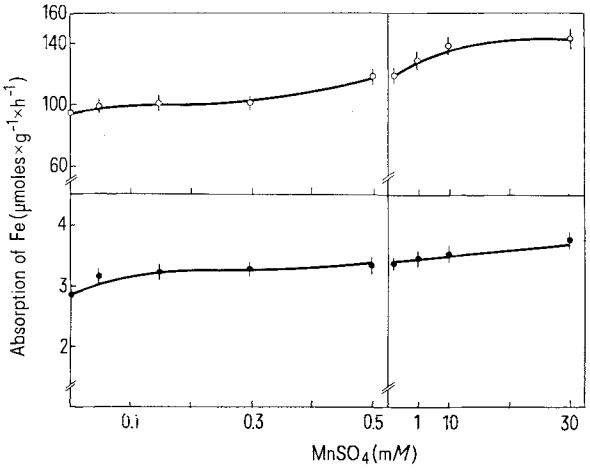
location from the root to the shoot[3]. The effects of Mn on the absorption of some monovalent cations were investigated[4], and it was found that Mn inhibited the uptake of Na from 0.1 m*M* NaCl and that of K from 5 m*M* KCl. Mn also reduced the absorption of Ca and Zn[5]. In contrast, the present study reveals that the effect of Mn on the absorption of Fe is different from those on other cations reported earlier[4,5].



Absorption of Fe from 0.1 m*M* (●—●) and 5 m*M* (○—○) $FeSO_4$ by excized rice roots, in the absence and presence of different concentrations of $MnSO_4$. The absorption medium contained 0.1 m*M* $CaSO_4$, besides others. The vertical bars are the standard errors of the mean.

Fe absorption was measured by suspending excized rice (*Oryza sativa* L. cv. I.R. 8) roots[6] in ^{59}Fe labelled $FeSO_4$ solutions (sp. act. 0.1 μCi/μmole, pH 5.5) containing various concentrations of $MnSO_4$. 5 replicate samples were used for each concentration. At the end of the experiment, the roots were desorbed in unlabelled cold (5 °C) solutions and radioassayed in a γ-ray spectrometer. The concentrations of $MnSO_4$ were similar to those of mechanisms 1 and 2 of EPSTEIN[7] and were chosen to understand the ion interactions.

The results (Figure) show that Fe absorption from 0.1 m*M* $FeSO_4$ is enhanced by the presence of $MnSO_4$ at concentrations above 0.1 m*M*. The absorption from 5 m*M* $FeSO_4$ is significantly increased by $MnSO_4$ above 0.5 m*M*. The findings suggest that rice roots, which are generally grown under submerged conditions, have a capacity to absorb Fe in a manner different from other plant roots. The exact mechanism, however, is not known. There is no evidence to suggest that Mn could bring about a change in the configuration of the ion-carrier and thus influence the uptake of other cations. However, this possibility is rather remote, because such an effect on the configuration would influence the uptake of other ions in a more or less similar manner, and our studies[4,5] reveal that this is not true. The present investigation further shows that chemically similar ions need not to be antagonistic to each other in their absorption, at least in some plant species.

[3] S. KANNAN and B. JOSEPH, Plant Physiol. *55*, 1006 (1957).
[4] S. RAMANI and S. KANNAN, Z. Pflanzenphysiol. *77*, 107 (1976).
[5] S. RAMANI and S. KANNAN, Z. Naturforsch. *31*, 12 (1976).
[6] S. KANNAN, Planta *96*, 262 (1971).
[7] E. EPSTEIN, Int. Rev. Cytol. *34*, 123 (1973).

Subcellular Distribution of Acetylcholinesterases in the Neural Lobe of the Bovine Pituitary Gland

H. VILHARDT[1] and RUTH V. BAKER

Department of Pharmacology, University of Oxford, Oxford (England), 13 March 1976.

Summary. Biochemical analysis of subcellular fractions of bovine neural lobe tissue indicated that non-specific acetylcholinesterase was principally associated with the pituicytes.

