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EXTRACELLULAR POTASSIUM CHANGES IN THE RAT NEUROHYPOPHYSIS DURING ACTIVATION OF THE MAGNOCELLULAR NEUROSECRETORY SYSTEM

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SUMMARY

1. Potassium-sensitive microelectrodes were used to measure extracellular [K^+] in the isolated rat neurohypophysis maintained *in vitro*. Electrical stimulation of the neurohypophysial stalk (20 Hz, 5 s) increased the inferred extracellular [K^+] by $9{\cdot}2 \pm 0{\cdot}4$ mM (mean $\pm$S.E. of mean; $n = 21$).

2. Veratridine (10 μM) enhanced the response to stalk stimulation, and at a higher concentration (50 μM) increased extracellular [K^+] in the absence of stimulation. By contrast, tetrodotoxin (1 μM) blocked the [K^+] increase completely and reversibly in each of five experiments, indicating that the increase was a consequence of action potential generation.

3. At the end of brief periods of stimulation, the raised extracellular [K^+] returned to pre-stimulation levels within 30 s. In the presence of ouabain (100 μM), the recovery was slower: the half-decay time was extended by 150–300% in each of three experiments.

4. Replacement of calcium in the medium with cobalt, cadmium or magnesium reduced the amplitude of the [K^+] increase by 26–30%, indicating that the [K^+] increase was largely independent of events subsequent to evoked release of hormone and/or transmitters.

5. Potassium-sensitive microelectrodes were placed in the neurohypophysis of rats anaesthetized with urethane. Electrical stimulation of the pituitary stalk (50 Hz, 5 s) produced transient voltage increases of $7{\cdot}6 \pm 0{\cdot}9$ mV (mean $\pm$S.E. of mean of seven experiments). These voltage increases were similar in magnitude to the response of the electrodes to the addition of $7{\cdot}6 \pm 1{\cdot}0$ mM-K^+ to rat plasma.

6. In seven lactating rats, the suckling of a litter of hungry pups evoked periodic reflex milk ejections, as detected by increases in intramammary pressure. Potassium-sensitive microelectrodes in the neurohypophysis recorded transient voltage increases prior to each milk ejection (0·4–5·5 mV). Each increase preceded an increase in intramammary pressure by 12–30 s.

7. Thus synchronized high-frequency activation of magnocellular neurones can produce large changes in extracellular [K^+]. The implications of these findings for stimulus–secretion coupling in the neurohypophysis are discussed in the light of

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previous reports that hormone release from the neurohypophysis is highly dependent on the frequency and pattern of electrical stimulation.

INTRODUCTION

Rat magnocellular vasopressin neurones fire phasically in response to stimuli which release vasopressin (for review, see Poulain & Wakerley, 1982). Quantitative radioimmunoassay studies have demonstrated that pituitary stalk stimulation which mimics the phasic patterns of vasopressin neurones is much more efficient for releasing vasopressin than continuous stimulation at a constant frequency (Dutton & Dyball, 1979; Bicknell & Leng, 1981; Shaw, Bicknell & Dyball, 1984; Bicknell, Brown, Chapman, Hancock & Leng, 1984; Cazalis, Dayanithi & Nordmann, 1985). A recent study using multiple-unit recording of axon terminals in the neurohypophysis suggests that the excitability of the terminals decreases during continuous stalk stimulation. It appears that phasic patterns may be more efficient for vasopressin release because such patterns provide enough time for recovery from the excitability decrease (Nordmann & Stuenkel, 1986). The mechanisms underlying the decrease in terminal excitability are poorly understood, but the very large changes in ionic environment that can be evoked by neuronal activation in various excitable tissues may themselves influence the excitability of the tissues (for review, see Somjen, 1979).

In the neurohypophysis, the largest changes in extracellular ionic concentrations under physiological circumstances are likely to occur during activation of oxytocin neurones. Reflex milk ejection is evoked by a pulsatile oxytocin release which is triggered by highly synchronized bursts of action potentials in magnocellular oxytocin neurones (Wakerley & Lincoln, 1973; Belin & Moos, 1986). The efficiency of electrical stimulation for hormone release from the excised neurohypophysis depends on the frequency of the stimulation, and the brief, high-frequency bursts (1–3 s at about 50 Hz) observed in oxytocin neurones during reflex milk ejection are particularly efficient for oxytocin release (Ishida, 1970; Dreifuss, Kalnins, Kelly & Ruf, 1971; Leng & Bicknell, 1984). The factors determining the efficiency of stimulus patterns for hormone release are poorly understood. However, high-frequency bursts may evoke a substantial increase in $[K^+]$ in the neurohypophysis, and high extracellular $[K^+]$ will facilitate release of posterior pituitary hormones (Douglas & Poisner, 1964*a*).

The purpose of the present study was to characterize the changes in extracellular $[K^+]$ in the neurohypophysis following stalk stimulation or following the synchronized burst of oxytocin neurones that occurs during reflex milk ejection. To analyse quantitative and pharmacological aspects of the $[K^+]$ response, we have also used an *in vitro* preparation. Some of these data have previously been communicated to the Physiological Society (Leng & Shibuki, 1987).

METHODS

Preparations

In vitro experiments. Thirty-five male rats (150–220 g body weight) were used for experiments *in vitro*. Each rat was stunned and decapitated, and the pituitary was removed and maintained in an

artificial medium of composition (mM): NaCl, 124; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; NaH_2PO_4, 1·25; $NaHCO_3$, 25; glucose, 10, bubbled continuously with 95% O_2–5% CO_2. The neural lobe was separated from the anterior and intermediate lobes, and cut at the mid-sagittal plane to facilitate diffusion. The neurointermediate lobe was also used in some experiments, but the results were not different from those obtained with the isolated neural lobe. The tissue was placed in a Plexiglass chamber covered with a piece of cotton mesh and perifused at 3 ml/min with medium warmed to 36 °C. Potassium-sensitive electrodes were inserted under visual control into the centre of the tissue.

