PKB) has been reported to regulate certain insulin responsive genes, but transcription factors regulated by PKB have yet to be identified. Genetic analysis in *Caenorhabditis elegans* has revealed that the forkhead transcription factor daf-16 is regulated by a pathway consisting of insulin receptor-like daf-2 and PI 3-kinase-like age-1. We have demonstrated that PKB directly phosphorylates and thereby inactivates AFX, a human orthologue of daf-16 (Kops et al. *Nature* **398**, 1999). We have furthermore shown that, next to PKB, insulin induced AFX phosphorylation and inactivation involves Ras signalling to the small GTPase Ral. We are currently investigating the mechanism by which PKB- and Ral-mediated phosphorylation of AFX inhibits its activity. We were able to show that at least in part regulation is through relocalisation of AFX from nucleus to cytoplasm but the details of AFX retention and the role of phosphorylation herein are still unclear.

As the pathway that regulates the activity of this forkhead regulates longevity in *C. elegans* we seek to determine what biological response is controlled by this pathway in mammalian cells, and we have obtained evidence that next to regulating transcriptional activity of metabolic enzymes, AFX may control cell cycle progression in part through regulation of the cyclin/cdk inhibitor p27.

**9 Phosphatidyl inositol 3-kinase, insulin signalling and growth control in *Drosophila*.** By D. WEINKOVE and S. J. LEEVERS. *Ludwig Institute for Cancer Research and the Department of Biochemistry and Molecular Biology*, *University College London*, *UK*.

Class $I_A$ phosphatidyl inositol 3-kinases (PI3Ks) are intracellular signalling molecules activated by insulin and insulin-like growth factors. Once activated, class $I_A$ PI3Ks produce 3′ phosphoinositides, and thereby stimulate signalling via a protein kinase cascade that includes the serine/threonine kinases PDK1, Akt/PKB and p70S6K. In mammalian cells insulin/PI3K signalling regulates cell survival, division and metabolism, whereas in the nematode worm, *C. elegans*, this pathway controls the decision to form dauer larvae and affects adult lifespan. We set out to investigate the function of class $I_A$ PI3Ks in the fruit fly *Drosophila* during the development of epithelial organs termed imaginal discs. *Drosophila* possess one class $I_A$ PI3K, Dp110, which associates with an SH2 domain-containing adaptor molecule, p60. We have investigated Dp110 and p60 function using mutants and transgenes. Our results demonstrate that Dp110 and p60 are essential genes that are required for imaginal disc cells to grow to their normal size and achieve their normal number. In addition, modulating Dp110 activity through the expression of Dp110 and p60 transgenes affects the growth and final size of imaginal discs and of the adult organs that they give rise to. Thus, in *Drosophila*, class $I_A$ PI3Ks regulate the growth of individual cells and of entire organs. Recent work from various laboratories has shown that other molecules on the insulin/PI3K pathway also regulate imaginal disc growth. These results will be discussed, together with the hypothesis that both the structure and the function of the insulin/PI3K signalling pathway has been conserved through evolution.

**10 Synucleins in neuron survival and degeneration.** By A. R. SAHA[1], N. N. NINKINA[1], D. P. HANGER[2], B. H. ANDERTON[2], A. M. DAVIES[1] and V. L. BUCHMAN[1]. [1]*School of Biology*, *University of St Andrews*; *and* [2]*Department of Neuroscience*, *Institute of Psychiatry*, *London*, *UK*.

The molecular and cellular mechanisms underlying neuronal loss in neurodegenerative diseases are unclear. It is generally thought that aggregation of mutated, abnormally

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press

modified or abnormally folded proteins leads to the accumulation of extracellular, intracellular or intranuclear deposits that severely compromise cell physiology, leading to the death of the affected neurons. However, there is growing evidence that neuronal apoptosis in the absence of obvious pathological deposits could have a serious impact in the pathogenesis of neurodegenerative diseases.

