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Activation of osmotically-activated potassium transporters after injection of mRNA from A6 cells in *Xenopus* oocytes

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Abstract

The different potassium pathways, under iso-osmotic or hypo-osmotic conditions, were examined in *Xenopus* oocytes that were micro-injected with mRNA from A6 cells. Hypo-osmotically stimulated ^{86}Rb (K^+) effluxes could be measured from intact oocytes 1–4 days after injection of 25 ng of poly $(A)^+$ RNA isolated from A6 cells. ^{86}Rb (K^+) effluxes were 2.2 times higher from oocytes micro-injected with 25 ng of poly$(A)^+$ RNA, than from water injected control oocytes. Water-injected oocytes themselves, however, were 7-fold more responsive to a hypo-osmotic shock than non-injected *Xenopus* oocytes. There was no significant effect of the different K^+ transport blockers tested (TEA, bumetanide, glybenclamide or quinidine) on the endogenous ^{86}Rb (K^+) effluxes from non-injected oocytes in either iso- or hypo-osmotic media. The ^{86}Rb (K^+) effluxes from water-and mRNA-injected oocytes in hypo-osmotic media were both inhibited by TEA. In mRNA-injected oocytes the increase in ^{86}Rb (K^+) transport following a medium dilution was also inhibited in the presence of glybenclamide or bumetanide. The present study reports that the activation of hypo-osmotically-activated potassium transporters in the oocytes of *Xenopus laevis*, after injection of mRNA from A6 cells differs quantitatively and in part qualitatively (glybenclamide-sensitivity) from the endogenous K^+ pathways of non-injected and of water-injected *Xenopus* oocytes.

Key words: mRNA expression; Potassium ion channel; Hypo-osmotic response; Oocyte; A6 cell; (*Xenopus*)

1. Introduction

A6 cells are designated as a continous cell line derived from the kidney of *Xenopus laevis* [1]. They form a polarised, highly differentiated epithelium of high electrical resistance when grown on a permeant support [2]. The two-barrier model developed by Ussing [3] can be applied to A6 cells, since these cells exhibit a transepithelial sodium transport directed from the apical to the basolateral side which is mediated by amiloride-sensitive Na^+ channels (located on the apical membranes) and by Na/K ATPase and K^+ channels located on the basolateral membranes [2,4–6].

When submitted to a hypo-osmotic medium on the serosal side, A6 cells swell and regulate their volume [7]. Small changes in the osmolarity of the serosal solution bathing A6 monolayers have also been found to affect the short-circuit current (transepithelial Na^+ transport) by changing the conductance of amiloride-sensitive Na^+ channels [8]. In such a situation, cells are faced with the problems of cell volume regulation and of coordination between ion entry and exit through the apical and basolateral membranes, respectively ('cross-talk' phenomena), resulting in modification of the K^+ permeability of the basolateral membranes.

The opening of a quinidine-sensitive K^+ channel located on the basolateral membrane was demonstrated under hypo-osmotic conditions in amphotericin-treated cells [4]. A recent study, using the isotope ^{86}Rb as a potassium analogue, characterised the presence of another K^+ channel, immediately opened by hypo-osmotic conditions which is TEA-inhibited [7].

The oocytes of *Xenopus laevis* have become a widely used expression system for mRNA encoding for ion-selective channels from other cells [9,10]; the apical, amiloride-sensitive Na^+ channel from A6 cells has already been expressed in this way [11]. We have

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followed the ^{86}Rb effluxes in non-injected, water-and A6 cell mRNA-injected oocytes of *Xenopus laevis* under iso-osmotic and hypo-osmotic conditions. In both conditions, the effect of different K^+ transporter blockers (TEA, bumetanide, quinidine and glybenclamide) was tested in order to distinguish between endogenous and functionally expressed hypo-osmotically-activated potassium transporters.

2. Materials and methods

2.1. RNA preparation

A6, a renal cell line of *Xenopus laevis* was a gift from Dr Rossier (Lausanne, Switzerland). The cells were originally obtained from the American Tissue Type Collection and subsequently cloned (clone A6–2F3) by limiting dilution [12]. Cells were grown between passages 88–89 at 28°C in a humidified atmosphere of 5% CO_2 in air. The amphibian cell medium (AM) [13], containing antibiotics, was supplemented three times weekly with 10% fetal calf serum (IBF, France) for cell nourishment. Cells grown until confluence were isolated by trypsination and calcium chelation, and quickly lysed in 4 M guanidinium thiocyanate. RNA was prepared by the acid guanidinium thiocyanate-phenol-chloroform procedure [14]. The total RNA obtained (0.5–1% of the original cell weight) was passed through an oligo DT cellulose column (type 3, Collaborative Biomedical Products, USA) following a modification of the procedure described in [15]: we omitted the detergent lauryl sulfate (SDS) in the final rinse because we found it had a detrimental effect on oocyte viability (estimated by cell K^+ concentrations) after injection. Poly(A)$^+$ mRNA, (typically 1–2% of total RNA), was concentrated by precipitation with 3 M sodium acetate and isopropanol and rinsed 2 or 3 times in 70% ethanol over 24 h to remove this salt. The final product was dissolved in diethyl pyrocarbonate (DEPC)-treated water and stored in small aliquots at −70°C. The quality of the samples of mRNA was assessed on 1% agarose (Life Technologies, USA) gels with 0.66 M formaldehyde and the quantity was measured by spectrofluorimetry (Uvikon 820, Kontron, France) at 260 nm.