In vivo experiments. Ten male Wistar rats (280–550 g body weight) were anaesthetized with urethane (ethyl carbamate; 1·25 g/kg body wt., I.P.) and the ventral surface of the pituitary and the stalk were exposed transpharyngeally. A bipolar stimulating electrode was placed on the stalk and a K^+-sensitive microelectrode was inserted into the neurohypophysis through the anterior lobe. Ten lactating rats (270–360 g) were anaesthetized with a lower dose of urethane (1·1 g/kg, I.P.) as the higher dose necessary for ventral surgery blocks reflex milk ejection. One inguinal mammary gland was cannulated with polyethylene tubing (o.d. ~ 1 mm) for monitoring intramammary pressure. A burr hole was drilled in the dorsal surface of the skull at the mid-line, 2 mm posterior to bregma, and a K^+-sensitive electrode was inserted stereotaxically into the neurohypophysis through the dorsal surface of the brain. After the operation propranolol was administered (100 μg/kg, I.V.) to facilitate the milk-ejection reflex (Tribollet, Clarke, Dreifuss & Lincoln, 1978), and ten pups separated overnight from their mother were allowed to suckle. At the end of each experiment, the electrode was withdrawn and the animal was killed with pentobarbitone (I.V.). The skull vault was opened, the brain removed, and the electrode lowered again to confirm the location of the electrode tip in relation to the remaining pituitary gland.

Stimulation

In all experiments, a bipolar stainless-steel stimulating electrode (model SNE 100, Rhodes Medical Instruments, CA, U.S.A) was placed on the neural stalk. Pulse trains were programmed using a Digitimer model D4030 pulse generator. For experiments *in vivo*, the outputs of two constant-voltage isolators (Devices, Mk IV) were combined to generate biphasic pulses (2 ms pulse width) with an intensity of 30 V peak-to-peak, which roughly corresponded to 1 mA current intensity. For experiments *in vitro*, outputs of 1 mA peak-to-peak from a pair of constant-current isolators (Grass, constant-current unit) were used after preliminary experiments had established that this current evoked maximal $[K^+]$ increases.

Potassium-sensitive microelectrodes

Glass micropipettes (tip diameter 5–20 μM) were siliconized with Repelcote (Hopkins & Williams) and filled with 150 mM-KCl. As a K^+-exchanger, 5% solution of potassium tetrakis (4-chlorophenyl) borate (Fluka) in 2-nitrophenyloctyl ether (Fluka) was used (Baum & Lynn, 1973). To increase the mechanical stability of the exchanger, a polyvinyl chloride adhesive (Vinyl Mender, Loctite) was added to the solution at a ratio of 2:1. This mixture was introduced into the glass micropipette by negative pressure for 100–300 μM from the tip. These electrodes showed stable K^+ sensitivity for at least 1 week. As a reference electrode, Ag–AgCl wire or a cotton wick attached to the neck muscle of the rat was used for *in vivo* experiments. For *in vitro* experiments, the reference electrode was Ag–AgCl wire, used in some experiments with a saline bridge consisting of a cotton wick saturated with normal medium and enclosed in plastic tubing (except for the tip of the wick). The voltage difference between the two electrodes was monitored through a preamplifier designed for ion-sensitive microelectrodes (Fig. 42 in Thomas, 1978).

The characteristics of the K^+-sensitive electrodes were tested in the Plexiglass chamber, during perfusion with artificial media containing 0, 5, 10 and 20 mM-KCl: the concentration of NaCl was varied inversely to keep the total concentration of KCl and NaCl constant. At least 5 min equilibration time was allowed following a change of medium before voltage measurements were taken, and at least 10 min was allowed following a change to 0 mM-K^+ medium. Voltage differences measured through the artificial media satisfied the equation:

$$V_2 - V_1 = \frac{RT}{F} \log_e \frac{\alpha[Na^+]_2 + [K^+]_2}{\alpha[Na^+]_1 + [K^+]_1}, \quad (1)$$

where the subscripts 1 and 2 correspond to two different media. The calculated values of α from this equation were 0·038±0·001 (mean ±S.E. of mean; $n = 10$ electrodes; calculated from the voltage differences recorded between 0 and 5 mM-K^+); 0·038±0·001 (between 5 and 10 mM-K^+); 0·041±0·001 (between 10 and 20 mM-K^+); and 0·038±0·001 (between 0 and 20 mM-K^+). The consistency of these values indicates that eqn (1) may be applied to a wide range of $[K^+]$ changes. Ten of these electrodes were further calibrated in eight neurohypophyses perifused with artificial media containing 0, 5, 10, 15, 20 and 25 mM-K^+. As only about 70% of the K^+ difference between

