and analysed by SDS/polyacrylamide-gel electrophoresis followed by autoradiography. None of these treatments released the [125I] labelled membrane anchor from the bulk of the enzyme. Also m-ACE was found to contain no inositol as analysed by g.c.-m.s. Thus, we conclude that ACE is not anchored in the membrane by a glycosyl-phosphatidylinositol moiety. In order to characterize the membrane anchor, m-ACE was labelled with [125I] TID, then the anchor was cleaved from the bulk of the protein with trypsin and purified as described in Table 1. The purified anchor was subjected to amino acid and protein sequence analysis. Amino acid analysis revealed a high proportion of glycine (16.4%/mol) and leucine (10.5%/mol) and the hydrophobic acids glycine, alanine, tyrosine, valine, methionine, isoleucine, leucine and phenylalanine accounted for 52% of the composition of the anchor. To date, the anchor has proved resistant to sequencing by both solid-phase and gas-phase techniques.

<table>
<thead>
<tr>
<th>Purification stage</th>
<th>Comments</th>
</tr>
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<tbody>
<tr>
<td>[125I]-m-ACE</td>
<td>Labelled with [125I] TID</td>
</tr>
<tr>
<td>Lisop-Nov 2.8 nm-Sepharose affinity chromatography</td>
<td>Removes labelled Triton X-100 and unreacted [125I] TID</td>
</tr>
<tr>
<td>Trp-Sep</td>
<td>Cleaves anchor from bulk of enzyme</td>
</tr>
<tr>
<td>Lisop-Nov 2.8 nm-Sepharose affinity chromatography</td>
<td>Separates anchor from uncleaved enzyme and catalytic fragment</td>
</tr>
<tr>
<td>Alkyl-Superose (f.p.l.c.)</td>
<td>Further purification of anchor without the use of organic solvents</td>
</tr>
<tr>
<td>Phenyl-Superose (f.p.l.c.)</td>
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</table>

This study reveals that the small (M, approx. 5000) hydrophobic membrane anchor of ACE can be selectively labelled with [125I] TID, and that the anchor is not a glycosyl-phosphatidylinositol moiety, but rather a peptide containing at least 50% hydrophobic amino acids. Thus, ACE may resemble intestinal lactase (Mantie et al., 1988), being anchored in the plasma membrane by a C-terminal transmembrane sequence of hydrophobic amino acids.

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Glucagon desensitization in hepatocytes: a cyclic AMP-independent process linked to activation of protein kinase C which does not involve G, 

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We have observed that glucagon-stimulated adenylyl cyclase activity was transiently desensitized in membranes prepared from hepatocytes that had been challenged with glucagon (Heyworth & Houslay, 1983; Murphy et al., 1987). This is a cyclic AMP-independent process (Heyworth & Houslay, 1983; Wakelam et al., 1986) and appears to involve a selective loss of coupling between glucagon receptors and G, (Houslay et al., 1987). Desensitization of glucagon-stimulated adenylyl cyclase can also be elicited by treatment of hepatocytes with the phorbol ester, tetradecanoyl phorbol acetate (TPA) (Heyworth et al., 1985), suggesting that activation of protein kinase C may be responsible for mediating the desensitization process.

Our studies have recently provided further evidence for this contention: in particular, the observations that desensitization can also be elicited by agonists acting through the diacylglycerol/protein kinase C signalling pathway (Wakelam et al., 1986; Murphy et al., 1987).

We have also shown that treatment of hepatocytes with pertussis toxin can completely obliterate the desensitization process (Heyworth et al., 1984). One well-defined action of pertussis toxin is to cause the NAD'-dependent ADP-ribosylation and inactivation of the G, subunit of the inhibitory protein G (Gilman, 1987; Heyworth et al., 1984; Houslay et al., 1989). As activation of G, leads to inhibition of adenyl cyclase activity, then such an action of pertussis toxin might suggest that desensitization could be mediated by the constitutive activation of G, We, however, demonstrate here that this is not the case.

