J. exp. Biol. ***195****, 19–34 (1994)*
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STIMULUS–SECRETION COUPLING IN THE NEUROHYPOPHYSIS OF THE JERBOA *JACULUS ORIENTALIS*

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Accepted 12 May 1994

Summary

1. In many mammals, severe dehydration is known to cause exhaustion of the vasopressin content of the neural lobe. Here, we have examined the physiological state of the neurohypophysis of the jerboa *Jaculus orientalis*, a rodent inhabitant of a semi-desert climate.

2. Isolated neurohypophyses and neurosecretory nerve endings were perfused *in vitro* and vasopressin and oxytocin release were determined by radioimmunoassay.

3. Electrical stimulation of the neurohypophysis with bursts of pulses mimicking the activity of hypersecreting neuroendocrine neurones induced similar increases of secretion in both control animals and animals dehydrated for up to 2 months. Neurohormone release was greatly potentiated when the bursts of pulses were separated by silent intervals.

4. Prolonged stimulation of neurohypophyses from both control and dehydrated animals induced a sustained increase of vasopressin release; in contrast, oxytocin release under similar conditions showed a biphasic secretory pattern consisting of a transient increase that subsequently decreased to a steady level whose amplitude was similar to that for vasopressin.

5. K^+-induced secretion was largely inhibited by the Ca^{2+} channel blockers nicardipine and ω-conotoxin, suggesting that in this neurosecretory system both L- and N-type calcium channels play a major role in stimulus–secretion coupling. Depolarization of isolated nerve endings using a fast-flow perifusion system showed that there was no difference in the amplitude and the time course of the secretory response in dehydrated and hydrated animals.

6. The results demonstrate that, despite the climatic conditions in which the jerboas live, their neural lobes retain the capacity to release, upon depolarization of the plasma membrane of the nerve endings, large amounts of neurohormone. It is concluded that the neurohypophyseal peptidergic release system in the dehydrated jerboa functions adequately even under extreme environmental stress.

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Key words: neurohypophysis, calcium channel, secretion, nerve endings, vasopressin, jerboa, *Jaculus orientalis*.

Introduction

The jerboa *Jaculus orientalis* is a rodent that lives in the semi-desert climate of the Middle Atlas mountains, East Morocco. In the wild, this animal has access for a period of approximately 4 months to water and plants (March–June) and it then has to live for more than 5 months under extremely arid conditions prior to hibernation. Elevated plasma vasopressin contents have been reported for this species (Baddouri *et al.* 1984; Butlen *et al.* 1984) which suggests, though does not prove, that the magnocellular neurones located in the hypothalamus are hyperactive and that this results from the arid environmental conditions under which the jerboa lives.

During the last decade, studies on the hypothalamo-neurohypophyseal system of the laboratory rat have yielded important information on the stimulus–secretion coupling mechanism at the nerve endings of the neurohypophysis. The main findings have been (i) that the patterning of the action potentials invading the nerve terminals is crucial for optimizing increases in (a) the inward calcium current (Fidler Lim *et al.* 1990; Jackson *et al.* 1991), (b) the internal calcium concentration, $[Ca^{2+}]_i$ (Brethes *et al.* 1987) and (c) the secretory process (Dutton and Dyball, 1979; Cazalis *et al.* 1985; Gainer *et al.* 1986); (ii) that the effect of impulse-evoked depolarization on the release of neuropeptides is more potent when interspersed by silent intervals (Cazalis *et al.* 1985); and (iii) that not only the absolute $[Ca^{2+}]_i$ at the nerve endings but also the variations in $[Ca^{2+}]_i$ are crucial for inducing neurohypophyseal hormone release (Cazalis *et al.* 1987*a*,*b*; Stuenkel and Nordmann, 1993). The aim of the present study was to investigate *in vitro* the capacity of the neurohypophysis, both from control animals and from animals subjected to severe dehydration, to release vasopressin (AVP) and oxytocin (OT) following depolarization of the nerve endings.

Materials and methods

The jerboa *Jaculus orientalis*, which lives in the Middle Atlas Mountains of East Morocco, was used in these studies. The climate of this region can be considered as semi-desert (the annual rainfall is less than 200 mm). The animals were captured in May and kept in the laboratory (Rabat, Morocco) for approximately 1 month. After capture, all animals were initially given barley, sunflower seeds and lettuce *ad libitum*, a condition considered similar to that observed during the ‘rainy season’ in the Middle Atlas Mountains. Two weeks after their capture, the animals were divided into two groups. One group of animals was fed on the above diet and was considered as ‘controls’ or ‘hydrated’ animals. The second group of animals was given only dry food consisting of barley and sunflower seeds, both of very low water content. The latter group (animals dehydrated for 4–9 weeks) produced a very hyperosmotic urine (3700±470 mosmol l^{-1}, N=13 *versus* controls 1900±320 mosmol l^{-1}, N=7) and hereafter are called ‘dehydrated’ animals. One month later, the animals were shipped to Strasbourg. All experiments were performed 6–12 weeks after capture.

