Assay of liver membrane insulin receptors in obese, hyperglycaemic (ob/ob) mice: stimulant effect of an oral antidiabetic sulphonylurea drug

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A reliable *in vitro* assay of insulin receptors has been developed, and a preliminary study of the *in vivo* effects of an antidiabetic sulphonylurea drug has been attempted.

Purified membranes were prepared from livers of male ob/ob 6 week-old mice and from normal, lean littermates by the method of Cuatrecasas (1972), using a buffer consisting of (mM): Tris HCl 50, $MgCl_2$ 10, NH_4Cl 5, bovine serum albumin 0.1% (w/v), pH 7.9. The homogeneity of the preparation was checked by electron microscopy. The protein concentration of the microsomal fraction was 1.9 ± 0.06 mg/ml: $n = 9$ (mean ± s.d.) for obese mice, and 1.38 ± 0.04 mg/ml: $n = 9$, for lean mice. Purified membranes were incubated at 13°C with 1.5 nM [^{125}I] insulin (Radiochemical Centre, Amersham) alone or in the presence of unlabelled insulin (50 μg/ml) to distinguish between receptor and non-saturably bound hormone. Incubates were layered over cushions of (0.3 M) sucrose in buffer in Beckman microfuge tubes and centrifuged. The supernatant was aspirated, tip of the tube cut off, and the radioactivity in the pellet counted.

Equilibrium between specifically bound and free [^{125}I] insulin was attained within 60 min of incubation at 13°C, and there was no apparent loss of binding sites during this period. Higher incubation temperatures appeared to destroy the binding sites. The binding affinity and capacity were determined from linear Lineweaver-Burke plots after incubating membranes with a range of concentrations of [^{125}I] insulin (0.02–1.5 M) alone or in the presence of excess unlabelled insulin. The molar dissociation constant (Kd) of the reaction and the binding capacity were, for ob/ob mice, 2.22 ± 0.6 × 10^{-10}M: $n = 4$ (mean ± s.e. mean), and 6.23 ± 3.6 × 10^8 binding sites per mg membrane protein respectively, and for lean mice, 3.94 ± 0.7 × 10^{-10}M: $n = 6$, and 5.47 ± 1.3 × 10^9 binding sites respectively.

After i.p. injection of the antidiabetic sulphonylurea drug Gliquidon (4 μg/g bodyweight; Boehringer Ingelheim Ltd) twice daily in 0.1 ml of lactomide for 7 days, there was a five-fold increase in liver membrane insulin receptors from ob/ob mice, when compared with the effects of the solvent alone. Bodyweights were unchanged during the course of the treatment.

It is concluded that insulin receptors can be measured under conditions which maintain the stability of the complex. Also, preliminary experiments suggest that sulphonylurea derivatives may exert their antidiabetic effects, at least in part, by increasing liver insulin receptors.

Reference

CUATRECASAS, P. (1972). Isolation of the insulin receptor of liver and fat-cell membranes. *Proc. Nat. Acad. Sci. USA.*, **69**, 318–322.

Comparison of the binding characteristics of tritiated opiates and opioid peptides

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Opiate receptor binding in homogenates of guinea-pig brain minus cerebellum was studied by a modification of the method of Pert & Snyder (1973), using [^{3}H]-D-Ala^2-D-Leu^5-enkephalin (44 Ci/mmol); [^{3}H]-D-Ala^2-leucine-enkephalin amide (17.6 Ci/mmol); [^{3}H]-D-Ala^2-methionine-enkephalin amide (15 Ci/mmol); [^{3}H]-etorphine (36 Ci/mmol); [^{3}H]-dihydromorphine (81 Ci/mmol) and [^{3}H]-morphine (28 Ci/mmol) as primary ligands. The brain tissue was homogenized in Tris buffer (pH 7.4, 0°C), centrifuged at 49,000 *g* for 10 min; the pellet was resuspended in Tris buffer (pH 7.4, 37°C) and incubated at 37°C for 45 min. The homogenate was then centrifuged again and the pellet resuspended in Tris buffer (pH 7.4, 25°C) to give a final concentration of 1 g tissue/100 ml. Aliquots of the homogenate were incubated with various concentrations of the primary ligands, without addition of Na^+, for 40 min at 25°C. Specific binding was the difference in counts obtained in the presence and absence of 50 nM of the antagonist (–)-2-(3-furylmethyl)-5,9-diethyl-2′-hydroxy-6,7 - benzomorphan (MR 2266). The kinetic parameters of the binding were determined by Scatchard analysis.

