The Pharmacokinetics and Metabolic Physiology of Leptin

A thesis submitted for the degree of Doctor of Philosophy of the University of New England

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Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis and all sources used have been acknowledged in this thesis.

Robert Allan Hart

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Abstract

Leptin is a cytokine hormone with multiple roles throughout the body. Previously leptin distribution and pharmacokinetics were poorly defined; here the pharmacokinetics of leptin has been examined in male and female mice at physiologic doses. These data were also used to create a predictive model for leptin distribution, allowing targeted research on leptin in the future.

The relationship between leptin and metabolic rate had not been well characterised to date. To explore this further, mice were administered a supraphysiologic dose of leptin or an anti-leptin antibody to examine the effects on oxygen consumption using indirect calorimetry. The administration of anti-leptin in fasted animals resulted in a modest, non-significant, reduction in oxygen consumption, whereas leptin prevented a reduction in metabolic rate. These data indicate that leptin has only a minor role in the regulation of metabolic rate. It may be that leptin is produced locally in peripheral tissues to exert an autocrine/paracrine effect, or perhaps in the central nervous system, both of which may maintain metabolic rate. These data, don't preclude leptin from a role in the central nervous system, but indicate that circulating leptin has a limited role in regulating metabolic rate and that it may have other roles in peripheral tissues.

To determine the targets of leptin, distribution of iodinated leptin was examined over a time course of 120 min following physiologic doses in female and male mice. In both sexes major target tissues included the liver, kidneys, skin, and digestive tract. Notably, in the digestive tract there was a difference in the amount of leptin recovered between the sexes, with up to 12.8 % of the dose recovered in females and up to 23.2 % recovered from the digestive tract in males. These data seem to indicate a sexual dimorphism for leptin distribution, which may translate to functional differences between the sexes.

Further examination of leptin distribution in both sexes revealed that the reproductive tract was a target tissue for circulating leptin in both sexes. In females, both the uterus and ovaries had a high recovery of administered leptin per gram of tissue, particularly in the first 30 min following

administration. In males, the amount of administered leptin recovered from the testes remained relatively stable over the duration of the experiment, but in both the seminal vesicles and the epididymides increases in the amount of leptin recovered were observed over the duration of the experiment. These findings indicate that leptin has an important role in reproductive physiology that has not been defined to date.

Using the leptin distribution data generated in female mice, a nine pool pharmacokinetic model was created using WinSAAM to predict leptin movement between the tissue pools over 120 min. The pools were selected as the tissues that accumulated the most leptin in the distribution study, the brain was included as the major postulated target for circulating leptin and the gall bladder was included to better fit the predicted transfer of leptin into the digestive tract. Two more pools were also generated to better fit the data, 'other', which included reproductive and musculoskeletal tissues, and a 'slow' turnover pool, from which leptin did not re-enter circulation over the 120 min examined. Major targets for circulating leptin were predicted to be the 'other' (several combined minor tissues) and 'slow' (a theoretical pool used to allow curve fitting), pools as well as the liver, kidneys and digestive tract. Major contributors of leptin to the circulation were predicted to be the liver, and skin, which may have represented enterohepatic circulation and subcutaneous adipose tissue, respectively. This initial model shows that more research is needed to fully elucidate the pharmacokinetics of leptin in the periphery.

As the digestive tract had been identified as a major target for circulating leptin, leptin pharmacokinetics were examined over a 120 min time course following oral and intravenous administration. Leptin was found to enter the digestive tract intact following intravenous administration, and following oral gavage was also found to enter the circulation intact. Subsequent to administration via both routes, leptin in the digestive tract was found to move aborally, but < 1 % of the administered dose was eliminated in the faeces. These data indicate a novel property of leptin, that it is recycled between the circulation and the lumen of the digestive tract. This suggests that leptin may have major roles in the digestive tract.

This thesis provides the most comprehensive examination of leptin pharmacokinetics to date, describing the first comparison between females and males, the most detailed model for leptin pharmacokinetics developed to date and identifying novel cycling of leptin between the gut and the circulation. It identifies novel findings and provides new avenues for leptin research.