Preparation of neurohypophyses and incubation conditions

Neurointermediate lobes were isolated after decapitation of anaesthetized animals

(weighing 80–140 g). The lobes were pinned down on a Sylgard (Dow Corning) resin-coated dish and the pars intermedia was carefully separated from the neural lobe. The neurohypophyses were then impaled on one probe of a paired electrode that was inserted within a Perspex chamber (Bicknell *et al.* 1982). The chamber had a volume of 50 μl and was perfused continuously with saline at a rate of 50 μl min^{-1}. Fractions (4 min each) were collected 60 min after the onset of the perfusion. Neuropeptide release was measured by radioimmunoassay as described previously (Cazalis *et al.* 1985). Vasopressin (AVP) and oxytocin (OT) antibodies were used at a final dilution of 1/300 000 and 1/40 000 respectively. The cross-reaction of OT in the AVP assay was less than 1:1000. Similarly, the cross-reaction of AVP in the OT assay was less than 1:200. The AVP and OT standards were obtained from Ferring (Malmö, Sweden) and Sandoz (Basel, Switzerland) respectively. [^{125}I]AVP and [^{125}I]OT were purchased from Amersham (Amersham, England). For both assays, the inter- and intra-assay variabilities were less than 10 %.

During the dissection period and the first 30 min of perfusion, the incubation medium contained (in mmol l^{-1}): NaCl, 140; $KHCO_3$, 5; $MgCl_2$, 1; $CaCl_2$, 2.2; glucose, 10; Hepes–Tris, 10; pH 7.1–7.2; and bovine serum albumin, 0.01 %. The medium was continuously gassed with 5 % CO_2 in O_2. Following this preincubation period, the concentration of NaCl was reduced to 40 mmol l^{-1}, the osmolarity being maintained with *N*-methyl-D-glucamine chloride (100 mmol l^{-1}; NMG). When the neurohypophyses were depolarized with K^+, the NMG concentration was reduced accordingly. Care was taken to maintain the osmolarity of all the media at 305–310 mosmol l^{-1}. In preliminary experiments performed on rats, we found that dimethylsulphoxide, which was used to dilute compounds such as nicardipine and A23187, had no effect on secretion from the neurohypophysis.

Electrical stimulation

Neurohypophyses were stimulated electrically with pulses that had a pattern of discharge similar to the bursting activity of AVP or OT cells (see Cazalis *et al.* 1985). The electrical activity of an AVP cell during dehydration and of an OT cell during lactation were recorded from the supraoptic nucleus of anaesthetized rats (kindly performed for us by Dominique Poulain). We modified a computer program originally written by D. Poulain (see Cazalis *et al.* 1985) which allowed us to use the activity within an AVP burst or an OT burst as a command to trigger a stimulator (ITT OX 710 C) and thus to generate a series of stimulus pulses (2 ms duration, 4 mA biphasic) with a pattern corresponding to that encountered *in vivo* (Dutton and Dyball, 1979). We used an Apple IIe computer with a Proclock card. The program has recently been adapted for IBM PC computers.

Preparation and perifusion of nerve terminals

Nerve endings were isolated as described previously (Cazalis *et al.* 1987*a*). Briefly, the pars intermedia was carefully removed and the neural lobe homogenized at 37 °C in a buffer containing 270 mmol l^{-1} sucrose, 2 mmol l^{-1} EGTA and 10 mmol l^{-1} Hepes–Tris, pH 7.2. The homogenate was centrifuged at 100 ***g*** for 1 min and the supernatant was then

centrifuged at 2400 ***g*** for 4 min. The pellet was resuspended in physiological saline at 37 °C (see above). A sample of the nerve endings was loaded onto a 0.2 μm filter (LC Acrodisk Gelman, Ann Arbor, MI, USA) and perfused at 25 μl min^{-1} for 45 min with physiological saline. It was then perfused with normal saline in which 100 mmol l^{-1} Na^+ had been substituted by NMG. After 15 min, the flow rate was increased progressively over the next 45 min until it reached 25 μl s^{-1}. Stimulation of AVP release was induced by perifusion with saline containing 50 mmol l^{-1} K^+. Osmolarity was maintained by reducing NMG accordingly. The half-time, $t_{0.5}$, of the concentration change at the filter was 4–6 s, as determined with ^{86}Rb (Lindau *et al.* 1992). Fractions of 7.15–7.25 s were then collected manually and AVP was determined by radioimmunossay.

Results

Effects of bursts of electrical pulses on AVP release

Isolated neural lobes were stimulated with bursts of pulses mimicking the activity of a vasopressin cell (hereafter called AVP-like bursts; see Discussion). Fig. 1 illustrates a typical experiment in which a neural lobe was stimulated three times with a train of four bursts. Whereas during the first and third periods of stimulation the bursts were separated by intervals of 30 s, they were given without any interval during the second period. AVP was collected in 4 min fractions. The groups of four bursts induced more AVP release when they were separated by silent intervals. In control animals, the series of four AVP bursts separated by 30 s intervals induced the release of 4.1 times the amount of AVP secreted following stimulation of the neural lobe with a single burst (1254±289 pg, *N*=6, *versus* 303±98 pg, *N*=4, mean ± S.E.M.). Indeed, the series of four AVP-like bursts delivered *without* silent periods induced the same amount (310 pg, *N*=2) of AVP release as that induced by a single burst. In animals dehydrated for 54 days, four AVP-like bursts separated by 30 s intervals induced the release of 1290 pg (*N*=2). Similar observations were made on the amount of OT secretion. One AVP-like burst gave rise to the release of 408 pg of OT (*N*=2) whereas four bursts separated by 30 s silent intervals induced the release of 1230±391 pg of OT (*N*=6). In another series of experiments, the neural lobes were stimulated with a pattern of pulses mimicking the electrical activity of an OT-containing cell during lactation (see Cazalis *et al.* 1985). Whereas one single OT-like burst released 173 pg of OT (*N*=2) and 171±60 pg of AVP (*N*=4), four OT-like bursts separated by 30 s intervals induced the release of 854 pg of OT (*N*=2) and 400 pg of AVP (*N*=2). Therefore, with both AVP-like and OT-like bursts, not only the number of bursts but also the silent period separating the bursts are of importance in potentiating the release of neuropeptides. Furthermore, no significant differences in the secretory response to the stimulation regimes were observed between control and dehydrated jerboas.

