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Rapid report

Functional expression of a Ca^{2+}-activated K^+ channel in *Xenopus* oocytes injected with RNAs from the rat testis

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Abstract

The present study investigated the feasibility of using *Xenopus* oocytes to express sperm ion channel by injection of RNAs extracted from the rat testis. The RNA-injected oocytes expressed an outwardly rectifying current which was dependent on K^+ concentration and inhibitable by K^+ channel blockers, charybdotoxin (CTX) and tetraethylammonium (TEA). The Ca^{2+} ionophore, ionomycin, could also stimulate current activation with similar current characteristics in the RNA-injected oocytes, suggesting the expression of a Ca^{2+}-activated K^+ channel. Immunolocalization indicated predominant Ca^{2+}-activated K^+ channel immunoreactivity associated with spermatogenic cells. Reverse transcriptase–polymerase chain reaction studies confirmed the expression of the Ca^{2+}-activated K^+ channel mRNA in isolated spermatogenic cells. Our results suggest that ion channels and/or receptors of spermatogenic cells could be investigated using the *Xenopus* oocyte as an expression system. The present study also suggests that sperm may possess a Ca^{2+}-activated K^+ channel which has been implicated in the process of sperm activation and gamete interaction.

Keywords: Sperm; Potassium ion channel; Calcium ion; Oocyte; (Rat); (*Xenopus*)

Although ion channels have been implicated in various sperm functions including sperm activation and sperm–egg interaction, direct characterization of the channels involved has been precluded by the small size of the sperm, rendering electrophysiological approaches infeasible. Attempts have been made to investigate sperm ion channels in a reconstituted system using the planar lipid bilayer technique [1–6]. However, the successful rate of channel protein being incorporated into lipid bilayer is low and the artificial membrane system limits further studies on the channel regulation.

We undertook the present study to investigate the feasibility of using *Xenopus* oocytes to express sperm ion channels by injection of RNAs from the rat testis. Instead of mature spermatozoa, the rat testis, which contains the precursors of the spermatozoa, was used as a source of spermatogenic RNAs because spermatozoa are highly differentiated cells lacking the machinery for protein synthesis and thus, many long-lived mRNAs that code for sperm proteins are made during spermatogenesis in the testis.

In 83% oocytes ($n = 105$) 2–5 days after injection of

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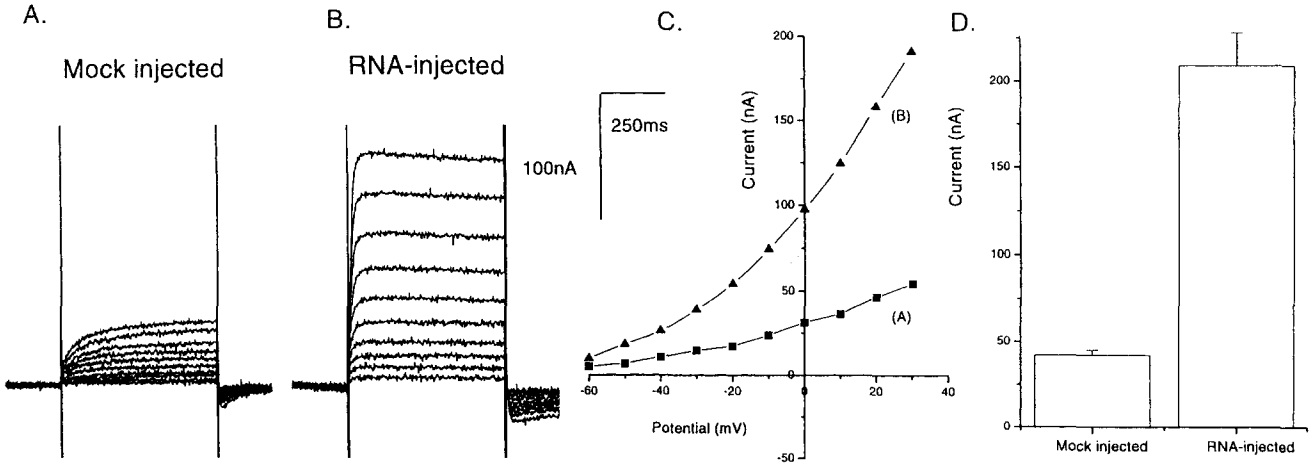