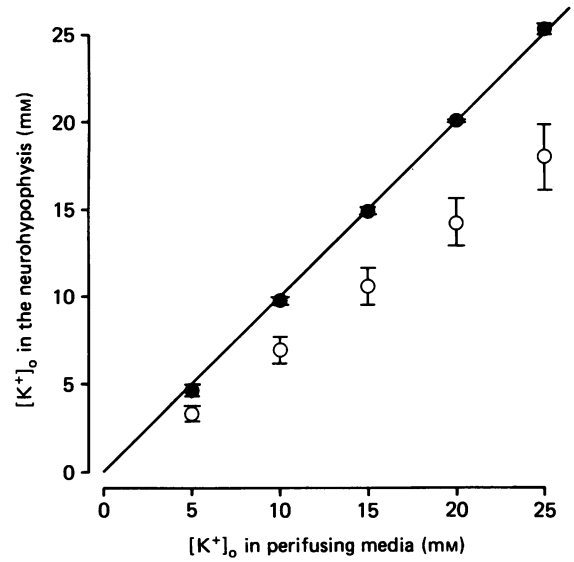

Fig. 1. Relationship between extracellular potassium ($[K^+]_o$) in the perifusate (abscissa) and that inferred in the neurohypophysis (ordinate). Neurohypophysial $[K^+]_o$ was calculated from the voltage change recorded through K^+-sensitive electrodes using eqn (1) in the text (○) or eqn (2) (●). The vertical bars show standard deviations of eight experiments. The straight line indicates equality of inferred $[K^+]_o$ and the actual concentration in the perifusate: eqn (2) provides a good fit to this line.

media was detected by these *in situ* measurements (Fig. 1), we inferred the presence of an interference factor (I). *In situ* the electrodes satisfied the modified equation:

$$V_2 - V_1 = \frac{RT}{F} \cdot \log_e \frac{\alpha[Na^+]_2 + [K^+]_2 + I}{\alpha[Na^+]_1 + [K^+]_1 + I}. \quad (2)$$

Thus the interference factor reduced the sensitivity of the electrodes to changes in $[K^+]$ in a manner similar to the additional presence of an unaccounted K^+ level. Assuming that I was equivalent to 2·5±0·2 mM-K^+ (mean ±S.E. of mean of estimates derived from eight experiments), the electrodes measured $[K^+]$ changes in the perifusate very accurately (Fig. 1).

Calibration of K^+-sensitive electrodes

Each electrode was calibrated in solutions containing 5 and 10 mM-KCl, and α was calculated from eqn (1). Voltage changes recorded in *in vitro* experiments were converted to $[K^+]$ changes using eqn (2), assuming that $I = 2{\cdot}5$ mM-K^+ and that changes in $[K^+]$ and $[Na^+]$ were reciprocal.

For *in vivo* experiments, it was inappropriate to use the value for I calculated from *in vitro* calibrations. The neurohypophysis lies outside the blood–brain barrier: the capillary vessels in the neurohypophysis are fenestrated (Broadwell & Brightman, 1976), and hence substances in plasma will penetrate the extracellular space. We therefore calculated a value for I corresponding to the behaviour of the electrodes in plasma. Trunk blood from five decapitated rats was pooled into a

heparinized beaker, and the plasma was immediately separated by centrifugation. The plasma was divided into five samples, and 0, 5, 10, 15 or 20 mM-KCl was added to each. From the voltage differences measured between each pair of samples with an electrode for which α had been calculated, and using a cotton wick reference electrode, the basal value for $[K^+]+I$ was calculated to be 16·5 mM-K^+ from eqn (2) (assuming a plasma concentration of 150 mM-Na^+). Voltage changes recorded *in vivo* were converted into $[K^+]$ changes using eqn (2) with these values.

Drugs

In different experiments, tetrodotoxin (1 μM; Sankyo), quinidine (100 μM; Sigma), veratridine (10, 50 μM; Sigma), ouabain (100 μM; Sigma) were added to normal medium.

Calcium replacement. In three experiments, the effects of replacing calcium in the medium with equimolar cadmium, magnesium or cobalt were studied. For these experiments the normal medium was modified (for cadmium the composition (mM) was: NaCl, 132; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; glucose, 10; HEPES, 10; pH = 7·4; and for cobalt it was NaCl, 127; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 5; NaH_2PO_4, 0·5; glucose, 10; HEPES, 10; pH = 7·4) to avoid precipitation. Calcium replacement did not affect the calibration K^+ sensitivity of the electrodes.

RESULTS

Potassium response evoked by stalk stimulation in vitro

By contrast with the recordings *in vivo* (see below), there was little difficulty about translating voltage responses measured from the isolated neurohypophysis *in vitro* into K^+ concentrations, as electrodes could be calibrated *in situ* (Fig. 1). Highly reproducible $[K^+]$ increases were evoked by stalk stimulation *in vitro* (Fig. 2*Aa*). Because a field potential component of only about 0·2 mV was recorded through micropipettes which contained no ion-exchanger (Fig. 2*Ab*), the voltage deflections

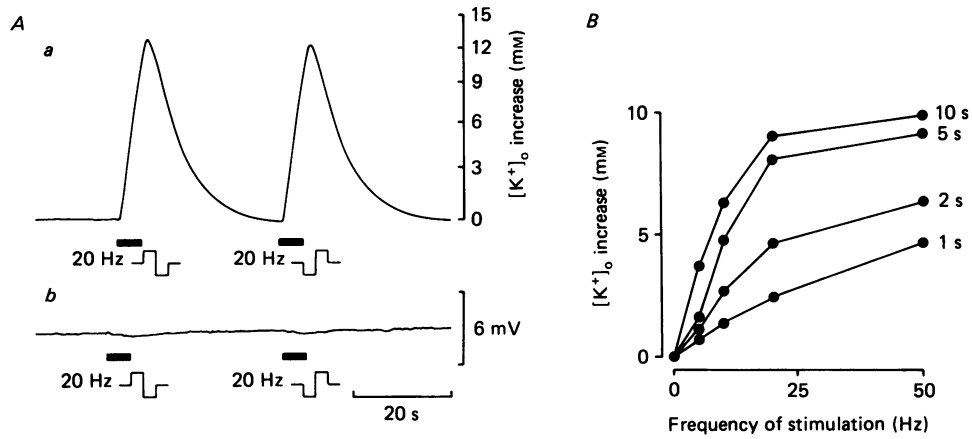

Fig. 2. Potassium ($[K^+]_o$) increases measured in the neurohypophysis during electrical stimulation of the neural stalk *in vitro*. *Aa*, record obtained through a K^+-sensitive electrode. *Ab*, control record obtained from the same preparation through a similar glass micropipette containing no ion-exchanger. The bars beneath each trace indicate timing of stalk stimulation (20 Hz, 5 s) of different polarities as indicated. Both traces share the same voltage scale. *B*, dependence of the $[K^+]_o$ response on the stimulus parameters. Abscissa, frequency of stimulus pulses. Ordinate, maximal K^+ concentration attained by stalk stimulation. The numbers at the right of each trace show duration of pulse trains. Each point represents an average of three trials in the stimulus condition. All data were obtained from one representative preparation.