Characterization of the Ca^{2+} channel type(s) involved in the stimulus–secretion coupling

In a series of three pilot experiments, isolated neural lobes from control animals were

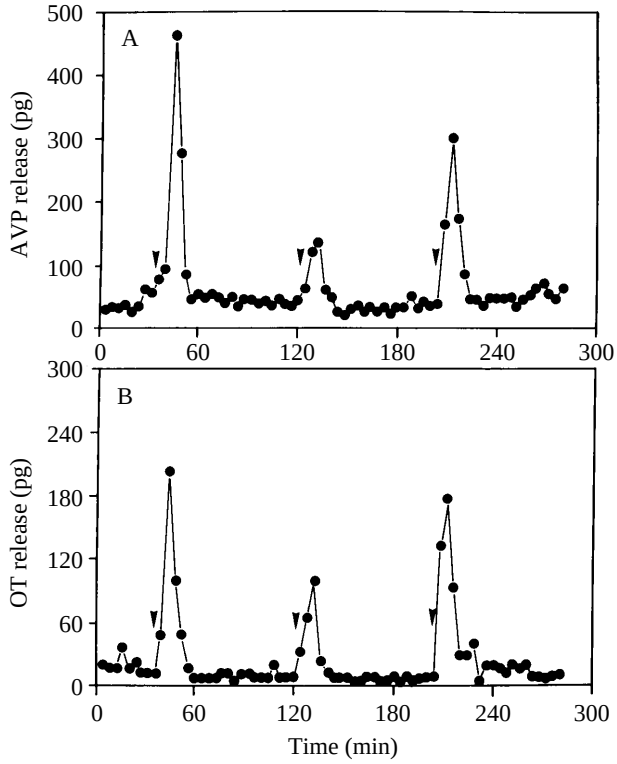

Fig. 1. Time course of vasopressin (AVP) and oxytocin (OT) release induced by electrical stimulation of the isolated neural lobe. The neural lobes were stimulated with four AVP-like bursts either separated (first and third stimuli) or not (second stimulation) by 30 s silent intervals. AVP (A) and OT (B) released into the perifusate were measured by radioimmunoassay. The arrowheads indicate the onset of the stimuli.

stimulated during three (S1, S2, S3) successive periods of 6 min with medium containing 100 mmol l^{-1} K^+. These depolarization periods were separated by 40 min intervals and, at the end of the experiments, the neurohypophyses were stimulated electrically (S4) with four AVP-like bursts separated by 30 s intervals. The amounts of evoked AVP release during the four stimulations were (mean ± S.E.M.): 1187±117 pg (S1), 1136±277 pg (S2), 1501±255 pg (S3) and 358±119 pg (S4) respectively. These results demonstrate that the neurohypophysis of the jerboa can be repetitively stimulated by K^+-induced depolarization and that each stimulus of a particular duration gives rise to the release of approximately the same amount of AVP. They also show that K^+-induced depolarization for a period of 6 min is a more potent stimulus than four AVP-like bursts.

To determine the concentration of external K^+ necessary to give half-maximal activation of AVP release, isolated neurohypophyses were stimulated with 50, 75 and 100 mmol l^{-1} K^+ and the amount of hormone released was determined. The two experiments gave values of 55 and 65 mmol l^{-1} for half-maximal activation of the secretory process.

Additional experiments have confirmed that, as in other neurosecretory systems, the release of neuropeptide depends largely on the presence in the extracellular medium of

calcium ions. In order to characterize the type(s) of voltage-dependent Ca^{2+} channels involved in the stimulus–secretion coupling mechanism in the neurohypophysis of the jerboa, secretory responses were compared in the absence and presence of specific Ca^{2+} channel antagonists. Neural lobes were initially perifused with medium containing 40 mmol l^{-1} Na^+, and secretion was triggered by increasing the extracellular K^+ concentration to 100 mmol l^{-1} for 6 min. After a 60 min recovery period in medium containing 40 mmol l^{-1} Na^+, the neural lobes were again depolarized for 6 min in the presence (experimental) or absence (control) of Ca^{2+} channel antagonists that had been added to the perifusion medium 20 min before the onset of the second stimulation. Fig. 2 illustrates representative results. The addition of nicardipine (10^{-6} mol l^{-1}) considerably reduced the amount of both AVP (67 % reduction, *N*=2) (Fig. 2B) and OT (78 % reduction, *N*=2) (not shown) released upon K^+-induced depolarization. Furthermore, when ω-conotoxin (3×10^{-8} mol l^{-1}) was added together with nicardipine (10^{-6} mol l^{-1}) to the incubating medium, the secretory response was completely abolished (Fig. 2C). Note that the AVP release induced by the Ca^{2+} ionophore A23187 was apparently unaffected by nicardipine, as the amount of AVP release, compared with controls, was not significantly different when the antagonist was added to the medium (Fig. 2B).

