ELSEVIER

Biochimica et Biophysica Acta 1324 (1997) 18–26

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Chlorpromazine activates chloride currents in *Xenopus* oocytes

Gary A. Quamme *

Department of Medicine, University of British Columbia, Vancouver Hospital, Koerner Pavilion, Vancouver, BC, Canada

Received 23 April 1996; revised 14 September 1996; accepted 24 September 1996

Abstract

Xenopus oocytes are frequently used for in vivo expression of DNA and RNA, especially those encoding ion channel proteins. Accordingly, it is important to understand the expression and control of endogenous conductances. Ionic currents were studied in native *Xenopus* oocytes with two-microelectrode voltage-clamp technique to characterize the actions of chlorpromazine (CPZ) and trifluroperazine (TFP), two widely used antipsychotic drugs. External application of CPZ or TFP markedly stimulated endogenous conductances in a dose-dependent and reversible fashion. The current-voltage (I–V) relationship was non linear and dependent on the presence of external chloride. The CPZ-activated currents were inhibited by Cl^- channel blockers. Although the removal of external Ca^{2+} had no effect on CPZ-induced conductances, the injection of BAPTA, a Ca^{2+} chelator, abolished endogenous activity. Thapsigargin also inhibited channel activity suggesting that CPZ acts through intraoocyte Ca^{2+} release. The calmodulin inhibitors, calmidazolium and W-7, failed to mimic the action of CPZ. These data provide evidence for external or internal phenothiazine receptors which when activated by CPZ induces Ca^{2+}-dependent Cl^- channel activity in endogenous native oocytes.

Keywords: Voltage-clamp; Fluorescence; Trifluopromazine; Intraoocyte Ca^{2+}

1. Introduction

Xenopus laevis oocytes are widely used to express plasma membrane proteins encoded in exogenously injected RNA or DNA [6,7,31]. Oocytes are also frequently used to characterize the electrophysiological properties of the expressed receptors, transporters, and channels [7,36]. These heterologous expressed transport proteins have to be differentiated from the endogenous ones present in the native oocyte. Several Cl^- currents are found in *Xenopus* oocytes [7]. These include the calcium-activated Cl^- channel [5,21,22,32] which is the major anionic conductance likely involved in fertilization potentials in mature oocytes [34]. These currents are very sensitive to niflumic acid and other Cl^- channel blockers but are not affected by multivalent cations. Another channel which has been reported to be present in native oocytes is a calcium-insensitive Cl^- channel which is activated by hyperpolarization [15,22,26]. This channel is blocked by barium and partially by the stilbene, SITS, but not by niflumic acid [15]. A number of Cl^- conductances involved with osmoregulatory functions have also been found [1,3]. Yang and Sachs provided evidence for a stretch-activated channel which was inhibited by the trivalent cations, gadolinium, and lanthanum [37]. Ackerman et al demonstrated a calcium-independent Cl^- current activated by hypotonicity which was present in native oocytes and

* Corresponding author. Fax: +1 604 8227879.

PII S0005-2736(96)00205-2

which may play a role in volume regulation [1]. These Cl^- channels were inhibited by the Cl^- channel blocker, NPPB, and lanthanum but not by niflumic acid. In addition to these channels which have been reported, there may be other Cl^- conductances in *Xenopus* oocytes.

Chlorpromazine (CPZ) and its congers are widely used in clinical medicine to control psychotic disorders. These agents have many cellular effects including inhibition of dopaminergic receptor actions [30], inhibition of Ca^{2+}-calmodulin processes [35], and inhibition of transport [8,14,18,24,25]. The importance of any of these effects on the use of phenothiazines in medicine is not fully understood.

In the present study, we describe a CPZ-activated Cl^- current in native oocyte membranes. Our data suggest that CPZ activates a Ca^{2+}-dependent Cl^- current through receptor-mediated increases in intracellular Ca^{2+} release.

2. Materials and methods

2.1. Materials

The Ca^{2+} chelator, BAPTA-AM, and the fluorescent dye, fura-2 acid were obtained from Molecular Probes, Eugene, OR. Trifluroperazine (TFP) was a gift of Smith, Kline, and French. NPPB, 5-nitro-2-(3-phenylpropylamino)-benzoic acid was from Research Biochemicals International, Natick, MA. DIDS, 4,4-diisothiocyanatostilbene-2, 2-disulfonic acid, and DMSO, dimethylsulfoxide, and other chemicals were purchased from Sigma, St. Louis, MO.

2.2. Isolation of oocytes

Adult *Xenopus laevis* were anaesthetized with 1.5/l 3-amino benzoic acid-ethylester methanesulfonate (Tricaine, Sigma) and several lobes of the ovary were removed. Fully grown oocytes (1.2–1.3 mm diameter, stages V and VI [9]) were selected after removal of the follicular cell layer by treatment with collagenase (3 mg/ml, type II, Sigma) in ORII buffer containing (in mM): NaCl, 82.5; KCl 2.0; $MgCl_4$, 1.0; Hepes/Tris 10; pH 7.4, for 3 h at 19°C with gentle continuous agitation. The oocytes were subsequently washed extensively with this solution and the remaining follicular cells manually stripped off. The oocytes were maintained for 2–5 days in ND96 solution (in mM): NaCl, 96; KCl, 2.0; $CaCl_2$, 1.8; $MgCl_2$, 1.0; Hepes-Tris, 5.0; pH 7.4 containing Na pyruvate, 2.5, and gentamycin (5 μg/ml) at 18°C with continuous gentle agitation. The solutions were changed on a daily basis. All studies were performed within 3–6 days following isolation of the oocytes.

