However, the monoclonal antibody failed to show this reaction. No change in the pattern of immunoreactivity was found in adrenalectomized rats or adrenalectomized rats injected with dexamethasone (1.25 mg/kg, intraperitoneally) 2 h prior to sacrifice. The intermediate lobe contained fibrocyte-like cells with numerous processes which were immunoreactive with both AS842 and MAB105. In the neurohypophysis, axonal varicosities as well as swellings (Herring bodies) were clearly labelled with AS842, although less intensively than cells in the anterior lobe. Once more, this staining occurred exclusively above secretory vesicles, but was not seen with MAB105. All immunostaining with AS842 or MAB105 was blocked after overnight preabsorption with human recombinant lipocortin-1 (50 μmol/l).

The AtT20/D1 cell line comprises a heterogeneous population of cells with regard to size. The largest of the cells stained very strongly (Fig. 1c), while smaller cells stained less intensely and non-uniformly. Other strains of AtT20 cells, 16/16 and 16v also stained positively for lipocortin-1, the distribution of label was much more uniform and less intense than in D1 cells.

Finally, cells prepared by peritoneal lavage from female Wistar rats were intensely immunopositive using MAB105 (Fig. 1d). Western blots of normal rat anterior pituitary gland with AS842 showed two immunoreactive bands. One co-migrated around 35 kDa with human recombinant lipocortin whilst the other was of lower molecular mass (approx. 22 kDa). The MAB105 detected only the 35 kDa band. Western blots of AtT20/D1 cells with AS842 or MAB105 gave one immunoreactive band co-migrating with recombinant human lipocortin-1. Rat hypophyli contained only the 35 kDa material using AS842, and no changes in intensity were found upon adrenalectomy or adrenalectomy and treatment with dexamethasone.

In summary, the present data show that a 22 kDa protein confined to secretory vesicles in rat adenohypophysis cross-reacts with lipocortin-1 antiserum 842 and do not support a role for lipocortin-1 in the glucocorticoid inhibition of pituitary ACTH secretion or hypothalamic corticotrophin-releasing factor production. In addition, these data suggest that transformed pituitary corticotrophs express lipocortin-1 whilst normal corticotrophs do not.

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Characteristics of lipocortin 1 binding to the surface of human peripheral blood leucocytes

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Study of the distribution and functions of the lipocortin/calpactin family of proteins spans diverse and extensive areas of cell biology [1]. Anti-inflammatory effects of recombinant human lipocortin 1 have been reported in rat paw oedema models [2] and there is evidence for production of the molecule by human monocytes in response to corticosteroid models (21 and there is evidence for production of the molecule by human monocytes in response to corticosteroid

Abbreviations used: mAb, monoclonal antibody; FITC, fluorescein isothiocyanate.
inhibit IgG binding to the type I Fc receptor. Freshly isolated monocytes, 24 h cultured monocytes or U937 were pre-incubated for 1 h at 37°C with 15 μg/ml lipocortin 1. Washed cells were then incubated in the presence of FITC-conjugated monomeric IgG at 4°C. Control samples run concurrently contained excess non-labelled IgG. Lipocortin 1 pre-incubation resulted in a 50% decrease in IgG binding to fresh monocytes. Inactivated lipocortin 1 showed no such effect. No significant inhibition of IgG binding was observed when 24 h precultured monocytes or U937 cells were incubated with lipocortin 1. Thus the differential, saturable binding of lipocortin 1 to freshly isolated monocytes, cultured monocytes and myelomonocytic cells appears to correlate with its ability to inhibit IgG binding to Fc receptor on these cells. This differential surface reactivity of a member of the lipocortin/calpastatin family of proteins with leucocytes could indicate a specific role for this protein in the modulation of the inflammatory response.

Abbreviation used: KpNPPase, K+-stimulated p-nitrophenyl phosphatase.

Isolation of human skeletal muscle sarcolemmal vesicles for the investigation of glutamine transport

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Glutamine is the most abundant free amino acid in the human body and the largest fraction of the body glutamine pool resides in skeletal muscle [1]. Glutamine has important roles in whole-body metabolism (for reviews see [1, 2]): it is a fuel for rapidly dividing cells (e.g. intestinal mucosa, lymphocytes), an important vehicle for carbon and nitrogen transport from muscle to viscera, a potential substrate for glutamine turnover can be controlled under physiological and pathophysiological circumstances. We have previously characterized the transport of glutamine in sarcolemmal vesicles isolated from rat skeletal muscle [5]. We have now used a similar isolation procedure [6] to obtain sarcolemmal vesicles from human skeletal muscle for use in the study of amino acid transport.

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