Prolonged dehydratation of Jerboa (*Jaculus orientalis*) inhibits the increase of intracellular concentration of calcium induced by vasopressin and prostaglandin E₂ in the renal medullary collecting ducts

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Abstract

The effect of dehydratation of Jerboas on the response to arginin-vasopressin (AVP), prostaglandin E₂ (PGE₂) and the muscarinic agonist carbachol was investigated in outer medullary collecting ducts (OMCD) micro-dissected from collagenase treated Kidney. The action of these agonists was tested on the variation of the intracellular calcium concentration ([Ca²⁺]ᵢ). On the OMCD micro-dissected from hydrated Jerboas, AVP induced an accumulation of adenosine 3',5'-cyclic monophosphate (cAMP) and an increase of [Ca²⁺]ᵢ. Tested in the same conditions, PGE₂ and carbachol increases the [Ca²⁺]ᵢ in Jerboa’s OMCD. When Jerboas were dehydrated for several days, we observed that: (1) the increase of both cAMP and [Ca²⁺]ᵢ induced by AVP was inhibited after 2 weeks of dehydratation; (2) the biphasic increase of [Ca²⁺]ᵢ induced by PGE₂ was markedly reduced by 86.7% after 2 weeks of dehydration; (3) the effect of carbachol on [Ca²⁺]ᵢ was not modified by the dehydration (only 14,3% of inhibition after 3 weeks of dehydration). These results indicate that dehydratation of Jerboa impairs the effect of AVP and PGE₂ in the renal OMCD.

Key Words: Micro-dissected ducts, cAMP accumulation, intracellular Calcium, dehydratation, desert Rodent.

Introduction

Desert Rodents including Jerboa (*Jaculus orientalis*) are able to excrete highly concentrated urine in response to chronic water deprivation. Two major processes are responsible for this physiological characteristic. The first was the reabsorption of NaCl, without H₂O by the thick ascending limb of Henle’s loop (Morel et al., 1987) and the second was the reabsorption of H₂O by the collecting ducts (Abramow et al., 1987) Brown, 1991) which generated cAMP. This action of AVP on cAMP was mediated by V₂ receptors (Jard, 1988).

The reabsorption of water was highly stimulated by antidiuretic hormone (AVP; AVP induces also in the collecting ducts an increase of [Ca²⁺]ᵢ. This effect is realized by occupancy of V₂ and V₁ receptors (Champigneulle et al., 1993, Imbert-Teboul et al., 1993).

The first purpose of this study is to determine the effect of AVP, PGE₂ and carbachol (muscarinic agonist) on the increase of [Ca²⁺]ᵢ in the OMCD of hydrated Jerboas. It was shown previously that during dehydration, Jerboas has a high level of circulating antidiuretic hormone above 400 pmol/l which is about 20-fold higher than hydrated Jerboa.

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(Baddouri et al., 1981). In the second part, we will investigate the variation of the response to these agonists after dehydration of Jerboas.

The results obtained show that in the OMCD of normal Jerboas AVP, PGE2 and carbachol increases the [Ca^{2+}]i. During dehydration of Jerboas, the responses to AVP and PGE2 were reduced but not the response to carbachol.

Materials and methods

Collagenase A (0.452 U/mg) and adenosine deaminase were purchased from Boehringer Mannheim (Germany). Bovine serum albumin (fraction V, fatty acid free) was from Miles Laboratories, Napperville, IL, USA. The phosphodiesterase inhibitor Ro 20-1724 [4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone] was from Paesel & Lorei Gmbh & Co. (Frankfurt, Germany). 125I - cAMP succinyl-tyrosine methyl ester and anti-cAMP antibody were obtained from ERIA Diagnostics Pasteur (Marnes-La-Coquette, France). Arginine-vasopressin was obtained from Ferring (AB Malmš, Sweden), Prostaglandin E2 (PGE2), 3-Isobutyl-1-methyl xanthine and carbachol were from sigma (St. Louis, Mo, USA). All other components were from Merck (Darmstadt, Germany).

cAMP accumulation experiments

The measurement of cAMP in isolated single tubules by radioimmunoassay was performed as described previously (Chabardès et al., 1990) and will be briefly recalled.