recorded by K^+-sensitive electrodes *in vitro* were assumed to be evoked almost solely by changes in extracellular $[K^+]$. Stalk stimulation (20 Hz, 5 s) increased the measured $[K^+]$ by $9{\cdot}2 \pm 0{\cdot}4$ mM (mean $\pm$ S.E. of mean, $n = 21$). The half-maximal K^+ concentration was attained 1–3 s after beginning stalk stimulation, and after the end of stimulation the K^+ concentration decreased to the half-maximal level within 3–10 s. After the $[K^+]$ returned to the pre-stimulation level, a small undershoot of $[K^+]$ was recorded (between 0 and 0·9 mM-K^+). Short pulse trains (1 s duration) evoked responses whose amplitude was approximately linearly proportional to the pulse frequency (Fig. 2*B*). With longer pulse trains, the responses saturated at an achieved extracellular $[K^+]$ of about 15 mM. During prolonged stimulation, the

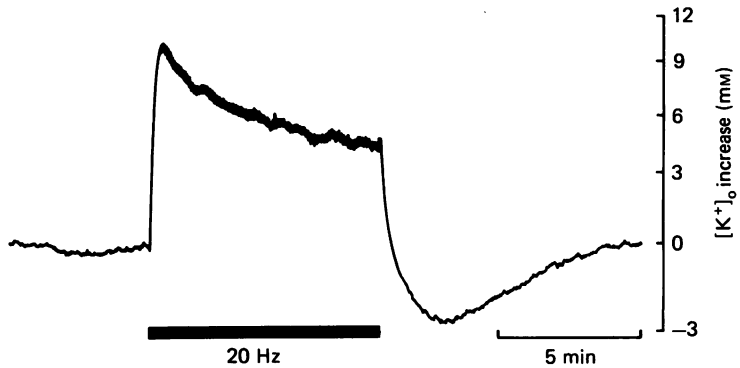

Fig. 3. Effects of continuous stalk stimulation (20 Hz, 8 min) on $[K^+]_o$ in the neurohypophysis *in vitro*. The maximal K^+ concentration was not sustained during prolonged stimulation. Note the marked undershoot of extracellular $[K^+]$ measured after the end of stimulation.

extracellular $[K^+]$ remained above the pre-stimulated level, but the maximal concentration was not maintained (Fig. 3): within about 5 min of stimulation at 20 Hz, the $[K^+]$ had decreased to the half-maximal level. Cessation of the stimulation evoked a consistent undershoot of 2–3 mM-K^+ for about 5 min.

Effects of sodium channel modulation on the $[K^+]$ *response to stalk stimulation*

Application of 1 μM-tetrodotoxin suppressed the $[K^+]$ response to stimulation gradually, and finally blocked it completely in each of four experiments (Fig. 4). In every case, after the perifusing medium was returned to normal medium, the response recovered, though incompletely. A second application of tetrodotoxin blocked the $[K^+]$ response faster and for longer (Fig. 4). In contrast to tetrodotoxin, veratridine (10 μM), which keeps sodium channels open, evoked a $[K^+]$ increase with a slow time course which followed the initial response evoked by stalk stimulation (Fig. 5*A*). Veratridine at a higher dose (50 μM) increased the basal K^+ concentration and within a few minutes evoked large $[K^+]$ oscillations (up to 37 mM; Fig. 5*B*). The effects of 50 μM-veratridine were completely abolished in the presence of 1 μM-tetrodotoxin. Veratridine did not affect the K^+ sensitivity of the electrodes *in situ*: the voltage response to a change from 5 to 10 mM-KCl was unaltered in the combined presence of 1 μM-tetrodotoxin and 50 μM-veratridine.

Effects of ouabain on the K^+ release

The time necessary for $[K^+]$ to change from 10 to 7·5 mM after a step change of $[K^+]$ in the medium from 10 to 5 mM was very slow (0·78 ± 0·06 min) compared with the half-decay time of the $[K^+]$ response to stimulation (3–10 s). We therefore looked for evidence of an active clearance mechanism responsible for the $[K^+]$ decrease following stimulation.

