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STEREOLOGICAL ANALYSIS OF THE RAT NEUROHYPOPHYSIS

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ABSTRACT

Variations in the volume density (V_v) of neurosecretory granules (NSG), microvesicles (MV) and smooth endoplasmic reticulum (SER) were analysed and correlated with variations in vasopressin content (VP) in the neurohypophysis during dehydration and subsequent rehydration. Neither changes in V_v (NSG) nor in V_v (SER) could be correlated with available data concerning the vasopressin content of the neurohypophysis. Variations in hormone concentration within NSG could explain discrepancies between V_v (NSG) and hormone assay data. SER seemed to be more likely involved in membrane recycling than in hormone transport. Some of the MV probably originated from the SER.

INTRODUCTION

Although it has been studied for a long time, the intracellular localization of neurosecretory products in nerve-endings of the neurohypophysis is still uncertain. It is firmly established (Weinstein et al. 1961) that most neuropeptides are located in neurosecretory granules (NSG) but the existence and location of another extragranular pool of neurohormones (Daniel and Lederis 1966, Ginsburg and Ireland 1966) remains open. Immunocytochemical methods have been used to localize oxytocin, vasopressin (VP) and neurophysin (Silverman 1976). However they can hardly provide

convincing images of localization in minute structures like smooth endoplasmic reticulum (SER) and microvesicles (MV). In the present study, volume density of NSG, MV and SER were correlated with the VP content of the neurohypophysis at successive steps of dehydration and rehydration.

MATERIAL AND METHODS

Groups of 3 rats each were sacrificed at : 2 and 4 days of dehydration and subsequently at 15, 30 minutes, 1, 3, 6, 12 h, 1, 2,5 days of rehydration. The neurohypophysis was dissected out and submitted to standard processing for electron microscopy. A two stage stereological analysis was performed by point counting (Weibel 1979). In the first stage, V_v of the neurohypophysis compartments was evaluated on 12 micrographs per animal (final magnification : 10 000 ; 600 point grid). In the second stage, V_v of the subcellular compartments of nerve endings were measured in two ways : for NSG at a final magnification of 20 000 using a 320 point grid (36 micrographs per animal) and for MV and SER at 50 000 magnification using a 720 point grid (72 micrographs per animal). Data from the 3 animals in each of the same experimental conditions were pooled after comparison of data variances.

Radioimmunoassays were performed on rats of the same strain and same weight using the method based on equilibrium dialysis (Cupo and Rougon-Rapuzzi 1981).

RESULTS

Vasopressin concentration in neurohypophysis :

Four days of dehydration reduced the VP concentration, to 2.5 % of the control. Between the fourth and sixth hour of rehydration VP content increased to 11 % of the control (Fig. 2).

Stereological data

No significant changes of axonal volume density were observed. Axons constituted about half of the gland volume. NSG constituted about 20 % of axonal volume. Dehydration induced a dramatic decrease in V_v (NSG) to 2 % of the control value after 4 days. During rehydration V_v (NSG) increased slowly and quite regularly and after 5 days of