2.2. Oocytes

The following methods to obtain defolliculated oocytes are based on those described previously [16]. Females of the clawed toad *Xenopus laevis* were anesthetized on ice and sections of the ovary removed and incubated in modified Barth's saline (MBS, see below) containing collagenase (1.1 U/ml, Serva, Germany), with gentle shaking at 18°C for 12–15 h overnight. The oocytes were then rinsed and washed for 1 h in Ca^{2+} free MBS with agitation to remove the surrounding follicular cell layer. Selected stage V or VI oocytes [17] in an apparently healthy state (regular shape and evenly coloured, well-defined animal and vegetal poles) were injected with 50 nl of DEPC-treated water or 25 ng of poly(A)$^+$ mRNA in 50 nl of DEPC-treated water, using a micro-injector system (Inject + Matic, Geneva). As significant differences of ^{86}Rb efflux were observed in non-injected and water-injected oocytes, the latter were used as controls for all mRNA expression. Neither our collagenase treatment, nor our injection procedures damaged the oocytes, as judged by the level of the internal K^+ concentration. Injected oocytes were incubated 24–48 h at 18°C in MBS supplemented with $^{86}RbCl$ (Amersham, England) to a final activity of approximately 10 μCi/ml.

2.3. Wash-out experiments

Survivors of the injection (80%) were selected after 2–4 days, rinsed twice in MBS and individual oocytes placed on a monofilament gauze (hole size 106 μm, Tripette et Renaud, France) at the base of a plexiglass tube. Five of these tubes were fixed through a plexiglass strip in such a way that each oocyte rested in 1.5 ml of bathing medium in a well of a 24-well cell culture tray (Nunc, Denmark). Successive periods of time of 15 min were choosen for wash-out experiments, each batch of 5 oocytes being quickly transferred to the wells containing the experimental medium. The ^{86}Rb (K^+) effluxes from the oocytes were measured over a 2-h period: in the first hour under isosmotic conditions and in the second under conditions of hypo-osmotic shock, the medium being diluted by 1/3 with buffered distilled water. At the end of the experiment, the oocytes were lysed mechanically in 2 ml of MBS. The samples, 10 μl of the charging medium and the oocytes were assayed for ^{86}Rb activity with 10 μl of Aqueous Counting Scintillant (Amersham) in a liquid scintillation counter (Packard Instruments, USA).

Owing to their extreme fragility, approximately 20% of the de-folliculated oocytes were damaged during experimentation resulting in considerable uncontrolled leakage of ^{86}Rb. When this was greater than 25% of the expected total internal activity the results was rejected. Since the oocytes were studied individually this source of error was eliminated.

^{86}Rb loss was expressed as a percentage of the amount of ^{86}Rb present in the oocyte at the beginning of each time period (i.e., at zero time for instance, this value is calculated from the amount measured in the eggs at the end of the experiment plus the total amount lost in the successsive washing periods). For each experiment, results are given as the mean ±S.E. of the

percentage of ^{86}Rb loss from 5 oocytes, as a function of time.

Our experimental protocol for comparing the effects of different experimental conditions, by using oocytes from the same donor studied on the same day, was designed to minimise the effects of the large variability in endogenous permeabilities typically observed in oocytes (see Ref. 18).

2.4. Solutions

The composition of MBS (in mM), was: 85 NaCl, 4 NaOH, 2.4 $NaHCO_3$, 1 KCl, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 10 Hepes (pH 7.4 adjusted with HCl). During the collagenase treatment and ^{86}Rb loading of the oocytes, penicillin and streptomycin were added at 0.5 U/ml, but these ionophores were omitted from the experimental medium and pre-experimental wash.

Tetraethylammonium (TEA) was used at a concentration of 10 mM, glybenclamide at 2.10^{-4} M, bumetanide at 10^{-4} M and quinidine at 200 μM. They were all purchased from Sigma, USA.

2.5. Statistics

Significance was calculated with Student's t-test on the means of unpaired data.

3. Results

3.1. ^{86}Rb wash-out from mRNA, water, and non-injected oocytes in iso- and hypo-osmotic media

In preliminary experiments we followed the kinetics of the ^{86}Rb (K^+) effluxes as a function of time in successive periods of 15 min over 2 h from water-injected and non-injected oocytes. A constant ^{86}Rb (K^+) efflux was observed with both batches of oocytes when the ^{86}Rb appearing in the external medium was plotted as a percentage of the ^{86}Rb remaining in the oocyte.