Secretory response of the neurohypophysis during sustained electrical stimulation

Neurohypophyses from normal and dehydrated animals (see Materials and methods) were stimulated over a period of 2 h with 150 AVP-like bursts separated by 30 s intervals. The results are expressed as a rate constant of AVP release (Fig. 3). This has the advantage of allowing the amount of AVP released into the medium, at any given time, to be compared with the AVP content of the neural lobe. Of primary interest is the observation that the rate constant of AVP release from neurohypophyses of animals dehydrated for 54 days (Fig. 3B) did not differ, under these experimental conditions, from that calculated from control experiments (Fig. 3A). In control animals, 150 AVP-like bursts separated by 30 s intervals induced the release of 4250±1272 pg of AVP per neural lobe (S.E.M.; *N*=6). This is equivalent to 28.3 pg of AVP per neural lobe per burst. In animals dehydrated for 54 days, the corresponding values were 6016±1390 pg of AVP per neural lobe (S.E.M.; *N*=5; 40 pg AVP per neural lobe per burst). In an additional experiment, 200 AVP-like bursts separated by 30 s intervals induced the release of 5590 pg of AVP per neural lobe (*N*=1; 28 pg of AVP per neural lobe per burst). These experiments clearly show that neural lobes from dehydrated animals release as much AVP as those from control jerboas. Fig. 4 shows the release of OT under similar conditions. It can be seen that dehydration (54 days) does not impair the secretory mechanism for OT. It should be noted that we systematically observed, during prolonged stimulation of the neural lobe from *Jaculus orientalis*, a large

Fig. 2. Effects of Ca^{2+} channel antagonists on the time course of vasopressin (AVP) release by isolated neural lobes. The neurohypophyses were depolarized with 100 mmol l^{-1} K^+ (heavy bars). Nicardipine (B, 10^{-6} mol l^{-1}), or nicardipine (10^{-6} mol l^{-1}) together with ω-conotoxin (C, 3×10^{-8} mol l^{-1}), was added 20 min before the onset of the second stimulus and were therefore present during the depolarizing period. The calcium ionophore A23187 (5×10^{-6} mol l^{-1}) was added in control (A) and nicardipine-treated (B) neural lobes as indicated.

initial transient increase of OT release which decreased with time, reaching a new 'steady' state after approximately 30 min. This is in contrast with the time course of AVP release (Fig. 3) which, except in few instances at the very beginning of the stimulation (see Fig. 6), remains relatively constant during the entire stimulation period. Note that, after the transient increase in OT secretion, the rate constants for AVP and OT are of similar magnitude. Taken together, these experiments show that dehydration of the jerboa for up to almost 2 months does not significantly impair the time course or the extent of the