In cholinergic transmission of nerve impulses, the acetylcholine released is inactivated by degradation by acetylcholinesterase. In the central nervous system, both specific acetylcholinesterase and non-specific acetylcholinesterase activity can be demonstrated[2,3]. The specific enzyme activity seems to be found mainly in neurons[2,4–6], whereas the non-specific esterase is principally located in the glial cells[3,7,8]. This is in accordance with the early finding by BÜLBRING et al.[9] that glial cell tumors are particularly enriched in the non-specific esterase.

The principal constituents of the neural lobe of the pituitary gland are the neurosecretory nerve endings and the pituicytes. The nerve endings are the distal projections of neurons of the hypothalamo-neurohypophysial system originating in perikarya located in the supraoptic and paraventricular nuclei of the hypothalamus. The nerve endings contain the hormones vasopressin and oxytocin, which are secreted from the neural lobe.

In the neural lobe, acetylcholinesterase activity has been demonstrated both biochemically[10,11] and at the ultrastructural level[12]. The concentration of non-specific acetylcholinesterase in neural lobes from rats and rabbits was generally found to be lower than that of the specific enzyme[10,11]. The histochemical study by BRIDGES et al.[12] indicated that in the rabbit neural lobe pituicytes mainly exhibited non-specific acetylcholinesterase activity, whereas nerve fibres identified as neurosecretory terminals occasionally displayed specific acetylcholinesterase activity.

In the present study, homogenates of bovine neural lobes were subjected to subcellular fractionation by ultracentrifugation. The distribution of acetylcholinesterases among the fractions was examined and compared with the distribution of other marker substances.

Materials and methods. Freshly collected bovine neural lobes were homogenized in ice-cold 0.3 *M* sucrose and subjected to differential centrifugation: 800 g_{av} for 15 min (I), 3000 g_{av} for 15 min (II), 5000 g_{av} for 15 min (III), 26,000 g_{av} for 15 min (IV), and 100,000 g_{av} for 60 min (V) leaving the final particle-free supernatant (VI). All operations took place at 4 °C.

The fractions were resuspended in 5 m*M* *Tris*-HCl (pH 7.5) and assayed for protein[13], vasopressin by the method of DEKANSKI[14] as described by VILHARDT and HOPE[15], succinate dehydrogenase[16] as modified by PORTEOUS and CLARK[17], lactate dehydrogenase[18] as modified by PICKUP and HOPE[19], $Mg^{2+}+Na^{+}+K^{+}$-AT-

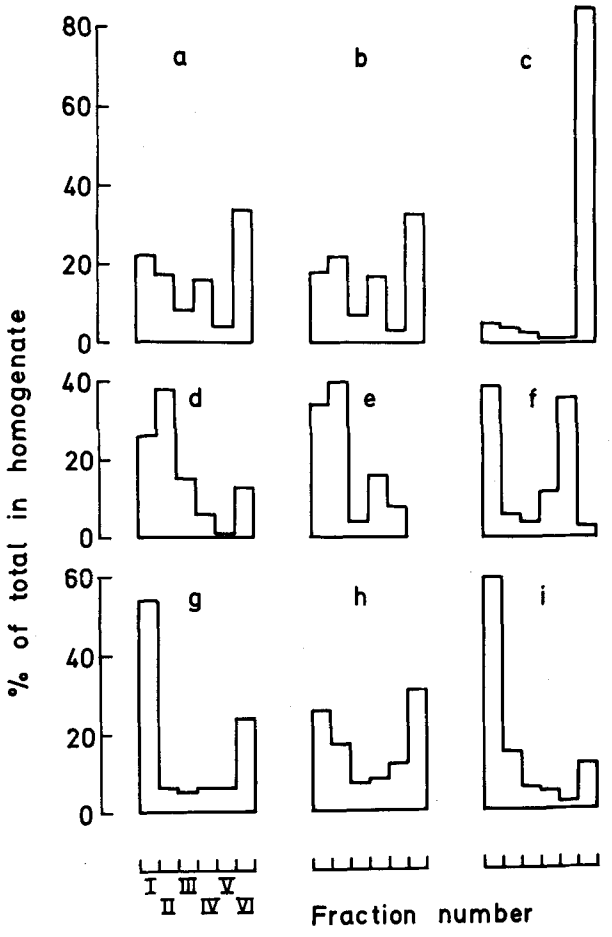


Fig. 1. Differential centrifugation of neural lobe homogenate. The fractions were assayed for a) protein, b) vasopressin, c) lactate dehydrogenase, d) succinate dehydrogenase, e) $Mg^{2+} + Na^{+} + K^{+}$-ATPase, f) 5′-nucleotidase, g) DNA, h) specific acetylcholinesterase, and i) non-specific acetylcholinesterase. Recoveries ranged between 80%–118%.

