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Isolation and Characterisation of Two
Amylin Responsive Proteins from Rat
Skeletal Muscle

Shao Chin Lee

A thesis submitted in partial fulfilment of the requirements for
the degree of Doctor of Philosophy in Biological Sciences, The
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To My Family

ABSTRACT

Two amylin responsive proteins, here designated ARP1 and ARP2, were discovered from rat skeletal muscle through two dimensional gel electrophoresis analysis. ARP1 was detected only in amylin-stimulated muscles where the insulin-stimulated glucose incorporation into glycogen was inhibited. This protein incorporated ^{32}P i but not [^{35}S]-methionine in the metabolic labelling experiments. Subsequent molecular characterisation revealed that ARP1 was a novel monomeric form (designated form 1) of protein p20, and two other monomeric forms (designated forms 2 and 3 respectively) of protein p20 were also characterised. The production of ARP1 was not affected by the presence of insulin, but calcitonin gene-related peptide (CGRP) was found to evoke the production of ARP1 in the presence or absence of insulin. In contrast, ARP2 was detected in both control and amylin-stimulated muscles. Amylin stimulation evoked incorporation of [^{35}S]-methionine but not ^{32}P i into the protein and increased its concentration significantly.

It is concluded that amylin elicits the production of ARP1 through phosphorylation and increases the protein biosynthesis of ARP2; the amylin-evoked production of ARP1 is insulin independent; amylin and CGRP share, at least in part, an intracellular signal transduction pathway; and ARP1 and 2 may be involved in the development of insulin resistance. It is suggested that ARP1 and 2 could potentially be used as molecular markers for the analysis of amylin action.

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ABBREVIATIONS

2D	Two dimensional
2 DE	Two dimensional gel electrophoresis
ARP1	Amylin responsive protein 1 (form 1 protein p20)
ARP2	Amylin responsive protein 2
CGRP	Calcitonin gene-related peptide
DMEM	Dulbecco's Modified Eagle Medium
DTT	Dithiothreitol
EDL	<i>Extensor Digitorum Longus</i>
EDTA	Ethylenediamine tetraacetic acid
GCG	Genetics computer group
HPLC	High performance liquid chromatography
IDDM	Insulin-dependent diabetes mellitus
IPG	Immobilised pH gradient
MS	Mass spectrometry
NIDDM	Non insulin-dependent diabetes mellitus
PMSF	Phenylmethylsulphonylfluoride
PMF	Peptide mass fingerprinting
SDS	Sodium dodecyl Sulfate
TFA	Trifluoroacetic acid
TNF	Tumour necrosis factor
TOF	Time of flight
Tris	Tri(hydroxymethyl)aminomethane

Abbreviations of units of measurement and of physical and chemical quantities are those recommended by the Journal of Biological Chemistry (*J. Biol. Chem.* 271, 1-4, 1996).

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