2.3. Electrophysiological measurements

For the electrophysiological measurements single oocytes were placed in a small Plexiglass chamber (0.5 ml) and were superfused constantly. Voltage-clamp experiments were performed by conventional two-microelectrode techniques and data acquisition and analysis were done using pClamp software (Axon Instruments, Foster City, CA). The microelectrodes were filled with 3M KCl and had resistances of 0.5 ~ 1.0 megohms. The basal resting membrane potential was initially determined 5–10 min after impalement. For most of the electrophysiology experiments, the oocytes were clamped at a holding potential of −70 mV and 750 ms voltage steps applied from −155 to +70 mV in 25 mV increments. Currents were measured without leak subtraction. Unless otherwise indicated, at the time of study, the oocytes were superfused with ND 96 buffer. All experiments were performed at 21°C.

2.4. Determination of cytosolic-free Ca^{2+}

Isolated oocytes were loaded with a final concentration of 100 μM fura-2 acid. The fluorescent dye, dissolved in dimethyl sulfoxide (DMSO), was injected into the oocyte with a micropipette with the aid of Pluronic acid (0.125%) and incubated for 20 min at 23°C. The final concentration of DMSO in the incubation medium did not exceed 0.01%. Glass cover slips, with cells loaded with fura-2, were mounted in a chamber containing 500 μl buffer placed on the mechanical stage of an inverted microscope (Diaphot; Nikon, Melville, NY). The fluorescence signal was monitored at 500 nm with excitation wavelengths alternating between 335 and 385 nm using a spectrofluorometer (Deltascan; Photon Technologies, Santa Clara, CA). Because of the large size of the oocyte, we were unable to calculate the abso-

lute Ca^{2+} concentration; accordingly, we report the 335/385 nm fluorescence ratio. The 335/385 nm ratio varies with the cytosolic Ca^{2+} concentration [12].

Pharmacologic agents were added directly to the bath from freshly made stock solutions. DIDS, NPPB, chlorpromazine, trifluoperazine (TFP) and thapsigargin were made up in DMSO; the final concentration of DMSO did not exceed 0.05% and did not affect basal current measurements. Unless specified the various drugs were applied 90 s prior to the addition of the phenothiazines.

2.5. Statistics

Data are presented as means ± SEM, where *n* indicates the number of separate experiments performed on separate oocytes.

3. Results

3.1. Phenothiazine-activated channel activity

The addition of chlorpromazine (CPZ) or trifluoperazine (TFP) to the bathing solution induced an oscillatory decrease in holding current in previously quiescent oocytes (Fig. 1). The onset of current activity was rapid beginning within 20 s of application of either drug. The oscillations lasted until agent was removed. Current activity was fully reversible at low CPZ concentrations but residual activity persisted with TFP and with high concentrations of CPZ. These phenothiazine-stimulated currents were observed in all oocytes studied throughout this study. Injection of 150 μM (final concentration) CPZ into the oocytes failed to illicite current activity. Accordingly, the CPZ-induced currents appear to be though activation of surface receptors rather than intraoocyte mechanisms.

3.2. Current-voltage (I–V) relationships of endogenous currents

In the absence of CPZ or TFP, there was a small background current of −147 ± 24 nA at −105 mV and 158 ± 25 nA at +70 mV with a slope conductance of 2.0 ± 0.2 μS at −11 mV ($n = 9$ oocytes, $N = 5$ frogs). Following the addition of 150 μM CPZ, the current increased to −316 ± 91 nA and 3524 ± 869 nA at −105 mV and +70 mV, respectively (Fig. 2). This current was voltage-dependent and showed a reversal about −20 mV which is suggestive for the presence of Cl^- conductances [2]. The slope conductance at −9 mV increased from 2.0 to 12.2 ± 3.5 μS with the administration of 100 μM CPZ. Fig. 3 illustrates the effect of TFP on the I–V relationship. The application of 150 μM TFP increased the current from −176 ± 16 to −788 ± 340