$\alpha$-synuclein is a small cytoplasmic protein of unknown function that is normally expressed in many neurons of the central nervous system. It has been implicated in the pathogenesis of Parkinson's disease and Alzheimer's disease because it is a constituent of the extracellular and intracellular deposits observed in these diseases, it is mutated in some families affected by hereditary early-onset Parkinson's disease and mutations in the gene promoter region increase the risk of acquiring a spontaneous form of Parkinson's disease. Three other members of the synuclein family ($\beta$-synuclein, persyn/$\gamma$-synuclein and synoretin) are structurally close to $\alpha$-synuclein and each other but have distinct patterns of expression and have not been implicated in neurodegeneration. Despite the considerable recent interest in $\alpha$-synuclein, very little is known about the biological functions of synucleins and the link between $\alpha$-synuclein and neurodegeneration is not understood. We demonstrated that overexpression of persyn, but not $\alpha$-synuclein, in cultured mouse sensory neurons influences the integrity of the neurofilament network. In contrast, overexpression of wild-type and mutant forms of $\alpha$-synuclein in these neurons causes apoptosis, possibly via interaction with Bad or other death-signalling molecules. These results suggest that abnormalities of $\alpha$-synuclein metabolism could lead to the neuronal loss occurring in certain forms of neurodegeneration before the formation of characteristic deposits.

**11 Pleiotropic effects of growth hormone and IGF-1 on biological ageing: inferences from moderately caloric restricted animals.** By W. E. SONNTAG. *Wake Forest University School of Medicine*, *North Carolina*, *USA*.

Research over the past 20 y clearly indicates that age related changes in cellular and tissue function are linked to decreases in the anabolic hormones, growth hormone and insulin-like growth factor (IGF)-1. Although there has been extensive research on the effects of these hormones on bone and muscle mass, the effects of these hormones on cerebrovascular and brain ageing has received little attention. We have also observed that in response to moderate caloric restriction (a treatment that increases mean and maximal lifespan by 30–40%), age related decreases in growth hormone secretion are ameliorated (despite a decline in plasma levels of IGF-1) suggesting that some of the effects of caloric restriction are mediated by modifying the regulation of the growth hormone/IGF-1 axis. Recently we have observed that microvascular density on the surface of the brain decreases with age and that these vascular changes are ameliorated by moderate caloric restriction. Analysis of cerebral blood flow paralleled the changes in vasculature in both groups. Administration of growth hormone for 28 d was also found to increase microvascular density in aged animals and further analysis indicated that cerebrovasculature is an important paracrine source of IGF-1 for the brain. In subsequent studies, administration of GHRH (to increase endogenous release of growth hormone) or direct administration of IGF-1 was shown to reverse the age-related decline in spatial working and reference memory. Similarly, antagonism of IGF-1 action in the brains of young animals impaired both learning and reference memory. Investigation of the mechanisms of action of IGF-1 suggested that this hormone regulates age-related alterations in NMDA receptor subtypes (e.g. NMDAR2A and R2B) and dopamine $D_2$ induced GTPase activity. The beneficial role of growth hormone and IGF-1 in ameliorating vascular and brain ageing are counterbalanced by their well-recognised roles in age related pathogenesis. Although research in this area is still evolving, our results suggest that decreases in growth hormone and IGF-1 with age have both beneficial and deleterious effects. Furthermore, part of the actions of moderate caloric restriction on tissue function and lifespan may be mediated through alterations in the growth hormone/IGF-1 axis.

**12 Evolution, stress, and longevity.** By T. B. L. KIRKWOOD. *Biological Gerontology Group*, *University of Manchester*, *UK*.