Solutions

The microdissection solution was composed of (mM): NaCl, 137; KCl, 5; MgSO4, 0.8; Na2HPO4, 0.33; KH2PO4, 0.44; MgCl2, 1; NaHCO3, 4; CH3COONa, 10; CaCl2, 1; glucose, 5; HEPES, 20, pH 7.4 and bovine serum albumin 0.1% (w/v). Collagenase was dissolved in microdissection solution. The incubation solution has the same composition as the microdissection solution but included 0.1% (w/v) bacitracin and 50 mM Ro 20-1724, inhibitor of phosphodiesterase type IV (Beavo, 1988) or 1 mM 3-isobutyl-1-methylxanthine (IBMX inhibitor of all phosphodiesterase activity). All solutions contained adenosine deaminase (0.5 U/ml) to prevent a possible accumulation of adenosine in the medium, indomethacin (5 μM) to inhibit the cyclooxygenase activity and to prevent the renal synthesis of prostaglandins.

Preparation and incubation of sample

The outer medullary collecting ducts (OMCD) were microdissected from collagenase-treated Jerboa Kidney. Adult Jerboas (Jaculus orientalis) of both sexes were captured in the middle Atlas of eastern Morocco and acclimatized for several months to usual laboratory conditions. Animals were separated and fed either a normal hydrated diet (sunflower grain ad libitum and fresh lettuce) or a dry diet (sunflower grain ad libitum only). During this period, animals had no access to drinking water.

Animals were anesthetized with pentobarbital (40 mg/kg of body weight, ip). After anesthesia, the left kidney was perfused with collagenase solution (0.14% w/v). Then, thin pyramids were incubated in collagenase solution (0.06% w/v) at 35°C during 30 min. Individual segments of OMCD (0.3-1.1 mm length) were transferred onto the hollow of a glass slide in 2 μl incubation medium and then photographed for the subsequent determination of their length.

Measurement of cAMP accumulation and calculations

The duration of the pre-incubation period of the tubules was of 10 min at 30°C, after which time the incubation period (4 min at 35°C) was initiated by addition of 2 μl incubation medium (final incubation volume of 4 μl). The reaction was stopped by rapidly transferring the samples with 1 μl incubation solution into a polypropylene tube containing 20 ml a mixture of formic acid in absolute ethanol (5%, v/v). Samples were evaporated to dryness overnight at 40°C and kept at –20°C until radio-immunoassay for cAMP content. Forty μl of potassium phosphate buffer (50 mM, pH
were added to the samples. After acetylation, \(^{125}\)I-labeled cAMP and specific antibody were added and the samples were incubated 23 h at 4°C. Separation of free cAMP and bound cAMP was carried out using polyethylene glycol (PEG 6000). This method allows the determination of about 2 to 80 fmol cAMP per sample. In our conditions, in the absence of stimulating hormone, the basal level of cAMP present in a single piece of tubule was close to the sensitivity threshold of the assay.

The amount of cAMP was expressed as femtomoles per millimeter of tubule length per 4 min incubation time at 35°C (fmol. mm\(^{-1}\).4 min\(^{-1}\)). In each experiment, different experimental conditions were tested in parallel on \(\text{n} \) replicate tubule samples microdissected from a same rat kidney (n = 6 to 8 per experimental condition). For each experimental series, results are given as the mean value calculated from individual means obtained in different experiments (N) ± standard error of the mean (SEM). Differences were tested using Student’s t-test for unpaired data.

**Measurement of intracellular concentration of calcium**

Intracellular concentration of calcium \([\text{Ca}^{2+}]_i\) was measured on single OMCD samples microdissected from collagenase-treated kidneys (protocol identical to that used for cAMP accumulation experiments, see above) using the calcium-sensitive fluorescence probe fura-2/AM as described previously (Champigneulle \textit{et al.}, 1993).