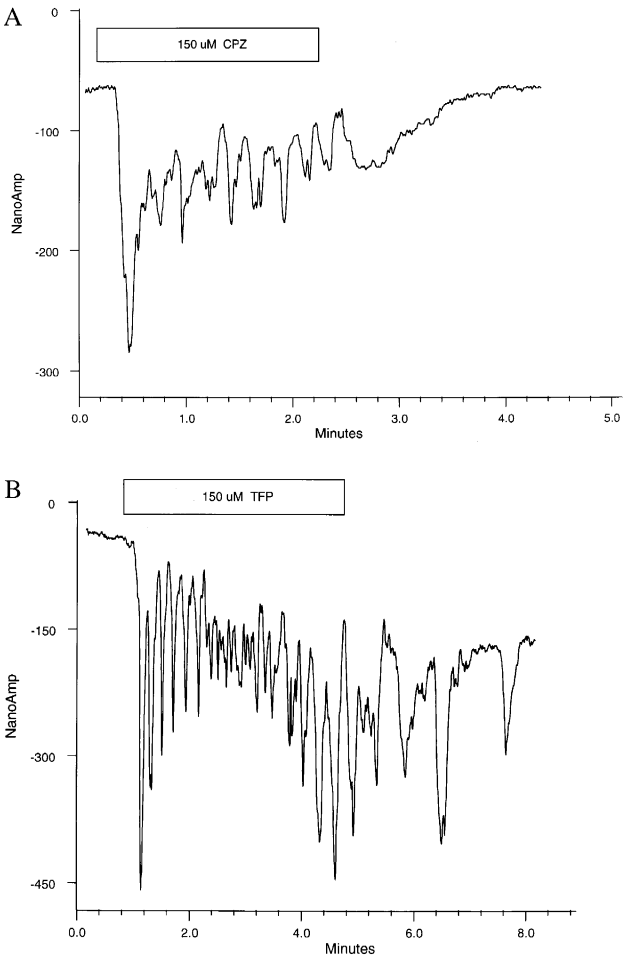

Fig. 1. Phenothiazine-induced current activity in previously quiescent *Xenopus* oocytes. Chlorpromazine (CPZ), in A, and, trifluoperazine (TFP) in B, were applied and removed where indicated at concentrations of 150 μM. The resting membrane potential was −50 mV (A) and −52 mV (B) and were voltage-clamped at a holding potential of −70 mV prior to addition of CPZ or TFP. Tracings are representative of 4 separate oocytes from 3 different frogs for each of the phenothiazines.

nA at −105 mV and from 190 ± 9 nA to 5323 ± 422 nA at +70 mV, respectively, and increased the slope conductance at −16 mV to 23.6 ± 4.1 μS.

3.3. Concentration-dependence of CPZ- and TFP-induced current activities

Experiments were performed to test the concentration-dependence of CPZ and TFP on current activation. CPZ activated endogenous currents in a concentration-dependent fashion. A concentration of 65 ± 18 μM was required to activate the current by half-maximal amounts as determined from the slope conductance versus the log function of the CPZ concentration (Fig. 4). Fig. 5 illustrates the concentration-dependence of TFP on stimulation of endogenous currents in oocytes. The concentration of TFP required to half-maximally stimulate current activity was 129 ± 24 μM.

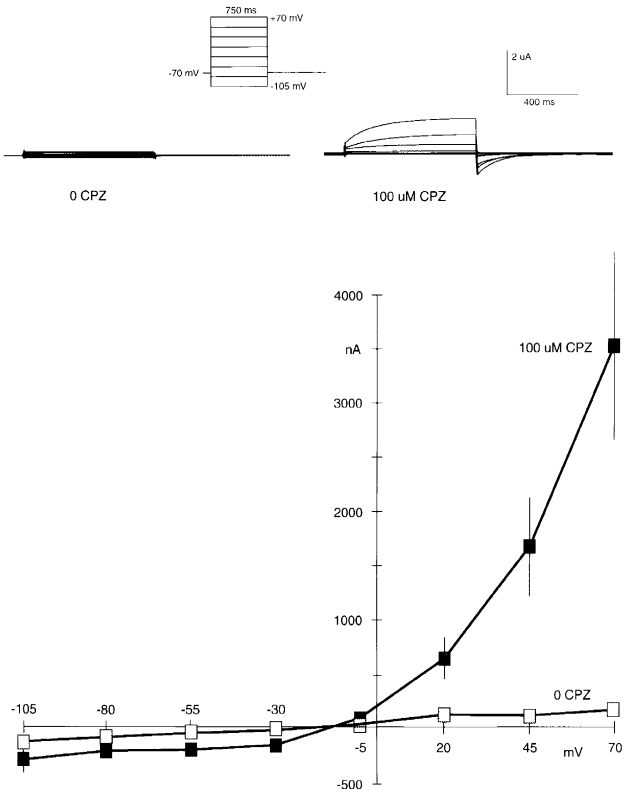

Fig. 2. The response of CPZ on the current-voltage (I–V) relationship of endogenous currents. Top panel shows the experimental protocol. Currents were measured with 750-ms pulses from −105 to +70 mV in 25 mV steps from a holding potential of −70 mV. CPZ (100 μM) was added 20 s prior to current measurements. Bottom panel illustrates the current-voltage relationship with and without the addition of CPZ (100 μM). The studies in the absence of CPZ were performed prior to those using CPZ on the same oocyte. Values are mean ± SEM, $n = 5$ oocytes.

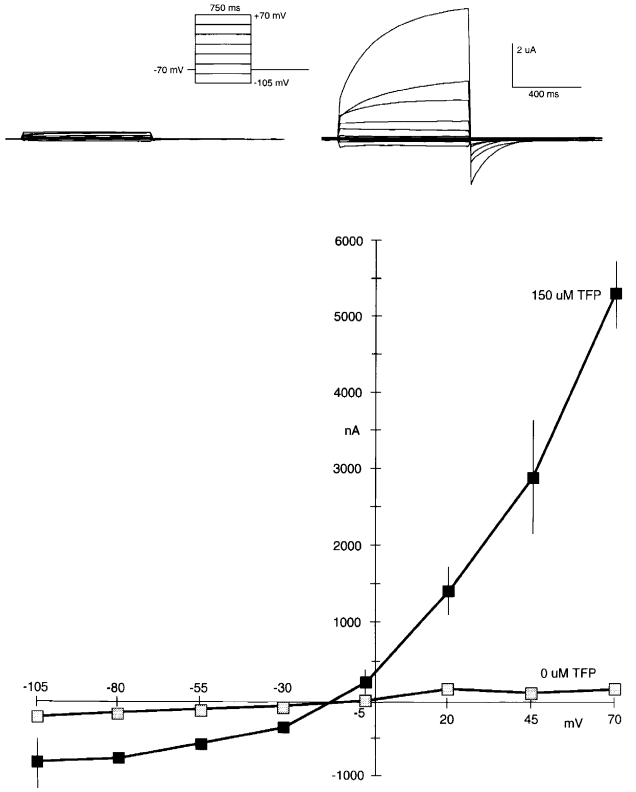

Fig. 3. The response of TFP on the current-voltage (I–V) relationship of endogenous currents. The studies were performed as given in the legend for Fig. 2 with 150 μM TPZ. The results with and without TPZ were performed on the same oocyte. Values are mean ± SEM, $n = 5$ oocytes.