We first compared the ^{86}Rb (K^+) effluxes from water-and non-injected oocytes in isosmotic media. The ^{86}Rb (K^+) efflux under isosmotic conditions from water-injected oocytes ($n = 64$) was 69% higher ($P < 0.01$) than that of non-injected oocytes ($n = 39$). However, no significant difference between the effluxes from mRNA and water-injected oocytes was detected under such isosmotic conditions (Fig. 1A,B).

The effect of a hypo-osmotic shock on the ^{86}Rb effluxes was then tested. After one hour in an isosmotic medium, the effluxes were measured over a further one hour period in a 1/3 diluted medium (Fig. 1A). A progressive increase in ^{86}Rb (K^+) efflux was observed for non-injected and water-injected oocytes

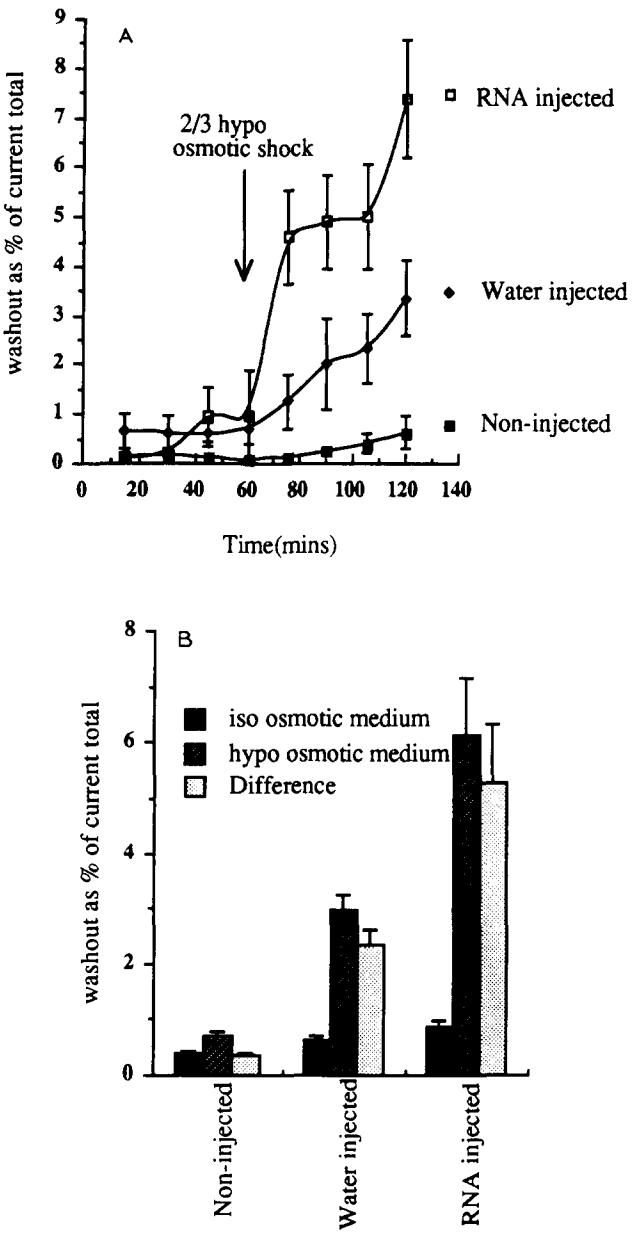

Fig. 1. ^{86}Rb effluxes from mRNA-, water-, and non-injected oocytes, under iso- and hypo-osmotic conditions. The values are percentages of the ^{86}Rb remaining in each oocyte at the beginning of the 15-min period. (A) A typical experiment in which the effect of a hypo-osmotic shock (1/3 dilution by buffered distilled water) is followed as a function of time by changing the bathing solution every 15 min. Each point is the mean of five oocytes. (B) Histogram of the ^{86}Rb efflux values of oocytes measured over 15-min periods, after 45-min treatment first in isosmotic and then in hypo-osmotic media and the difference between these values representing the increase in ^{86}Rb efflux in response to the hypo-osmotic shock. Values for mRNA, water-and non-injected oocytes are means from 65, 65, and 39 oocytes, respectively.

while an immediate (significant after 20 min) and large increase was found with mRNA-injected oocytes (Fig. 1A). In all groups of oocytes, a clearly marked increase in ^{86}Rb (K^+) efflux was observed after 45 min in a hypo-osmotic medium (Fig. 1B). This increase being

