Antagonism Between Ca and Na Ions at Neurohypophysial Nerve Terminals

DOUGLAS and POISNER[1] were first to demonstrate the importance of Ca^{2+} ions in the release of octapeptide hormones from the neurohypophysis. Stimulation of neural lobes in vitro by increasing the external $[K^+]$ or by electrical currents is ineffective if $CaCl_2$ is omitted from the bathing solution, while the liberation of hormones is progressively increased if $[CaCl_2]$ is raised to 3–4 mM; the efficiency of stimulation declines above this range.

It is generally held that the release process in vivo is initiated by action potentials which depolarize the nerve fibre endings, and that Ca^{2+} diffuses into these depolarized terminals along its electrochemical gradient, thus reaching sites which, when occupied by calcium, would in some way promote hormone release. Although Na^+ is required for the generation and propagation of action potentials, it is not essential for the release process, since liberation of hormones can be evoked in Na^+-free solutions[1] or in the presence of tetrodotoxin[2]. Rather, a given secretory stimulus seems to become more effective as the concentration of Na^+ is lowered, which suggests that Na ions may hinder the influx of Ca^{2+} during depolarization and/or may compete for the postulated binding sites. In frog heart muscle, it has been described that the intensity of muscle contraction increases as $[Na^+]$ is lowered, but remains fairly constant if the ratio $[Ca^{2+}]/[Na^+]^2$ is maintained[3,4]. LÜTTGAU and NIEDERGERKE[4] have suggested that in heart muscle Na^+ and Ca^{2+} compete for a negatively charged carrier molecule which moves inward during membrane depolarization. A similar mechanism has been postulated to account for the increase in transmitter release which follows the depolarization of presynaptic nerve terminals[5]. In this study, a similar ionic interaction has been found.

Isolated neurohypophyses were obtained, following decapitation, from adult rats of both sexes. The cut end of the pituitary stalk was tied to a platinum wire and the preparation immersed in small test tubes containing 1.0 ml of modified Locke solution (cf. ref.[1]), maintained at 37 °C and gassed with 95% O_2–5% CO_2. After a 20 min preincubation period the neural lobe was transferred into fresh media every 10 min by means of a micromanipulator. Hormone release was evoked by a 10-fold rise in [KCl] (to 56 mM) at NaCl concentrations of either 50, 100 or 150 mM. In certain experiments, the concentration of $CaCl_2$ was also modified in order to yield predetermined ratios of $[Ca^{2+}]/[Na^+]^2$. After incubation, media were stored at 4 °C overnight and their milk ejection activity determined in a rat bioassay[6]. Results are expressed in fractions of international units (μl) of synthetic oxytocin (Syntocinon, Sandoz Ltd.).

Figure 1 shows 2 typical experiments in which each preparation was studied during 13 consecutive incubation periods. Hormone secretion was evoked by KCl (56 mM) during the 2nd, 6th and 10th incubation period, ionic strength and iso-osmolarity in the medium being maintained by the addition of choline chloride; the $[CaCl_2]$ was kept constant at 2.2 mM throughout the experiments.

[1] W. W. DOUGLAS and A. M. POISNER, J. Physiol. Lond. *172*, 1 (1964).
[2] J. J. DREIFUSS, I. KALNINS, J. S. KELLY and K. B. RUF, J. Physiol. Lond. *215*, 805 (1971).
[3] W. WILBRANDT and H. KOLLER, Helv. physiol. pharmac. Acta *6*, 208 (1948).
[4] H. C. LÜTTGAU and R. NIEDERGERKE, J. Physiol., Lond. *143*, 486 (1958).
[5] J. DEL CASTILLO and B. KATZ, J. Physiol., Lond. *124*, 553–559 (1954).
[6] G. W. BISSET, B. J. CLARK, J. HALDAR, M. C. HARRIS, G. P. LEWIS and M. ROCHA E SILVA, Br. J. Pharmac. Chemother. *31*, 537 (1967).