3.4. Inhibition of CPZ-induced currents by Cl^- channel blockers

Extracellular DIDS, NPPB, and niflumic acid, known inhibitors of Cl^- channels, inhibited the conductance in a voltage and concentration-dependent fashion further supporting the notion that the current induced by CPZ was carried by chloride (Fig. 6). Although members of the Cl^- channel family possess a significant structural similarly there are several functional differences among them, one being the sensitivity to inhibitors [1,15,22,26]. Concentrations

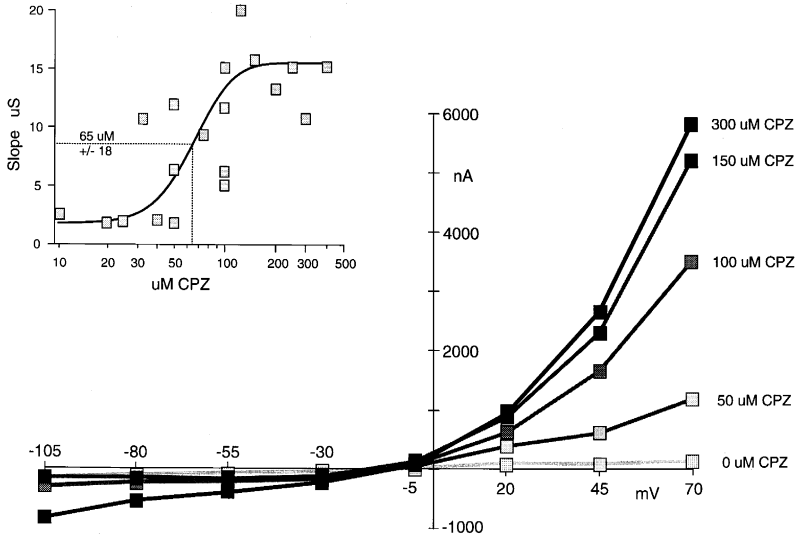

Fig. 4. The concentration-dependent effects of CPZ on current activity. Currents were measured with 800 ms pulses from −105 to +70 mV in 25 mV steps from a holding potential of −70 mV. CPZ was added 20 s prior to current measurements. The inset illustrates the slope conductance for each of the concentrations. The I–V curves at each concentration was a mean of 3–9 oocytes. The concentration of CPZ required to induce half-maximal stimulation was obtained using the Gompertz model as described by Richards [29]. The CTZ concentration for half-maximal current stimulation was 65 ± 18 μM.

of 100 μM DIDS, NPPB, and niflumic acid inhibited CPZ-stimulated conductance by 88 ± 7, 91 ± 5, and 70 ± 11% respectively at 70 mV. The concentration of DIPS NPPB, and niflumic acid which inhibited CPZ-stimulated current by 50% was 84 ± 17, 31 ± 8, 70 ± 11 μM, respectively. The current appeared to

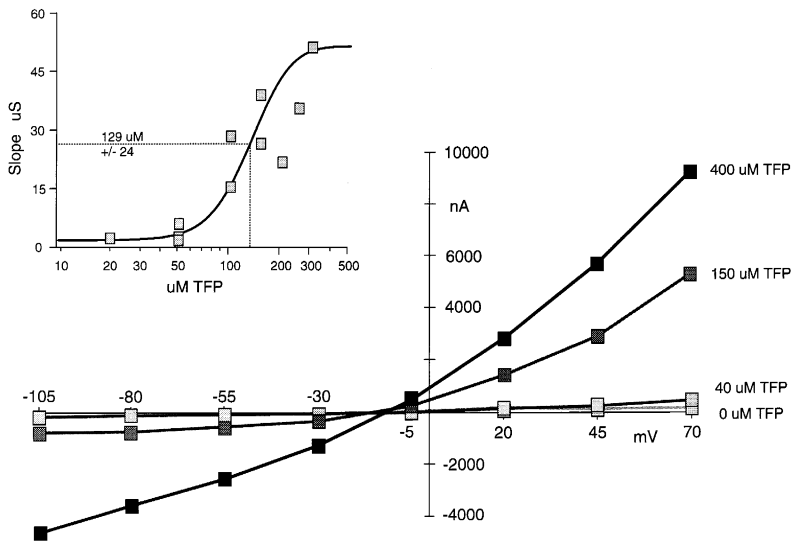

Fig. 5. The concentration-dependent effects of TFP on current activity. Currents were measured with 800 ms pulses from −105 to 70 mV in 20 mV steps from a holding potential of −70 mV. TFP was added 20 s prior to current measurements. The insert shows the slope conductance as a function of TFP concentration. The TFP concentration for half-maximal stimulation of current was 129 ± 24 μM. The I–V curve at each concentration was a mean of 2–4 oocytes.