The samples were loaded for 30-60 min with 10 \(\mu\)M fura-2/AM at room temperature in dark. Each fura-2-loaded tubule was then transferred to a superfusion chamber fixed on the stage of an inverted fluorescence microscope (model IM 35; Zeiss, Oberkochen, Germany). The tubule was maintained close to the bottom of the superfusion chamber by two holding pipettes and superfused at 37°C at a rate of 10-12 ml/min corresponding to an exchange rate of about 10 exchanges per min. The composition of the superfusion medium was similar to that of the microdissection medium used in cAMP experiments, except that indomethacin, adenosine deaminase and bovine serum albumin were not added since the superfusion medium was flushed continuously. After a 5 to 10 min equilibration period, medium containing agonists was superfused on single OMCD. Due to the dead space of the superfusion setup, the time necessary to achieve full equilibrium concentration in the chamber was of about 15 to 20 s. Fluorescence measurements were carried out using a standard photometric setup (MSP 21, Zeiss) assisted by a microcomputer. By means of a circular diaphragm, an area of 60 \(\mu\)m diameter was selected over the tubule image by observation with transmitted light (x400 magnification). The fluorescence intensity emitted from this area (during brief excitation periods at 340 and 380 nm alternatively, at a maximal rate of 30 cycles/min) was continuously recorded. Autofluorescence was measured on homologous OMCD samples not loaded with fura-2 and ranged from 0.2% of the signal measured at the beginning of the experiment.

After substraction of the corresponding autofluorescence value, \([\text{Ca}^{2+}]_i\) was calculated from the successive 340/380 ratios as previously detailed (Champigneulle \textit{et al.}, 1993).

**Results**

\textbf{I - Effect of AVP, PGE\textsubscript{2} and carbachol on the hydrated Jerboa OMCD}

The effect of AVP, carbachol and PGE\textsubscript{2} is tested on the variation of \([\text{Ca}^{2+}]_i\) in OMCD loaded by Fura-2 in the presence of 2 mM extracellular \textit{Ca}\textsuperscript{2+}. The Basal \([\text{Ca}^{2+}]_i\) measured in these conditions is 191.3 ± 9.5 nM \([\text{Ca}^{2+}]_i\), (n = 12). The addition of 100 nM AVP induces a monophasic increase of \([\text{Ca}^{2+}]_i\) (figure 1). The absolute increase over basal value at peak phase is: \(\Delta[\text{Ca}^{2+}]_i = 209,1 ± 44,7\) nM, \(n=7\). A second superfusion with 100 nM AVP did not increase \([\text{Ca}^{2+}]_i\) (figure 1).
C

\[ \text{[Ca}^{2+}]_i \text{ (nM)} \]

A

\[ \text{[Ca}^{2+}]_i \text{ (nM)} \]

B

\[ \text{[Ca}^{2+}]_i \text{ (nM)} \]

C

**Figure 1.** Patterns of increase of \([\text{Ca}^{2+}]_i\) induced by AVP, PGE2 and carbachol in hydrated Jerboa OMCD. OMCD were microdissected from hydrated Jerboa and loaded with Fura-2. Tubules were then superfused with medium containing 2 mM extracellular \(\text{Ca}^{2+}\). The horizontal bars indicates the addition of 100 nM AVP (A), 0.3 mM PGE2 (B) and 100 nM carbachol (C).

In the same conditions, the addition of different concentrations of PGE2 (3nM, 30nM and 300nM) to OMCD induced a progressive increase of \([\text{Ca}^{2+}]_i\). The addition of 3 nM PGE2 induced a very small increase of \([\text{Ca}^{2+}]_i\): \(\Delta[\text{Ca}^{2+}]_i = 12.8 \pm 2.8 \text{nM}, \ n=5\). For higher concentrations (30nM and 300 nM) of PGE2, we observed a biphasic increase of \([\text{Ca}^{2+}]_i\) with a Peak followed by a more sustained Plateau (figure 1). The mean increase of \([\text{Ca}^{2+}]_i\) at the peak was : \(\Delta[\text{Ca}^{2+}]_i = 42.1 \pm 7.1 \text{nM}, \ n=5\) under 30 nM PGE2 and \(108.8 \pm 11.4 \text{nM}, \ n=7\) under 300 nM PGE2.

In these conditions, we tested the effect of carbachol on the \([\text{Ca}^{2+}]_i\) in Jerboas OMCD. The results indicate that 100 µM carbachol induced a monophasic increase of \([\text{Ca}^{2+}]_i\) in the presence of 2mM extracellular \(\text{Ca}^{2+}\) less than that observed with AVP and PGE2 (figure 1).