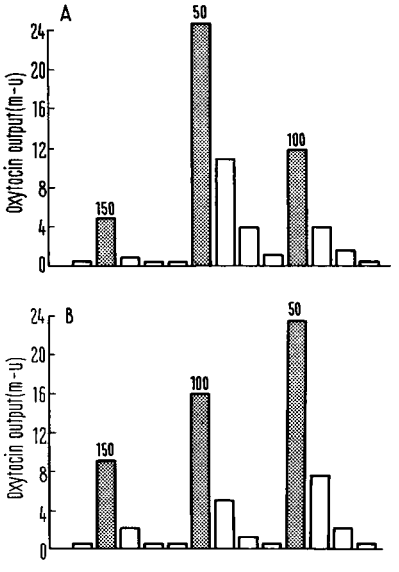

Fig. 1. Effect of partial replacement of external NaCl by choline chloride on evoked hormone relase from 2 (A, B) neural lobes in vitro. Each column represents hormone output from a single rat neurohypophysis during consecutive 10 min incubation periods; open columns: resting state; stippled columns: excess potassium (56 mM). The concentration of $CaCl_2$ was kept constant at 2.2 mM troughout. Numbers above columns indicate concentrations of NaCl (mM), iso-osmolarity being maintained with choline chloride.

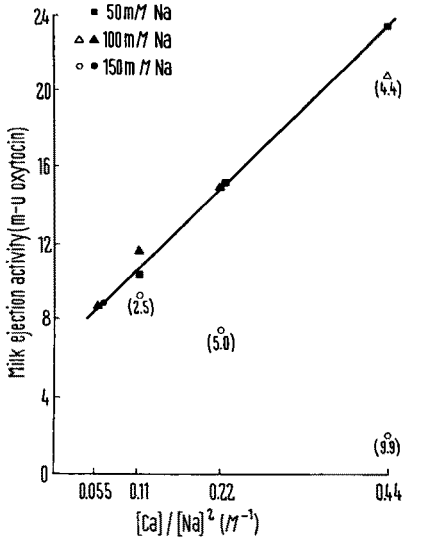

Fig. 2. Hormone release from isolated rat neural lobes as a function of the $[Ca^{2+}]/[Na^+]^2$ ratio of the bathing solution. Ratios were calculated for NaCl concentrations of 50, 100 and 150 mM (see also Table), the osmolarity of the media being adjusted with choline chloride where required. Black symbols: 0.28 mM < $[CaCl_2]$ ⩽ 2.2 mM; open symbols: $CaCl_2$ > 2.2 mM (concentrations in parenthesis). Each point represents mean output of 4–6 individual neural lobes. Note that with $[CaCl_2]$ ⩽ 2.2 mM, output varies roughly linearly with the $[Ca^{2+}]/[Na^+]^2$ ratio, while output is lower with $[CaCl_2]$ > 2.2 mM.

It is readily apparent that the standard stimulus is most effective at the lowest [NaCl] tested (50 mM),and is still more effective at an intermediate (100 mM) than at the normal concentration (150 mM). This graded response was obtained with all possible permutations of the experimental design and cannot be ascribed to the addition of choline, since the replacement of NaCl by LiCl gave similar results.

These observations could be explained by assuming that both Na^+ and Ca^+ have an affinity for the hypothetical cellular binding sites (X), but that only the complexes Ca-X are able to promote release. In media containing a high NaCl concentration, Na ions might combine with either or both of postulated anionic groups of X so as to form inactive complexes.

If such an antagonism did exist, the release of hormones might depend on the $[Ca^{2+}]/[Na^+]^2$ ratio over a wide range of Ca^{2+} concentrations. In order to test this possibility, experiments were done in which this ratio in the bathing solution was either kept constant, or varied deliberately, ionic strength and iso-osmolarity being maintained in each case by the addition of choline chloride.

Results are summarized in the Table and in Figure 2, and show that for $CaCl_2$ concentrations between 0.28 and 2.2 mM, the release of hormones increases linearly with the rises of the $[Ca^{2+}]/[Na^+]^2$ ratio.

Whereas release is indeed determined by this ratio over an approximately 10-fold range of $[Ca^{2+}]$, the relationship does not hold for $CaCl_2$ concentrations above approximately 3 mM, which is in keeping with the observation of DOUGLAS and POISNER[1] that vasopressin release decreases in this range. Similarly, BROWN and FELDBERG[7] have described that the release of acetylcholine from the perfused cervical superior ganglion by raised [KCl] is depressed by high Ca^{2+} concentrations; and GAGE and QUASTEL[8] have reported that the frequency of miniature end plate potentials in rat diaphragma increases with $[Ca^{2+}]$ in the range of 0.32 to 2.0 mM, but diminishes with a further rise in $CaCl_2$. It has been proposed that the partial inactivation of the release process in high $[CaCl_2]$ may be due to the formation of inactive Ca_2-X complexes.