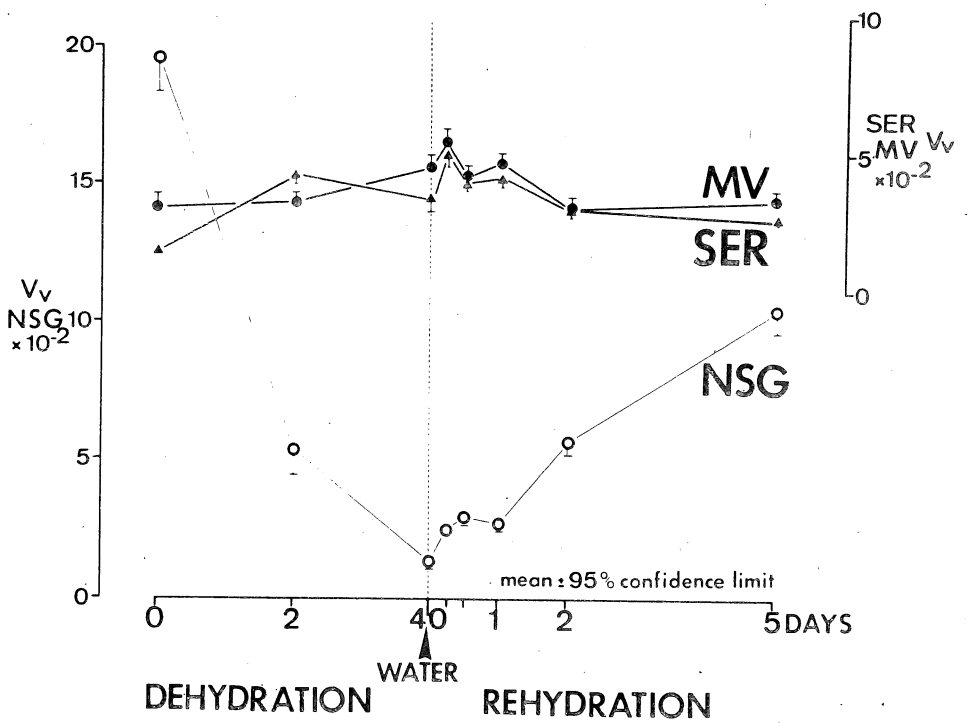

Fig. 1. Time-related variations in volume density of NSG, MV and SER throughout the dehydration and rehydration.

rehydration reached only about half of the control value (Fig. 1). However both V_v (MV) and (SER) increased during dehydration (Fig. 1) and showed rapid and marked variations during the initial stage of rehydration (Fig. 2). After 2 days, V_v values for these compartments reached control values.

DISCUSSION

Changes in V_v of the subcellular compartments could not be correlated to the vasopressin content of the neurohypophysis.

There was no variation in VP concentration in the neurohypophysis that paralleled the variations of (MV) and (SER) during the beginning of rehydration. Thus, SER and MV seemed to be more likely involved in membrane supply and