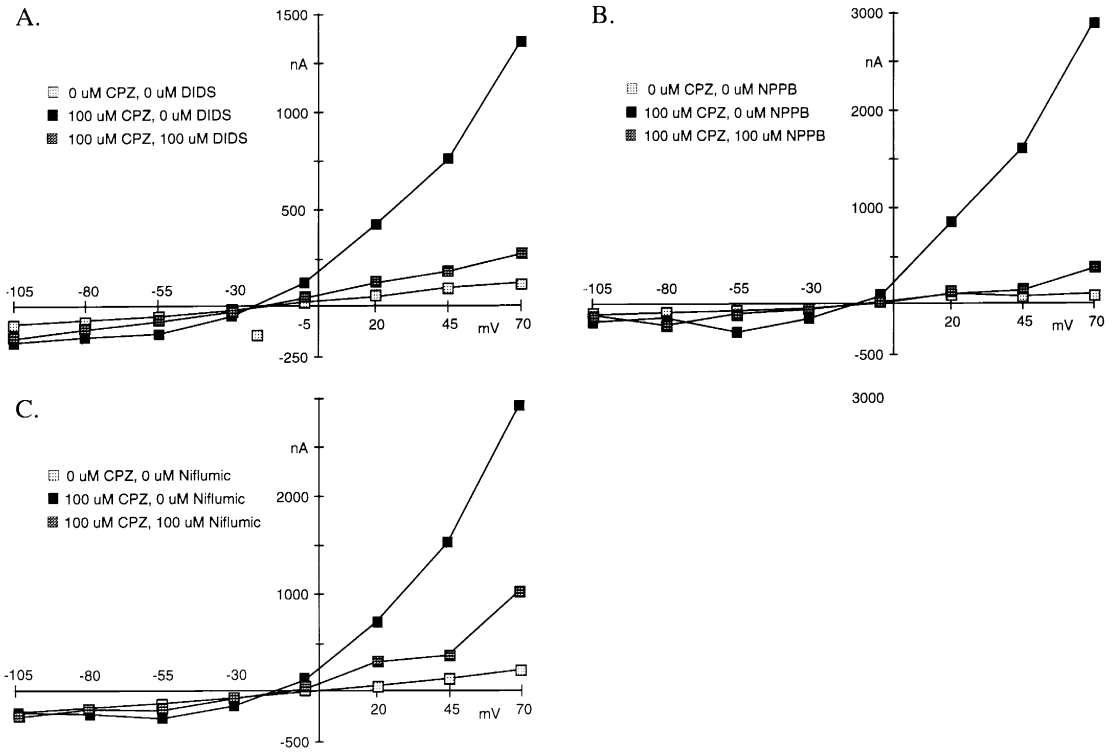

Fig. 6. Effect of chloride channel blockers on CPZ-activated currents. The effect of 100 μM DIDS (A), NPPB (B), or niflumic acid (C) on I–V relationship of CPZ-activated Cl^- currents. Currents were measured in bath solutions as given in legend to Fig. 3, 10 min after adding DIDS, 30 s after adding NPPB or 30 s after adding niflumic acid. I–V plots are representative experiments performed on 2–5 oocytes.

be equally sensitive to the channel blockers used here.

The trivalent cation, lanthanum, inhibited hypotonicity-activated Cl^- currents of *Xenopus* oocytes in a concentration-dependent manner [1]. In the present studies, lanthanum did not inhibit CPZ-induced Cl^- currents in oocytes; -256 ± 18 nA at -105 mV and 4046 ± 215 nA at 70 mV, $n = 3$ (data not shown). Barium also had no effect on the CPZ-induced currents -254 ± 26 nA at -105 mV and 4220 ± 414 nA at 70 mV (data not shown). These cations, La^{3+} and Ba^{2+} did not affect the basal currents 152 ± 10 nA and 182 ± 21 nA at 70 mV, $n = 3$, respectively observed in the native oocyte [1].

3.5. Characterization of CPZ activation of Cl^- currents

In order to determine the basis of CPZ-activation, we first assessed the importance of external Ca^{2+} on Cl^- currents. Removal of Ca^{2+} from the bathing solution had no effect on the activation of Cl^- currents by CPZ (Fig. 7a). These observations suggest that CPZ induction of Cl^- currents is not dependent on the presence of extracellular Ca^{2+}.

In order to differentiate the CPZ-induced Cl^- current among the various Cl^- channels, we clamped intraoocyte Ca^{2+} concentration ($[Ca^{2+}]_i$) with the injection of BAPTA. In these oocytes, CPZ failed to activate Cl^- currents suggesting that intracellular Ca^{2+} is required to induce currents (Fig. 6). These observations further suggest that CPZ may activate Cl^- channels through a calcium-dependent mechanism.

Next, we used thapsigargin, a specific inhibitor of microsomal Ca^{2+}-adenosine triphosphatase (Ca^{2+}-ATPase) that has been shown to deplete endoplasmic reticulum Ca^{2+} stores, to assess the basis of CPZ-induced Cl^- currents [11]. Pretreatment of the oocytes with thapsigargin abolished the CPZ-activated cal-

