

$\text{Na}^{+7}$ . We found in this study that 15 mM  $\text{CaCl}_2$  did not significantly increase the activity of either the  $\text{Val}^5\text{-A II}$  or  $\text{Ile}^5\text{-A II}$  (Table II). Previous caution that our results could be influenced by the intrinsic activity of  $\text{Ca}^{++}$  in modifying the bp activity appears to be unfounded, at least at the  $\text{CaCl}_2$  concentration used. Moreover, the fact that  $\text{CaCl}_2$  enhanced the bp activity of  $\text{Asn}^1, \text{Val}^5\text{-A II}$  but failed to significantly enhance the bp activity of the  $\text{Val}^5\text{-A II}$  and  $\text{Ile}^5\text{-A II}$ , is further evidence against the possibility that the peptides reach the vascular receptors in a 'bolus', not unlike the results of an earlier investigation<sup>7</sup>.

This investigation shows that the degree of enhancement of activity of a given angiotensin II peptide varies with the ion present. In addition it is apparent that the activity of the naturally occurring angiotensins is affected by a given ion in a different manner than the amide derivative. These results lend further support to the hypothesis of a selective interaction of each ion with the hormone or its analogs leading to an alteration of the equilibrium of the preferred conformation which is detected by changes in the bp activity<sup>5,8</sup>.

**Zusammenfassung.** Nachweis, dass die Blutdruckaktivität von  $\text{Val}^5\text{-}$ ,  $\text{Ile}^5\text{-}$  und  $\text{Asn}^1, \text{Val}^5\text{-Angiotensin II}$  durch  $\text{NaCl}$  gesteigert wird. Die Aktivität der natürlichen Hormone wird jedoch von  $\text{CaCl}_2$  nicht signifikant erhöht, obwohl das Ion die Aktivität von  $\text{Asn}^1, \text{Val}^5\text{-Angiotensin II}$  stark vermehrt.

G. SCHAECHTELIN, DASHA SUROVEC and R. WALTER<sup>9</sup>

*Department of Physiology and Biophysics, Mount Sinai School of Medicine of the City University of New York, Fifth Avenue and 100th Street, New York (N.Y. 10029, USA), 12 November 1974.*

<sup>8</sup> H. GLOSSMANN, A. BAUKAL and K. J. CATT, *Science* 185, 281 (1974).

<sup>9</sup> Present address: Department of Physiology and Biophysics, University of Illinois Medical Center, P.O. Box 6998, Chicago, Illinois 60680, USA.

## Morphological Aspects of the Functional Synchronization of Supraoptic Nucleus Neurons

It is well-known that the neurons of the supraoptic nucleus are involved in the synthesis, transport and liberation of the neurohormones ADH and oxytocin<sup>1,2</sup>. The secretory activity of the hypothalamic-neurohypophyseal system in vertebrates has been studied in both normal and experimental conditions, the latter producing hypo- and hyperfunction<sup>3</sup>. To evaluate the functional state of the neurosecretory neurons, the nuclear and nucleolar volumes and the development of Nissl substance were used as parameters by ZAMBRANO and MORDOH<sup>4</sup>. They demonstrated periods of asynchronic secretory activity in neurosecretory neurons of normal rats, during which

different zones in the supraoptic nucleus could vary their functional state, although the degree of activity within each zone was maintained constant. This functional zonal asynchrony is maintained in animals stimulated by dehydration, although a certain degree of synchronization of all neurosecretory neurons during rehydration can be observed<sup>5,6</sup>. In our study we wish to analyze some ultrastructural aspects of the neurosecretory neurons in the supraoptic nucleus, suggesting the existence of functional coordination and synchronization processes among these neurons.

**Materials and methods.** The ultrastructure of hypothalamic supraoptic nucleus in both sexes of Wistar rats was studied. The rats were fixed by perfusion of 3% glutaraldehyde and the hypothalamic blocks were post-fixed in 2% osmium tetroxide. Both fixatives were buffered at pH 7.4 in 0.12 M phosphate buffer. The hypothalamic blocks were embedded in Durcupan (Fluka) as usual and stained with 1% aqueous uranyl-acetate and then lead citrate.

**Results.** The present ultra-structural study of normal rat supraoptic nucleus neurons is in accord with studies previously described<sup>7,8</sup>. Briefly, the neurons have a large nucleus with a prominent nucleolus. The cytoplasm presents 2 well-defined regions: a perinuclear region with a very well developed Golgi apparatus, containing lysosomes and neurosecretory vesicles; and a marginal cytoplasm, very rich in free ribosomes and cisterns of granular endoplasmic reticulum.

Different functional states of the neurons of the supraoptic nucleus are reflected by the variations seen in the

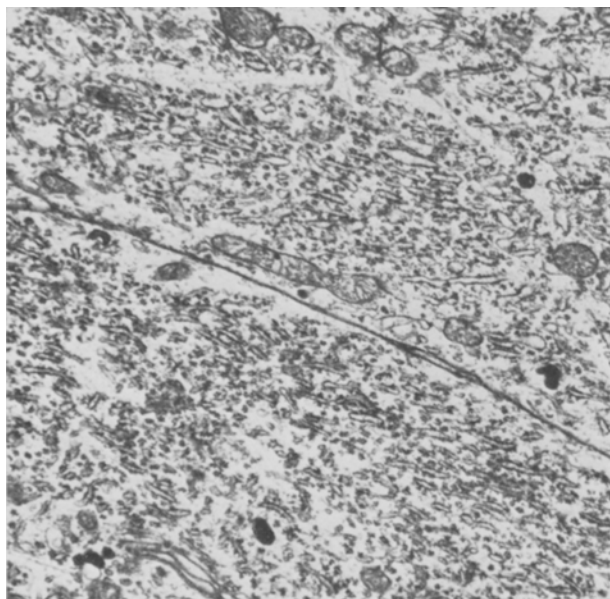


Fig. 1. Wide zone of apposition without interposition of a glial barrier in 2 neurosecretory neuron somas. Numerous granular endoplasmic reticulum cisterns are seen in the marginal cytoplasm of these neurons.  $\times 12,000$ .