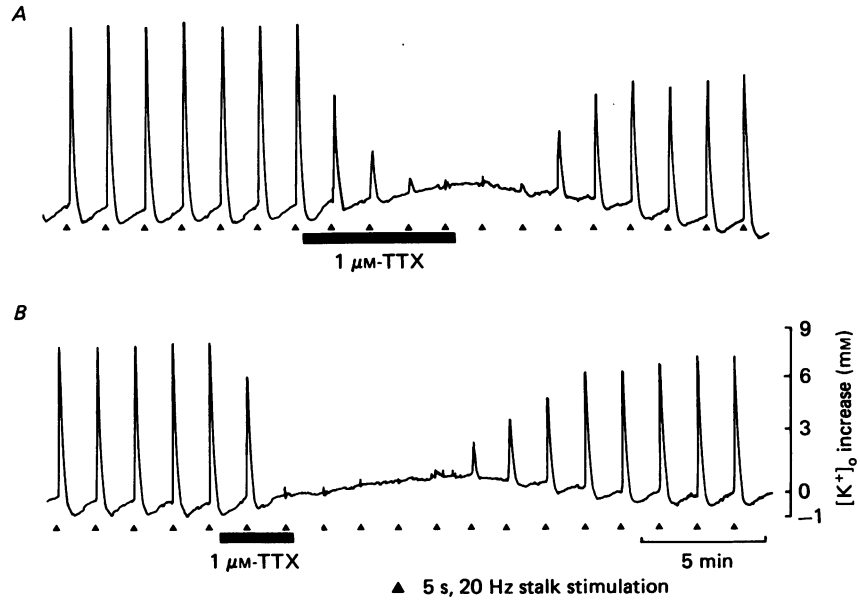

Fig. 4. Continuous traces showing the effects of tetrodotoxin (TTX) on the $[K^+]$ response evoked by stalk stimulation *in vitro*. Arrowheads indicate the initiation of stalk stimulation (20 Hz, 5 s) given at 90 s interval: each stimulation produces a large rise in $[K^+]_o$ followed by an undershoot after the end of stimulation. The bars beneath the traces indicate the application of 1 μM-TTX. Tetrodotoxin reversibly and repeatably blocked both the rise in $[K^+]_o$ evoked by stalk stimulation, and the undershoot following the end of stimulation. Thus the $[K^+]_o$ changes evoked by stalk stimulation *in vitro* are the result of action potential generation.

It appeared most likely that K^+ would be cleared from the extracellular medium by Na^+–K^+ pump activity in the terminal membranes. To test this possibility, the effects of the Na^+–K^+ pump blocker ouabain on the response to stimulation were studied (Fig. 6). Ouabain did not affect the K^+ sensitivity of the electrodes under calibration conditions when no tissue was present. In each of three experiments the half-decay time of the $[K^+]$ decrease was extended, reversibly and repeatably, by 150–300% in the presence of ouabain (100 μM). Furthermore, both the basal and the maximal evoked K^+ concentrations were increased by 2·2–3·0 and 0·9–5·0 mM respectively. Thus it seems that the Na^+–K^+ pump determines, at least in part, the time course of the $[K^+]$ decrease and the saturation level of the K^+ concentration achieved by stalk stimulation.

Effects of calcium replacement on the [K⁺] response to stalk stimulation

Although most of the K^+ released during stimulation is likely to result from action potentials in the neurosecretory axons and terminals, it is possible that the pituicytes or the terminals themselves may also release K^+ through events which occur following hormone or transmitter release. In three experiments we blocked hormone and

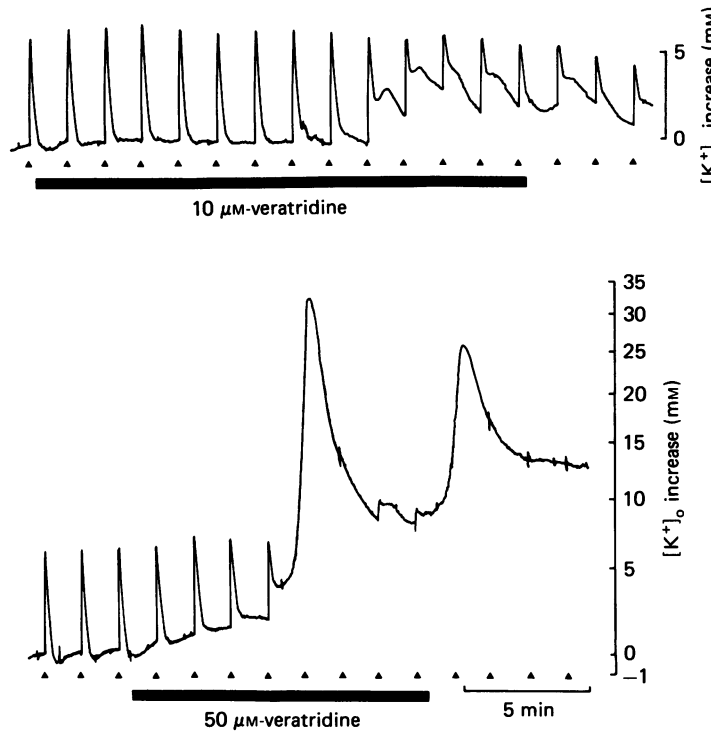

Fig. 5. Effects of veratridine on the [K⁺] response to stalk stimulation *in vitro*. *A*, application of 10 μM-veratridine (bar). *B*, application of 50 μM-veratridine. Arrowheads mark periods of electrical stimulation of the neural stalk (20 Hz; 5 s; 1 mA).

transmitter release by replacing calcium in the perifusing medium with equimolar cobalt, cadmium, or magnesium (one experiment for each condition). In each case, calcium replacement suppressed the [K^+] response to stimulation only by 26–30%, and had no effect upon the measured basal extracellular [K^+]. This indicates that at least 70% of the K^+ release occurred before hormone or transmitter release (Fig. 7). The soma membrane of supraoptic neurones has been reported to contain calcium-activated K^+ channels (Andrew & Dudek, 1984). However, it seems unlikely that the reduced [K^+] response to stimulation was the result of blockage of this channel, since in one experiment quinidine (100 μM), which blocks calcium-activated K^+ channels at the soma, had no effect on the [K^+] response to stimulation (data not shown).