### II- Effect of dehydration on the variation of \([\text{Ca}^{2+}]_i\) induced by AVP, PGE2 and carbachol

The response to AVP, PGE2 or carbachol measured on OMCD microdissected from dehydrated Jerboas was compared to that obtained from hydrated Jerboas.

To this end, Jerboas were maintained on dry diet during one to five weeks. Every week, OMCD were microdissected and used for the measurement of \([\text{Ca}^{2+}]_i\) variations in the presence of either 100 nM AVP, 0.3 mM PGE2 or 100 mM carbachol. The effect of 10 nM AVP was studied also on cAMP accumulation.

1) **Effect of AVP:**

It has been previously shown that the OMCD of Jerboa possesses receptors to AVP coupled to stimulation of adenylyl (Baddouri *et al.*, 1984). In this study, we measured the cAMP generated by on non permeabilized OMCD. The level of cAMP determined by this method (in the presence of IBMX) was equivalent to that measured previously (Baddouri *et al.*, 1984).

In OMCD, AVP induced an accumulation of cAMP and increased the \([\text{Ca}^{2+}]_i\). Figure 2 shows the variation of these two parameters during dehydration of the Jerboas compared to the responses obtained in the same conditions but on dehydrated Jerboa. We observed that the accumulation of cAMP induced by 10 nM AVP decreased to 64.4% of AVP control response after 1 week of dehydration, to 45.8% after 3 weeks of dehydration and then still unchanged. The increase of \([\text{Ca}^{2+}]_i\) induced by AVP is also reduced during dehydration (figure 2).
Figure 2. Variation of AVP response during dehydration of Jerboa. OMCD were microdissected from hydrated Jerboas (control) or Jerboas maintained on a dry diet for 1 to 5 weeks. * p<0.05 vs AVP control response. Left panel: cAMP accumulation values measured in OMCD. Values are the means ± SEM calculated from 4 experiments performed in the presence of 10 nM AVP. Right panel: Increase of [Ca2+]i over basal value (Δ[Ca2+]i) measured in OMCD. Tubules were loaded with Fura-2 then superfused with medium containing 2 mM extracellular Ca2+ and stimulated by 100 nM AVP. Values are the means ± SEM from N= 5 Jerboas.

The response to AVP was not significantly modified after one week of dehydration but decreases after two weeks of dehydration. Then it did not change for more time of dehydration (Δ[Ca2+]i: AVP, 1 week = 222.3 ± 69.2 nM, n = 3, NS; AVP, 2 weeks = 76.5 ± 16.7, n = 4; p<0.005; 65.6% of inhibition).

2) Effect of PGE2:

Figure 3 shows the mean values obtained after addition of 300nM PGE2 to OMCD of Hydrated (control) and dehydrated jerboas. We observed that the result to PGE2 was not modified after one week of dehydration but decreased markedly to 16.3% of the control after two weeks and remained very low even with more dehydration.

3) Effect of carbachol

In the same experiments, we tested the variation of the increase of [Ca2+]i induced by 100 µM Carbachol during dehydration. Ever the responses to carbachol are of low magnitude compared to that obtained with AVP and PGE2 on hydrated Jerboas. We did not observe any significant difference between hydrated and dehydrated Jerboas (figure 4). These results are in opposite to that obtained with PGE2 and AVP.

Discussion

The aim of this study was to investigate the hormonal responses in the OMCD of Jerboa kidney and their variations during dehydration.

The results obtained in the Jerboa OMCD show that (1) AVP induced an
accumulation of cAMP which was impaired by dehydration; (2) AVP, PGE$_2$ and carbachol stimulated an increase of [Ca$^{2+}$]i; (3) during dehydration, the increase of [Ca$^{2+}$]i induced by AVP and PGE$_2$ were markedly reduced but not the response induced by carbachol.

It has been previously shown that the OMCD of Jerboa possesses receptors to AVP coupled to stimulation of adenylyl (Baddouri et al., 1984). In this study, we measured the cAMP generated by adenylyl cyclases on non-permeabilized OMCD. The level of cAMP determined by this method (in the presence of IBMX) was equivalent to that measured previously (Baddouri et al., 1984).

