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Lysosomal Fragility in Hypothalamic Neurosecretory Neurones

by

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Although histochemical studies may indicate the degree of enzymatic activity, and hence of metabolic activity of cells, the quantitative histochemical methods in common use have several disadvantages. The assessment of the degree of enzymatic activity by observing the intensity of colouration obtained after the corresponding histochemical test depends, amongst other factors, on the thickness of the section. It is known that microtome sections show considerable variation from the intended thickness. Although biochemical estimation of enzyme content in weighed pieces of tissue is more accurate, admixture with cells other than those under study inevitably leads to errors. The most accurate method available is cytochemical analysis of individual cells, but this is a tedious process for the study of a large population of cells.

The secretory cells of the magnocellular neurosecretory nuclei of the mammalian hypothalamus (i.e. the supraoptic and paraventricular nuclei) are known to be rich in acid phosphatase (Eranko, 1951 ; Sloper, 1955 ; Rinne & Kivalo, 1958). Electron microscopic studies of these cells have shown the presence of large granules (4,000 Å to 1 μm in diameter) which are thought to be lysosomal in nature (Fig. 1). In the cytoplasm most of the acid phosphatase together with several other acid hydrolases is believed to exist within lysosomes (de Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955).

A useful and accurate technique for comparison of activity of lysosomal enzymes between the neurones of the supraoptic nucleus and those of the paraventricular nucleus, under different physiological and experimental conditions, is described in this report.

MATERIALS AND METHODS

Twelve female rats from 3 sister litters were divided into 3 equal groups. In each group 1 rat served as a normal control, 1 was dehydrated by the substitution of 2.5% sodium chloride for drinking water for 20 days, 1 was a lactating rat which had suckled 10 pups for a period of 24 days, and 1 was water loaded by the administration of 10 ml distilled water by stomach tube 5 times daily for 7 days. These procedures were so carried out that, in each group, all the animals could be killed simultaneously by rapid decapitation without anaesthesia. Immediately after decapitation the brain was removed and a thick slice containing the hypothalamus frozen with dry ice. Cryostat sections were cut at 15 μm thickness

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in the coronal plane from that region of the hypothalamic block containing both supraoptic and paraventricular nuclei, these areas having been first identified by rapid haematoxylin staining. The Gomori lead nitrate method for acid phosphatase (Pearse, 1961) was carried out simultaneously on representative sections obtained from all 4 animals in each group in the following manner. Eight sections from the series mounted from each brain were introduced into the incubation medium at 37°C staggered at 15 min intervals. This enabled the sections to be incubated for periods of time varying from 15 min to 120 min, and yet permitted simultaneous withdrawal of all the slides from the incubation medium for subsequent treatment. It was thus possible to determine the shortest period of incubation which resulted in a clear positive reaction for acid phosphatase within the neurosecretory cells of each nucleus in each brain.

RESULTS

In accordance with previous observations (Eranko, 1951 ; Sloper, 1955 ; Rinne & Kivalo, 1958) the strongly positive reaction for acid phosphatase displayed by hypothalamic neurosecretory cells was found to impart a prominence to the supraoptic and paraventricular nuclei in sections treated by this method.

TABLE 1.

Gomori lead nitrate reaction for acid phosphatase in rat hypothalamic neurosecretory neurones. (SON = supraoptic nucleus ; PVN = paraventricular nucleus ; — = no reaction ; + = granular reaction ; D = diffused reaction. In each cage, the results from groups 1, 2 & 3 are indicated in that order).

Animal reference	Nucleus	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min
Normal control	SON	— — —	— — +	— + +	+ + D	DDD	DDD	DDD	DDD
	PVN	— — —	— — +	— + +	+ + D	DDD	DDD	DDD	DDD
Lactating	SON	— — —	— — +	— + +	+ + D	DDD	DDD	DDD	DDD
	PVN	— + +	+ + +	+ + D	DDD	DDD	DDD	DDD	DDD
Dehydrated	SON	+ + +	+ + +	+ + D	DDD	DDD	DDD	DDD	DDD
	PVN	— + +	+ + +	+ + D	DDD	DDD	DDD	DDD	DDD
Water loaded	SON	— — —	— — +	— — +	+ + +	+ + D	DDD	DDD	DDD
	PVN	— — —	— — +	— — +	+ + +	+ + D	DDD	DDD	DDD

Two stages could be observed in the cytoplasmic reaction (Figs. 2-4). At first dark discrete granules appeared, whereas with longer periods of incubation the reaction became diffuse throughout the cytoplasm. When compared with the normal controls, both phases of the reaction appeared in the neurosecretory cells of the dehydrated and lactating rats with shorter periods of incubation, and in those of the water loaded rats with longer periods of incubation (Table 1). No clear cut differences could be observed in the time of onset of either the granular or the diffused reaction between the cells of the supraoptic nucleus

and those of the paraventricular nucleus in all the rats except in the lactating animals, in whom both reactions appeared earlier in the paraventricular nucleus. The optimum period of incubation, under the conditions used, was found to be 30 to 45 min.

