A STUDY OF

INSULIN RECEPTORS IN

NORMAL AND NEOPLASTIC

CELLS

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ABSTRACT

The characteristics of insulin binding to cultured monolayers of normal human adult and foetal fibroblasts and human tumour cell lines were assessed as the basis for a study of regulation of insulin receptors between normal and tumour cells. Insulin bound specifically to both normal and transformed cells with similar dependence on temperature, time, and pH. There were fewer high affinity and more low affinity receptors on tumour cells compared with normal adult fibroblasts. The affinity of insulin receptors in normal foetal fibroblasts was less than in normal adult fibroblasts while transformed foetal fibroblasts had receptors of even lower affinity.

The sensitivity of insulin receptors to down regulation by insulin was measured in the human breast tumour cell lines MCF-7 and T-47D and the human colon tumour cell line HCT-8 and was compared with measurements in normal human adult fibroblasts. Adult fibroblasts were sensitive to down regulation (40% loss of binding after 2h exposure to 17nM insulin) whereas breast tumour cells were resistant to down regulation (15-17% loss of binding after 4h exposure to 170nM insulin). HCT-8 cells were sensitive to down regulation after 4h exposure to 3.8nM insulin but the extent of down regulation lessened at higher concentrations of insulin. This paradoxical result appeared due to an increase in the affinity of receptors on HCT-8 cells for insulin following exposure to hormone; by comparison, the affinity of receptors on fibroblasts significantly decreased during down regulation. Insulin-induced down regulation of receptors was also compared between normal and transformed foetal fibroblasts. Normal foetal fibroblasts were less sensitive to insulin-induced receptor down regulation than were adult fibroblasts. Transformed foetal fibroblasts had lost all high affinity receptors so that receptor down regulation was too low to measure.

Biological responses to insulin (insulin-stimulated [^{3}H]leucine incorporation into protein and [^{3}H]thymidine incorporation into DNA) were measured in parallel with studies of receptor regulation in order to assess the effect of

down regulation on cell metabolism. Fibroblasts were more responsive to insulin-stimulated leucine and thymidine incorporation than were tumour cells. Down regulation of insulin receptors decreased the responsiveness of fibroblasts but not tumour cells to insulin-stimulated leucine incorporation. Responsiveness of tumour cells to insulin-stimulated leucine incorporation paradoxically increased with lengthy exposure to insulin. The responsiveness of fibroblasts to insulin-stimulated thymidine incorporation was unchanged following receptor down regulation and insulin did not induce a change in cell number. Insulin had variable effects on thymidine incorporation in tumour cells and in most instances induced parallel changes in cell growth.

Tumour cells thus differ from normal cells in their expression and regulation of insulin receptors and their responsiveness to insulin. These differences may well contribute to the metabolic and growth advantage that tumour cells have over normal tissue.

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ABBREVIATIONS

B/F	bound/free
B/T	bound/total
^{14}C	carbon 14
Ca^{2+}	calcium
cDNA	complementary DNA
Ci	curie
cm	centimeter
CURL	compartment for the uncoupling of receptor-ligand complexes
CV	coefficient of variation
df	degrees of freedom
DME	Dulbeccos modified eagle medium
DNA	deoxyribonucleic acid
DNAase	deoxyribonuclease
DR	down regulated
EDTA	ethylenediaminetetra-acetic acid
EGF	epidermal growth factor
F_{12}	Hams F_{12} medium
FCS	foetal calf serum
FDGF	fibroblast derived growth factor
g	gram

Abbreviations cont'd

h	hour
^{3}H	tritium
HCl	hydrochloric acid
^{125}I	iodine 125
k_1, k_{-1}, k_2	rate constants
K_a	binding affinity constant
KCl	potassium chloride
K_M	Michaelis-Menten constant
KRH	Krebs Ringer buffer
l	liter
LDL	low density lipoprotein
M	molar
mCi	millicurie
MEM	minimal essential medium
Mg^{2+}	magnesium
$MgSO_4$	magnesium sulphate
min	minute
ml	milliliter
mM	millimolar
mm	millimeter
mRNA	messenger ribonucleic acid

Abbreviations cont'd

N	non-specific binding
Na^+	sodium
$NaC_2H_3O_2$	sodium acetate
NaCl	sodium chloride
NaCN	sodium cyanide
NaOH	sodium hydroxide
NaF	sodium fluoride
NH_4HCO_3	ammonium bicarbonate
NS	non-significant
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate buffered saline
PDGF	platelet derived growth factor
R	binding site concentration
ros	UR2 sarcoma viral oncogene
RV	residual variance
SD	standard deviation
SDS	sodium dodecyl sulphate
SE	standard error
sec	second
SEM	standard error of the mean
src	Rous sarcoma viral oncogene
SS	sum of squared deviations
SV40	Simian virus 40
TCA	trichloroacetic acid
tRNA	transfer ribonucleic acid

Abbreviations cont'd

µCi	microcurie
µg	microgram
µl	microliter
µm	micrometer
Vmax	maximum velocity
v/v	volume per volume
w/v	weight per volume
Zn^{2+}	zinc
%	percentage
°C	degrees celsius