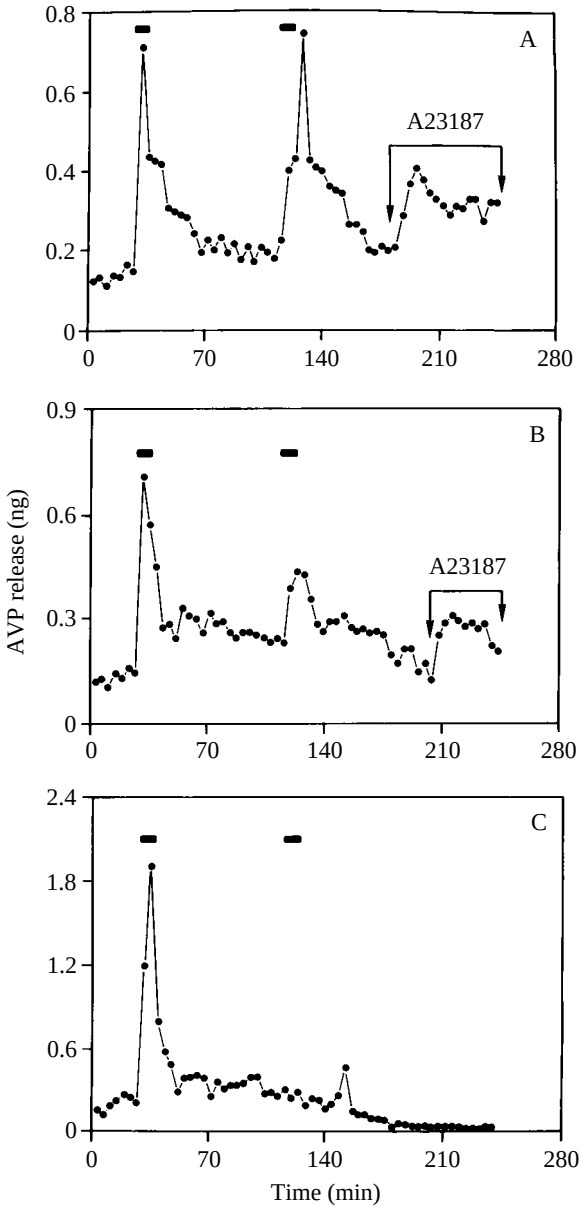

Fig. 2

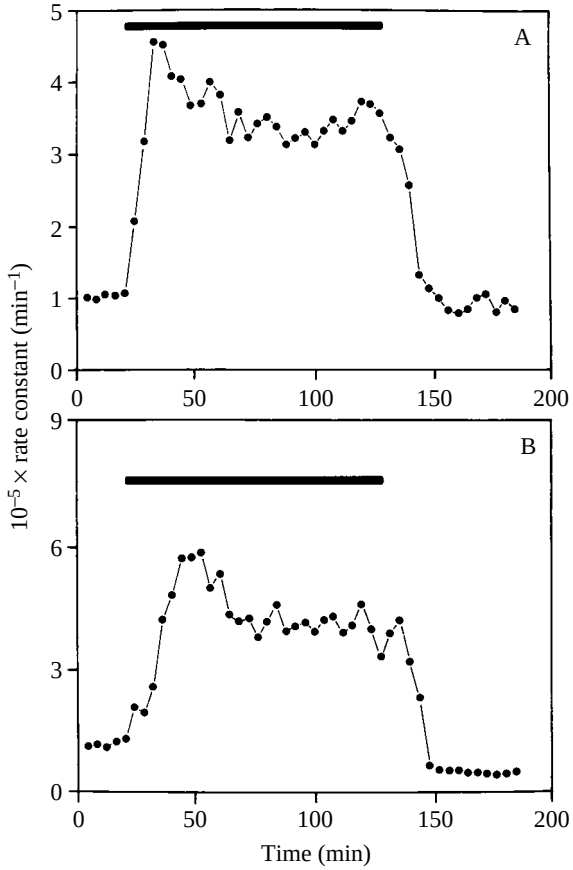

Fig. 3. The effect of prolonged electrical stimulation on the rate of release of vasopressin (AVP) from neurohypophyses isolated from control animals (A) and animals dehydrated for 54 days (B). The neural lobes were stimulated with 150 AVP-like bursts separated by 30 s silent intervals (bars). The results are expressed as the rate constant of AVP release, which was calculated as follows: rate constant (min^{-1})=$H/(\Delta t \times H_t)$, where H represents the amount of hormone released in the time interval Δt, and H_t is the neural lobe hormonal content at the mid-point of the interval Δt (see Cazalis *et al.* 1985, for details).

secretory response induced by prolonged electrical stimulation. Furthermore, the amount of AVP released per burst (see above) is similar regardless of whether the tissue is stimulated with 200 bursts or with 150 bursts, suggesting that, during prolonged stimulation, each burst is equally able to trigger release of neuropeptides.

Capacity of the neural lobe to release AVP and OT during prolonged electrical stimulation and K^+-induced depolarization

During the course of the present study, we often tested the viability of the neurohypophyses at the end of an experiment by stimulating them with perifusate containing 100 mmol l^{-1} K^+ either for a short (6 or 8 min) or for a prolonged (40 or 60 min) period. The amounts of AVP and OT released during 6 min or 40 min of

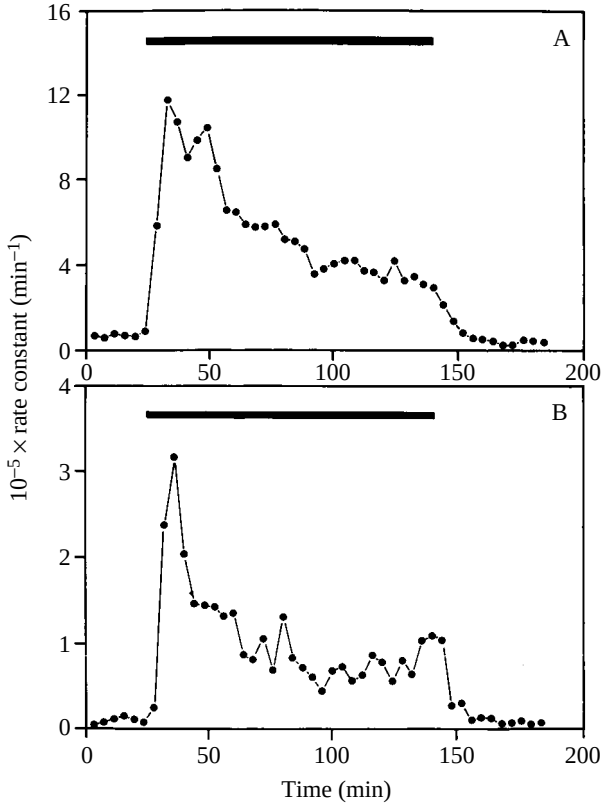

Fig. 4. The effect of prolonged electrical stimulation on the rate of release of oxytocin (OT) from neurohypophyses isolated from control animals (A) and animals dehydrated for 54 days (B). The neural lobes were stimulated with 150 AVP-like bursts separated by 30 s silent intervals (bars). The results are expressed as the rate constant of OT release, which was calculated as described in the legend to Fig. 3.

depolarization did not differ significantly from the amounts released by 8 min or 60 min depolarization periods, respectively. Because of this and the relatively small number of animals available (see Discussion), the results presented above are pooled data. Following a 6–8 min depolarization, neural lobes from dehydrated animals (54 days) released as much AVP as neurohypophyses from control animals. Furthermore, in control animals, 6–8 min of depolarization induced the release of approximately 64 % (AVP) and 74 % (OT) of the neurohormone released after 40–60 min of stimulation. A similar value for AVP (68 %) was observed for neural lobes isolated from dehydrated animals. Thus, as in the rat (see Discussion), a large part of the neurohormone released during sustained K^+-induced depolarization is secreted during the first minutes of stimulation.