Table 1

		Control	Glibenclam.	Control	Bumetanide	Control	TEA	Control	Quinidine
A	iso-osmotic	0.15 ± 0.02	0.21 ± 0.02	0.37 ± 0.07	0.46 ± 0.12	0.17 ± 0.03	0.28 ± 0.05	0.16 ± 0.02	0.22 ± 0.04
non-injected	hypo-osmotic	0.51 ± 0.08	0.51 ± 0.08	1.01 ± 0.17	0.86 ± 0.16	0.45 ± 0.05	0.46 ± 0.06	0.43 ± 0.05	0.49 ± 0.13
	difference	0.37 ± 0.08 ***	0.30 ± 0.07 ***	0.64 ± 0.13 ***	0.39 ± 0.14 **	0.28 ± 0.04 ***	0.18 ± 0.03 ***	0.26 ± 0.04 ***	0.27 ± 0.12 *
B	iso-osmotic	0.52 ± 0.07	0.79 ± 0.12	0.60 ± 0.12	0.58 ± 0.12	0.67 ± 0.12	0.62 ± 0.12	0.65 ± 0.14	1.07 ± 0.28
water-injected	hypo-osmotic	3.92 ± 0.50	3.41 ± 0.67	2.65 ± 0.31	1.99 ± 0.35	2.38 ± 0.36	1.17 ± 0.21	1.89 ± 0.30	3.37 ± 0.81
	difference	3.39 ± 0.47 ***	2.62 ± 0.67 ***	2.05 ± 0.26 ***	1.41 ± 0.34 ***	1.71 ± 0.32 ***	0.55 ± 0.22 **	1.24 ± 0.23 ***	2.28 ± 0.75 **
C	iso-osmotic	0.73 ± 0.17	0.47 ± 0.07	1.21 ± 0.42	0.46 ± 0.10	1.07 ± 0.26	0.33 ± 0.06	1.10 ± 0.21	1.67 ± 0.28
mRNA-injected	hypo-osmotic	6.93 ± 0.98	3.71 ± 0.87	4.91 ± 1.10	2.09 ± 0.48	5.35 ± 1.26	0.66 ± 0.17	3.38 ± 0.98	3.65 ± 0.79
	difference	6.20 ± 0.97 ***	3.24 ± 0.84 ***	3.69 ± 0.88 ***	1.64 ± 0.41 ***	4.28 ± 1.17 ***	0.33 ± 0.18 n.s.	2.28 ± 0.86 **	1.99 ± 0.82 *

Effect of different drugs on the ^{86}Rb effluxes from non-injected oocytes (A), water/DEPC-injected oocytes (B) and mRNA-injected oocytes (C) under isosmotic or hypo-osmotic conditions (2:3 dilution by buffered distilled water). Number of oocytes were in A (non-injected): glybenclamide 2.10^{-4} M, $n = 22$; bumetanide 10^{-4} M, $n = 10$; TEA 10 mM, $n = 14$; quinidine 200 μM, $n = 15$; in B (water/DEPC-injected oocytes), the number of oocytes were of 48, 20, 18, and 24, and in C (mRNA-injected oocytes) the numbers were of 24 and 34, 16, 11 and 10 for glybenclamide, bumetanide, TEA and quinidine, respectively; t-tests: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s., non different.

6.8 times greater in water-injected than in non-injected oocytes ($n = 39$, $P < 0.001$). An even greater hypo-osmotic response occured in the mRNA-injected oocytes ($n = 64$) which showed a further 2.2 fold increase in ^{86}Rb efflux ($P < 0.005$) above that of the water-injected oocytes (Fig. 1B). In mRNA-injected oocytes, the effluxes values often but not always showed an apparent plateau between 75 and 105 min, followed by a second, variable, incremental phase (Fig. 1A). Preliminary attempts to follow this second phase over a longer time period showed no further sign of levelling off. In short, the hypo-osmotically-induced increase in ^{86}Rb (K^+) transport following the injection of mRNA was greater than that following injection of water, which in turn was greater than that in non-injected oocytes.

3.2. Effects of K^+ transporter blockers on ^{86}Rb effluxes

We adopted a pharmacological approach to the identification of the various ^{86}Rb (K^+) pathways present under the two osmotic conditions. To draw valid conclusions from such experiments it was essential to know that changes of K^+ effluxes were due to the opening of new K^+ pathways and not to damage to the membranes of hypo-osmotically shocked oocytes. Tetraethylamonium (TEA), glybenclamide, and quinidine are frequently used K^+ blockers (for review see Ref. 19), while bumetanide is known to inhibit the Na/Cl and Na/K/2Cl co-transporters [20,21]. The effects of these drugs were therefore tested on the ^{86}Rb (K^+) effluxes from mRNA-, water-, and non-injected oocytes.