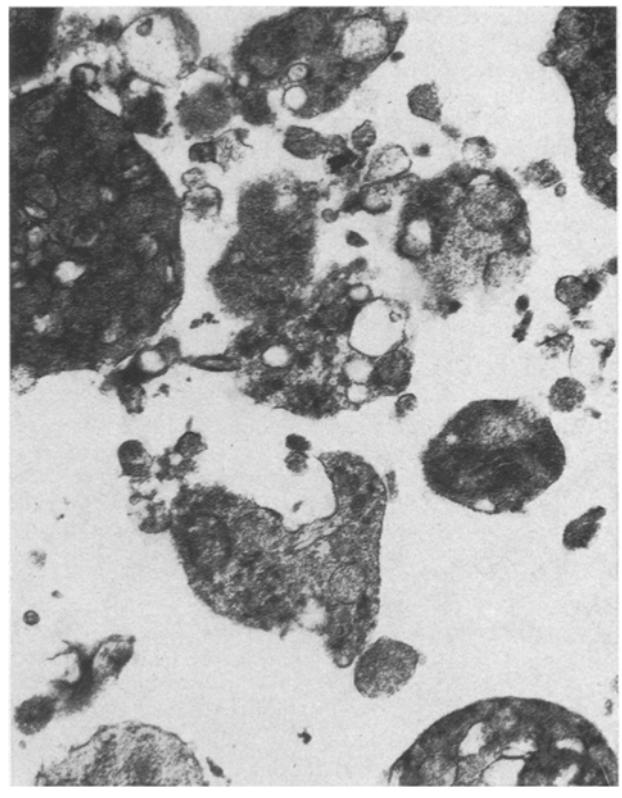


Fig. 2. Electron microscopic appearance of fraction II. After isolation the resuspended fraction was centrifuged at 100,000 g_{av} for 60 min before fixation. The fraction contains large numbers of broken off nerve endings characterized by their contents of neurosecretory vesicles. ×40,000.

Pase[15], DNA[20] and 5′-nucleotidase[21]. Acetylcholinesterase (acetylcholine acyl-hydrolase, EC 3.1.1.8) activity was determined by the method of Ellman et al.[22] with acetyl-thiocholine as a substrate. BW 284c51 (Burroughs Wellcome, final concentration 1.5×10^{-6} *M*) was used as an inhibitor of specific acetylcholinesterase activity and eserine sulphate (Sigma Chemical Co., final concentration 10^{-5} *M*) as general inhibitor of cholinesterase activity.

Results and discussion. Both specific and non-specific acetylcholinesterase activity could be demonstrated in homogenates of bovine neural lobes. The non-specific activity constituted 71.7 ± 13.6% of the specific acetylcholinesterase activity (6 experiments).

The distribution among subcellular fractions of enzymes and other substances is shown in Figure 1. The soluble cytoplasmic enzyme lactate dehydrogenase is mainly located in fraction VI, indicating that the homogenization procedure has led to a thorough disruption of the tissue. Part of the enzyme found in fraction VI may, however, have leaked out of the cells during the preparation[23].

Fraction I contains high amounts of the nuclear marker DNA. Since the neuronal elements of the neural lobe do not contain nuclei, DNA may be used as a marker for the pituicytes. The concomittant occurrence in fraction I of the plasma membrane marker 5′-nucleotidase indicates

[1] Present address: Institute of Medical Physiology C, 71 Raadmandsgade, DK-2200 Copenhagen N, Denmark.