The time course of the secretory response during the first seconds of depolarization was studied using a fast perifusion system, as described in the Materials and methods. The isolated neurohypophyseal nerve endings from control animals and animals dehydrated for 65 days responded to successive, brief (20 s) or prolonged (60 s) depolarization

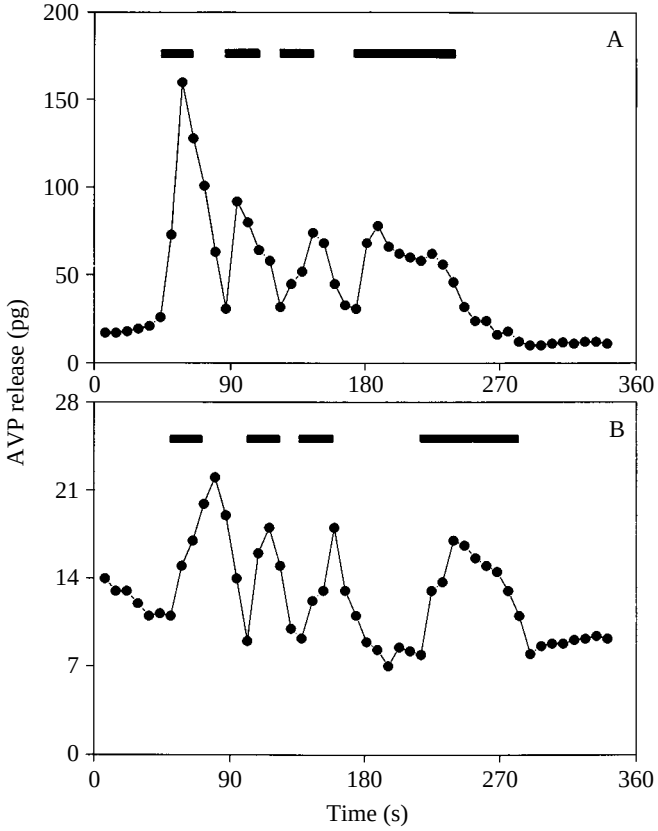

Fig. 5. Vasopressin (AVP) release from isolated nerve endings from control animals (A) or animals dehydrated for 65 days (B) measured using a fast-flow technique. The nerve endings were depolarized with saline containing 50 mmol l^{-1} K^+ (bars).

periods and the time course of the secretory response was similar in both hydrated and dehydrated animals (Fig. 5). This suggests that the membrane properties and the availability of secretory granules at the site of exocytosis in nerve endings from dehydrated animals are very similar to those in control animals.

Fig. 6 illustrates experiments in which neurohypophyses from hydrated animals (Fig. 6B) and animals dehydrated for 22 days (Fig. 6B) were stimulated electrically with 150 AVP-like bursts separated by 30 s silent intervals. At the end of the stimulation period, the neural lobes were returned to saline containing 40 mmol l^{-1} Na^+, and 60 min later they were depolarized for a prolonged period with saline containing 100 mmol l^{-1} K^+. The main results of these experiments are: (i) prolonged intermittent electrical stimulation of neural lobes from control and dehydrated animals induced a maintained release of AVP, whereas continuous K^+ stimulation triggered a robust increase in AVP secretion that decreased over time (Fig. 6); (ii) in both hydrated and dehydrated animals, the release of AVP was much larger during the first minutes of K^+-induced depolarization than during the delivery of bursts of electrical pulses. However, in both control and dehydrated animals, the total amounts of AVP release during electrical or K^+ stimulation