The disposable soma theory suggests that longevity is determined through the setting of longevity assurance mechanisms so as to provide an optimal compromise between investments in somatic maintenance (including stress resistance) and in reproduction (Kirkwood & Rose, *Phil. Trans. Roy. Soc. Lond. B* **332**, 1991). A corollary is that species with low extrinsic mortality are predicted to invest relatively more effort in maintenance, resulting in slower intrinsic ageing, than species with high extrinsic mortality. We tested this prediction in a comparative study of stress resistance in primary skin fibroblasts and confirmed that cells from long-lived species are indeed more resistant to a variant of stressors (Kapahi et al. *Free Rad. Biol. Med.* **26**, 1999). A widely studied example of within-species variation in lifespan is the rodent calorie restriction model. Food restricted animals show elevations in a range of stress response mechanisms and it has been suggested that this is an outcome of natural selection for life history plasticity. We have developed a theoretical model for dynamic optimisation of the allocation of effort to maintenance and reproduction in response to fluctuations in food availability (Shanley & Kirkwood, unpublished). The model supports the suggestion that the response to calorie restriction may be an evolutionary adaptation, raising interesting questions about the hierarchy of genetic control of multiple stress response systems. The model identifies ecological factors likely to support such an adaptation that may be relevant in considering the likely relevance of a similar response to calorie restriction in other species.

**13 Reproduction and the evolution of ageing in *Drosophila*.** By L. PARTRIDGE. *Galton Laboratory*, *Department of Biology*, *University College London*, *UK*.

The force of natural selection against mutants with deleterious effects declines for older age cohorts. Ageing can therefore evolve as a result of mutation accumulation

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press

and pleiotropy. The pleiotropy theory posits the occurrence of mutants with beneficial effects in the young, but deleterious effects at later ages. The cost of reproduction is likely to be an important source of this kind of pleiotropic gene action. Artificial selection and environmental manipulation of reproduction in *Drosophila* have been used to demonstrate that reproduction acts with a time lag to produce a subsequent wave of mortality through the population. Mortality associated with earlier reproduction appears to be the main evolutionary cause of ageing in this species. The wave of mortality may explain the phenomenon of mortality rate deceleration, in which the age-related increase in mortality slows down at later ages.

## POSTERS

**P1 Ageing, protein oxidation and carnosine.** By C. BROWNSON and A. R. HIPKISS. *Molecular Biology and Biophysics Group, King's College London, UK.*

Carnosine is a naturally occurring dipeptide ($\beta$-alanyl-L-histidine) found especially in long lived tissues (e.g. muscle and brain) at concentrations up to 20 mM in humans. Intramuscular concentrations of carnosine appear to correlate with maximal lifespan in mammals, but may decline with age. Functions ascribed to carnosine include antioxidant, free radical scavenger, buffer and transition metal ion chelator. Carnosine (but not antioxidants such as vitamins C and E) can delay senescence of cultured human fibroblasts and rejuvenate senescent cells; the mechanism(s) of these effects are unknown.

A symptom of ageing at cellular and whole organism level is the accumulation of aberrant polypeptides containing carbonyl groups. Oxygen free radicals are potential sources of protein carbonyls either by their direct action on polypeptides or via generation of reactive end products of lipid peroxidation, e.g. malondialdehyde and hydroxynonenal. Our previous studies have shown that carnosine reacts with small aldehydes (e.g. malondialdehyde) and thereby prevent their deleterious effects on proteins. In the present study we have attempted to determine whether carnosine reacts with carbonyl groups present in a protein. Protein (ovalbumin) was treated with methylglyoxal (MG), a naturally occurring metabolite, to generate carbonyl groups on the polypeptide. Using $^{14}$C-labelled carnosine we find that carnosine reacts with MG-modified protein. Carnosine also inhibited crosslinking of the MG-treated protein to lysine, N-($\alpha$)-acetyl-lysine methyl ester and a normal protein ($\alpha$-crystallin).

We therefore propose that, in addition to its documented activities, carnosine is (1) a general aldehyde scavenger and (2) reacts with 'aged' proteins bearing carbonyl groups forming protein-carbonyl-carnosine adducts. This 'carnosinylation' of oxidised proteins, should it occur in vivo, might either produce a form of lipofuscin (the age pigment) or promote the selective degradation of the modified polypeptide via the proteosome system or via the scavenger and/or AGE receptors (RAGEs) present on macrophages, endothelial cells, fibroblasts, etc. This proposal may partly explain the rejuvenating effects of carnosine on senescent cultured human fibroblasts.