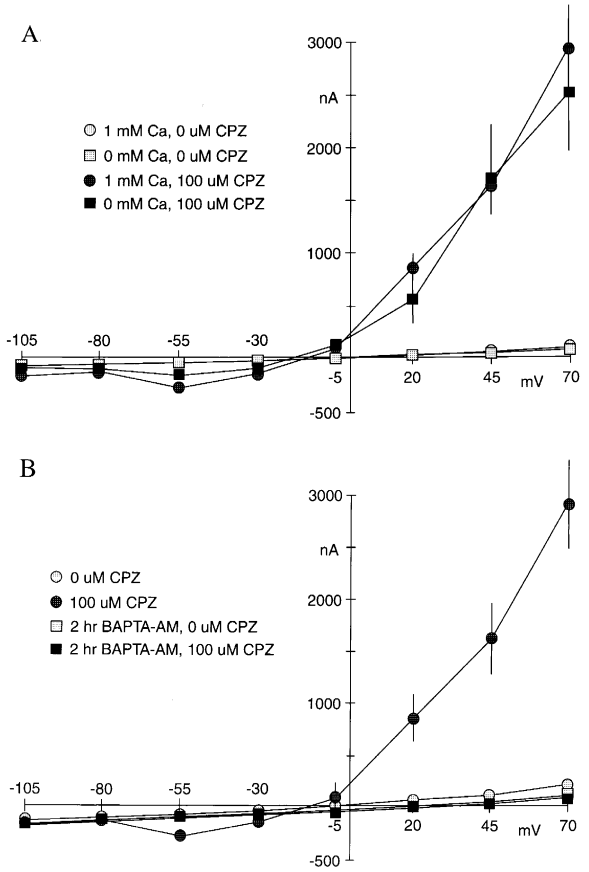

Fig. 7. Characterization of CPZ activation of Ca^{2+}-dependent Cl^- currents. A. Effect of external Ca^{2+} on CPZ-induced Cl^- currents. I–V profiles were determined as given in legend to Fig. 2 with and without Ca^{2+} in the bathing solution. $CaCl_2$ was replaced in the ND96 solution by isosmotic substitution with NaCl.

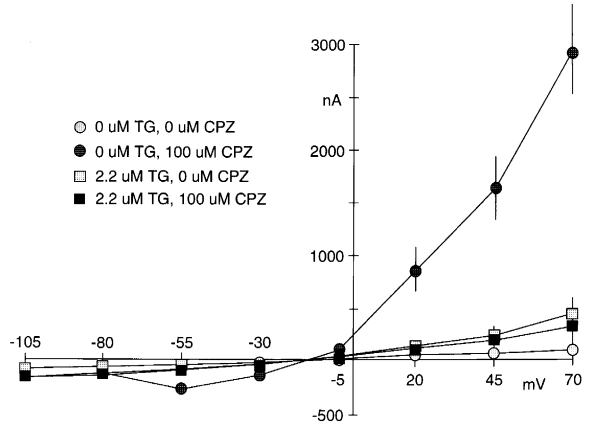

Fig. 8. Effect of thapsigargin on CPZ-induced Cl^- currents. The oocytes were pretreated (20–25 min) with thapsigargin (2.2 μM) prior to determining CPZ-induced I–V relationships. Values are means ± SE, $n = 3$ oocytes. CPZ-induced Cl^- currents require an increase in $[Ca^{2+}]_i$. Oocytes were injected where indicated with 15 nl BAPTA (1 M) about 120 min prior to the application of CPZ (100 μM), and an I–V protocol performed as given in legend to Fig. 2. Tracings are mean ± SE of 3 oocytes.

cium-dependent Cl^- channels (Fig. 8). Thapsigargin did not alter basal Cl^- currents in native oocytes (data not shown). The interpretation of these results predicts that CPZ increases Ca^{2+} within the oocyte likely through release from the endoplasmic reticulum which in turn activates surface Cl^- channels.

Microfluorescence was used with the Ca^{2+}-sensitive dye, fura-2, to determine whether CPZ induced an increase in intraoocyte Ca^{2+} which may activate endogenous currents. Fig. 9 shows a representative fluorescent tracing. The application of CPZ to *Xenopus* oocytes led to a marked increase in intraoocyte Ca^{2+} concentration as reflected by the increase in the 335/380 nm fluorescence ratio. These studies support the notion that CPZ leads to an enhanced channel activity through the release of intracellular Ca^{2+}.

The phenothiazines, including CPZ and TFP, are potent calmodulin inhibitors [35]. To test the hypothesis that CPZ may stimulate channel activity through

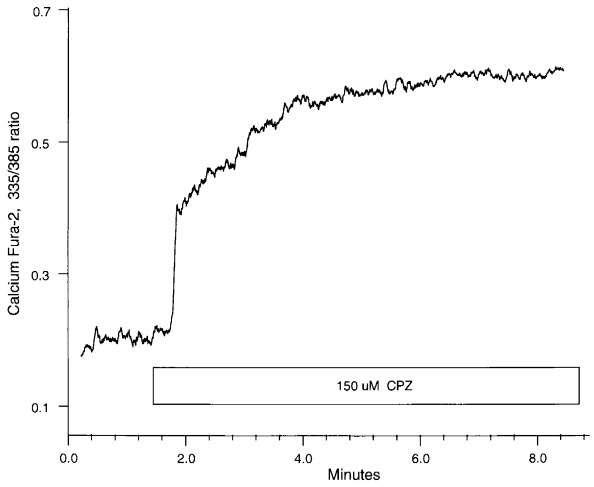

Fig. 9. CPZ-induced changes in intracellular Ca^{2+} concentration in *Xenopus* oocytes. CPZ, 100 μM was added where indicated. $[Ca^{2+}]_i$ was determined by microfluorescence with fura-2 as reflected by the 335/380 excitation ratio [11]. Results are representative of 4 cells.

calmodulin inhibition we pretreated oocytes with the calmodulin inhibitor, calmidozolium, and performed current-voltage experiments. Calmodulin had little effect on endogenous current activity; 154 ± 20 nA at -105 mV and 83 ± 18 nA at 80 mV with 25 μM calmidazolium compared to 124 ± 11 nA and 67 ± 11 nA, $n = 5$, respectively, without the inhibitor. Additionally, another calmodulin inhibitor W-7, 100 μM, failed to stimulate channel activity; 196 ± 7 nA at -105 mV and 247 ± 6 nA at 70 mV, $n = 4$.