There was no significant effect of these drugs on the

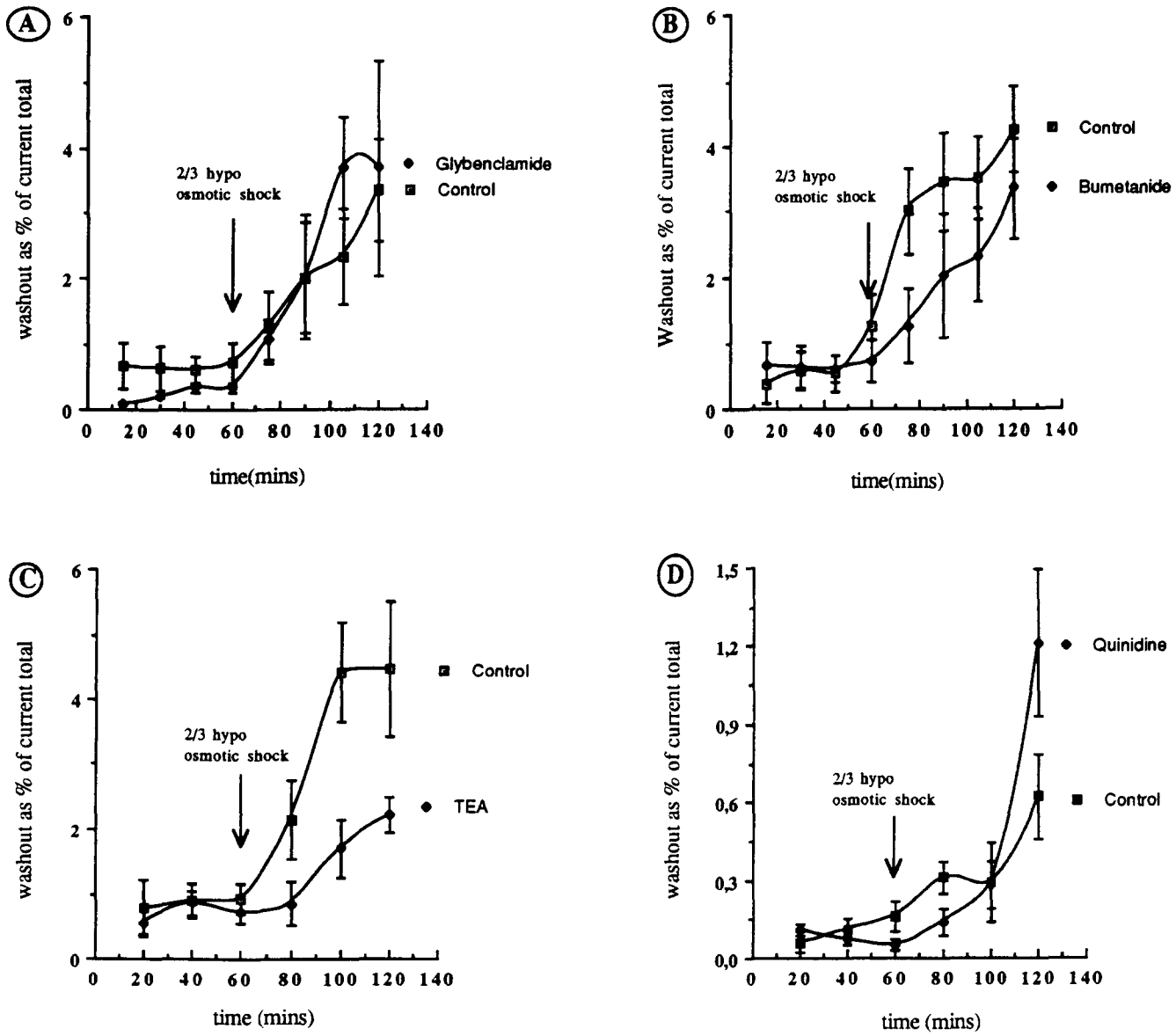

Fig. 2. Typical experiments with water/DEPC-injected oocytes showing the effect of a hypo-osmotic shock (2/3 dilution by buffered distilled water) in the presence and absence of glybenclamide (A), bumetanide (B), TEA (C), and quinidine (D) at the same concentrations as in Table 1.

