Studies on Vesiculated and Non-Vesiculated Hypothalamic Neurosecretory Cells in the Dog

Some cells of the hypothalamic neurosecretory nuclei of the dog are noteworthy for the presence of large (20–100 μm) perikarionic vesicles. JEWELL[1] believed them to be osmoreceptors but more recent ultrastructural data are against this[2]. BARGMANN[3] refers to these neurons as cells undergoing degeneration. Somatic vesiculation is known to take place in response to axotomy[4] and to diseases affecting the neurites[5]. Neurosecretory axons are, interestingly enough, involved in mechanisms of neuroapocrine secretion[6,7], recently revealed, in electron microscope studies, as processes of degeneration and regeneration[8]. It was considered possible, therefore, that cell vesiculation could develop as a somatic reaction to a hormone-releasing mechanism involving the casting off and rebuilding of axon structures. Since the chromatolytic reaction, vacuole and vesicle formation, etc., is most severe the closer the site of axotomy lies to the neuron soma, it was believed that meaningful data could be obtained by correlating neurosecretory axon length with vesicle size and by comparing retrograde degeneration rates of vesiculated and non-vesiculated cells following pituitary stalk section. On this basis, the following hypotheses were formulated: 1. The ratio non-vesiculated/vesiculated cells should be relatively low in the nuclei with short-axoned neurons, i.e., supraoptic pars ventromedialis, and high in the distant paraventricular nuclei; 2. vesicle surface and volume should decrease as the length of the axon increases; 3. Vesiculated neurons must degenerate at a faster rate than non-vesiculated ones following surgical interruption of the hyphophysial stalk.

Material and methods. Stalk section was carried out succesfully in 5 adult dogs through a sub-temporal approach and the animals sacrificed later at different post-operative time lengths (7, 83, 106, 128 and 206 days). The hypothalami of these and of 23 normal dogs were analyzed in seriated sections stained by the Nissl, paraldehide-fuchsin, Nauta-Gygax and Bielschowsky-Gross techniques. Counts of normal and of residual neuron populations were made in the ventromedial and dorsolateral parts of the supraoptic (SON-Vm; SON-Dl), the paraventricular (PVN) and the accessory supraoptic (SON-Ac) nuclei of the right hypothalamus. Mean vesicle diameter was calculated by averaging major and minor vesicle axis, volume by the formula of the rotating ellipsoid, $V = \pi/6\ ab^2$ and axon lengths of neurosecretory neurons deducted from the seriated histological sections.

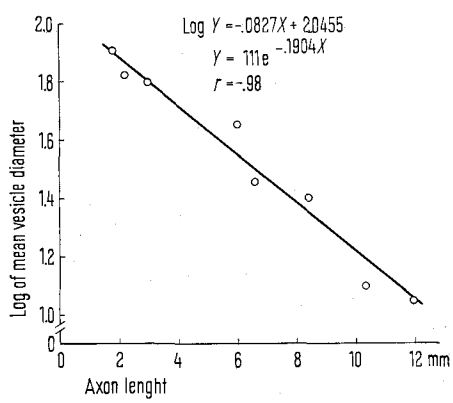


Fig. 1. Mean vesicle diameter of normal neurosecretory cells plotted as a function of axon length.

Results and discussion. Non-vesiculated/vesiculated cell ratios in normal dogs were 200 for SON-Vm, 677 for SON-Dl and 1411 for PVN. In this same group, when the logarithm of mean vesicle diameter was plotted against axon length, a linear relationship appears to exist between the two (Figure 1). The straight line on the graph represents the regression of log mean vesicle diameter (Y) on axon length (X) and was located according to the equation $Y = -0.0827\ X + 2.0455$. The degree of association between the two variables was very high: $r = -0.98$ and $P = < 0.01$. Vesicle volume and axon length were similarly related, $Y + 36.178\ (k) = -0.0288\ X + 5.2339$ and $r = -0.81$. When plotting percent of remaining neurons against survival time in the stalk-sectioned dogs, the shape of the curve for non-vesiculated cell population showed an exponential trend, that is, the number of residual neurons decreased progressively with the length of survival; a similar curve has been described for the human neurosecretory nuclei[9]. Figure 2 shows this data in a semilogarithmic scale. Degeneration rates of vesiculated neurons were much faster, since none of these cells were found in the stalk sectioned animals. In addition, hypothalami of normal dogs stained by the silver-impregnation techniques presented unmistakable features of degenerative and regenerative processes all along the neurosecretory pathway, identical with those described in a recent electron microscope study[8]. These results indicate that a highly significant negative correlation exists between vesicle size and axon length and that vesiculated neurons degenerate at a much faster pace than non-vesiculated ones following axotomy. As already mentioned, neurosecretory vesicles seem to be generated by a process of cytoplasmic cytolisis of unknown cause[2]. It is tempting to suggest that, in the dog, such a mechanism