4. Discussion

CPZ and its congers are widely used in clinical medicine for a variety of psychiatric disorders. The alleged basis for their use and action are also widespread involving, inhibition of Ca^{2+}-calmodulin [35], and inhibition of enzyme activities such as Na^+/K^+-ATPase, Ca^{2+}-ATPase, acetylcholinesterase [2], phosphodiesterase, [3,13], and inositol [1,4,5] triphosphate-5-phosphatase activities [10]. CPZ acting through these enzymes or directly on the membrane may inhibit many transport functions such as Ca^{2+}-dependent K^+ currents in canine smooth muscle cells [18], rat hippocampal pyamidal neurons [8] and Madin-Darby canine kidney (MDCK) cells [27], ATP-sensitive K^+ currents [14] and divalent cation channels [16] in rat ventricular cells [14], Na^+ currents [25], and Ca^{2+} currents [24] in mouse neuroblastoma cells, sodium gating currents in squid giant axons [13], and nicotinic acetylcholine receptors in mouse muscle cells [17] and *Torpedo* neurons [28].

In the present study, we show that the phenothiazines, CPZ and TFP, induce an endogenous calcium-activated Cl^- current in native oocytes likely by activating surface receptors as intraoocyte injection in contrast to external application failed to illicite current activity. However, CPZ injected into the oocyte may not reach the same intracellular calcium stores as CPZ that is applied to the surface. Nevertheless, the data indicate that phenothiazine receptors are present either on the surface or in the oocyte which releases intracellular Ca^{2+}. CPZ and TFP induced currents in a dose-dependent fashion which is reversible at low agonist concentrations. The evidence for activation of a Cl^- current is persuasive. The reversal current was in the order of -20 mV which is suggestive for chloride. Additionally, the CPZ-activated currents were inhibited by the commonly used Cl^- channel blockers, DIDS, NPPD, and niflumic acid. The evidence also supports the notion that this is a Ca^{2+}-dependent Cl^- current in that chelation of intraoocyte $[Ca^{2+}]$ with BAPTA abolished the CPZ-activated currents. Accordingly, CPZ, in addition to its many effects on the nervous system, also activates endogenous Ca^{2+}-dependent Cl^- currents in *Xenopus* oocytes.

The mechanisms of CPZ on channel proteins are presently not known. It has been speculated that many of the effects of CPZ are due to unspecific membrane actions on channel proteins [14]. CPZ is an amphiphilic agent which may interact with membrane phospholipids to alter the lipid phase transitions [17,23,33]. It is unlikely that the lipomonetic effects of phenothiazines play a role in activating Cl^- currents in the present study. First, chelation of intraoocyte Ca^{2+} with BAPTA abolished the effects of CPZ. Second, the inferential inhibition of intraoocyte Ca^{2+} release with thapsigargin mitigated the action of CPZ-activated Cl^- currents. The basis for the CPZ-induced increase in intraoocyte Ca^{2+} concentration is not fully understood. Cl^- currents were not dependent on external Ca^{2+} in the bathing solution (Fig. 7). For these reasons, we speculate that CPZ and TFP increase intraoocyte Ca^{2+} through release of Ca^{2+} from endoplasmic reticulum stores. Phenothiazine have been reported to inhibit sarcoplasmic reticulum Ca^{2+}-ATPase through an inositol trisphosphate (IP_3) independent manner in a sufficiently rapid fashion to increase Ca^{2+} efflux to regulate physiological events in the cell [20]. de Meis and Inesi have shown that Ca^{2+} efflux through the Ca^{2+}-ATPase is sensitive to thapsigargin [19]. On the other hand, thapsigargin also inhibits IP_3-induced Ca^{2+} release from endoplasmic reticulum and sarcoplasmic reticulum [11]. Accordingly, chlorpromazine may increase intraoocyte Ca^{2+} by binding to receptors and generation of IP_3 with subsequent release of intraoocyte Ca^{2+}. This would require activation of phospholipase C likely through receptor-mediated or G-protein-dependent mechanisms. Further studies are required to define the specificity of this receptor.

Phenothiazines, including CPZ and TFP, are potent calmodulin inhibitors [4,35]. Accordingly, it is attractive to speculate that the Cl^- currents reported

here are regulated by Ca^{2+}-calmodulin, i.e. Ca^{2+}-calmodulin inhibited channels that may be activated by calmodulin inhibitors [27]. This is unlikely however as the CPZ concentration for Ca^{2+}-calmodulin inhibition is much less than the IC_{50} determined for activation of Cl^- currents [35] (Fig. 2). Second, intraoocyte Ca^{2+} chelation abolished CPZ-activation whereas calmodulin inhibitors, W-7 and calmidazolium, did not have similar effects as CPZ. For these reasons it would seem that Ca^{2+}-calmodulin inhibition is not directly involved in CPZ-activation of Cl^- currents.