Voltage changes recorded with K^+-sensitive electrodes following stalk stimulation in vivo

Potassium-sensitive electrodes placed in the rat neurohypophysis after ventral

exposure of the pituitary recorded repeatable voltage increases following brief, high-frequency stimulation of the neural stalk (1–5 s at 50 Hz). The voltage changes were independent of the polarity of the electrical stimulation, and similar glass micropipettes which contained no ion-exchanger recorded a much smaller voltage decrease in response to stimulation (Fig. 8*A*). The responses recorded using K^+-sensitive

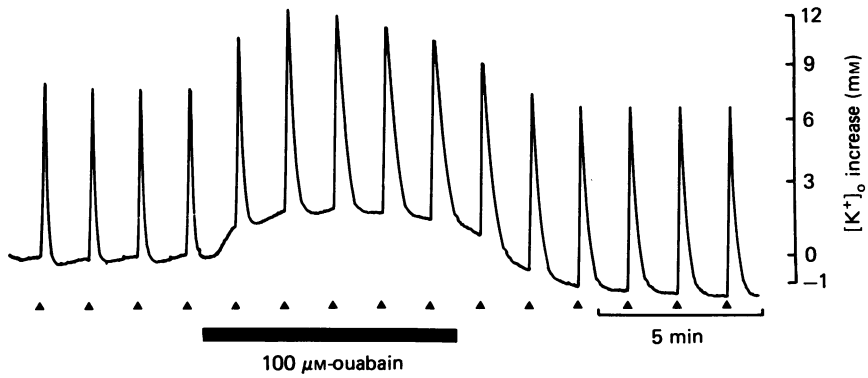

Fig. 6. Effects of 100 μM-ouabain application on the $[K^+]_o$ response to stalk stimulation *in vitro*. During ouabain exposure, the basal extracellular concentration is elevated, as is the response to stimulation, and the decay time of the raised $[K^+]_o$ is prolonged. Arrowheads mark periods of electrical stimulation of the neural stalk (20 Hz; 5 s; 1 mA).

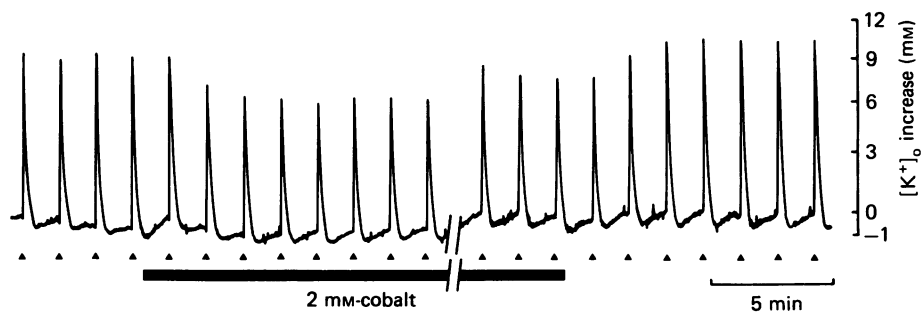

Fig. 7. Effects of replacement of calcium with cobalt on the $[K^+]_o$ response to electrical stimulation of the neural stalk *in vitro*. Calcium replacement resulted in a 28% reduction on the $[K^+]_o$ increase evoked by stimulation (arrows; 20 Hz, 5 s, 1 mA). As such calcium replacement completely blocks stimulus-evoked hormone release from the neurohypophysis, we conclude that most of the K^+ release in response to stimulation occurs prior to any hormone release.

electrodes appeared to consist of two components: a rapid, small negative voltage change similar to that recorded through normal glass pipettes, and a slower, more persistent voltage increase (Fig. 8*B*). Assuming that the former was a recording of a field potential and that the latter reflected $[K^+]$ changes, we calculate that the field effects may cause approximately 11–12% underestimate of the $[K^+]$ changes resulting from stimulation, but the following data are uncorrected for this factor.

In seven rats stalk stimulation at 50 Hz for 5 s resulted in a voltage increase of $7{\cdot}6 \pm 0{\cdot}9$ mV (mean $\pm$S.E. of mean) as measured with K^+-sensitive electrodes placed in the neurohypophysis. The maximal voltage was attained within 2–3 s, and after-

wards a gradual decrease followed (Fig. 8*Aa*). After cessation of the stimulation the voltage returned to pre-stimulation levels within 2–3 s, followed by a voltage undershoot of between 1·2 and 2·8 mV. Thus the decline in extracellular [K^+] following the end of stimulation was much more rapid *in vivo* than *in vitro* (cf. Figs 1 and 8). The voltage response of K^+-sensitive electrodes following stalk stimulation was recorded only at a very narrow range of depths from the ventral surface of the pituitary: this range corresponded closely with the location of the neurohypophysis (Fig. 8*B*).

