G. NON TECHNICAL SUMMARY (NTS)

Project title: Molecular mechanisms controlling Salt and Potassium homeostasis
Duration of project - years: 5
Duration of project - months: 0

Purpose of the project (as in ASPA Section 5C(3)):
(a) basic research: YES
(b) translational or applied research with one of the following aims:
   (i) avoidance, prevention, diagnosis or treatment of disease, ill-health or other abnormality, or their
   effects, in man, animals or plants: YES
   (ii) assessment, detection, regulation or modification of physiological conditions in man, animals or
   plants: NO
   (iii) improvement of the welfare of animals or of the production conditions for animals reared for
   agricultural purposes: NO

(c) development, manufacture or testing of the quality, effectiveness and safety of drugs, foodstuffs
and foodstuffs or any other substances or products, with one of the aims mentioned in paragraph (b): NO
(d) protection of the natural environment in the interests of the health or welfare of man or animals: NO
(e) research aimed at preserving the species of animal subjected to regulated procedures as part of
the programme of work: NO
(f) higher education or training for the acquisition, maintenance or improvement of vocational skills: NO
(g) forensic inquiries: NO

Keywords:
Hypertension (high blood pressure), kidney, dietary salt

Describe the aims and objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed):
Hypertension (high blood pressure) is a major public health problem affecting more than a billion people
worldwide with complications including stroke, heart failure and kidney failure. It is most frequently
caused by inappropriate salt retention by the kidney. We have learnt a lot about the mechanisms in the
kidney for regulating the balance of salt loss and salt retention through the study of rare inherited forms
of blood pressure. One of these (Gordon Syndrome or FHH) causes salt retention by activating a
specific salt transporter in the kidney called NCC that is blocked by thiazide diuretics (probably the most
widely used blood pressure lowering drugs world-wide). A second group of inherited syndromes (and
also the commonest non-inherited and potentially curable forms of hypertension) cause salt retention
by causing the adrenal gland to secrete excessive amounts of aldosterone, which is the hormone
controlling salt retention in the kidney. In addition, around 10% of essential human hypertension is
thought to be caused by excess aldosterone production and in a third of cases it derives from small
benign nodules in the adrenal glands. These offer the prospect of a potential long-term cure through
removal of the affected adrenal gland by key-hole surgery. Yet, there are still significant gaps in our
understanding of how NCC is regulated and what drives the adrenal gland to over-secrete aldosterone.
This project will address both these issues.

What are the potential benefits likely to derive from this project (how science could be advanced
or humans or animals could benefit from the project)?:
The pathway regulating the NCC protein in the kidney can be potentially blocked by small chemical molecules (drugs). This would allow us to generate new drugs to act as diuretics ('water tablets') and lower blood pressure. A better understanding of the adrenal causes of hypertension may similarly enable new drugs to be developed and/or allow better understanding of the processes driving the formation of the small benign tumours in the adrenal gland (which are often the source of the excess aldosterone driving the blood pressure).

What types and approximate numbers of animals do you expect to use and over what period of time?:
The project will only use mice and may use up to 3550 mice in its 5-year duration.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected levels of severity? What will happen to the animals at the end?:
We will breed some genetically modified mice to mimic the genetic changes seen in human hypertension syndromes. These do not have an adverse effect on the mice except for causing hypertension. Most of the procedures done on these genetically modified mice are mild and wherever possible are non-invasive (e.g., measurement of the blood pressure from a tail BP cuff or cardiac function by surface Doppler imaging as in human ECHO tests). All animals will be euthanised.

Application of the 3Rs
Replacement:
Blood pressure and other measures of kidney function can only be observed in a whole living animal. However, in using the mouse as a model of human genetic hypertension we will generate the mice by modern gene editing methods (CRISPR), which represents the most efficient method in terms of minimising the numbers of animals needed. We will also explore the functions of the human mutations we are interested in tissue culture and other cell-based systems before exploring them in a mouse. This will allow us to explore a lot of the biology of the human mutations before we look at their effect on the BP and kidney function.

Reduction:
We are very keen to reduce the numbers of mice used. Hence, all of our experiments will be designed to ensure they have adequate (but not excessive) statistical power. So that if there is a biological effect in the mice we will be able to detect it - that is to minimise the risk of a false negative result. The experiments will also use randomisation and blinding to ensure that the results are as robust and reproducible as possible. With these safeguards, we will use the minimum number of animals to observe the effects we are looking for with an appropriate level of precision.

Refinement:
In this project we will be using where ever possible non-invasive methods of measure the BP of the mice and the performance of their hearts. This will involve using tail-cuff measurements (as used in the human measurement of BP) and heart ECHO cardiograms (again as used in humans and involves simply bouncing sound waves off the heart.)