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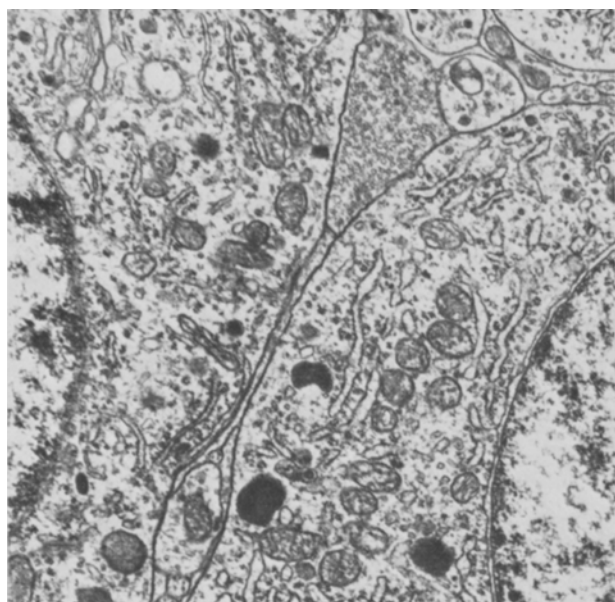


Fig. 2. A synaptic bouton containing cholinergic synaptic vesicles, and establishing synapses with 2 neurosecretory neurons.  $\times 12,000$ .

number and distribution of the cellular organoids of the perikaryon in diverse zones of this nucleus. Nevertheless, this asynchrony is preferentially zonal, and within the same zone the neurosecretory neurons show ultrastructural characteristics which are fairly constant.

In our ultrastructural observations we have frequently found wide zones of apposition of the neuronal somas without the interposition of a glial barrier (Figure 1). In these zones the membranes of the neuronal somas establish intimate contact or are separated by a narrow interstitium of about 200 Å. The neurons interconnected by these appositional zones of their somas present identical morphological characteristics in both quantity and distribution of their organoids, suggesting that they may be in the same functional state.

The other morphological aspect which we wish to emphasize is the presence of cholinergic axo-somatic synapses, with synaptic boutons which are shared by 2 neural somas (Figure 2), i.e., the same synaptic bouton forms a synapse with the somas of 2 juxtapositioned neurosecretory neurons.

### Membrane Filters do not Prevent Cell Contacts

Experimental embryologists studying induction systems have made extensive use of membrane filters to separate two cell populations<sup>1,2</sup>. The filters were originally employed in an attempt to distinguish between a chemical inducer<sup>3</sup> and induction due to cellular contacts<sup>4</sup>. The majority of these studies have utilized Millipore filters (Millipore Ltd.) to separate the two cell groups. Several studies using these filters have reported the presence of cellular extensions along the borders of the filter<sup>5,6</sup>. However, some authors still hope that thicker filters might prevent cellular contact<sup>6</sup>.

The present paper describes the results obtained when thick Millipore filters were used in a primary neural

*Discussion.* Extensive contact zones between the neural somas, without the interpositioned glial barrier, have been observed in other cerebral locations, especially during fetal and postnatal development<sup>9</sup>. In the supraoptic nucleus of the adult rat these zones are frequently seen. Although the presence of 'tight junctions' has not been observed, it is very possible that some type of interneuronal communication exists in these narrow appositional zones of the neuronal membranes. This communication could function in a manner analogous to the 'gap junctions' seen in other localities of nervous tissue<sup>10,11</sup>. This hypothesis seems to be strengthened by the fact that these interconnected neurons present identical morphological characteristics.

We feel that the appositional zones of the neuronal somas, as well as the presence of synaptic boutons shared by 2 neurons, are morphological structures which may permit an interneuronal coordination and a synchronized discharge. In this sense, the groups of interconnected neurons, within each zone of the supraoptic nucleus, would behave as functional units composed of various synchronized neurons. The asynchrony of the secretory activity of the supraoptic nucleus neurons would be zonal, i.e., the diverse zones of the supraoptic nucleus may present different functional states which could be cyclically modified<sup>4</sup>. Within the same zone, the neurons maintain similar morphofunctional characteristics. The zonal asynchrony is interpreted as a mechanism which permits the maintenance of a high activity state in the supraoptic nucleus during long periods of time without fatigue<sup>6</sup>.

*Resumen.* El estudio ultraestructural de las neuronas del núcleo supraóptico de la rata demuestra amplias zonas de aposición de los somas neuronales y botones sinápticos que son compartidos por dos somas. Los autores postulan que estas estructuras morfológicas pueden ser mecanismos de coordinación y sincronización inter-neuronal.

M. LAFARGA, G. PALACIOS and ROSA PEREZ

*Department of Histology, Faculty of Medicine, Autonomous University of Barcelona, Hospital San Pablo, Avenida San Antonio M. Claret 167, Barcelona 13 (Spain), 23 October 1974.*

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