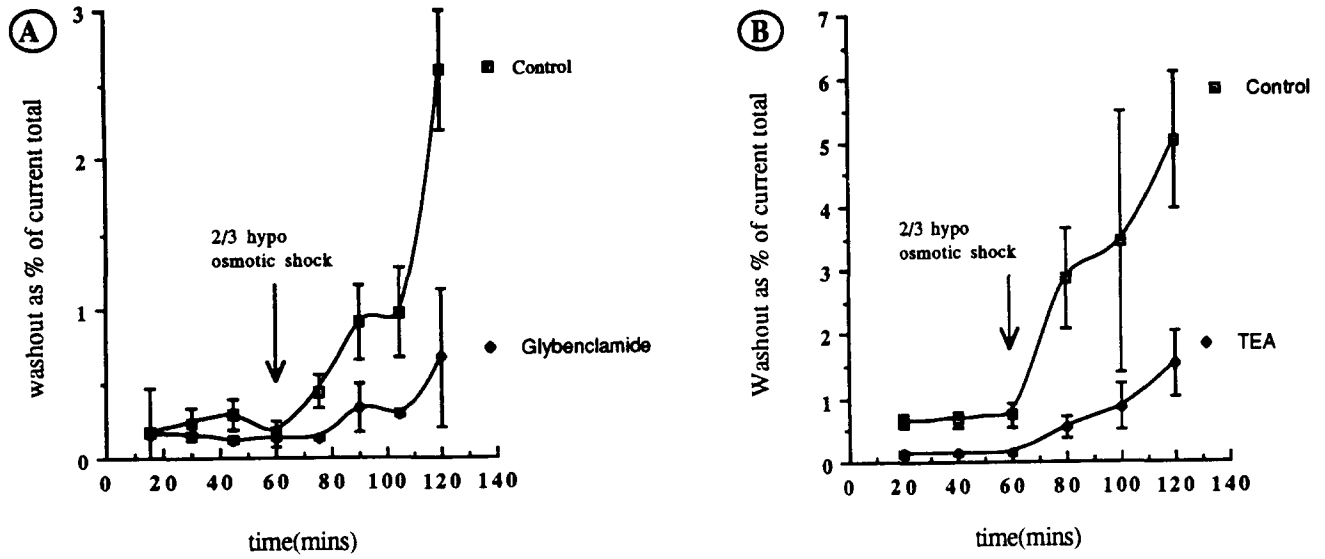

Fig. 3. Typical experiments with 25 ng mRNA-injected oocytes showing the effects of a hypo-osmotic shock (2/3 dilution by buffered distilled water) in the presence and absence of glybenclamide (A) and TEA (B), at the same concentrations as in Table 1.

endogenous ^{86}Rb (K^+) effluxes from non-injected oocytes in either iso-or hypo-osmotic media (Table 1A): the hypo-osmotic response i.e., stimulation of ^{86}Rb (K^+) effluxes remained unchanged.

Similar experiments were made with water-injected oocytes. As in non-injected oocytes, no inhibitory effect of the various drugs tested could be detected under isosmotic conditions and glybenclamide (2. 10^{-4} M) also had no significant effect on the hypo-osmotically induced stimulation of the $^{86}Rb(K^+)$ effluxes (Fig. 2A, Table 1B). On the other hand bumetanide blocked the hypo-osmotic response by 59% immediately after the shock (Fig. 2B, $P < 0.05$ $n = 13$) although this effect was no longer statistically significant after 45 min of oocyte subjection to the dilute medium (Table 1B). In contrast, TEA (10 mM) clearly inhibited the response to a hypo-osmotic shock by 51% ($P < 0.01$, $n = 18$, Table 1B and Fig. 2C). Quinidine apparently increased $^{86}Rb(K^+)$ effluxes under both iso- and hypo-osmotic conditions (Fig. 3D) although these differences were not statistically significant in either osmotic medium (Table 1B and Fig. 2D).

The effects of the different agents were then tested on mRNA-injected oocytes under both osmotic condi-

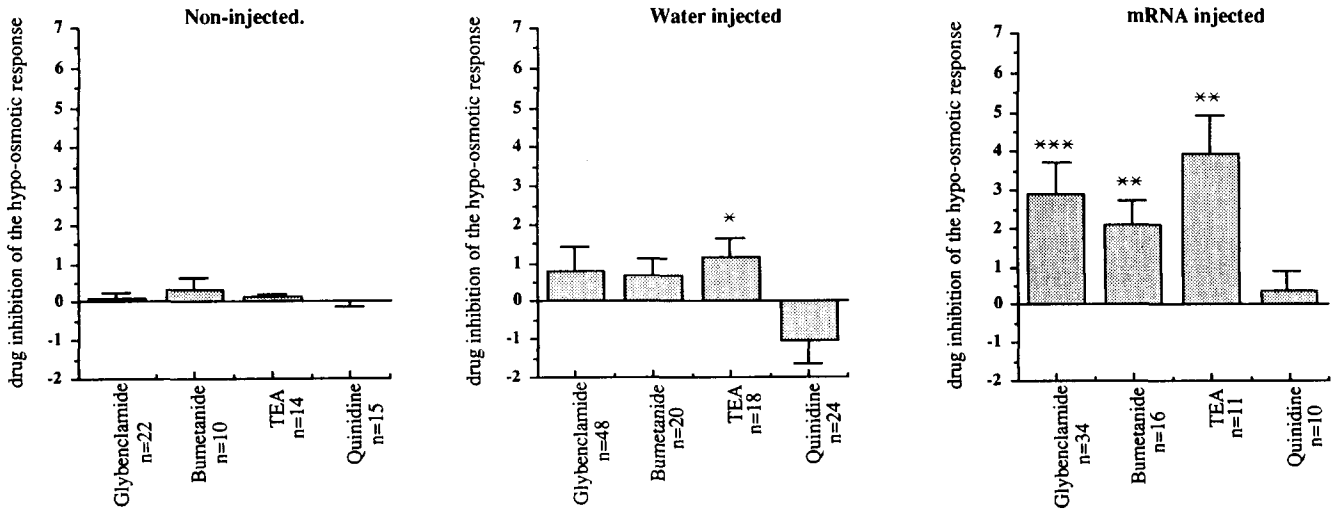

Fig 4. Histograms summarizing the inhibitory effects of different drugs on the responses to a hypo-osmotic shock of non-injected, water-injected, and mRNA-injected oocytes. The columns represent the differences between the hypo-osmotically-stimulated ^{86}Rb effluxes, in isosmotic and hypo-osmotic media, due to the presence of the drug, and are expressed in the ordinate as the 'drug inhibition of the hypo-osmotic response'. *t*-test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

