A cytoskeleton from synaptosomes which is rich in a spectrin-like protein

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There is an increasing amount of evidence for the linking of intracellular cytoskeletal structures to membrane proteins of the plasma membrane in both the lymphocyte and erythrocyte and such complexes can be separated from other membrane components by extraction with non-ionic detergents (Koch & Smith, 1978; Branton et al., 1981; Mescher et al., 1981; Geiger, 1983). The purpose of the present work is to identify and characterize cytoskeletal-membrane links in synaptosomes, pinched-off nerves-endings which can be prepared from brain by subcellular fractionation (Whittaker, 1969).

Synaptosomes were prepared from rat cerebral cortex as described previously (Hesketh et al., 1977), harvested by centrifugation and resuspended in 0.32 M sucrose. After incubation in 10 mM Tris/0.15 M NaCl/1% Nonidet P-40/1 mM MgCl₂/1 mM-EGTA/1 mM-phenylmethylsulphonyl fluoride for 30 min at 0-4°C (Hesketh et al., 1983), the samples were centrifuged at 35000 g for 30 min and the pellet of insoluble cytoskeletal proteins was either resuspended in a small volume of 10 mM-Tris buffer, pH 8.0, or fixed in 2.5% glutaraldehyde in 30 mM-Pipes (1,4-piperazinediethanesulfonic acid), pH 7.2, and processed for electron microscopy. SDS/polyacrylamide-gel electrophoresis was carried out using 7.5% acrylamide gels in a discontinuous system with Tris/glycine buffer (Laemmli, 1970). After electrophoresis gels were either stained with Coomassie Brilliant Blue to reveal proteins or the proteins were transferred to nitrocellulose paper (Towbin et al., 1979) for either detection of glycoproteins using a biotin/avidin system (Gordon-Weeks, 1983) or immunological detection of spectrin-like protein.

SDS/polyacrylamide-gel electrophoresis showed the Nonidet residue to contain seven major polypeptide bands, the approximate molecular weights of which were 225, 158, 78, 70, 56, 40 and 45 kilodaltons. Densitometry showed the 225-kilodalton component to comprise about 50% of the protein content. Transfer of the protein to nitrocellulose sheets and incubation with antibodies against spectrin showed this component to be a spectrin-like protein (Hesketh et al., 1983). This protein is presumably identical to fodrin, a spectrin-like protein recently isolated from brain (Burridge et al., 1982). PSD contain a doublet of polypeptides of molecular weight very similar to fodrin and which react with anti-fodrin antibodies (Carlin et al., 1983). However, the Nonidet synaptosome cytoskeleton appears quite distinct from such PSD; the polypeptide composition is quite different, there being much more spectrin present in the Nonidet residue and very little of the 51-kilodalton PSD-specific protein. In addition the residue contained very little PSD material as judged by electron microscopy but consisted of fine filaments and amorphous, electron-dense material.

Using the biotin/avidin blotting technique several concanavalin A-binding glycoproteins were found present in the Nonidet residue; the major binding protein was of 178 kilodaltons, with other components of approximate molecular weight 200, 157, 141, 116, 104 and 58 kilodaltons.

In conclusion Nonidet treatment of synaptosomes has allowed the preparation of a synaptosome cytoskeleton which contains primarily fodrin, a spectrin-like protein, and also actin. By comparison with the erythrocyte, it is likely that this cytoskeleton underlies the synaptosomal plasma membrane. The presence of glycoproteins associated with these cytoskeletal proteins suggests that the actin-spectrin network is linked to membrane proteins. Such links may function in the response of the nerve cell to external stimuli via membrane receptors involved in nerve growth or synaptic activity.

Abbreviations used: SDS, sodium dodecyl sulphate; PSD, post-synaptic densities.
Substrate dependency of gastric acid secretion: effect of metabolic substrates on aminopyrine accumulation by rat parietal cells

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Measurement of arteriovenous differences across the fundic mucosa of pentagastrin-injected rats also demonstrated a net uptake of glucose, D-3-hydroxybutyrate, glutamine and branched-chain amino acids (Anderson & Hanson, 1983a,b). Metabolism of these substrates could provide energy to support gastric acid secretion. The extent to which a particular substrate will be metabolized by parietal cells will depend on its availability (determined by its concentration), and on the activity of the pathway metabolizing the substrate. The ability of substrates, supplied separately at physiological concentrations, to support acid secretion has been investigated here by determining the accumulation of aminopyrine by parietal cells in response to different substrates. Aminopyrine is a weak base, which, when protonated, is trapped inside the parietal cell, and its accumulation therefore measures the sequestration of acid within the cell, and not necessarily acid secretion. Nevertheless, there is good evidence that aminopyrine accumulation is a reliable index of the response of isolated parietal cells to stimulation (Soll, 1980). Furthermore as other stomach cells do not accumulate aminopyrine (Sonnenberg et al., 1979), it is possible to perform experiments with a crude parietal cell fraction.