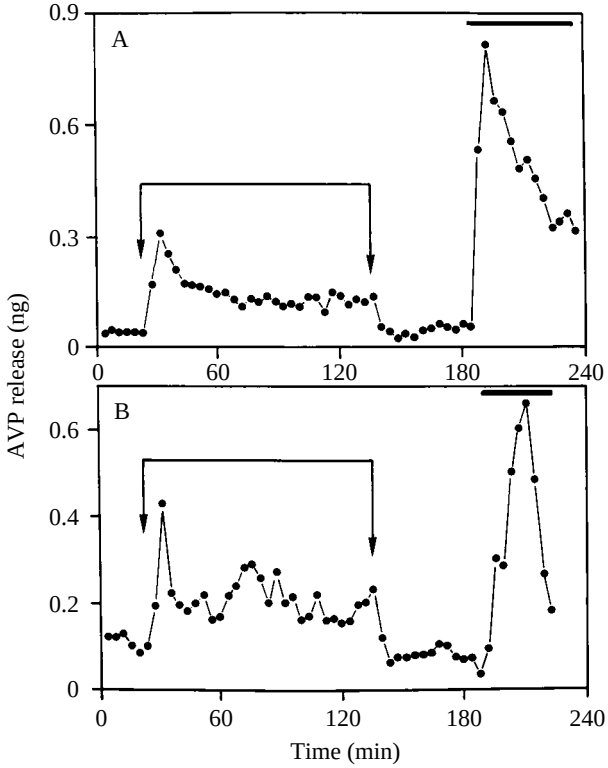

Fig. 6. Effects of prolonged electrical and K^+-induced stimulation on the amount of vasopressin (AVP) released from neural lobes isolated from control (A) and dehydrated (B) animals. The neurohypophyses were stimulated by 150 AVP-like bursts separated by silent intervals (between the arrows). They were further depolarized with saline containing 100 mmol l^{-1} K^+ (bars).

were very similar (values given above). In conclusion, prolonged K^+-induced depolarization gave rise to a large, but not sustained, release of AVP, whereas bursts of electrical pulses separated by silent intervals gave rise to a smaller, but maintained, release of vasopressin. The decrease of secretion during maintained K^+-induced stimulation was not due to exhaustion of the tissue stores of AVP because we found that, during a 40–60 min K^+-induced depolarization, the neurohypophysis released only approximately 0.26 % (controls) or 0.20 % (dehydrated) of its vasopressin content.

Discussion

The aim of the present paper was to study *in vitro* the capacity of the neurohypophysis from control and dehydrated jerboas (*Jaculus orientalis*) to release AVP and OT. The dehydrated animals had access to a dry diet only and were subject to dehydrating stress as judged by the osmolarity of their urine. Jerboas are difficult to catch, so we had only small numbers of animals to work with. It was not, therefore, always possible to apply

meaningful statistics to the results. In the following discussion we therefore include, when necessary, conclusions from some of our previous work and those of others on studies on the rat neural lobe. The main conclusion of this paper is that, despite a very pronounced dehydrated physiological state, the jerboa possess an AVP (and OT) release system that can function adequately even under conditions in which most animals so far studied would have lost their capacity to release neurohypophyseal hormones.

Secretion of neuropeptide can be inhibited (by approximately 70 %) by preincubating the neural lobe with the dihydropyridine nicardipine, a calcium channel antagonist (Fig. 2B). Furthermore, we have also found that *ω*-conotoxin, a toxin isolated from the snail *Conus geographus* (see Olivera *et al.* 1990; Sher and Clementi, 1991; Takeda and Nordmann, 1992), abolishes the release of AVP from depolarized neural lobes (Fig. 2C). This finding is important because it shows that the secretory response in neuropeptidergic nerve endings is dependent upon the activation of both N- and L-type calcium channels. This contrasts with, for instance, what has been found in cerebral synaptosomes, where it seems that the release mechanism is largely dependent upon the activation of the N-type channels (Hirning *et al.* 1988). We also found that half-maximal secretion of AVP occurs at a $[K^+]_o$ of approximately 60 mmol l^{-1}. This value is slightly larger than that calculated using the data obtained from the rat neural lobe (Cazalis *et al.* 1987*a*). Although this could represent some kind of 'protection' against cellular calcium loading in these hyperactive cells, it should be kept in mind that the data were obtained from only two animals and should therefore be treated with caution. The present findings are relevant to any attempt to classify different types of Ca^{2+} channels (for a review, see Tsien *et al.* 1988). Briefly, high-voltage-activated calcium channels have been classified into two subtypes (Bean, 1989; Plummer *et al.* 1989; Swandulla *et al.* 1991; Wang *et al.* 1992). One group, the N-type channels (Nowycky *et al.* 1985), exhibit rapid and complete inactivation that is voltage-dependent; the second type, in contrast, inactivates extremely slowly and is known as the L-type channel. However, this classification, based solely on Ca^{2+} current kinetics, has recently been questioned (Plummer *et al.* 1989; Regan *et al.* 1991) and another high-threshold channel type has been described (Llinas, 1988). In view of recent pharmacological and electrophysiological studies, one could simply classify the high-threshold channels as 'L' (DHP-sensitive), 'P' [funnel web spider toxin (FTX)-sensitive] and '*ω*' (*ω*-conotoxin-sensitive) channels (Sher and Clementi, 1991). The results described above suggest that, in the neural lobe, not only L- but also N-type channels are involved in the stimulus–secretion coupling mechanism. It should be pointed out that the isolated nerve endings from the rat neurohypophysis have been shown to have high-affinity binding sites for *ω*-conotoxin (Dayanithi *et al.* 1988). However, both types of neurohypophyseal high-voltage-activated channels, termed 'Nt' and 'L' (Lemos and Nowycky, 1989), are inhibited, although at 10-fold different concentrations, by *ω*-conotoxin (Wang *et al.* 1992). It is therefore difficult to differentiate unequivocally between Ca^{2+} channels and to force them into preconceived classes based on nomenclature. Molecular biological studies (e.g. Lester *et al.* 1989) should allow more convincing differentiation between channels in the future. In any event, our data extend our previous knowledge of the stimulus–secretion coupling mechanism in the neural lobe (Dayanithi *et al.* 1988) and show that the depolarization-induced secretion of

neurohypophyseal peptides is largely dependent on the activation of DHP-sensitive channels, but that another type of channel also contributes to the activation of the secretory process.