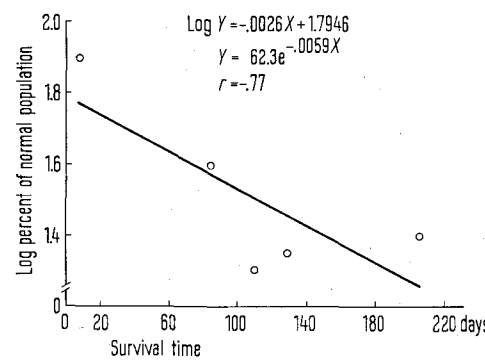


Fig. 2. Number of residual non-vesiculated magnocellular neurons following pituitary stalk section expressed as log percentages of the mean normal population.

[1] P. A. JEWELL, J. Physiol., Lond. *121*, 167 (1953).
[2] D. ZAMBRANO and E. DE ROBERTIS, Z. Zellforsch. *81*, 264 (1967).
[3] W. BARGMANN, in *The Neurohypophysis* (Ed. H. HELLER; Academic Press, N.Y. 1957), p. 11.
[4] K. R. SMITH, J. comp. Neurol. *116*, 105 (1961).
[5] E. BECK, P. M. DANIEL and H. B. PARRY, Brain *87*, 153 (1964).
[6] D. BODIAN, Bull. J. Hopkins Hosp. *118*, 282 (1966).
[7] J. D. GREEN and V. L. BREEMEN, Am. J. Anat. *97*, 177 (1955).
[8] H. D. DELLMANN and E. M. RODRIGUEZ, Z. Zellforsch. *111*, 293 (1970).
[9] A. MORTON, Brain 93, 329 (1970).

could in turn be triggered by a microapocrine releasing activity at the axon level, similar to that described in the neurosecretory system of several other species[10–12].

Resumen. El tamaño y el número de neuronas vesiculadas en los núcleos neurosecretores del perro están significativamente correlacionados con el largo axonal. Despues de la sección del tallo hipofisiario, las células vesiculadas degeneran más rapidamente que las no-vesiculadas. Estos resultados permiten suponer que las vesículas neurosecretoras se originan como resultado de mecanismos de secreción neuroapocrina en los axones.

H. Ferreyra Moyano and J. S. de Olmos

Instituto de Investigaciones Médicas M. y M. Ferreyra, Casilla de Correo 389, Córdoba (Rep. Argentina), 15 November 1971.

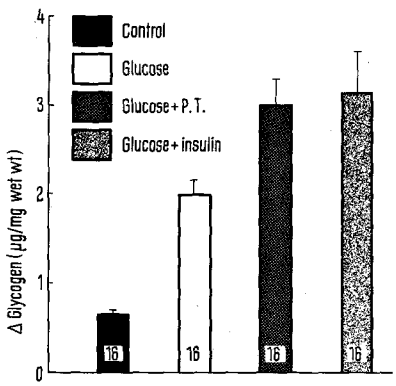
[10] A. R. Daniel and K. Lederis, J. Endocrin. *34*, 91 (1966).
[11] G. Friedberg, E. S. Nishioka, H. A. Bern and W. R. Fleming, J. exp. Zool. *162*, 311 (1966).
[12] C. G. Smoller, Science *147*, 882 (1965).