tions. In an isosmotic medium, only TEA had a significant effect, blocking the ^{86}Rb (K^+) efflux by 69% ($P < 0.05$ $n = 11$ see Table 1C). When mRNA-injected oocytes were submitted to a hypo-osmotic medium they showed higher stimulatory responses than water-injected oocytes (see Fig. 1). Glybenclamide (2.10^{-4}M) blocked this response by 46.5% ($P < 0.05$ $n = 34$, see Table 1C and Fig. 3A). and bumetanide (10^{-4}M) also reduced the increase by 57% ($P < 0.05$ $n = 16$, see Table 1C). The largest inhibitory effect on the osmotic response, was that of TEA 88% ($n = 11$, $P < 0.005$, see Table 1C and Fig. 3B). Quinidine did not block the ^{86}Rb (K^+) effluxes in either isosmotic or hypo-osmotic media in mRNA-injected oocytes (Table 1C).

We investigated the possibility that the inhibitory effect of the co-transport blocker bumetanide is cumulative with the effects of the K^+ channel blockers glybenclamide and TEA, by running paired experiments in the presence of either an individual inhibitor or a combination of inhibitors, with mRNA-injected oocytes. In these experiments, TEA blocked the osmotic response by 92%, and TEA + bumetanide blocked it by 82% (the difference between these two inhibitions is not significant $n = 10$). Glybenclamide inhibited the response by 58%, and glybenclamide + bumetanide by 54% (this difference is not significant $n = 9$). The simultaneous addition of two blockers, therefore, had no cumulative effects.

Histograms summarising the inhibitory effects of the drugs on the hypo-osmotically induced increase in ^{86}Rb (K^+) in the three groups of oocytes are given in Fig. 4. The results are not shown in percentage of inhibition, in order to indicate the absolute values of the ^{86}Rb (K^+) effluxes found in this study, and to emphasize the variability of level of ^{86}Rb (K^+) permeability occuring in the different groups of oocytes.

4. Discussion

Since their introduction as an expression system for foreign mRNA [22], *Xenopus laevis* oocytes have become an essential tool for the in vivo expression of mRNA transcripts encoding for functional proteins from a variety of tissues. Of these, A6 transcripts such as the amiloride-sensitive sodium channel present in the apical membrane of these cells have also been successfully translated and identified [11,23].

In this study, we found that the ^{86}Rb (K^+) effluxes of non-injected oocytes increased slighty when submitted to a hypo-osmotic shock. The resting ^{86}Rb (K^+) transport and the osmotic response (^{86}Rb (K^+) increase) were much higher in water-injected oocytes than in non-injected oocytes. The former being 69% and 314% more permeable to ^{86}Rb under isomotic and hypo-osmotic conditions, respectively, than the latter. The simplest explanation for this increased transport is that the process of injecting distilled water (50 nl) into the oocytes damages or weakens the membrane. This explanation however, must be discounted for the following reasons. Firstly, water injection did not significantly alter the equilibrium ^{86}Rb (K^+) content of the oocytes compared with that of non-injected oocytes (70462 ± 12735 cpm and 73100 ± 12127 cpm for both oocytes, respectively, $n = 13$). Secondly, although the ^{86}Rb (K^+) wash-out was higher from water injected oocytes than from non-injected oocytes in an isosmotic medium, it was nevertheless constant over a 1 h period and much lower than that of dead or damaged oocytes (see Materials and methods for rejection criteria). In addition, the increased hypo-osmotic response could not have been due to leaks caused by stretch damage to the membrane (following the expected cell swelling), since blockers such as TEA and bumetanide reduced this hypo-osmotically stimulated increase in ^{86}Rb (K^+), which they could not have done had the increased permeability been due to a leak. The highest ^{86}Rb (K^+) transport increase upon hypo-osmotic shock was found in mRNA-injected oocytes in which the ^{86}Rb (K^+) efflux rose 220% of that of water-injected oocytes.

The immediate questions which arise concern the causes of the increase in ^{86}Rb (K^+) wash-out under hypo-osmotic conditions observed in all batches of oocytes, and the origins of the differences between them. In de-folliculated oocytes, several K^+ pathways have been detected, mainly by electro-physiological techniques. Yang et al. [24] have characterised a non-selective stretch-activated ion channel, which is not inhibited by TEA. A K^+ current activated by depolarisation, and TEA-sensitive has been reported by Parker et al. [25], and a voltage and quinine-sensitive K^+ channel was noted by Lu et al. [26]. The relative importance of each of these pathways in the overall K^+ permeability under resting or under stimulated conditions has not been investigated to our knowledge. All of these channels may be opened under hypo-osmotic conditions. An expected event upon dilution of the medium is membrane depolarisation. This may directly affect the activity of any of these channels, as well as changing the electro-chemical gradient itself. Indeed the entry of water would be expected to induce cell swelling, and therefore dilute the intracellular K^+ concentration, thereby modifying its elecro-chemical gradient. In many cell types in which regulatory volume decrease (RVD) has been described after hypo-osmotic shock, depolarisation has also been reported. This is considered to be due to opening of non-selective SA cation channels (permitting the entry of Na^+) and/or the activation of Cl^- channels [27–30]. In this study the low endogenous response in non-injected oocytes was apparently not inhibited by any of the blockers tested, although some small effects could have been

masked by the inherent variability of the individual oocytes.