Fig. 1. Basal current in testis RNA-injected *Xenopus* oocytes. Current recordings from the mock-injected (A) and RNA-injected oocyte (B) with corresponding *I–V* curves (C). (D) Comparison of corresponding current magnitudes at +30 mV. Currents were elicited by voltage steps from −60 mV to +30 mV with 10 mV increments. RNAs were extracted from the testes of sexually mature Sprague–Dawley rats (8–10 weeks old, 320–350 g) using Trizol reagent (Gibco-BRL, Grand Island, NY, USA) as described by Monstein et al. [16]. *Xenopus* oocytes, treated with collagenase (2 mg/ml) to remove follicle cell layer, were injected with 50 nl total RNA (1 μg/μl). Currents were recorded 2–5 days after injection under two-electrode voltage-clamped configuration. The pipette solution contained 3 M KCl. The bath solution contained (mM): NaCl, 95; KCl, 1; $MgCl_2$, 1; $CaCl_2$, 1; Hepes, 5.

testicular RNAs, an outwardly rectifying whole-cell current was observed under two-electrode voltage-clamped configuration, with an averaged current magnitude of 209 ± 19 nA at +30 mV as compared to 42 ± 3 nA in the mock-injected oocytes ($n = 18$, Fig. 1), excluding the possibility that the current observed was due to the activation of endogenous channels [7]. As shown in Fig. 2A–D, the current could be substantially inhibited by Ca^{2+}-dependent K^+ channel blockers, tetraethylammonium (TEA) [8] and charybdotoxin (CTX) [9]. The reversal potential of the current, measured by the tail currents, shifted to the new K^+ equilibrium upon changes in external K^+ concentration, as indicated by a linear dependence on the $[K]_o$ (Fig. 2E). These data indicated possible expression of a Ca^{2+}-activated K^+ channel in the RNA-injected oocytes.

In the RNA-injected but not the mock-injected oocytes, the Ca^{2+} ionophore, ionomycin (0.5 μM), was able to stimulate an increase in current after the run-down of the initially observed basal current. An increase of 87 ± 9 nA ($n = 69$, Fig. 3), which also exhibited an outwardly rectifying *I–V* relationship (Fig. 3C), was induced by ionomycin. The ionomycin-activated current could be blocked by TEA (1 mM) by 64 ± 5% ($n = 9$, not shown). The ionomycin-activated current was also sensitive to CTX, as indicated by the concentration-dependent inhibition of the current (Fig. 3D). The above results further indicated the expression of a Ca^{2+}-activated K^+ channel in the RNA-injected oocytes.

Since several cell types are present in the testis, namely the spermatogenic cells, Sertoli and Leydig cells, we performed immunohistochemical studies using an antibody raised against the α subunit of a 'Maxi' Ca^{2+}-activated K^+ channel to confirm its localization in the rat testis. As shown in Fig. 4A, immunoreactivity was found to be predominantly associated with spermatogenic cells of later development stages, indicating that the channels are expressed in spermatogenic cells including the spermatozoa.

To further confirm the presence of the Ca^{2+}-activated K^+ channel in spermatogenic cells, the reverse transcriptase–polymerase chain reaction (RT–PCR) was performed to investigate the expression of the Ca^{2+}-activated K^+ channel mRNA in these cells. Spermatogenic cells were isolated from the testis as described [10] and the RT–PCR product of these cells was 437 bp (Fig. 4C) as expected of the Ca^{2+}-activated K^+ channel. A similar RT–PCR product was also observed in adrenal glands where the pres-

