

The tissues, from Wistar rats, were homogenized in 0.25 M sucrose containing 0.1 M 2 mercaptoethanol at pH 7.3 (0.01 M phosphate); cytosol prepared by centrifugation (110,000 g for 1 h) was stored at -15°C . Binding of [^3H]-oestradiol to cytosol components after incubation at 30°C was measured by the method of Mester, Robertson, Feherty & Kellie (1970), with minor modifications.

Binding of oestradiol was detected in cytosol from all tissues, equilibrium being reached in 3 min with hypothalamus and after about 8 min with uterus. Dissociation of the complexes was followed by continued incubation for various times after addition of adsorbent; two exponential components were seen in the dissociation curves with uterine and female hypothalamic cytosols, while in cytosols of other tissues (including male hypothalamus) only one component of low affinity was detected. Dissociation half-times for high affinity sites were 100 min for hypothalamus and 70 min for uterus. The amount of high affinity binding at equilibrium was obtained by extrapolation and the values obtained (oestradiol concentration, $0.5 \times 10^{-8}\text{M}$ – $0.25 \times 10^{-9}\text{M}$) were plotted by Scatchard's 1949 method; the rectilinear plots indicated homogeneity of the sites. The dissociation constants of both the uterine and female hypothalamic sites were of the order of $3 \times 10^{-10}\text{M}$ (agreeing with values reported by Feherty, Robertson, Waynforth & Kellie, 1970, and did not vary with the sexual cycle. The number of available binding sites was of the order of 1.5×10^{10} per hypothalamus in oestrous and dioestrous rats and in prepubertal females. Preliminary results indicate that the number of available high affinity sites in the hypothalamus is reduced in pro-oestrous.

The work was supported by an M.R.C. grant. N.J.M. receives a grant from Pfizers Limited.

REFERENCES

- EISENFELD, A. J. (1970). ^3H -estradiol: *in vitro* binding to macromolecules from the rat hypothalamus, anterior pituitary and uterus. *Endocrinology*, **86**, 1313–1318.
- FEHERTY, P., ROBERTSON, D. M., WAYNFORTH, H. B. & KELLIE, A. E. (1970). Changes in the concentration of high affinity oestradiol receptors in rat uterine supernatant preparations during the oestrus cycle, pseudopregnancy, pregnancy, maturation and after ovariectomy. *Biochem. J.*, **120**, 837–844.
- MESTER, J., ROBERTSON, D. M., FEHERTY, P. & KELLIE, A. E. (1970). Determination of high affinity oestrogen receptor sites in uterine supernatant preparations. *Biochem. J.*, **120**, 831–836.
- SCATCHARD, G. (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

Accumulation of neurophysin in the median eminence and the cerebellum of sheep with natural scrapie

B. G. LIVETT and H. B. PARRY* (introduced by D. B. HOPE)

Department of Pharmacology and Nuffield Institute for Medical Research, University of Oxford

A feature of neuronal degeneration in the central nervous system of aged and scrapie sheep is the accumulation of neurosecretion-like material (NSLM) and aggregations of electron dense bodies, 200–500 nm in diameter, within axons and presynaptic terminals in the cerebellum and hypothalamo-neurohypophyseal system (NHS) (Bignami, Beck & Parry, 1970). The immunofluorescence technique for demonstrating neurophysin (Livett, Uttenthal & Hope, 1971) made possible a study of the nature of this NSLM.

Pure porcine neurophysin-II (Uttenthal & Hope, 1970) was injected into rabbits at intervals of approximately 2 months over a year to produce an antiserum which

reacted with neurophysin in cryostat sections of sheep posterior pituitary. The use of ethanol-fixed tissues embedded in paraffin (Holborow & Johnson, 1967) enabled immunofluorescence staining to be followed, after postfixation in Bouin's solution, by conventional histochemical reactions for NSM.

In normal sheep, fluorescence was confined to the supraoptic and paraventricular nuclei, and to the neural portion of the HNS. In the median eminence and lower infundibular stem, the fluorescence appeared as fine fibres. In the pars infundibularis, the proximal portion showed intense fluorescence of globular appearance; in the central portion the fluorescence was more diffuse and its intensity low, but the intensity was high in the most distal portion.

In sheep with natural scrapie there was a reduction in specific fluorescence in the hypothalamic nuclei, but at the level of the median eminence the fluorescence was dramatically increased. In the distal tract there was a considerable reduction, particularly in the central portion of the neural lobe. This pattern is similar to that found for NSM by Beck, Daniel & Parry (1964).

Intense foci, 0.5–5 μ m in diameter, of neurophysin-specific immunofluorescence were observed outside the HNS in the granular layer of the cerebellum and in the region of the III cranial nerve. Subsequent staining of the same sections by the Gomori CAH method revealed NSLM in precisely the same sites. Similar NSLM was found by Bignami *et al.*, in degenerating cerebellar bouton terminaux, which showed at the ultrastructural level electron dense bodies within Herring body-like structures.

These observations suggest that degenerating bouton terminaux in the cerebellum contain neurophysin or a protein sufficiently similar to cross-react with this cross-species reactive neurophysin antiserum.

We thank Mr. L. O. Uttenthal for the purified porcine neurophysin-II and for assistance in the preparation of the antiserum. This work was supported in part by a Research Grant from the Medical Research Council to Dr. D. B. Hope. B.G.L. is a Nuffield Dominions Demonstrator (Australia).

REFERENCES

- BECK, E., DANIEL, P. D. & PARRY, H. B. (1964). Degeneration of the cerebellar and hypothalamo-neurohypophyseal systems in sheep with scrapie; and its relationship to human system degenerations. *Brain*, **87**, 153–176.
- BIGNAMI, A., BECK, E. & PARRY, H. B. (1970). Neurosecretion-like material in the hindbrain of ageing sheep and sheep affected with scrapie. *Nature, Lond.*, **225**, 194–196.
- HOLBOROW, E. J. & JOHNSON, G. D. (1967). Immunofluorescence. In: *Handbook of Experimental Immunology*, ed. WEIR, D. M., pp. 571–596. Oxford: Blackwell.
- LIVETT, B. G., UTTENTHAL, L. O. & HOPE, D. B. (1971). Localization of neurophysin-II in the hypothalamo-neurohypophyseal system of the pig by immunofluorescence histochemistry. *Phil. Trans. Roy. Soc. B.*, **261**, 371–378.
- UTTENTHAL, L. O. & HOPE, D. B. (1970). The isolation of three neurophysins from porcine posterior pituitary lobes. *Biochem. J.*, **116**, 899–909.

Definition of the histaminic component of the bronchoconstrictor and cardiovascular effects of anaphylatoxin in the guinea-pig

A. C. SACKEYFIO (introduced by R. HICKS)

Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, Yorkshire

Anaphylatoxin (AT) is a potent histamine liberator in the guinea-pig. Heparin, histaminase and catecholamines are also involved in AT-induced effects *in vivo*. How-