[2] J. D. Utley, Biochem. Pharmac. *15*, 1 (1966).
[3] D. B. Tower and O. M. Young, J. Neurochem. *20*, 269 (1973).
[4] C. C. D. Shute and P. R. Lewis, Z. Zellforsch. mikrosk. Anat. *69*, 334 (1966).
[5] A. Kokko, H. G. Mantner and R. J. Barrnett, J. Histochem. Cytochem. *17*, 625 (1969).
[6] L. Rechardt, in *Progress in Brain Research* (Ed. O. Eränkö; Elsevier Publ. Comp., Amsterdam 1971), vol. *34*, p. 423.
[7] E. Giacobini, Acta physiol. scand. *45* suppl., 156 (1959).
[8] R. M. Torack and R. J. Barrnett, Expl Neurol. *6*, 224 (1962).
[9] E. Bülbring, F. J. Philpot and F. D. Bosanquet, Lancet *2*, 865 (1953).
[10] F. S. LaBella and S. Shin, J. Neurochem. *15*, 335 (1968).
[11] K. Lederis and A. Livingston, J. Physiol., Lond. *201*, 695 (1969).
[12] T. E. Bridges, A. W. Fisher, J. L. Gosbee, K. Lederis and R. C. Santolaya, Z. Zellforsch. mikrostk. Anat. *136*, 1 (1973).
[13] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1961).
[14] J. Dekanski, Br. J. Pharmac. Chemother. *7*, 567 (1952).
[15] H. Vilhardt and D. B. Hope, Biochem. J. *143*, 181 (1974).
[16] B. T. Pennington, Biochem. J. *80*, 649 (1961).
[17] J. W. Porteous and B. Clark, Biochem. J. *96*, 159 (1965).
[18] F. Wróblewski and J. S. LaDue, Proc. Soc. exp. Biol. Med. *90*, 210 (1955).
[19] J. C. Pickup and D. B. Hope, Biochem. J. *123*, 153 (1971).
[20] J. M. Kissane and E. Robins, J. biol. Chem. *223*, 184 (1958).
[21] P. L. Ipata, Analyt. Biochem. *20*, 30 (1967).
[22] G. L. Ellman, K. D. Courtney, V. Andres jr. and R. M. Featherstone, Biochem. Pharmac. *7*, 88 (1961).
[23] R. V. Baker, H. Vilhardt and D. B. Hope, J. Neurochem. *24*, 1091 (1975).

the presence in this fraction of a substantial amount of pituicytes. Nearly 60% of the total non-specific acetylcholinesterase activity is also sedimented in fraction I, strongly suggesting an association between this enzyme and the pituicytes. However, since the neural lobe also contains endothelium and blood cells, it cannot be excluded that part of the enzyme activities demonstrated in fraction I originates in these structures.

Vasopressin and $Mg^{2+}+Na^{+}+K^{+}$-ATPase may be used as markers for the nerve endings of the neural lobe[24]. The distribution by differential centrifugation of these substances indicates that the neurosecretory nerve terminals mainly accumulate in fraction II, but also to some extent in fraction I. Electron microscopic examination of fraction II demonstrates the presence in this fraction of large quantities of broken off nerve endings identified by their contents of neurosecretory granules (Figure 2). The concentration in fraction II of non-specific acetylcholinesterase, however, is much lower than that of fraction I and it is reasonable to conclude that the nerve endings only contain small amounts of that enzyme if any at all. The non-specific esterase activity observed in fraction II could be due to the presence in that fraction of a small number of pituicytes.

The specific acetylcholinesterase is found in relatively high concentrations in both fraction I and II and cannot be specifically associated with either the pituicytes or the nerve endings, but it may be a constituent of both particles. A much larger part of this enzyme was found in a soluble form (fraction VI) than was the case for the non-specific acetylcholinesterase.

In conclusion, this investigation indicates that the non-specific acetylcholinesterase may be primarily located in the pituicytes of the neural lobe. In this respect the pituicytes resemble the glial cells of brain tissue. The nerve endings display specific acetylcholinesterase activity and very little non-specific activity.

[24] H. VILHARDT, R. V. BAKER and D. B. HOPE, Biochem. J. *148*, 57 (1975).