In the Jerboa OMCD, the addition of AVP in the presence of 2mM extracellular Ca$^{2+}$ induced an increase of [Ca$^{2+}$]i similar to that observed in the rat OMCD (Champigneulle et al., 1993; Imbert-Teboul et al., 1993). This increase of [Ca$^{2+}$]i presented an homologue desensitization like that observed on rat isolated nephron segments (Dublineau et al., 1990). It has been shown previously that in the kidney membranes, vasopressin presented a desensitization related to V2-receptors which is responsible of cAMP formation (Butlen et al., 1984). However, the authors did not perform measurement of V1-receptors of vasopressin that are responsible of calcium increase. Our results complete the first result obtained (Butlen et al., 1984) by indicating that the desensitization concerns also V1-receptors.

Addition of PGE$_2$ to OMCD induced a biphasic increase of [Ca$^{2+}$]i. The first phase with very high decline rapidly was probably due to the breakdown of the phosphoinositides. The second phase more sustained was probably due to an entry of Ca$^{2+}$ from extracellular medium. The effect of PGE$_2$ on [Ca$^{2+}$]i suggest the presence of EP1 receptors in Jerboa OMCD like those described in rat (Aarab et al., 1993; Aarab et al., 1999).

In the same experiments, we observed that the addition of carbachol, muscarinic agonist, led to an increase of [Ca$^{2+}$]i of lower magnitude than that observed with AVP and PGE$_2$. This response of carbachol is equivalent to that obtained previously in rat OMCD (Marchetti et al., 1990) and rabbit CCD (Breyer et al., 1991).

In the second part of this study, we have examined the variation of responses described before during prolonged dehydration of Jerboas. We observed that (1) the increase of cAMP and [Ca$^{2+}$]i induced by AVP decreased from one week of dehydration; (2) the response to PGE$_2$ was markedly reduced where the response to carbachol was not significantly modified.

During dehydration of Jerboas, urinary flow is reduced to 20-25ml/h (Baddouri et al., 1981) with a high osmotic pressure (3000-4000 mosmole/l) and a high plasma level of AVP (average 450 pmol/l compared to 50 pmol/l in normal jerboas (Baddouri et al., 1981; Baddouri et al., 1984; El-Husseini and Haggag, 1974). It has been shown previously that adenylyl acyclase activity stimulated by AVP in the medullary thick ascending limb of Henle’s loop (MTAL) was reduced during dehydration but not that observed in OMCD (Baddouri et al., 1984; Morel et al., 1987). In this study we observed a reduction of AVP response in the OMCD
equivalent to that observed in MTAL (Baddouri et al., 1984). The reduced amount of cAMP formed under vasopressin stimulation in the jerboas fed to a dry diet might indicate some physiological "down regulation" of the number of vasopressin-specific receptors in the kidney as a result of the huge ADH concentration present in blood plasma under these conditions.

Our results are in agreement with the measurements of AVP receptors in Jerboa OMCD which showed a reduction in binding sites (Baddouri et al., 1984), since we observed a reduction of AVP response which concern cAMP and [Ca$$^{2+}$$]i increases. This result confirms that the reduction of AVP response was due to the diminution of AVP receptors (see above) which is probably a consequence of homologue desensitization due to high level of plasma AVP.

The experiments realized in this study cannot explain the reduction in PGE$_2$ response during dehydratation. But this reduction is very likely related to the water deprivation of Jerboas. It was shown before that high concentration of AVP stimulates PGE$_2$ synthesis in Rabbit collecting ducts (Kirschenbaum et al., 1982; Schlondorff et al., 1985). If this regulation is also present in Jerboa OMCD, the synthesis of PGE$_2$ would be very high due to the high AVP concentration. So the reduction in the PGE$_2$ response might be explained by a prolonged occupation of PGE$_2$ leading to a depletion of intracellular Ca$$^{2+}$$ stores.

In summary, in Jerboa OMCD, AVP, PGE$_2$ and Carbachol increases [Ca$$^{2+}$$]i. The response to carbachol was not impaired during dehydratation of Jerboa. By contrast, the PGE$_2$ and AVP responses were markedly reduced.

References


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