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Release of Neurophysin Together with Vasopressin by a Vasopressin by a Ca<latex>$^{2+}$</latex> Dependent Mechanism

L. O. Uttenthal, B. G. Livett and D. B. Hope

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Release of neurophysin together with vasopressin by a Ca^{2+} dependent mechanism

By L. O. Uttenthal, B. G. Livett and D. B. Hope
Department of Pharmacology, Oxford University

Oxytocin and vasopressin are stored with their binding proteins, the neurophysins, within neurosecretory vesicles in the nerve endings of the mammalian neurohypophysis. Depolarization of the nerve terminals, either by the arrival of a nerve impulse *in vivo* or by immersion of the gland in solutions of high K^+ concentration *in vitro*, brings about a release of the hormones into the extracellular space. Douglas & Poisner (1964) have shown that this release is dependent on the entry of Ca^{2+} into the nerve endings, and have proposed that Ca^{2+} is necessary for coupling the stimulus of depolarization to the secretory process. Whereas Douglas (1967) suggests that Ca^{2+} plays a part in emptying the neurosecretory vesicles by an exocytotic mechanism, the finding of Smith & Thorn (1965) that Ca^{2+} dissociates the hormone-neurophysin complex suggests that secretion may take place by diffusion of the hormones through the vesicular and cell membranes after liberation from their binding proteins. A biochemical method of distinguishing between these two mechanisms is to study whether other macromolecular constituents of the neurosecretory vesicles are specifically released by depolarizing stimuli. Fawcett, Powell & Sachs (1968) have previously shown by tracer experiments in dogs that a protein cross-reacting with a rabbit antiserum to bovine neurophysin is released from neurohypophyses stimulated by high K^+ solutions *in vitro* or by haemorrhage *in vivo*, but their technique did not allow a quantitation of the protein in relation to the amount of hormone released. A parallel release of neurophysin and hormone would be expected if exocytosis plays a part in the secretory mechanism.

In our experiments, freshly dissected porcine posterior pituitary lobes were cut sagitally to facilitate oxygenation and escape of secretory products, and incubated in various modified Locke solutions gassed with 95 % O_2 and 5 % CO_2. The incubation medium was changed at 10 min intervals and assayed for vasopressin by the rat pressor method of Dekanski (1952) and for porcine neurophysin-II (Uttenthal & Hope 1970) by immunological techniques. Initial experiments showed that washing in Locke solution brought the escape of hormone to a low resting level, after which incubation in high K^+ (56 mmol/l) Locke solution elicited an increase in the pressor activity of the medium. Pre-incubation of the glands in Ca^{2+}-free Locke solution inhibited the K^+-stimulated response. When samples of the incubation media were tested by immunodiffusion in agar gel containing 40 mg/ml Dextran 10, precipitin lines were obtained with a rabbit antiserum to porcine neurophysin, the strongest reactions occurring with the samples of highest pressor activity. This gave a preliminary indication of a parallel release of vasopressin and porcine neurophysin-II.

Quantitation of the porcine neurophysin-II released was achieved by micro-complement fixation analysis of the samples with a rabbit antiserum specific to this protein (see figure 1). In the absence of Ca^{2+}, high K^+ (56 mmol/l) failed to evoke a release of the hormone or protein above the resting level. However, when Ca^{2+} was restored to the medium, 56 mmol/l K^+

increased the rate of release of both vasopressin and porcine neurophysin-II. This was followed by a return to the resting level after 20 min in normal Locke solution.

A ratio of 1.5 ± 0.3 ($\pm$S.D.) μg porcine neurophysin-II/μg lysine vasopressin released was obtained for the results shown in figure 1, and further work is in progress to establish more precisely the ratio in which the hormone and protein are released.

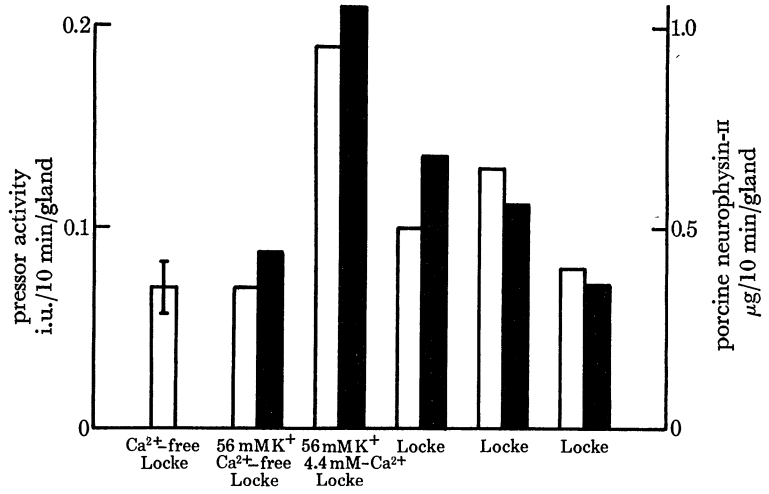

FIGURE 1. Release of vasopressin and porcine neurophysin-II from isolated porcine posterior pituitary lobes in response to high K^+ in the presence of Ca^{2+}. Open bars show the pressor activity and blocked bars the amount of porcine neurophysin-II in samples of the incubation medium. The first bar shows the resting level of hormone release ($\pm$S.D., $n = 5$) in Ca^{2+}-free Locke solution; the following bars represent successive 10 min incubation samples.

In recent ultrastructural studies on the neurohypophysis of the hamster, Douglas *et al.* (1971) observed neurosecretory vesicles fused with the cell membrane in Ω-figure profiles suggestive of exocytosis. The present biochemical findings show that in the pig, neurophysin-II and lysine vasopressin are released in parallel by a K^+-stimulated, Ca^{2+}-dependent mechanism, supporting the view that neurosecretion from the posterior pituitary involves exocytosis.

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Note added in proof (*February* 1971). Release of neurophysin by physiological stimuli known to release vasopressin has recently been shown to occur *in vivo* by K. W. Cheng & H. G. Friesen (1970) Physiological factors regulating secretion of neurophysin. *Metabolism* **19**, 876–890.

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