**P2 Mechanisms of action of immunoregulatory small peptides, during ageing.** By V. Kh. KHAVINSON, V. G. MOROZOV and V. V. MALININ. *Institute of Bioregulation and Gerontology of the North-Western Department of the Russian Academy of Medical Sciences, St Petersburg, Russia.*

The purpose of this investigation consisted of studying experimentally the impact of the synthetic dipeptides thymogen (Glu-Trp) and vilon (Lys-Glu) on cellular and humoral immunity, nonspecific resistance of the organism and processes of anti-oxidation defence, regeneration of tissues and carcinogenesis.

Administration of thymogen and vilon to mice and rats suffering immune depression promoted the enhancement of cellular and humoral immune response to thymus dependant antigens. At the same time vilon produced more pronounced action on chemotaxis and phagocytic reactions of neutrophilic leucocytes of the blood.

Long term exposure of mice to the dipeptides under study prolonged animal life by 20–30% and decreased the incidence of spontaneous tumours significantly. Thymogen and vilon increased the lifespan of *Drosophila melanogaster* and enhanced the activity of enzymes responsible for antioxidation defence (catalase, superoxide dismutase). Nevertheless, vilon produced more pronounced action, as compared to thymogen, on the processes of cellular regeneration in lymphoid organs and intestine of x-irradiated rats. The results of thymogen and vilon comparative study in the culture of thymic and spleen cells of rats of various age revealed differences in the action of dipeptides under study on the processes of lymphoid tissue growth and synthesis of nucleic acids and protein in lymphocytes.

Vilon produced stronger action in comparison with thymogen on the expression of thymocyte receptors in guinea pigs against the background of age related involution of thymus. The data obtained confirm that immunomodulating properties of small peptides depend on age related peculiarities of immune reactivity of the organism, as well as structural and functional characteristics of the drugs.

**P3 Basal and stress-induced hypothalamo-pituitary-adrenal (HPA) activity in aged male and female rats.** By D. JULIAN, R. J. WINDLE, S. A. WOOD, Y. M. KERSHAW, S. L. LIGHTMAN and C. D. INGRAM. *Departments of Anatomy and Medicine, University of Bristol, UK.*

The HPA axis regulates secretion of corticosteroid hormones and a variety of metabolic and homeostatic processes, particularly in response to stressful stimuli. Plasma corticosteroid levels are tightly regulated and show both ultradian pulses of basal secretion, which display diurnal variation in amplitude, and a rapid increase and decline in response to acute stress. This control of the HPA axis has been reported to become dysfunctional during ageing leading to elevated basal levels and prolonged responses to stress. To characterise the changes to the dynamic activity of the HPA axis during ageing we have undertaken repetitive blood sampling from young and aged rats.

Male Wistar rats (4, 12 and 24 mo old) were sampled every 10 min for 24 h using an automated blood sampling system. In young animals corticosterone levels showed

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press

pulses of secretion separated by interpulse periods when levels fell to near zero. Mean levels showed a diurnal rhythm due to a decreased amplitude during the early light phase and an increased amplitude in the early dark phase. Both groups of aged animals showed a similar pattern of secretion except that no diurnal variation in pulse characteristics was detected. Regardless of age, a psychological stress (10 min white noise 114 dB) caused a rapid increase in corticosterone levels which peaked 25 min after onset and rapidly declined to pre-stress levels about 50 min later.

Female rats (3 and 20 mo old) were also sampled, and a comparison was made between the Lewis and the hypersecretory Fischer-344 rat strains. Both strains showed pulses of corticosterone secretion with a similar frequency across the day. In both young and aged Lewis rats the pulses showed a diurnal variation in amplitude but this was severely attenuated in the aged animals. The young F-344 rats showed a pattern of secretion with no diurnal variation in their high amplitude pulses. These persistent high amplitude pulses were lost in the aged F-344, leading to a greater diurnal variation. Neither group of aged animals showed any difference in the onset or duration of the response to noise stress compared with their respective young controls, but the amplitude was reduced in the aged Lewis animals.