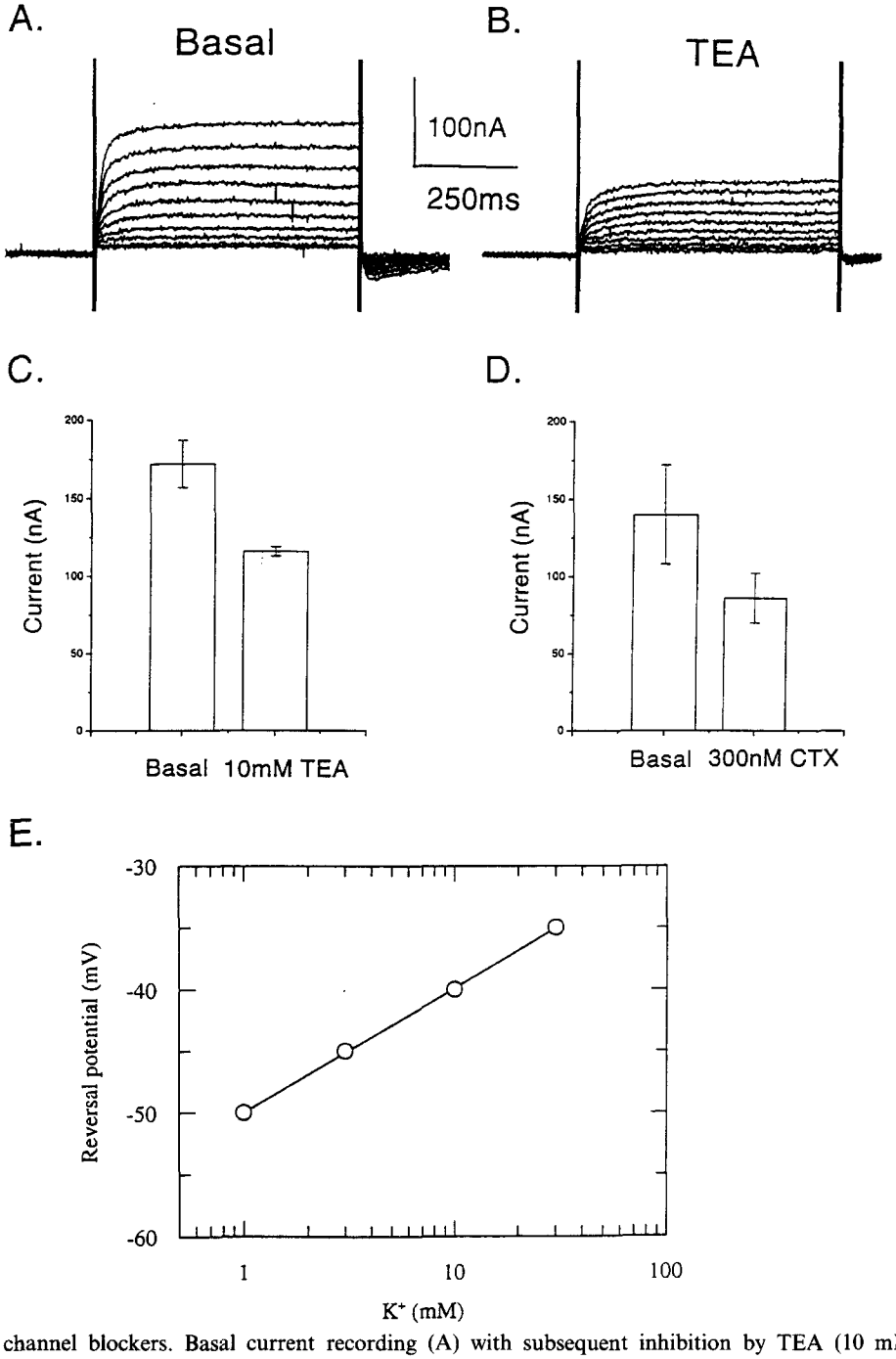

Fig. 2. Effect of K^+ channel blockers. Basal current recording (A) with subsequent inhibition by TEA (10 mM, B) obtained from RNA-injected oocytes. Summary of effect of TEA (C) and CTX (D) with corresponding current magnitudes at +30 mV. (E) Dependence on external $[K^+]$. Reversal potentials, measured by the tail currents, are plotted against K^+ concentrations in the bath ($[K^+]_o$). NaCl in the bath was fixed at 35 mM and KCl was varied from 1 to 60 mM with corresponding NMDG-Cl of 59 to 0 mM.

ence of a Ca^{2+}-activated K^+ channel has been demonstrated [11].