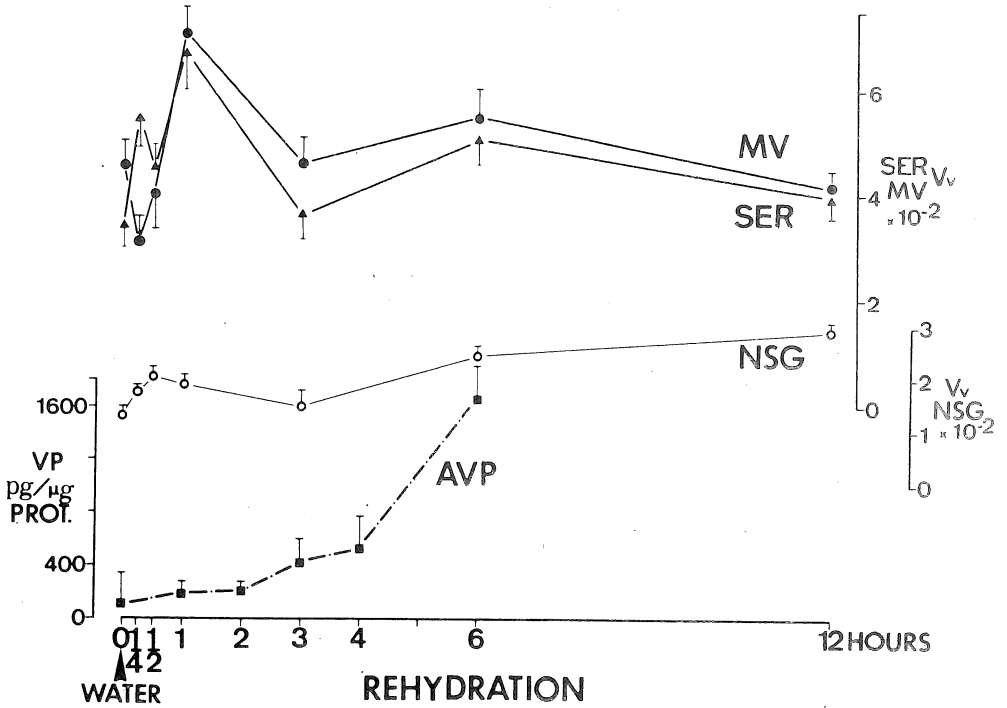

Fig. 2 . Time-related variations in volume density of NSG, MV and SER during the first 12 hours of rehydration (mean ∓ 95% confidence limit). The variations in VP content were reported for comparison sake (mean ∓ SD). Control value of AVP concentration : 7489 ∓ 1702.

recycling than in hormone transport.

There were no parallel variations of VP concentration and NSG either. Recovery of NSG during rehydration was linear although recovery of VP concentration is logarithmic (Fig. 3). Variations in hormone concentration within NSG during rehydration could explain this result.

REFERENCES

Cupo A, Rougon-Rapuzzi G, Delaage MA. Immunochemical detection of vasopressin pre cursors : artificial processing and quantification along the hypothalamo-hypophysial axis. Eur J Biochem 1981 ; 115 : 169-174

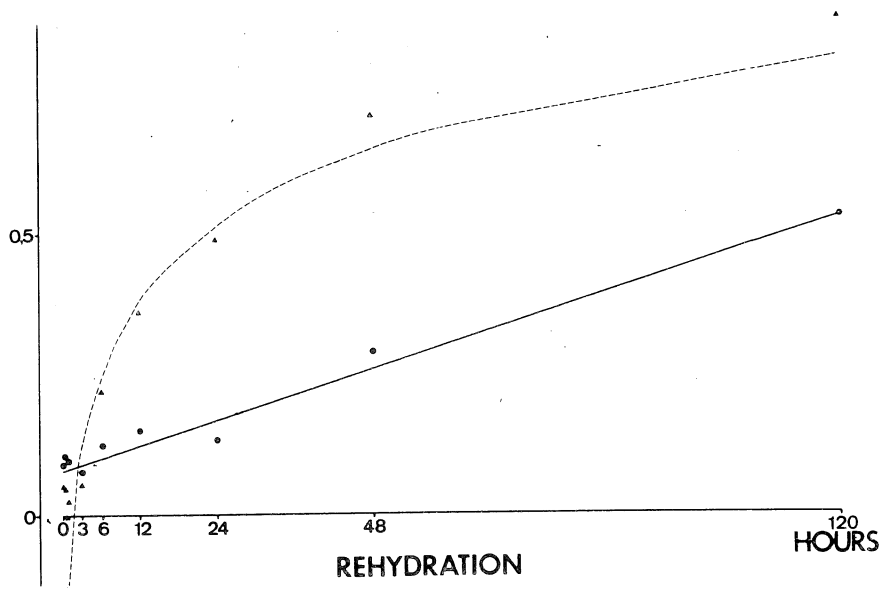

Fig. 3 . Experimental values of VP content ( ) and NSG volume density ( ) during the rehydration expressed as fractions of controls. Solid line (for NSG) and dotted line (for VP) are the theoretical curves fitted to the points.

Daniel AR, Lederis K. Effects of anesthesia and hemorrhage on hormone storage and ultrastructure of the rat neurohypophysis. J Endocrinol 1966 ; 34 : 91-104.

Ginsburg M, Ireland M. The role of neurophysin in the transport and release of neurohypophysial hormones. J Endocrinol 1966 ; 35 : 289-298.

Silverman AJ. Ultrastructural studies on the localization of neurohypophysial hormones and their carrier proteins. J Histochem Cytochem 1976 ; 24 : 816-827.

Weinstein H, Malamed S, Sachs H. Isolation of vasopressin containing granules from the neurohypophysis of the dog. Biochem Biophys Acta 1981 ; 50 : 386-389.