Thus the ultradian but not the diurnal pattern of corticosterone secretion is retained during ageing. Furthermore we have not found any evidence of impairment of the feedback mechanisms, as shown by the rapid response in activation and inhibition of the HPA axis after an acute psychological stress stimulus. The flattening of the diurnal variation in pulse amplitude may cause or contribute to pathological changes during senescence.

Supported by the Wellcome Trust, the Neuroendocrinology Charitable Trust and the Special Trustees for the United Bristol Hospitals.

**P4 Qualitative and quantitative morphology of the rat phrenic nerve in young and old rats.** By R. H. M. KING, J. R. MUDDLE, M. NOURALLAH, Y. ZOUKOS and P. K. THOMAS. *Department of Clinical Neurosciences, Royal Free and University College Medical School, London, UK.*

Peripheral nerve pathology ascribed to a normal ageing process has been reported both in human and animal peripheral nerves. In man a mild degree of peripheral neuropathy is common in older age groups; clinical manifestations are usually restricted to a loss of ankle jerks and a reduction in vibration sense.

Animal studies have shown the occurrence of massive intramyelinic oedema in a proportion of fibres in spinal roots. Other studies have reported distal axonal loss. As some of these studies were flawed by the use of nerves liable to entrapment or injury, we chose to examine the phrenic nerve as it is protected from accidental injury. 60 rats aged 3–34 mo were deeply anaesthetised and then perfused with 1% paraformaldehyde plus 1% glutaraldehyde in PIPES buffer. The whole length of the phrenic nerve was removed bilaterally, conventionally processed and embedded in epoxy resin. Sections from the level of the thoracic outlet and just proximal to the diaphragm were cut and stained. These were analysed morphometrically and combined in 4 groups, 3–10, 11–18, 19–26 and 27–34 mo. A small but significant loss of myelinated fibres and an increase in fascicular area were found in the oldest animals. No statistically significant axonal atrophy was demonstrated. Electron microscopy of selected animals confirmed fibre degeneration.

Median fibre size increased up to 19–26 mo and then diminished slightly in the oldest rats. Myelin thickness increased slightly with age up to 26 mo but did not change thereafter. There was no increase in the proportion of thinly myelinated fibres in old age. The most prominent of the alterations seen in the myelin sheath was intramyelinic oedema (myelin bubbles). Although a striking change morphologically, this only affected a small proportion of fibres.

In conclusion we found loss of axons in old rats. Intramyelinic oedema has been considered to be a manifestation of demyelination secondary to axonal atrophy but we were unable to confirm the occurrence of atrophy.

**P5 Increased expression of the p75 NGF receptor in ageing enteric neurons: a possible role in age-related neuron cell death.** By V. SOUBEYRE[1], J. M. SAFFREY[2] and T. COWEN[1]. [1]*Department of Anatomy and Developmental Biology, Royal Free and University College Medical School, London and* [2]*Department of Biology, The Open University, Milton Keynes, UK.*

Over the last decade, it has become clear that loss of function in the ageing nervous system is not associated with extensive neuron cell death. However, some populations of neurons have been shown to be selectively vulnerable, for example, those of the myenteric plexus of the ileum in aged guinea pigs and rats. We have recently demonstrated that this loss of enteric neurons is diet-dependent. In aged rats fed on a restricted diet, no neuron loss was observed whilst ad lib fed rats displayed a loss of about 60%. Cholinergic neurons are likely to be the subpopulation of the enteric neurons affected. A candidate for the regulation of survival in adult neurons is p75 NGF To investigate the possible involvment of p75 in the survival of ageing neurons we looked at its expression in the enteric nervous system using immunohistochemistry and in situ hybridisation in 6 mo and 24 mo old Sprague–Dawley rats killed by cervical dislocation while heavily anaesthetised with Sagatal. A monoclonal antibody against the receptor was used to examine the distribution of p75 protein in whole mount preparations of the enteric nervous system and the intensity of neuronal immunostaining was determined using light microscopy and image analysis.

