INFLUENCE OF THE PITUITARY GLAND FROM THE HOMOZYGOTE (+/+) AND HETEROZYGOTE (ob/+) LEAN MOUSE ON INSULIN SECRETION IN VITRO

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1. Introduction

In a previous publication from this laboratory [1] it was shown that perifusing the pituitaries of lean and obese mice in series with isolated pancreatic islets produced a rapid stimulation of insulin secretion from the lean mouse but not from the obese mouse islets. In subsequent investigations it was observed that this stimulatory effect on insulin secretion was less consistent with the pituitaries of lean mice than with the obese mice. As the obese hyperglycaemic syndrome in these mice is due to a recessive gene ob [2] the lean littermates are of a mixed genotype ob/+ or +/+. Therefore it was of interest to ascertain whether the variability of the results described above with the lean mice could be associated with these two distinct genotypes. For this purpose a breeding programme, described in this paper, was set up in order to separate lean homozygotes +/+ from the lean heterozygotes ob/+. Experiments reported here show that under the experimental conditions previously described [1] the pituitary glands from the lean ob/+ but not the lean +/+ stimulated insulin secretion.

2. Methods

2.1. *Breeding of homozygote lean animals*

The obese mouse colony originated from the Jackson Memorial Laboratory, Bar Harbor, Maine USA, and was introduced into local mixed colonies (i.e. not pure strains), in Edinburgh and Birmingham, from which our original stocks were obtained. They have since been maintained in this Department as a random bred, closed colony. The animals are housed in plastic cages 11 × 8 inches (5 animals per cage) and given free access to food (oxoid breeding diet) and tap water. Artificial lighting is maintained on a 12 hr cycle.

Male obese mice are placed on a restricted diet to produce suitable animals for breeding, termed ob/ob R.D., [3], the females are completely infertile. Lean females, which are genetically ob/+ or +/+ were crossed with ob/ob R.D. males. Females which had not produced any obese offspring after 3 litters were presumed to be +/+ and then used to start the colony. 12 putative +/+ females were mated with lean males. The F1 generation were all lean animals, but were a mixed population of +/+ and ob/+, depending upon the genotype of the male parent. The F1 females were then back-crossed with ob/ob R.D. males, those producing no obese offspring were thus demonstrated to have been born from +/+ parents. There is the problem that the F1 males could not be crossed with obese animals due to the infertility of the obese female. Therefore F1 males were crossed with known ob/+ females, those producing no obese offspring after 3 litters were thus shown to have been born from +/+ parents.

The animals produced from these matings were not used for experimental work, serving only as a method of ensuring the absence of the ob gene. The F1 animals known to originate from homozygous parents were then paired, taking care to avoid mating of siblings. The F2 and subsequent generations being used for experimental work.

Since such breeding would eventually result in an inbred strain, lean animals were periodically removed

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from the main stock, screened for the obese gene as described, and then introduced into the homozygous colony.

2.2. *Experimental procedure and insulin assay*

The experimental procedure for the preparation of pituitary glands and isolated pancreatic islets, the perifusion system and the insulin assay method were as previously described [1]. All the islets were prepared from the homozygote lean mice.

3. Results and discussion

The results given in fig.1 show that the perifusates of the obese mice pituitaries produced a rapid marked stimulation of insulin secretion from islets of the homozygote lean mice (as previously reported). The pituitaries of the homozygote +/+ lean mice had no effect whereas those of the lean ob/+ heterozygotes produced a stimulation similar to that observed with the ob/ob animals. These results suggest that the ob gene is associated with a higher concentration or an elevated release of the pituitary factor responsible for the stimulation of insulin secretion, and this could be either a primary defect or secondary to some other genetic dysfunction. Whether the stimulatory factor is present at lower concentrations in the pituitaries of the lean homozygotes mice or completely absent has not been established. These experiments show clearly defined biological perameters separating the two genotype populations in the lean mice. In this respect it is of interest that an almost bimodal distribution of values for the levels of ACTH in the pituitaries of the adult lean mice which could be a partial expression of the ob gene in the ob/+ animals has been reported. Furthermore it has been demonstrated [5] that the rate of glucose oxidation to CO_2 is much lower in adipose tissue of ob/ob mice than that of homozygote +/+ animals and the rate in the adipose tissue of lean heterozygotes ob/+ is intermediate. Similarly oxygen consumption in ob/ob and lean pre-weaned mice has