DISCUSSION

The amount of a particular enzyme present in a tissue or cell need not be indicative of the state of physiological activity of the metabolic pathway concerned, since many enzymes may be present in excess of physiological requirements (Adams, 1965). A change in the enzyme content induced experimentally or pathologically, however, can reasonably be regarded as indicative of a real alteration in the metabolism of the tissue. In an analysis of all the criteria which determine the quantitative nature of a histochemical assay, Glenner (1965) concludes that " the time required to obtain a visible colouration under identical incubating conditions, at a particular substrate concentration, is perhaps the most objective index of activity available".

Bitensky (1963) has devised a ' fragility test ' which determines for each cell type, the maximum time of exposure lysosomal membranes can withstand before they are rendered permeable to the glycerophosphate substrate. This time would depend, for a given set of conditions, on two factors : (1) the conditions that existed in the cell before death ; (2) the mode of death. Rapid decapitation was found by this same worker toresult in remarkably little variation in the stability of liver lysosomes. Both physiological and pathological variations in life were shown to affect their stability. Cohen, Bitensky, Chayen and Russell (1964) observed that fragility became more marked in the lysosomes of the glandular epithelium of the human endometrium during its secretory phase, when secretory activity was very high in these cells. This test can be usefully applied for comparing acid phosphatase activity in similar lysosome containing cells under different conditions. Bitensky (1963) has shown, however, that fragility of lysosomes varies in different types of cells under the same conditions, and even in the different types of cells found in the same organ. Hence this method is not applicable to comparing such activity in different types of cells.

Chronic dehydration increases the level of synthetic activity in hypothalamic neurosecretory cells, the site of formation of the neurohypophysial hormones. The reverse process of water loading would have the opposite effect. The hormone mainly affected in these states is antidiuretic hormone (vasopressin). In the lactating rat the stimulus of suckling results in a predominant secretion of oxytocin from the neurohypophysis with increased synthetic activity in the hypothalamic neurosecretory neurones. This study has indicated that in dehydration, when the secretory activity in hypothalamic neurosecretory cells was very high, both granular and diffused reactions occurred sooner than in the normal controls. In lactation both reactions appeared sooner in the paraventricular nucleus than in the supraoptic nucleus. In the water loaded animals both reactions were delayed compared to the normal controls. These results add to the already existing evidence that the paraventricular nucleus is predominantly concerned with the synthesis of oxytocin (Olivecrona, 1957 ; Nibbelink, 1961 ; Brooks, Ishikawa, Koizumi & Lu, 1966 ; Heller, 1966).

SUMMARY

The time of onset of the histochemical reaction for acid phosphatase was used as an index of synthetic activity in the hypothalamic neurosecretory neurones of normal, dehydrated, lactating and water loaded rats. The reaction, confined to cytoplasmic granules initially, was later diffused due to fragility of lysosomal membranes. When compared with normal controls, both phases occurred faster in dehydrated and lactating rats, and more slowly in water loaded rats. In lactating rats alone was a time difference observed between supraoptic and paraventricular neurones, the latter reacting faster. This was construed as further evidence that the paraventricular nucleus is more concerned with oxytocin synthesis.

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REFERENCES

ADAMS, C. W. M. (1965). In *Neurohistochemistry*, Ed. Adams, C. W. M. p. 253, Amsterdam: Elsevier.

BITENSKY, L. (1963). The reversible activation of lysosomes in normal cells and the effects of pathological conditions, in *Lysosomes*, Eds. de Reuck, A. V. S. & Cameron, M. P. pp. 362-383, London : Churchill.

BROOKS, C. M., ISHIKAWA, T., KOIZUMI, K. & Lu, H. H. (1966). Activity of neurones in the paraventricular nucleus of the hypothalamus and its control. *J. Physiol., (London)*, **182**, 217-231.