The results also highlight the importance of interburst intervals for maintaining a steady hormone release. We stimulated neurohypophyses with the pattern of electrical activity recorded from magnocellular neurones of the rat hypothalamus under two conditions, dehydration and lactation. These patterns have been shown to potentiate greatly the increase of $[Ca^{2+}]_i$ following depolarization of the nerve terminals (Brethes *et al.* 1987) and AVP/OT secretion from isolated neural lobes (Cazalis *et al.* 1985). We do not know whether the AVP-containing cells in the dehydrated jerboa have a similar pattern of firing, but we have assumed that this is the case. This is because most of the electrical recordings from hypersecreting neurosecretory cells in both vertebrates (see, for example, Poulain *et al.* 1977; Poulain and Wakerley, 1982) and invertebrates (Gillary and Kennedy, 1968; Gainer, 1972; Stuenkel and Cooke, 1988) have so far shown typical bursting activity, and it is likely therefore that jerboa magnocellular neurones also have a similar pattern of firing. The release of neurohormones following electrical depolarization of the jerboa neural lobe results in two major effects. First, when the neurohypophyses are stimulated with four AVP-like bursts separated by silent intervals, the amount of AVP released is equal to four times that induced by one single burst. However, when four identical bursts are delivered without silent intervals, the amount of neuropeptide secreted is not significantly different from that induced by one single burst. Therefore, as has already been observed in the rat neural lobe, the intervals between bursts are crucial for potentiating the mechanism of neurosecretion (see also Gillary and Kennedy, 1968). Our results strengthen the hypothesis that not only $[Ca^{2+}]_i$ (for reviews, see Nordmann, 1983; Augustine *et al.* 1987) but also the changes in $[Ca^{2+}]_i$ are crucial for inducing a robust and sustained secretory response (Knight and Baker, 1982; Lindau *et al.* 1992). For example, in the present study, we found that prolonged depolarization with K^+ gave rise to a large AVP release but that the rate of release was not maintained (Fig. 6). In contrast, bursts of pulses delivered with silent intervals elicited a lower but sustained secretion. Therefore, it appears that nature has developed in neurosecretory systems a firing pattern that allows sustained release of neuropeptides from nerve endings.

We have found in preliminary experiments that the plasma osmotic pressure of dehydrated jerboas is not significantly different from that of control animals (A. Raji and J. J. Nordmann, in preparation). These results, and those of the present study, show that the neural lobe in dehydrated jerboa is well adapted to the semi-desert conditions in which the animals live. It is of major interest to observe that, even after prolonged (up to 54 days) dehydration, the neural lobe can be electrically stimulated for hours and does not show any sign of fatigue. Although the rate constant for the release of OT at the beginning of the stimulation is transiently higher than that for AVP, both secretions have a similar magnitude during prolonged stimulation (Figs 3 and 4). We do not yet know whether this is due to intrinsic properties of the plasma membrane of the OT-containing nerve terminals or to a larger ‘readily releasable’ pool of granules. It is, however, worth mentioning that the same observation has been made in the rat except that, in this case, OT is released steadily whereas AVP secretion has an initial large amplitude and then

decreases to reach a new steady value (Bicknell *et al.* 1984; Cazalis *et al.* 1985). Furthermore, we have used a recently developed fast-flow perifusion technique (Lindau *et al.* 1992), which allows an analysis of the time course of the secretory response at the onset of depolarization, to show that the membrane properties of the isolated nerve endings from dehydrated and control animals are very similar. In addition, the rate of increase in AVP release from isolated nerve endings obtained from dehydrated animals suggests that, even under conditions of increased secretory activity, granules are available at or near the site of exocytosis.

In conclusion, this work which, to our knowledge, is the first study *in vitro* on the capacity of the neural lobe of a desert rodent to release a neurohormone, demonstrates an adaptation of the magnocellular neurone that allows it to release, even after prolonged dehydration, sustained and large amounts of neurohormones. One explanation would be that there is an increase in the rate of AVP secretion, without exhaustion, of a releasable pool of neuropeptides. Taking into account the above results and studies in progress in our laboratory on the turnover of neuropeptides, on the AVP content of the plasma and on the kinetics of exo- and endocytosis, we postulate that, under dehydrated conditions, the jerboa's water balance must largely be maintained as the result of an adaptation of cellular mechanisms at the level of the kidney and not (or only marginally) because of an increased rate of AVP release from the neural lobe. It is worth pointing out that, in the jerboa dehydrated for a prolonged period, specific alterations in kidney Na^+/K^+-ATPase activity have been observed (Baddouri *et al.* 1984; Doucet *et al.* 1987). Further studies at the level of the kidney are necessary to clarify the mechanisms that allow the jerboa to live in such a desert climate.

We thank the shepherds in the Middle Atlas Mountains for much help in catching the jerboas. We also thank John Bicknell and A. P. F. Fink for the gift of AVP and OT antisera, respectively. We are indebted to José Lemos and Ed Stuenkel for helpful suggestions concerning the manuscript. Finally, we thank Marouan Duchenu Boukli and Myriem Balapneu Boukli (Rabat, Morocco) for their patience.

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