The data show that the level of expression of p75 protein is significantly ($P<0.01$) increased, by about 30%, in the myenteric plexus of 24 mo rats suggesting that the upregulation of p75 may contribute to age related death of enteric neurons. Further studies are required to investigate the effect of diet on p75 expression.

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press

**P6 Phosphoinositide 3-kinase modulates NGF-induced responses in sympathetic neurons from young, adult and aged rats.** By N. ORIKE and T. COWEN. *Department of Anatomy and Developmental Biology*, *Royal Free and University College Medical School*, *London*, *UK*.

Sympathetic neurons are dependent on target derived neurotrophins for their growth and survival during early development. However, as they mature they become less dependent and by adult life exhibit survival independence, whilst still retaining growth responsiveness to neurotrophic factors. Understanding the conditions which determine survival of adult neurons may throw light on the selective vulnerability which is characteristic of the ageing nervous system.

We have previously shown that levels of target derived NGF, a key extrinsic regulator of growth and survival, does not decline with age in tissues where neurons undergo atrophy and die (Cowen et al. *NeuroReport* 7, 1996). Thus, we are interested in identifying potential intracellular regulators of age-related neurodegeneration.

Recent studies have suggested a pivotal role for PI 3 kinase (PI 3-K) in growth and survival of neurons including mediation of responses initiated by phosphorylation of Trk by NGF. We have carried out preliminary experiments to examine the effects of PI 3-K blockade on the growth and survival responses of sympathetic neurons from rats of different ages (1 wk, 12 wk and 17 mo old) killed by cervical dislocation while deeply anaesthetised with Sagatal. We cultured these neurons in 10 μM of the PI 3-K inhibitor LY294002 (LY) in the presence of NGF (1 ng/ml) and found that NGF-induced growth and survival was inhibited in young but not older neurons. This differential effect was dose dependent, since we found that growth of both young and old neurons was inhibited when cultured in higher concentrations of LY (50 μM). Survival of young neurons was also inhibited.

These results suggest that older neurons are less sensitive to PI 3-K blockade than younger neurons, particularly in relation to survival. This may result from increased constitutive activity of PI 3-K in older neurons which could also account for the acquisition of survival independence. Alternatively, older neurons may acquire additional extracellular regulators of growth and survival.

**P7 Calcium binding protein distribution in aged rat autonomic ganglia.** By U. V. BOOLAKY and R. M. SANTER. *Anatomy Unit*, *Cardiff School of Biosciences*, *Cardiff University*, *UK*.

The occurrence and distribution of the calcium binding proteins calbindin-D28k, calretinin and parvalbumin have been investigated by indirect immunofluorescence and enzyme histochemistry in autonomic ganglia of young (3 mo) and aged (24+ mo) male Wistar rats killed by cervical dislocation while deeply anaesthetised with ether. The purpose of the study was to determine whether there may be evidence to implicate alterations in intracellular calcium buffering capacity as a contributing factor to age-associated neuronal changes in the autonomic nervous system. In the major pelvic ganglion calbindin was present in sympathetic (tyrosine hydroxylase-positive) but not in parasympathetic (NADPH-diaphorase-positive) neuronal somata. The number of calbindin-positive sympathetic neurons was markedly reduced in the aged group of animals. Calbindin-positive intraganglionic nerve processes were similarly reduced in number in the aged group. Immunofluorescence for calretinin and parvalbumin was much lower in neuronal somata and processes and did not alter with age. Calbindin immunofluoresecence was strong in large clusters of small intensely fluorescent cells in the major pelvic ganglion but calretinin and parvalbumin staining was very weak. In the superior cervical ganglion calbindin immunmoreactivity was confined to a small population of neurons scattered throughout the ganglion, some of which were still detectable at 24 mo. Calretinin was not detectable in the majority of neuronal somata but present in fibres forming perineuronal baskets around many neurons whereas parvalbumin was only present in neuronal somata. By 24 mo the levels of calretinin and parvalbumin immunofluoresence were barely detectable. In the coeliac-superior mesenteric ganglion complex immunostaining for all three proteins was very weak in neuronal somata and undetectable at 24 mo. Calretinin immunofluorescent perineuronal baskets were a prominent feature of many neurons in this ganglion at 3 mo but very scarce at 24 mo. It is not known whether the perineuronal baskets represent preganglionic or gastrofugal fibres or both. There was no immunostaining in small intensely fluorescent cells within the superior cervical or coeliac-superior mesenteric ganglia. It is evident from this study that the staining intensity and distribution of the 3 calcium binding proteins investigated do not remain constant throughout life in rat postganglionic autonomic neurons. The distinct decreases in immunofluorescence observed in neuronal somata and processes suggest that intracellular calcium buffering may be perturbed in these aged neurons.