Effect of a Potent Hypolipemic Agent on Glycogen Metabolism

The presence in blood of high levels of cholesterol, non esterified fatty acids (NEFA) and tryglycerides is a common feature of several diseases. Besides the metabolic disturbance that this abnormal levels reflect, they also interfere with other metabolic functions, i.e. the high levels of serum NEFA impair glucose uptake in muscle[1]. Therefore, pharmacologists and internists are continuously searching for compounds able to drop down the abnormally high levels of circulating lipids. In 1968, Pereira et al.[2,3] described a new compound, 5-(3-pyridyl) tetrazole (3-PT) with a chemical structure similar to the nicotinic acid one. Like this acid, 3-PT has a potent in vivo hypolipemic effect. In the present experiment we have tested the effect of 3-PT upon carbohydrate metabolism in muscle and compared it with the insulin one, using an in vitro experimental model.

Material and methods. Female mice of the C3H-S strain, 9 weeks old, were used throughout the experiments. These animals were provided by the Instituto de Embriología, Biología e Histología, Facultad de Ciencias Médicas, Universidad Nacional de La Plata. They were caged in groups of 10 in a room ad hoc at a temperature of 25 ± 1°C with water and food ad libitum and illumination (fluorescent light 40 W) from 06.00 to 18.00 h alternating with 12 h darkness.

In the present experimental design, lots of 18 animals each were killed by cervical dislocation and decapitation at 16.00 h. In each animal the diaphragm was quickly and carefully dissected, washed with cool buffer in a Petri dish and middly excized. One hemidiaphragm was kept as a non-incubated control while the corresponding pair was treated as it will be described: Following a 20-min preincubation period in a flask with a 4°C medium, the hemidiaphragms were transferred and incubated in a second flask containing a medium at 37°C for 90 min in a Dubnoff shaker. In both periods, the flasks were continuously gassed with 95%O_2–5% CO_2. The preincubation medium contained bovine albumin (100 mg/100 ml) and glucose (300 mg/100 ml) in Krebs-Ringer-Bicarbonate (KRB) with the addition of glutamate, fumarate and pyruvate. The 37°C incubation medium had the same composition, but in some cases, either (3-PT) or cristalline insulin was added in a concentration of 1.25 mg/100 mg and 1 mU/ml, respectively. In other cases a combination of both compounds was simultaneously studied. At the end of the incubation period both the non-incubated as well as the incubated hemidiaphragms were treated for glycogen extraction and determination, according to Seifer's method[4]. The results were expressed as the quotient obtained substracting the glycogen value achieved in the control and non-incubated hemidiaphragm from the one attained in the paired incubated one.



Each bar represents average ± S.E.M. In circles, number of cases. P between control and glucose < 0.001. P between glucose and 3-PT < 0.005. P between 3-PT and insulin N.S.

Results. The Figure shows the results obtained expressed as μg of glycogen per mg of wet weight tissue. The incubated hemidiaphragms present significantly larger glycogen values when compared with the non-incubated ones ($P < 0.001$). Otherwise, the addition of either insulin or 3-PT produces a further increase above the one elicited by glucose alone ($P < 0.005$). When insulin and 3-PT were simultaneously tested, the tissue behaved as if it were in the presence of a single compound (data not shown). On the other hand, the 2 compounds – in the concentration employed – produce similar changes in the tissue glycogen content of the incubated hemidiaphragms.

Discussion. Muscle glycogen increases when the tissue is incubated in the presence of high levels of glucose[5]. Furthermore, this in vitro synthesis of glycogen can be enhanced by the addition of insulin to the incubation medium. Our results indicate that 3-PT in a concentration

[1] P. B. Garland and P. J. Randle, Biochem. J. *93*, 678 (1964b).
[2] G. F. Holland and J. Pereira, J. med. Chem. *10*, 149 (1967).
[3] J. N. Pereira, G. F. Holland, F. A. Hochstein, S. Gilgore, S. Defelice and R. Pinson, J. Pharmac. exp. Therap. *162*, 148 (1968).
[4] S. Seifer, S. Dayton, B. Novic and E. Muntwyler, Arch. Biochem. Biophys. *25*, 191 (1950).
[5] A. C. Wardlaw and P. J. Moloney, Can. J. Biochem. Physiol. *39*, 695 (1961).