[NaCl] (mM)	$[CaCl_2]$ (mM)	$[Ca^{2+}]/[Na^+]^2$ (M^{-1})	Mean hormonal output (mU oxytocin) Mean S.D.M.	No. of experiments
150	1.24	0.055	8.6 ± 0.6	5
100	0.55	0.055	8.8 ± 1.5	5
150	2.48	0.11	9.3 ± 1.5	5
100	1.10	0.11	11.6 ± 1.5	5
50	0.28	0.11	10.2 ± 1.6	6
150	4.95	0.22	7.4 ± 0.7	5
100	2.20	0.22	14.9 ± 2.3	5
50	0.55	0.22	15.0 ± 3.1	4
150	9.90	0.44	2.0 ± 0.0	5
100	4.40	0.44	20.9 ± 3.5	6
50	1.10	0.44	23.3 ± 1.6	5

The cellular localization of the ionic binding sites X is still unknown. Our finding that release is governed by the $[Ca^{2+}]/[Na^+]^2$ ratio in the bathing solution suggests the existence of sites not far removed from the cell surface, viz. within the membrane, which is readily accessible to the incubation medium. If the sites were intracellular, release ought to be depressed by an increase in intracellular $[Na^+]$; no such depression was found by DICKER[9] when active sodium transport was blocked with cardiac glycosides.

It remains to be determined whether the inhibitory action of Na ions on neurohypophysial hormone release is specific or is shared by other univalent cations. In frog cardiac muscle, an increase in the tension of contracture occurs when NaCl is replaced by sucrose, and lithium, choline or *tris* chlorides[4], suggesting that the effect of lowering $[Na^+]$ is a specific action of Na ions. In contrast, KELLY[10] has suggested that at frog neuromuscular junctions the competition between Na^+ and Ca^{2+} may not be chemically specific, but charge specific.

The fact that the release mechanism does inversely depend on the external Na^+ concentration – in contrast to the height of the action potential[11] – suggests that the release process for octapeptide hormones can function independently of the generation of action potentials (cf. ref.[2]). Moreover, since under normal conditions, a major part of the action currents flowing at the level of the axon terminals in the neural lobe are in all likelihood carried by Na ions, it may be concluded that, both in vivo and in standard Locke solution, the release mechanism may not be fully operational[12].

Résumé. La secrétion hormonale de neurohypophyses isolées de rat a été estimée à l'aide d'un test d'éjection du lait. Au moment de la dépolarisation des terminaisons neurosecrétrices, il y a compétition entre le sodium et le calcium externes pour d'hypothétiques sites membranaires. Pour des concentrations de calcium inférieures ou égales à la concentration physiologique, la libération hormonale est fonction du rapport $[Ca^{2+}]/[Na^+]^2$ dans le milieu externe.

J. J. DREIFUSS, J. D. GRAU and RITA E. BIANCHI

Department of Physiology, University of Geneva Medical School, CH-1211 Genève 4 (Switzerland), 18 June 1971.

[7] G. L. BROWN and W. FELDBERG, J. Physiol., Lond. *86*, 290 (1936).
[8] P. W. GAGE and D. M. J. QUASTEL, J. Physiol., Lond. *185*, 95 (1966).
[9] S. E. DICKER, J. Physiol., Lond. *185*, 429 (1966).
[10] J. S. KELLY, Q. J. exp. Physiol. *53*, 239 (1968).
[11] A. L. HODGKIN and B. KATZ, J. Physiol., Lond. *108*, 37 (1949).
[12] This work was supported by grants from the Swiss National Science Foundation No. 5340.3 and 3.556.71 and the F. Hoffmann-La-Roche Foundation No. 117.

Peroxidase Uptake by the Fat Body of a Millipede *Spirostreptus asthenes* (Diplopoda, Myriapoda)

A recent study on the fat body of a millipede *Spirostreptus asthenes* shows the appearance of tyrosine-rich protein granules in the premolt stage and their disappearance following molt.[1]. That this protein appears prior to cuticle formation and disappears during the formation of cuticle may suggest its utilization in the cuticle formation.

Studies on insect fat body show that the fat body protein could be formed either by synthesis or sequestration.