The larger ^{86}Rb (K^+) wash-out found from water -injected oocytes, as compared with non-injected oocytes, was inhibited by TEA under hypo-osmotic conditions (but not in isosmotic conditions). The difference in scale and pharmocology between water-and non-injected oocytes is presumably due to ^{86}Rb (K^+) pathway(s) which are either already present in an inactive form, or expressed from the endogenous genome after the injection of water. Their activation or expression may be caused by the effects of an internal hypo-osmotic shock from the injection of distilled water (1/10 of the total cell volume) or by the inflow of external ions, for example Ca^{2+}, at the point of injection.

Conversely to water- or non-injected oocytes, in mRNA-injected oocytes, the ^{86}Rb (K^+) wash-out was found to be inhibited by TEA (69% inhibition) under isosmotic conditions. This finding is similar to that reported in A6 cells, in which TEA blocked ^{86}Rb (K^+) wash-out under isosmotic conditions [7]. Futhermore, the large and immediate increase in K^+ transport following a hypo-osmotic shock was sensitive to TEA and also to glybenclamide, but not to quinidine. Such a glybenclamide-sensitivity was not found with water-injected oocytes or non-injected oocytes. Honore and Lazdunski [31] have noted that a glybenclamide-sensitive K^+ channel exists in the follicular cells of *Xenopus* oocytes, but that it is not present in the oocyte membrane of defolliculated oocytes. In A6 cells, we also found a quinidine-insensitive, but TEA and glybenclamide inhibited, osmotically activated K^+ permeability (7, and unpublished results). This could give evidence that the increase in ^{86}Rb (K^+) permeabiltiy was due to the expression of the mRNA originating from A6 cells. However, it has to be noted that the injection of total mRNA from A6 cells could have expressed a 'signal' involved in the osmotic response (expression of another channel, i.e., Ca^{2+} channel, Cl^- channel, water channel.....). Therefore, it could be objected that the observed increase in ^{86}Rb effluxes in mRNA-injected oocytes was not due to the direct expression of mRNA from A6 cells coding for the K^+ transporter(s) but to an indirect effect, i.e., stimulation of silent endogenous transporter(s) by the expression of a signal linked to the total A6 cell-mRNA injection. Distinction between these possibilities remains the major problem of mRNA expression in any expression system.

Bumetanide has been found to block a Na/K/2Cl cotransporter in *Xenopus* oocytes measured by ^{86}Rb uptake in the inward direction under isosmotic conditions; it was also shown that the activity of this cotransporter was reduced by hypo-osmotic solutions [32]. From electrochemical gradient calulations in our hypo-osmotic solutions it would be expected to function in the reverse direction (i.e., outward). In water-injected oocytes, the ^{86}Rb (K^+) wash-out was partially inhibited by bumetanide under hypo-osmotic conditions (although only transiently), while in mRNA injected oocytes, ^{86}Rb (K^+) wash-out was also inhibited under both isosmotic and hypo-osmotic conditions. This result may confirm the presence of such a cotransporter, and its involvement in cell volume regulation. Furthermore, in A6 cells a Na/K/Cl cotransporter located in the apical membrane has also been shown to be blocked by bumetanide [33]. Under hypo-osmotic conditions a greater inhibition of the ^{86}Rb (K^+) effluxes from mRNA-injected oocytes, than from water-injected oocytes was found with bumetanide. This effect could be due to the expression of mRNA from A6 cells coding for a cotransporter, superimposed on the presence of the endogenous cotransporter, as the possibility that this pathway had also been expressed in our oocytes cannot be ruled out. The inhibition of the osmotic response (^{86}Rb (K^+) increase) by bumetanide however was not cumulative with that of TEA or glybenclamide, and as yet we have no explanation for this phenomenon.

In conclusion, this study reveals an increase in ^{86}Rb (K^+) transport in oocytes submitted to hypo-osmotic media. This process could be one of the mechanisms involved in cell volume regulation, and it is probable that stimulation of a Cl^- permeability is also involved. We were also able to demonstrate an enhanced osmotic stimulation of K^+ transport after injection with mRNA from A6 cells and the pharmocological properties of the stimulated ^{86}Rb (K^+) pathways were very similar to those described in A6 cells under similar conditions. This finding may represent a step forward in the indentification and cloning of cDNA for the channels involved.

5. Acknowledgments

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6. References

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