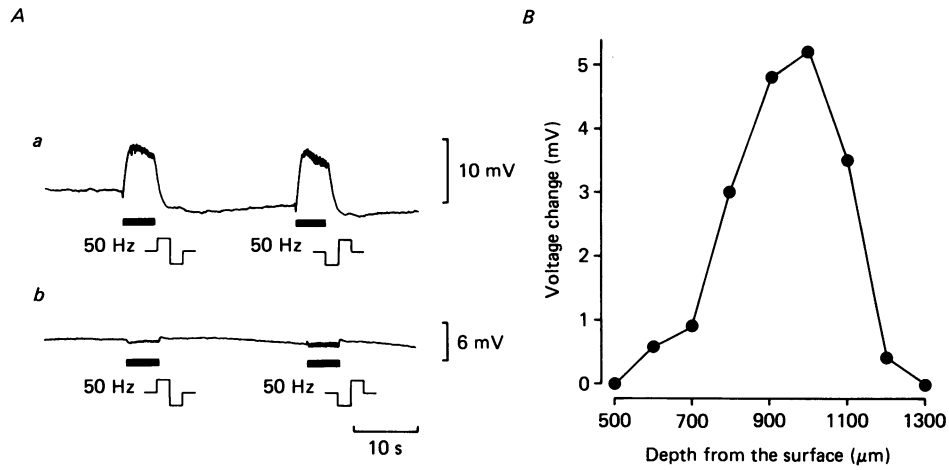

Fig. 8. Extracellular [K^+] increase in the neurohypophysis evoked by stalk stimulation *in vivo*. *Aa*, record obtained through a K^+-sensitive electrode in the neurohypophysis. Bars beneath the traces show timing of stalk stimulation (50 Hz, 5 s). The stimulus polarity was reversed for the second stimulation. *Ab*, control record obtained through a normal glass micropipette filled with 150 mM-KCl. Both *a* and *b* share the same voltage scale as shown at the right of *b*. The vertical scale in *a* represents voltage change evoked by $[K^+]_o$ increase from 5 to 10 mM and recorded through the K^+-sensitive electrode used. *B*, depth profile of $[K^+]_o$ responses evoked by stalk stimulation (50 Hz, 2 s) obtained from one rat. Abscissa, depth of the electrode tip from the ventral surface of the pituitary. Ordinate, maximal voltage response to stalk stimulation.

The measured voltage changes are consistent with a large change in extracellular [K^+], but a number of factors make it impossible for us to express accurately the observed voltage changes in terms of [K^+] changes. Most importantly, the sensitivity of our K^+-sensitive electrodes to changes in plasma [K^+] was much less than their sensitivity to changes in the K^+ concentration of artificial medium, indicating that unidentified factors in plasma interfered with the ion-exchanger. Assuming that the K^+-sensitive electrodes in the neurohypophysis behaved similarly to electrodes in plasma, then the maximal response observed to electrical stimulation corresponds to an increase of $7{\cdot}6 \pm 1{\cdot}0$ mM-K^+. Making the more conservative assumption that the electrode sensitivity was similar to that recorded *in vitro*, stimulation produced a rise of $4{\cdot}4 \pm 0{\cdot}6$ mM-K^+. These values are also underestimated by 11–12% if the field potential effects are taken into account.

Potassium increases measured during the milk-ejection reflex

In seven out of nine lactating rats which showed reflex milk ejection under urethane anaesthesia, a K^+-sensitive electrode placed in the vicinity of the neurohypophysis recorded voltage increases of 0·4–5·5 mV (2·5 ± 0·7, mean ± S.E. of mean) at 5–20 min intervals (Fig. 9). Again, these responses to suckling could only be

Fig. 9. Polygraph records showing voltage changes recorded with a K^+-sensitive microelectrode in the neurohypophysis (upper trace) and simultaneously recorded intramammary pressure changes (lower trace) during reflex milk ejection in a lactating rat suckled by ten hungry pups. Each milk ejection, marked by a rise in intramammary pressure, was preceded 12–30 s earlier by voltage changes indicative of a large rise in $[K^+]_o$.

recorded from a narrow range of depths (400–600 μM at about 9 mm from the dorsal surface of the brain). Although there was a large variance in the amplitude between animals, each rat showed very consistent voltage responses at any one electrode depth. There is a non-uniform distribution of oxytocin terminals within the neurohypophysis (Van Leeuwen, De Raay, Swaab & Fisser, 1979): thus the largest measured voltage increase (5·5 mV) which is near to that achieved by 50 Hz stalk stimulation (7·6 mV) probably most accurately reflects the actual $[K^+]$ increase achieved by excitation of a high density of oxytocin terminals. Each voltage increase was followed by a voltage decrease (between 0·1 and 1·2 mV) to below the basal level (Fig. 2), and the total duration of the biphasic response was between 25 and 35 s. Each voltage change was followed by an increase in intramammary pressure characteristic of reflex milk ejection with a latency of between 12 and 30 s. The high-frequency burst discharge of oxytocin cells precedes an increase in intramammary pressure by 15–20 s (Wakerley & Lincoln, 1973) and is followed by several seconds of electrical quiescence: hence the observed $[K^+]$ changes were apparently coincident with the electrical activation of the oxytocin system.

Assuming that the K^+-sensitive electrodes in the neurohypophysis behaved similarly to electrodes in plasma, then the maximal response observed during reflex milk ejection corresponds to an increase of 5·3 mM-K^+. Making the more conservative assumption that the electrode sensitivity was similar to that recorded *in vitro*, the milk-ejection reflex was accompanied by rises of up to 3·2 mM. Again, these values are underestimated by 11–12% if the field potential effects are taken into account.

DISCUSSION

Potassium-sensitive microelectrodes placed in the rat neurohypophysis *in vivo* recorded reproducible voltage changes following electrical stimulation of the neural stalk. These changes were independent of the stimulus polarity, were evoked by stimulus currents similar to those required to activate magnocellular neurones antidromically, and were restricted to the neurohypophysis itself. Previous studies of extracellular [K^+] changes in brain have used double-barrelled electrodes to subtract the field potential component from the voltage change resulting from [K^+] change (Vyskočil & Kříž, 1972). In the neurohypophysis, the contribution of the field potential was very small (10% *in vivo*, 2% *in vitro*), and so an immediately adjacent reference electrode was considered unnecessary.

The discrepancy between the absolute magnitude of the voltage increases recorded *in vivo* and those recorded *in vitro* following stalk stimulation probably arises from a number of factors in addition to the demonstrated interference by substances in plasma. Firstly, the field potential interference was greater *in vivo*, leading to a greater degree of underestimation of the [K^+] change. Secondly, the relatively large electrode tip will measure *in vivo* a K^+ concentration that is a weighted mean of the blood concentration and the extracellular concentration. Finally, and most importantly, wash-out of extracellular K^+ must be more efficient in the presence of normal vascular perfusion of the neurohypophysis. In particular, this last factor would explain the more rapid return to normal of extracellular [K^+] following stimulation *in vivo* compared to *in vitro*.