The present study has demonstrated the feasibility of using *Xenopus* oocytes to express RNAs extracted from the rat testis. Using this expression system, together with other techniques, we have been able to characterize a Ca^{2+}-activated K^+ channel predominantly present in spermatogenic cells. The RNA-in-

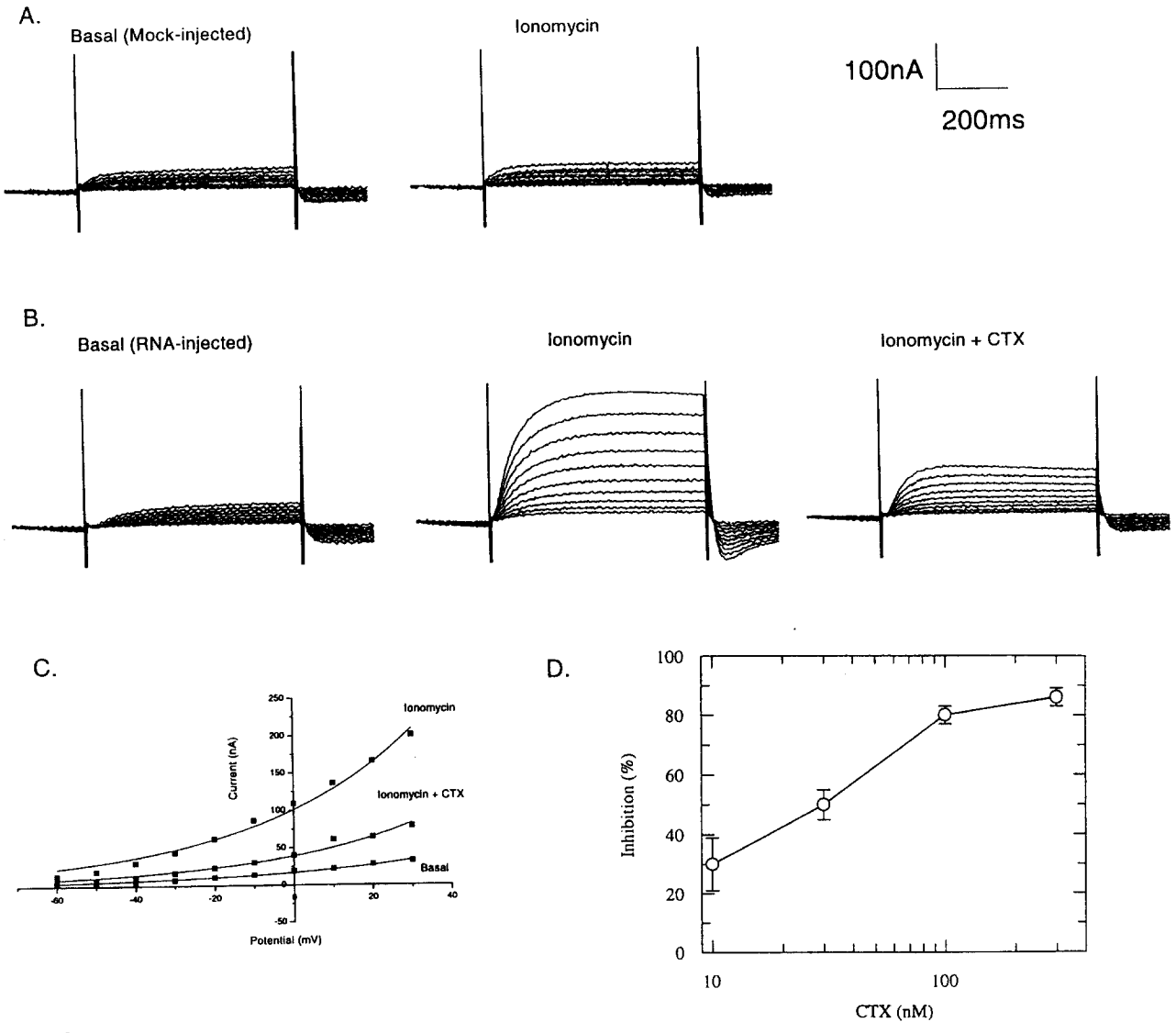

Fig. 3. Ca^{2+} ionophore-induced current and its sensitivity to K^+ channel blocker. (A) Current recordings before and after addition of ionomycin (0.5 μM) from a mock-injected oocyte. (B) Ionomycin (0.5 μM)-activated current recordings with subsequent inhibition by CTX (300 nM), and (C) corresponding *I–V* curves obtained from RNA-injected oocyte. (D) Concentration dependence of the CTX effect. Inhibition of the basal current in RNA-injected oocytes by CTX is plotted against the concentration of CTX used.

jected but not the mock-injected oocytes exhibited an observable basal current without stimulation, indicating overexpression of the channels as reported for many other ion channels expressed in *Xenopus* oocytes. Upon stimulation with the Ca^{2+} ionophore, ionomycin, the RNA-injected oocytes also responded with an increased current which also exhibited similar characteristics. These current characteristics include an outward rectification, a sensitivity to the Ca^{2+}-activated K^+ channel blockers, CTX or TEA, and a dependence on external K^+ concentration. The fact that the current can be activated by the Ca^{2+} ionophore indicates a Ca^{2+} dependence of the current. Taken together, the present results suggest the expression of a Ca^{2+}-activated K^+ channel in oocytes injected with RNAs from the rat testis. The type of the channel as well as its cellular localization have been further illustrated by immunohistochemical and RT–PCR studies using specific antibody and oligonucleotide primers, respectively, designed based on a 'Maxi' Ca^{2+}-activated K^+ channel sequence [12]. These studies confirm that a 'Maxi' Ca^{2+}-activated