**P8 Environmental control of physiological ageing and longevity.** By M. R. J. SHEEHY. *Department of Biology*, *University of Leicester*, *UK*.

Stochastic theories of ageing are often overlooked in favour of adaptive theories. While much gerontological research has employed insect models, little is known about ageing in *Crustacea*. This group provides an excellent opportunity for comparative studies because it contains some of the shortest and longest lived of all invertebrates. The European lobster *Homarus gammarus* for example has a natural longevity exceeding 50 y. This long lifespan can be explained in an evolutionary context: once past the vulnerable planktonic larval stage, relatively low natural mortality is achieved by highly cryptic juvenile behaviour and heavy protective armouring of a relatively large body in adults. This would reduce natural selection pressure for rapid maturation while a strong positive correlation between adult body size and reproductive performance could select for lengthened lifespan. On the other hand, senescence by random cellular damage accumulation in the metabolising postmitotic tissues of this large, sedentary, cool-water decapod would be expected to occur much more slowly than in shorter lived migratory tropical species, for example. Could stochastic theory also explain the lobster's long life?

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press

In the present study, I explore the relationship between environmental temperature, longevity and rate of physiological ageing in wild and laboratory populations of 5 related (pleocyemate) crustaceans with widely differing lifespans, from diverse natural habitats. These include European lobster, Western Australian rock lobster (*Panulirus cygnus*), signal crayfish (*Pacifastacus leniusculus*), redclaw crayfish (*Cherax quadricarinatus*) and yabby (*C. cuspidatus*). As a cellular biomarker of the free radical induced oxidative damage that is considered to be one of the major causal mechanisms underlying ageing, I use neuronal lipofuscin accumulation, quantified by confocal image analysis of its in situ yellow autofluorescence, as a simple but dependable index of physiological ageing.

Results were: (1) In laboratory crayfish populations (*C. quadricarinatus*) rate of physiological ageing is positively correlated with environmental temperature in a complex nonlinear format that suggests a thermal maximum and thermal midrange metabolic compensation. (2) In wild lobster populations (*H. gammarus*) rate of physiological ageing varies between geographical locations and is positively, but nonlinearly, correlated with average ambient sea temperatures. (3) For the 5 pleocyemates so far examined, average annual habitat temperature positively correlates with species-specific rate of physiological ageing which in turn inversely correlates with longevity.

In conclusion: (1) The observed associations between environmental temperature, longevity and rate of physiological ageing in these poikilotherms are underpinned by a strong physicochemical rationale for the influence of body temperature on physiological rate processes in general, and which can be assumed to include those specific oxidative and other biochemical reactions responsible for the cumulative manifestations of ageing. (2) The results highlight the likelihood that, in conjunction with inherited genotypic influences, observed natural variability in life history and population parameters result from underlying differences in species and population-specific rates of physiological ageing that are strongly influenced by ambient extrinsic environmental factors.