The neurohypophysis contains glial cells (pituicytes) but has no diffusion barrier between the extracellular space and capillary vessels (Broadwell & Brightman, 1976). Elsewhere, glial cells are believed to serve as a spatial buffering system for controlling the extracellular ionic environment (Orkand, Nicholls & Kuffler, 1966). According to the studies of Gardner-Medwin and his co-worker (Gardner-Medwin, 1983; Gardner-Medwin & Nicholson, 1983), about 80% of the K^+ flux evoked by an electrochemical gradient through the rat brain passes through glia rather than through the extracellular space. The pituicytes extend their processes both between the terminals and the space surrounding capillary vessels (Hatton, Pearlmutter, Salm & Tweedle, 1984), and thus probably provide a major, and highly efficient route of diffusion from the extracellular space to the circulation *in vivo*. Our observations that the [K^+] increase evoked by 50 Hz stalk stimulation *in vivo* is smaller than that evoked by 20 Hz stimulation *in vitro* certainly suggest that K^+ clearance into the blood is both rapid and efficient *in vivo*.

The maximal extracellular [K^+] measured in the neurohypophysis *in vitro* was attained within a few seconds of stimulation at 20 Hz, but was not maintained during continued stimulation. The observed profile was very similar to the profile of extracellular [K^+] changes surrounding photoreceptors of the drone compound eye during photic stimulation (Coles & Tsacopoulos, 1979; Coles & Orkand, 1983). In these studies, the intracellular [K^+] was also measured, and showed a marked fall during photic stimulation with a time course similar to that of the decline in extracellular [K^+]. Such large extracellular and intracellular [K^+] changes will be accompanied by reciprocal changes in [Na^+]. In percentage terms, extracellular [K^+]

and intracellular [Na^+] will change much more than intracellular [K^+] and extracellular [Na^+], respectively, and these larger changes will have most effect upon the excitability of axons and terminals. Thus sustained stimulation of the neural stalk is likely to evoke (a) a sustained depolarization of the terminal membranes in the neurohypophysis as a result of the rise in extracellular [K^+] which develops within a few seconds of the onset of stimulation, and (b) a decrease in amplitude of action potentials as a result of a rise in intracellular [Na^+] which develops over minutes rather than seconds. We know from release studies *in vitro* that, for brief periods of stimulation, release of hormone from the neurohypophysis is facilitated at high frequencies of stimulation, and that this facilitation is followed by 'fatigue' of stimulus–secretion coupling (Bicknell *et al.* 1984; Shaw *et al.* 1984; Cazalis *et al.* 1985; Nordmann & Stuenkel, 1986): during prolonged stimulation the high initial rates of secretion decline dramatically within 1–2 min. We suggest therefore that frequency facilitation and fatigue of hormone release are, at least partly, consequences of changes in terminal excitability that result from rises in extracellular [K^+] and intracellular [Na^+], respectively.

Our recordings using K^+-sensitive microelectrodes *in vivo* indicate that, during suckling-induced reflex milk ejection, the extracellular [K^+] in the rat neurohypophysis can rise by between 3·2 and 5·3 mM (values from alternative calibrations): even the lower value is one of the largest [K^+] increases ever recorded under physiological conditions in mammals (Somjen, 1979). Although high K^+ concentrations inhibit synaptic transmission at the squid giant axon (Erulkar & Weight, 1977), high extracellular [K^+] increases both the frequency of miniature end-plate potentials and the quantal content of end-plate potential evoked by stimulation at the neuromuscular junction of the frog (Takeuchi & Takeuchi, 1961) and the rat (Parsons, Hofmann & Feigen, 1965).

In the rat neurohypophysis, what consequences will this transient [K^+] increase have for hormone release? High extracellular [K^+] may facilitate hormone release either by generation of additional action potentials in neurohypophysial axons by direct depolarization of axonal membranes, or more probably by sustaining raised intracellular calcium levels by slowing the repolarization of the terminal membrane after an action potential. Spike generation by high potassium may explain the [K^+] response observed during veratridine application (Fig. 5): such mechanisms have been suggested to explain oscillatory changes of [K^+] in the rat optic nerve (Connors & Ranson, 1984). On the other hand, high K^+ concentrations are known to stimulate hormone release in the neurohypophysis in a calcium-dependent manner (Douglas & Poisner, 1964*a*, *b*). Although the threshold of K^+ level for hormone release is higher than that evoked by stalk stimulation, a calcium increase inside the terminals evoked by subthreshold K^+ level may facilitate hormone release evoked by stalk stimulation. Finally, under conditions requiring chronically elevated hormone secretion from the neurohypophysis such as lactation, parturition or dehydration, the morphology of the pituicytes alters dramatically (Tweedle & Hatton, 1980, 1982; Hatton *et al.* 1984). In the normal, unstimulated, condition the neurosecretory axons are enveloped by pituicyte processes, and many terminals are completely engulfed by pituicyte cytoplasm. However, in the lactating rat, for example, these processes are retracted away from the terminal regions of the neurosecretory axons. If, like glial cells

elsewhere, one role of pituicytes is to buffer the changes in extracellular [K^+] induced by neurosecretory activation, it seems likely that their ability to buffer such changes will change with the morphological reorganization. We may thus expect that, if extracellular [K^+] changes do facilitate hormone release, such facilitation will be more marked in the lactating or parturient animal, when greatest hormone release is demanded.

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