First, we have been able to show that the G,-mediated guanine nucleotide-dependent inhibition of adenyl cyclase is not observed using membranes from glucagon-desensitized hepatocytes. This appears to be due to the functional inactivation of G, through a process which is mediated by protein kinase C. Such an observation would be in accord with studies showing that purified G, can be phosphorylated and inactivated by purified preparations of protein kinase C (Katada et al., 1985). Secondly, glucagon is able to elicit the rapid desensitization of adenyl cyclase activity in hepatocytes from streptozocin-diabetic rats in the same manner as that seen using hepatocytes from mature animals. This is despite the absence of detectable G, activity present in hepatocytes from diabetic animals (Gawler et al., 1987).

Thirdly, normally levels and kinetics of desensitization are seen in hepatocytes from young animals which express levels of G, which are some 50% of that found in adult animals.
Mature Sz-diabetic Mature Young

Animal source

Fig. 1. Pertussis toxin blocks glucagon desensitization in hepatocytes from mature, diabetic and young rats

Young animals were 6 weeks old; mature animals were 225–250 g in size and diabetes was induced using streptozocin (Sz) (Gawler et al., 1987). Hepatocytes were prepared and desensitization monitored as before (Heyworth & Houslay, 1983; MurDRhv et al., 1987). They were me-

Such experiments provide strong indications that G\(_{\text{i}}\) is not involved in mediating desensitization caused by glucagon. How then does pertussis toxin block this process? Interestingly, pertussis toxin can still block desensitization in hepatocytes from both streptozotocin-diabetic and young rats, despite the absence of functional G\(_{\text{i}}\) in the former and reduced G\(_{\text{i}}\) expression in the latter. As pertussis toxin did not attenuate receptor-stimulated inositol phospholipid metabolism we suggest it is possible that this toxin may inhibit the functioning of (a) protein kinase C (isoenzyme).


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Fig. 1. Pertussis toxin blocks glucagon desensitization in hepatocytes from mature, diabetic and young rats

Young animals were 6 weeks old; mature animals were 225–250 g in size and diabetes was induced using streptozocin (Sz) (Gawler et al., 1987). Hepatocytes were prepared and desensitization monitored as before (Heyworth & Houslay, 1983; Murphy et al., 1987). They were pre-

Picomolar \(^{125}\text{I}-\text{W-conotoxin binding to brain and spinal N-type Ca}^{2+}\text{-channels: effects of cations and endothelin and visualization by autoradiography}

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Three types of voltage-sensitive calcium channels, namely, L-, T-, and N-type, exist on animal cells (Miller, 1988). The N-type channels are believed to be solely associated with neuronal cells and are not influenced by organic Ca\(^{2+}\)-agonists/antagonists such as BAY K-8644, verapamil and nitrendipine, which act at the voltage-sensitive dihydropyridine-binding sites (Miller, 1988).

A polypeptide toxin, W-conotoxin GVIA (CTX), isolated from fish hunting snails has been shown to bind with high affinity to chick (Abe et al., 1986) and rat brain (Cruz & Olivera, 1986) membranes and shown to block specifically the N-type Ca\(^{2+}\)-channels (Abe et al., 1986). However, an endogenous agonist for the voltage-sensitive Ca\(^{2+}\)-channels has not been found, although Yanagisawa et al. (1988) have proposed that a 21-residue-containing vasoconstrictor peptide (endothelin), derived from endothelial cells, may be one such Ca\(^{2+}\) facilitator.

In view of the relative lack of information on the characteristics and modulation of CTX-labelled N-type Ca\(^{2+}\)-channels and the recent availability of suitable radioligands for labelling these channels, we have examined the binding of \(^{125}\text{I}-\text{CTX} to homogenates of guinea-pig spinal cord, cortex and hippocampus. In addition, we have studied the effects of Ca\(^{2+}\) and La\(^{3+}\) ions and endothelin on CTX binding in order to assess their relative activity at the N-type Ca\(^{2+}\)-channels.

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