Parietal cells were prepared according to Trotman & Greenwell (1979), and were incubated at 37°C in a Krebs-Ringer bicarbonate medium gassed with O2/CO2 (95:5), and containing 30g of fatty acid-free bovine serum albumin/l, 0.1μCi of [14C]aminopyrine/ml, 0.4μCi of [3H]poly(ethylene glycol)/ml (M, 4000) and appropriate amino acids. Acid secretion was stimulated by the addition of carbachol (10-4M), histamine (10-5 × 5M) and 3-isobutyl-1-methylxanthine (10-4M). After 30min, when aminopyrine accumulation reached a plateau, portions of the cell suspensions were centrifuged for 30s at 10000g. The cell pellet was digested overnight in Protosol, and the amounts of 14C and 3H in the pellet and supernatant were determined by liquid scintillation counting. The amount of aminopyrine in the pellet was corrected for extracellular aminopyrine [poly(ethylene glycol) acted as marker of extracellular volume], and was divided by the intracellular water to give the intracellular aminopyrine concentration, which in turn was expressed relative to the supernatant aminopyrine concentration.

Addition of secretagogues in the absence of exogenous substrates stimulated aminopyrine accumulation, which was important to establish whether this effect of exogenous substrates was specifically linked with the provision of energy for acid secretion or was a general one, related to the maintenance of parietal cell viability. When resting or stimulated parietal cells were preincubated without added substrates for 30min, the subsequent aminopyrine response to secretagogue stimulation in the presence of exogenous substrates was not different from that of non-preincubated cells. Thus, incubation of the cells for 30min without substrates did not impair their viability as judged by the subsequent secretory response, and the effect of added substrates can be presumed to be specifically associated with the provision of energy for acid secretion.

The effect of substrates on aminopyrine accumulation was investigated in control (non-preincubated) cells, and in those at least partially depleted of endogenous substrates by preincubation for 30min without added substrates. Glucose (5mM), oleate (0.6mM), butyrate (0.25mM), lactate (0.6mM), D-3-hydroxybutyrate (0.55mM) and isoleucine (0.068mM) all stimulated aminopyrine accumulation in the presence of secretagogues in comparison with cells incubated without exogenous substrates. In substrate-depleted cells all of the substrates effective with control cells plus acetooacetate (0.35mM) and valine (0.117mM), stimulated aminopyrine accumulation when compared with cells incubated without added substrate. In neither control nor preincubated cells did acetate (0.25mM), leucine (0.09mM) or glutamine (0.5mM) stimulate aminopyrine accumulation.

Aminopyrine accumulation with glucose as substrate was greater than that found with the other substrates mentioned above in both control and preincubated cells. Data relating the concentration of isoleucine to aminopyrine accumulation by parietal cells could be fitted to an expression of the form similar to the Michaelis–Menten equation, and a K0.5 of 0.15mM was obtained. The relationship between the concentration of D-3-hydroxybutyrate and aminopyrine accumulation was approximately linear up to a peak of 2.0mM-substrate, but 5.0mM-D-3-hydroxybutyrate gave a different aminopyrine ratio below this peak level, possibly because the high concentration had perturbed cellular metabolism. The ability of glucose to support aminopyrine accumulation by parietal cells was near maximal at 5.0mM-substrate, and was unaffected by insulin (100nM) over a range of glucose concentrations (0.25–5.0mM).

In summary, a number of substrates, with the notable exception of glutamine, when presented to parietal cells at physiological concentrations appear to be able to support acid secretion. When resting physiological substrate concentrations are used glucose appears to be the best substrate, but since both isoleucine and D-3-hydroxybutyrate can produce higher aminopyrine ratios when supplied to the cells at supra-normal concentrations it is possible that the predominance of glucose at normal physiological concentrations reflects its greater relative availability.

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