**P9 Reactive oxygen species as effectors of aluminium toxicity in vitro.** By H. R. GRIFFITHS and M. LAI. *Department of Pharmaceutical Sciences, Aston University, Birmingham, UK.*

Aluminium (Al) is a neurotoxic agent and has been suggested to be an aetiological factor in a number of brain related disorders. Several pathophysiological effects have been identified both in vivo and in vitro which suggests the cellular response is widespread. Moreover, many of the observed altered physiological functions are interrelated implying that a cascade of neuronal responses may occur to a primary lesion by Al. Such responses include generation of reactive oxygen species (ROS), and displacement of critical metal ions. Ageing is associated with increased ROS activity, either due to increased production or ineffective scavenging. To further study the mechanism by which Al exerts its toxicity and may contribute to accelerated ageing, and to investigate ROS production as an initiating event, 2 important cellular targets have been examined in the rat cholinergic neuronal line, B50.

Toxicity of aluminium in neuronal cells was investigated using 3 different aluminium complexes, where significant toxicity (lactate dehydrogenase leakage) was achieved by 1 mM Al-transferrin per $10^6$ cells after 24 h ($30.4 \pm 4.4\%$ compared with $9.7 \pm 2.8\%$; $P < 0.01$). Significant protection against toxicity was observed by addition of antioxidants. To examine the effects of aluminium on DNA conformation, nucleoids were extracted from B50 cells treated with increasing Al-transferrin for 24 h. Extracted nucleoids were stained with ethidium bromide and light scattering properties were evaluated by FACS. Forward scatter (size) was significantly reduced following Al-transferrin in a dose dependent manner from 1–5 mM per $10^6$ cells, indicative of chromatin condensation and reduced transcriptional activity. Using laser scanning confocal microscopy the expression of selected proteins following Al treatment over a period of up to 14 d was investigated, since pathological lesions arising from Al intoxication in animals have been shown to develop between 5 and 14 d. The B50 cells showed staining for $\beta$-tubulin, MAP-2 and amyloid precursor protein. By d 7, significant increases in fluorescence intensity were observed for $\beta$-tubulin ($4.36 \pm 1.28$ vs $2.4 \pm 1.08$; $P < 0.05$) and amyloid precursor protein ($4.2 \pm 0.9$ vs $2.69 \pm 0.43$; $P < 0.02$) but not MAP-2 ($0.99 \pm 0.11$ vs $1.00 \pm 0.26$; $P > 0.05$). This effect increased up to 14 d exposure.

These data suggest that aluminium toxicity is mediated at least in part by ROS, and that the nucleus may represent an important target for the action of aluminium. Since amyloid precursor protein expression is regulated by the redox sensitive transcription factor AP-1, oxidants may be important mediators in the pattern of protein expression in the ageing brain.

**P10 Are molecular chaperones major determinants of ageing rate in *Caenorhabditis elegans*?** By G. A. WALKER and G. J. LITHGOW. *University of Manchester, UK.*

Mutation of any one of 10 genes in *C. elegans* extends the adult lifespan by approximately 100%. In addition to conferring increased lifespan, these Age mutations also influence stress resistance. It is well established that molecular chaperones are important in cellular stress response, less clear is their influence on ageing. Molecular chaperones are induced during ageing in invertebrates, however the induction of these genes by stress is reduced with age in mammals. This deficit may be responsible for the accumulation of conformationally altered protein observed during ageing and consequently the acceleration of mortality rates.

Here we demonstrate that a putative insulin signalling pathway regulates the molecular chaperone gene *hsp-16*. This suggests that molecular chaperone levels may impact on ageing. Currently *hsp-16* transgenic lines are being used to define the role of molecular chaperones in retarding ageing in long-lived mutants of *C. elegans*. Results so far indicate overexpression of *hsp-16A* confers resistance to lethal thermal stress. Lifespan analysis is being undertaken.

https://doi.org/10.1046/j.1469-7580.2000.19620293.x Published online by Cambridge University Press