Factors Affecting High-Frequency Fungal Protoplast Fusion

L. FERENCZY, F. KEVEI, M. SZEGEDI, A. FRANKÓ and I. ROJIK

Department of Microbiology, and Laboratory of Electron Microscopy, Attila József University, P. O. Box 428, H-6701 Szeged (Hungary), 19 March 1976.

Summary. Factors influencing the fusion frequency of protoplasts have been examined with auxotrophic mutants of *Aspergillus nidulans*. The optimum conditions were a total of 5 to 15 million protoplasts per ml, and 25% polyethylene glycol (PEG) 4000 or 6000 as fusogenic agent in 10 to 100 m*M* $CaCl_2$ solution.

Formerly we had developed methods for fusion of fungal protoplasts[1–3], including a high-frequency procedure carried out with polyethylene glycol (PEG) and Ca ions[4]. Details of PEG-induced protoplast fusion were given elsewhere[5], and independently by ANNÉ and PEBERDY[6–8].

Here we present results concerning 1. the concentration of the protoplasts involved in the aggregation and fusion by PEG, 2. the stabilizing, aggregating and fusogenic capacities of PEG preparations of different molecular weights at different concentrations, and 3. different inorganic materials as osmotic stabilizers and promoters or inhibitors of protoplast fusion. Appropriate control of these factors renders the fusion method more reliable for 'genetic transfusion', i.e. for transfer of genetic material via protoplast fusion.

Materials and methods. Aspergillus nidulans[9] mutants requiring lysine (SzMC 0443) and methionine (SzMC 0442) were used. The methods of protoplast formation, fusion, nutritional complementation and heterokaryon formation were similar to those described previously[2,4], except that no buffer was included in the stabilizing 0.6 *M* KCl solution; prior to PEG treatment the protoplasts were suspended in 0.05 ml osmotic stabilizer and then PEG solution was added to give a total of 1 ml; after PEG treatment for 15 min the samples were diluted with 0.6 *M* KCl and plated. The fusion frequency is expressed as the number of colonies developing on minimal medium as a percentage of those growing on nutritionally-sufficient medium, in both cases after PEG treatment.

PEG preparations (Fluka and Hoechst) with molecular weights of 400, 1540, 4000, 6000 and 20,000 were used for comparison at different concentrations (%, w/v).

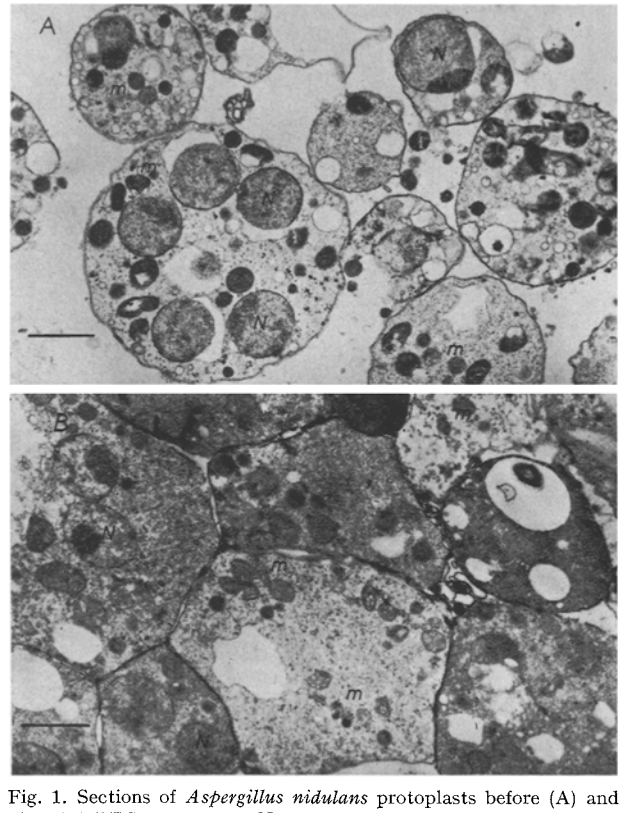


Fig. 1. Sections of *Aspergillus nidulans* protoplasts before (A) and after (B) PEG treatment. N, nucleus; m, mitochondria. Markers represent 2 μm.