In summary, CPZ and TFP activate endogenous Cl^- currents in native oocytes. The evidence suggests that CPZ-activation is through intraoocyte Ca^{2+} release which in turn stimulates surface Cl^- channels. The source of Ca^{2+} is likely the endoplasmic reticulum but the presence of phenothiazine receptors and/or Ca^{2+} release pathways are unknown. Also unknown is what physiological role CPZ-induced Cl^- currents play in the *Xenopus* oocyte. It is evident that the processes involved in stimulating these currents must be considered in the use of expression studies and the physiology of *Xenopus* oocytes.

Acknowledgements

We gratefully acknowledge the excellent secretarial assistance of Susanna Lau in the preparation of the manuscript. This work was supported by a research grant from the Medical Research Council of Canada (MT-5793).

References

[1] Ackerman, M.J., Clapham, D.E. and Wickman, K.D. (1994) J. Gen. Physiol. 103, 153–179.
[2] Adhikary, G., Nandy, P., Chandra, S., Kdar, P.S. and Sen, P.C. (1991) Biochem. Inter. 25, 951–961.
[3] Arellano, R.O. and Miledi, R. (1993) J. Gen. Physiol. 102,833–857.
[4] Asano, M. and Hidaka, H. (1984) Biopharmacological properties of naphthalenesulfonamides as potent calmodulin inhibitors. In: Calcium and Cell Function, Vol. 5 (Cheung, W.Y., ed.), pp. 123–164.
[5] Barish, M.E. (1983) J. Physiol. 342, 309–325.
[6] Benoit, P. and Changeux, J.P. (1993) Neurosci. Lett. 160, 81–84.
[7] Dascal, N. (1987) CRC Crit. Rev. Biochem. 22, 317–387.
[8] Dinan, J.G., Kelly, J.S. and Crunelli, V. (1987) Brain Res. 407, 159–162.
[9] Dumont, J.N. (1972) J. Morphol. 136, 153–180.
[10] Fowler, C.J. and Brannstrom, G. (1992) Methods Findings Exp. Clin. Pharmacol. 14, 629–636.
[11] Glennon, M.C., Kwan, C-Y., Bird, G.S.J. and Putney, J.W. Jr. (1992) J. Biol. Chem. 267, 8230–8233.
[12] Grynkiewicz, G., Peonie, M. and Tsien, R.Y. (1985) J. Biol. Chem. 260, 3440–3450.
[13] Ichikawa, M., Urayama, M. and Matsumoto, G. (1991) J. Memb. Biol. 120, 211–222.
[14] Kon, K., Krause, E. and Gögelein, H. (1994) J. Pharm. Exp. Ther. 271, 632–637.
[15] Kowdley, G.C., John, J.E., III, Moorman, J.R., Jones, LR. and Ackerman, S.J. (1994) J. Gen. Physiol. 103, 217–230.
[16] Lefevre, T., Coulombe, A., Ghazi, A. and Coraboeuf, E. (1995) J. Memb. Biol. 147, 147–158.
[17] Lejoyeux, M., Daveloose, D., Adés, J., Maziére, J.C. and Viret, J. (1992) Life Sci. 52, 7–11.
[18] McCann, J.D. and Welsh, M.J. (1987) J. Gen. Physiol. 89, 339–352.
[19] Meis de, L. and Inesi, G. (1992) FEBS Lett. 299, 33–35.
[20] Meis de, L. (1991) J. Biol. Chem. 266, 5736–5742.
[21] Miledi, R. and Parker, I. (1984) J. Physiol. 357, 173–183.
[22] Miledi, R. and Woodward, R.M. (1989) J. Physiol. 416, 601–621.
[23] Minetti, M. and Di Stasi, A.M.M. (1987) Biochemistry 26, 8133–8137.
[24] Ogata, N. and Narahashi, J. (1990) J. Pharmacol. Exp. Ther. 252, 1142–1149.
[25] Ogata, N., Narahashi, J. and Yoshi, M. (1990) J. Physiol. 420, 165–183.
[26] Parker, I. and Miledi, R. (1988) Proc. R. Soc. (Lond.) B233, 191–199.
[27] Paulmichl, M., Wöll, E., Lang, F., Weiss, H. and Waldegger, S. (1991) J. Cell. Physiol. 148, 314–319.
[28] Revah, F., Ledever, F., Giraudat, J., Galzi, J.L., Changeux, J.P. and Haumont, P.Y. (1990) Proc. Nat. Acad. Sci. USA 87, 4675–4679.
[29] Richards, F.J. (1959) J. Exp. Bot. 10, 290–300.
[30] Seeman, P. (1991) Pharmacol. Rev. 32, 229–313.
[31] Sigel, E. (1990) J. Memb. Biol. 117, 201–221.
[32] Takahashi, T., Neher, E. and Sakmann, B. (1987) Proc. Nat. Acad. Sci. USA. 84, 5063–5067.
[33] Tanji, K., Sekimizu, K., Kawato, S., Natori, S., Mizushima, T. and Ohta, Y. (1992) J. Pharm. Pharmacol. 44, 1036–1037.
[34] Webb, D.J. and Nuccitelli, R. (1985) Dev. Biol. 107, 395–406.
[35] Weiss, B., Wallace, T.L. and Prozialeck, W.C. (1982) Biochem. Pharmacol. 31, 2217–2226.
[36] Wright, E.M. (1993) Am. Rev. Physiol. 55, 575–589.
[37] Yang, X.C. and Sachs, F. (1987) Science 243, 1068–1071.