COHEN, S., BITENSKY, L., CHAYEN, J. & RUSSELL, J. K. (1964). Histochemical studies on the human endometrium. *Lancet*, **2**, 56-58.

DE DUVE, C., PRESSMAN, B. C., GIANETTO, R., WATTIAUX, R. & APPELMANS, F. (1955). Tissue fractionation studies : 6. Intracellular distribution patterns of enzymes in rat-liver tissue. *Biochem. J.*, **60**, 604-617.

ERANKO, O. (1951). Histochemical evidence of intense phosphatase activity in the hypothalamic magnocellular nuclei of the rat. *Acta Physiol. Scand.*, **24**, 1-6.

GLENNER, G. G. (1965). Enzyme histochemistry, in *Neurohistochemistry*, Ed. Adams, C. W. M. pp. 109-160, Amsterdam : Elsevier.

HELLER, H. (1966). The hormone content of the vertebrate hypothalamo-neurohypophysial system. *Br. med. Bull.*, **22**, 227-231.

NIBBELINK, D. W. (1961). Paraventricular nucleus, neurohypophysis and parturition. *Am. J. Physiol.*, **200**, 1229-1232.

OLIVECRONA, H. (1957). Paraventricular nucleus and pituitary gland. *Acta physiol. scand.*, **40**, Suppl. 136.

PEARSE, A. G. E. (1961). In *Histochemistry : Theoretical and Applied*, 2nd ed. p. 881, London : Churchill.

RINNE, U. K. & KIVALO, E. (1958). Effect of dehydration and rehydration on the acid phosphatase activity of the hypothalamic magnocellular nuclei. *Annls. Med. exp. Biol, Fenn.*, **36**, 350-355.

SLOPER, J. C. (1955). Hypothalamic neurosecretion in the dog and cat, with particular reference to the identification of neurosecretory material with posterior lobe hormone. *J. Anat.*, **89**, 301-316.

EXPLANATION OF PLATE

Fig. 1. Electron micrograph of a part of a neurosecretory cell from the supraoptic nucleus of a normal rat, showing large dense granules (L) which are believed to be lysosomal in nature. Osmium tetroxide, followed by uranyl acetate and lead citrate. (X 6,800.)

Fig. 2. Granular reaction for acid phosphatase in rat hypothalamic neurosecretory neurones after short periods of incubation. Gomori lead nitrate method. (X 1,000.)

Fig. 3. Onset of the diffused reaction for acid phosphatase in rat hypothalamic neurosecretory neurones after longer periods of incubation.
Gomori lead nitrate method. (X 1,000.)

Fig. 4. Markedly diffused reaction for acid phosphatase in rat hypothalamic neurosecretory neurones, after very long periods of incubation.
Gomori lead nitrate method. (X 1,000.)

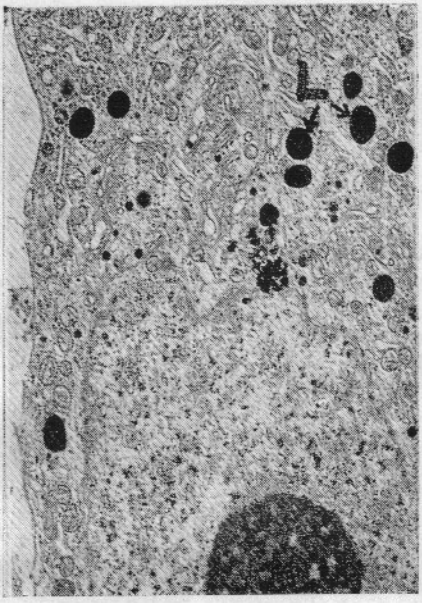

Fig. 1

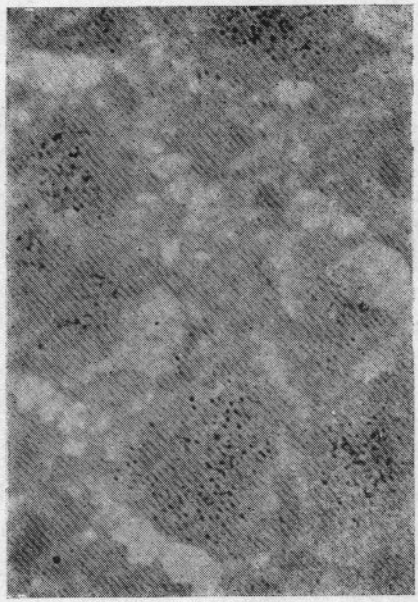

Fig. 2

Fig. 3

Fig. 4