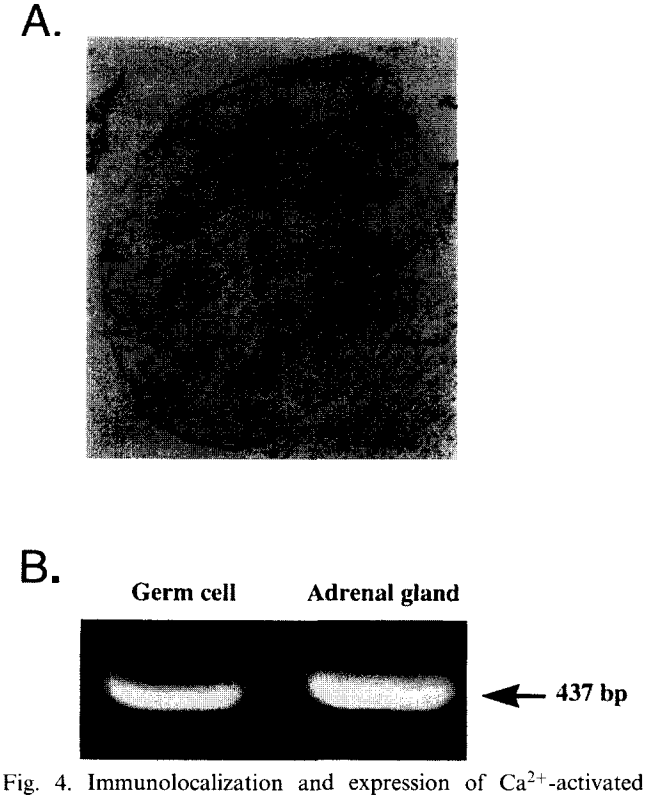

Fig. 4. Immunolocalization and expression of Ca^{2+}-activated K^+ channel. (A) Immunoreactivity, as indicated by the arrows, was predominantly associated with spermatogenic cells (×160). (B) RT–PCR products of 437 bp obtained from spermatogenic cells and adrenal gland (positive control). A polyclonal antiserum was raised against residues 913–926 of the *slowpoke* [12] using the sequence: VNDTNVQFLDQDDD. The immunogenic peptide was synthesized on a lysine core linked to a solid-phase peptide synthesis support. After cleavage, 2 μM of peptide emulsified in complete Freund's adjuvant was injected into two rabbits. The procedure was repeated 2 weeks later, and serum was collected 2 weeks afterwards. Immunostaining methods have been described previously [17]. To each RT reaction, 5 μg of total RNA were dissolved in 10 μl water and 1 μl of oligo(dT) primer (0.5 μg/μl). For the PCR, 20 μl was used in a total of 100 μl. The conditions were: denaturation at 94°C for 60 s; annealing at 53°C for 90 s; extension at 72°C for 90 s, 30 cycles. The specific oligonucleotide primers for the Ca^{2+}-activated K^+ channel were: CAAGATGGATGCGCTCATCA (sense) and TAGAAATTCTGGCAGGATTC (antisense), corresponding to nucleotides 78–515 with expected cDNA of 437 bp [12].

K^+ channel, as well as mRNA, is predominantly present in spermatogenic cells including the sperm.

The confirmation of the presence and localization of the Ca^{2+}-activated K^+ channel in spermatogenic cells including the sperm indicates that this channel may be involved in sperm function. The present finding lends support to the recent implication of Ca^{2+}-activated K^+ channels in hamster sperm acrosome reaction [13] and the initiation of sperm motility in Salmonid fishes [14]. Activation of K^+ channels, which was shown to be sensitive to TEA, by the egg peptide speract has also been demonstrated in patch-clamped hypoosmotically swollen intact sea urchin sperm [15]. The present demonstration of the presence of the Ca^{2+}-activated K^+ channel in sperm suggests that this channel may be involved in one or more sperm functions mentioned above. However, the exact role of the Ca^{2+}-activated K^+ channel in sperm functions remains to be elucidated. Studies are currently under way in our laboratory to investigate the modulation of the Ca^{2+}-activated K^+ channel by factors regulating sperm function and sperm–egg interaction.

The present study offers a methodology by which testicular ion channels, particularly those in spermatogenic cells, can be expressed and directly analyzed and characterized by electrophysiological techniques. Since spermatozoa are highly differentiated cells lacking the machinery for protein synthesis, the testis, which contains spermatogenic cells producing many long-lived mRNA for sperm proteins, would be the logical source of RNAs. However, there are other cell types, e.g. Sertoli and Leydig cells, present in the testis. It would be necessary to confirm the cellular localization of the ion channel expressed as demonstrated in the present study. On the other hand, the present methodology may also offer an easy access to ion channels or receptors in these testicular cell types without going through tedious cell isolation procedures, provided that the precise origin of the channel/receptor can be confirmed by other methods. In fact, we have also observed other types of channels and receptors, e.g. NMDA, kainate, GABA and acetylcholine, expressed in testis RNA-injected oocytes; however, the origin of these channels and receptors awaits further investigation. The co-expression of ion channels, receptors and perhaps other regulatory factors allows further investigation of ion channel regulation mediated by receptors, which would could not possibly be conducted in the reconstituted bilayer system.

In summary, the present investigation has demonstrated the feasibility of using the *Xenopus* oocyte as an expression system to probe ion channels/receptors

in spermatogenic cells and/or spermatozoa, which would be crucial for elucidating detailed mechanisms governing the processes of spermatogenesis, sperm maturation and activation, and sperm–egg interaction.

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