There is apparently only one binding site for D-Ala^2-D-Leu^5-enkephalin in guinea-pig brain homogenate. At this site, the ligand has a K_d of 1.27 ± 0.16nM ($n = 5$) and the number of binding sites corresponds to 7.4 ± 0.31 pmol/g wet wt ($n = 5$). Under the same conditions, only one binding site was detected for D-Ala^2-leucine-enkephalin amide or D-Ala^2-

methionine-enkephalin amide. The corresponding K_D's were 2.52 ± 0.48 and 1.96 ± 0.17 nM ($n = 5$ and the numbers of binding sites were 12.4 ± 0.93 and 12.8 ± 2.22 pmol/g wet wt ($n = 5$). Etorphine was found to have a similar number of binding sites (15.4 + 2.4 pmol/g wet wt ($n = 3$)) but a higher affinity (0.37 ± 0.03 nM ($n = 3$)) than the D-Ala^2-enkephalin amide. However, with both morphine and dihydromorphine two binding sites were found; the total numbers of binding sites were 3.7 ± 0.59 and 4.3 ± 0.36 pmol/g wet wt ($n = 4$), respectively.

When the binding of D-Ala^2-D-Leu^5-enkephalin, dihydromorphine and a mixture of the two ligands in a ratio of 3:1 was compared in the same homogenate, it was found that the mixture yielded a larger number of cpm's (10232 ± 366) than the maximal number of cpm's produced by either D-Ala^2-D-Leu^5-enkephalin (7812 ± 237) or dihydromorphine (6585 ± 267) alone ($n = 3$, $P<0.025$).

The above evidence suggests that there are at least two different binding sites, one of which recognizes morphine-like ligands and one which recognizes enkephalin-like ligands. The larger number of binding sites found for the D-Ala^2-enkephalin amides and etorphine may indicate that these compounds can interact with both binding sites.

Reference

PERT, C.B. & SNYDER, S.H. (1973). Properties of opiate-receptor binding in rat brain. *Proc. Nat. Acad. Sci. U.S.A.*, **70**, 2243–2247.

The use of ADTN (2-amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene) as a ligand for brain dopamine receptors

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Dopamine (DA) receptors in human brain have been assessed *in vitro* using [^{3}H]-apomorphine (APO) as ligand (Lee, Seeman, Tourtellotte, Farley & Hornykiewicz, 1978), although the high proportion of non-specific binding encountered makes APO an unsatisfactory ligand. We have therefore, evaluated ADTN, a DA agonist, as a ligand for DA receptors, and compared [^{3}H]-ADTN binding in preparations of putamen from schizophrenics and controls.

Initial studies with calf striatal membranes (Burt, Creese & Snyder, 1976) showed that the relative potencies of DA agonists and antagonists in displacing high affinity [^{3}H]-ADTN binding (Table 1) were similar to those reported for [^{3}H]-DA and [^{3}H]-APO binding (Burt *et al.*, 1976; Seeman, Lee, Chau-Wong, Tedesco & Wong, 1976). Incubations contained membrane preparation (1mg protein), [^{3}H]-ADTN (10Ci/m mol) 7.5nM, and drugs in 50 mM tris/HCl pH 7.4 + 100 mM NaCl + 0.1% ascorbic acid. After incubation for 20 min at 37°C, bound radioactivity was separated by filtration and quantified by scintillation counting. Specific binding was defined as that displaced by DA (1μM) or (+)-butaclamol (1μM).

In calf striatal preparations specific [^{3}H]-ADTN binding (approximately 50% of total binding) was saturable and of high affinity. Scatchard analysis of saturation data yielded a K_D of 9nM and B_{MAX} of 180 fmol/mg protein.

In human putamen preparations specific binding was 25–30% of total binding. [^{3}H]-ADTN binding in preparations of putamen from controls ($n = 17$) and schizophrenics ($n = 19$) was (mean ± s.e. mean) 42 ± 6 fmol/mg and 49 ± 4 fmol/mg respectively.

Diagnostic criteria and details of storage data and neuroleptic medication have been described elsewhere (Owen, *et al.*, 1978).

Table 1 Inhibition of [^{3}H]-ADTN binding by drugs

	Drug	*Ki(nM)*	*Hill coefficient*
Agonists:	Dopamine	11	1.4
	Apomorphine	5	1.4
	ADTN	7	1.1
Antagonists:	(+)-Butaclamol	45	0.61
	(–)-Butaclamol	>10,000	0.6
	Fluphenazine	100	0.48
	Haloperidol	240	0.61
	Spiperone	360	0.65
	Chlorpromazine	530	0.48

Our results suggest that (a) ADTN, DA and APO bind to similar sites *in vitro* (b) with human brain preparations ADTN has advantages over APO as a ligand, and (c) the increased binding of DA antagonists in striata of schizophrenics (Owen, Cross, Crow, Longden, Poulter & Riley, 1978) is not associated with increased binding of agonists.

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