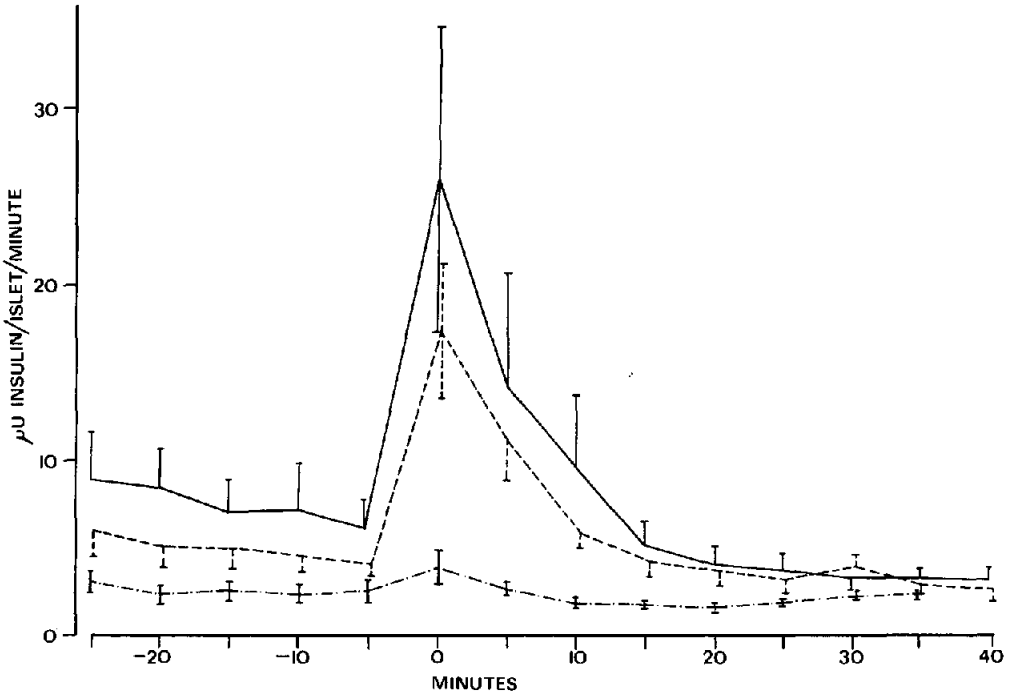

Fig.1. The effect of the perifusate from pituitary glands of ob/ob mice, +/+ lean mice and ob/+ lean mice on isolated islets from +/+ lean mice: (- - -) ob/ob, n = 8; (——) ob/+, n = 5; (. – . –) +/+, n = 5. Vertical bars indicate SEM. Pituitary glands introduced into the system at zero time.

been measured [6] and it was suggested that there is a gradient of oxygen consumption with ob/ob animals at the low end +/+ animals at the high end and ob/+ individuals in the middle of the gradient.

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References

[1] Beloff-Chain, A., Edwardson, J. A. and Hawthorn, J. (1975) J. Endocrin. 65, 109–116.
[2] Ingalls, A. M., Dickie, M. M. and Snell, G. D. (1950) J. Hered. 41, 317–318.
[3] Eastcott, A. (1972) Laboratory Animals 6, 9–18.
[4] Edwardson, J. A. and Hough, C. A. M. (1975) J. Endocr. 65, 99–107.
[5] Yen, J. T., Lowry, L. and Steinmetz, J. (1968) Biochem. Biophys. Res. Comm. 33, 883–887.
[6] Kaplan, M. L. and Leveille, G. A. (1974) Am. J. Phys. 227, 912–915.