

## Tamoxifen Blocks Chloride Channels

### A Possible Mechanism for Cataract Formation

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#### Abstract

Tamoxifen is an antiestrogen frequently used in the treatment of breast cancer and is currently being assessed as a prophylactic for those at high risk of developing tumors. We have found that tamoxifen and its derivatives are high-affinity blockers of specific chloride channels. This blockade appears to be independent of the interaction of tamoxifen with the estrogen receptor and therefore reflects an alternative cellular target. One of the clinical side effects of tamoxifen is impaired vision and cataract. Chloride channels in the lens of the eye were shown to be essential for maintaining normal lens hydration and transmittance. These channels were blocked by tamoxifen and, in organ culture, tamoxifen led to lens opacity associated with cataracts at clinically relevant concentrations. These data suggest a molecular mechanism by which tamoxifen can cause cataract formation and have implications for the clinical use of tamoxifen and related antiestrogens. (*J. Clin. Invest.* 1994. 94:1690–1697.) **Key words:** tamoxifen • cataract • lens • P-glycoprotein • chloride channel

#### Introduction

Tamoxifen is an antiestrogen frequently used in the treatment of breast cancer (1) and is being assessed as a prophylactic treatment for those at high risk of developing breast tumors

(2). Tamoxifen interacts with the estrogen receptor (3–5), and this is generally considered to be the mechanism by which its pharmacological effects are mediated. The apparent specificity of tamoxifen for the estrogen receptor, and the absence of other known cellular targets, is one of the reasons tamoxifen and its derivatives are considered suitable for long-term, prophylactic treatment. However, tamoxifen is not without side effects. Rats fed tamoxifen develop cataract (6), and visual impairment and cataract have been reported in patients undergoing long-term tamoxifen treatment (7–9).

Tamoxifen has been reported to reverse multidrug resistance in P-glycoprotein-expressing cell lines (10–13), and tamoxifen binds to P-glycoprotein (Callaghan, R., and C. F. Higgins, manuscript submitted for publication). P-glycoprotein is a 170-kD polypeptide that confers multidrug resistance by pumping hydrophobic compounds out of cells, reducing their intracellular concentrations and, hence, toxicity (14). Expression of P-glycoprotein is also associated with cell volume-regulated chloride channels (15–17), and several compounds that reverse multidrug resistance, such as verapamil and 1,9-dideoxyforskolin, have been found to block these channels (16, 17). We therefore examined the effects of tamoxifen on chloride channel activity in P-glycoprotein-expressing cells.

Tamoxifen and tamoxifen derivatives were found to be reversible, high-potency blockers of cell volume-regulated chloride currents in several cell lines. Because cataract is one of the few reported side effects of long-term tamoxifen treatment, we examined the role of chloride channels, and their interaction with tamoxifen, in the lens. Chloride channels were found to be important for maintaining lens clarity. These channels were blocked by tamoxifen, and, in organ culture, tamoxifen caused opacification of lenses consistent with cataract formation. These findings suggest a molecular basis for the side effects of tamoxifen on visual impairment.

#### Methods

**Cells and expression system.** NIH3T3 *MDR1* cells are derivatives of NIH3T3 mouse fibroblasts that have been permanently transfected with the human *MDR1* gene; these cells express high levels of human P-glycoprotein (18–20). S1/1.1 cells are a derivative of the human S1 lung carcinoma line that has been permanently transfected with the human *MDR1* gene (21). We have shown previously that the cell volume-activated chloride channels in both these cell lines are associated with P-glycoprotein expression (15–17). MCF7<sup>Adr</sup> cells have amplified P-glycoprotein expression after selection for adriamycin resistance (22). Cells were grown in 35-mm plastic Petri dishes in Ham's F10 medium

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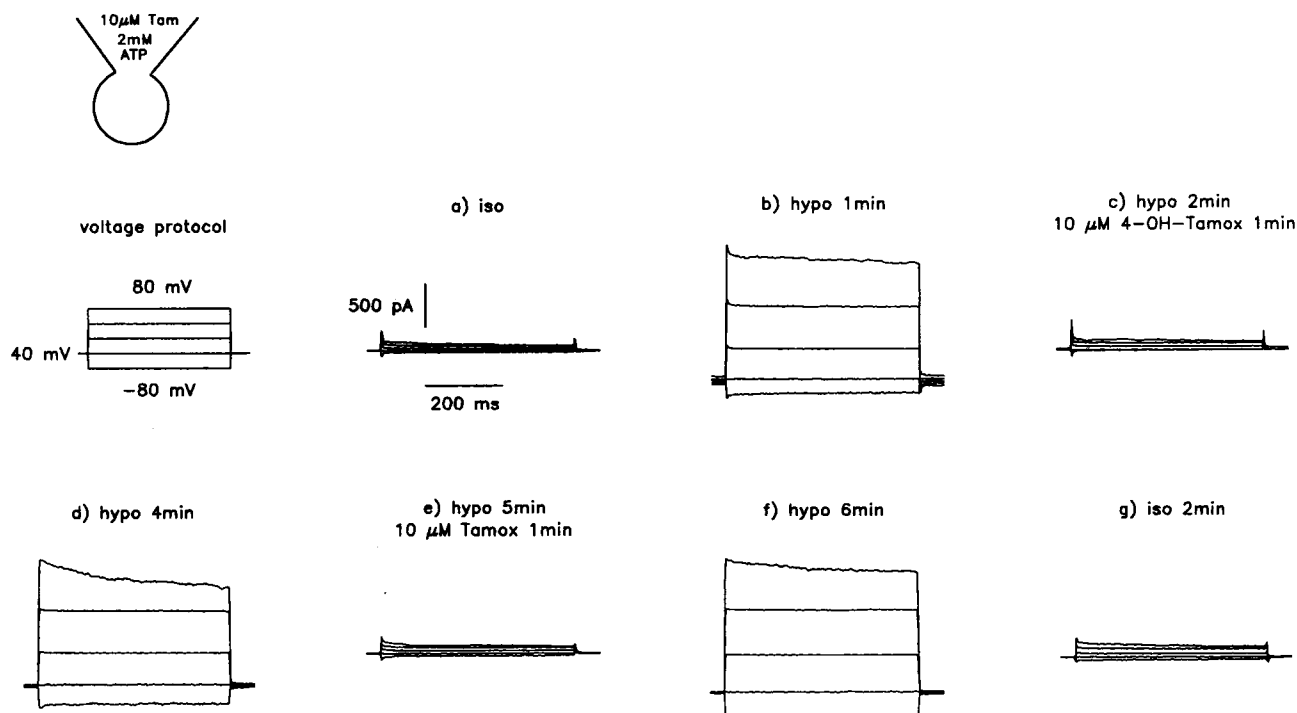
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**Figure 1.** Inhibition of volume-regulated chloride channels by tamoxifen and 4-hydroxy-tamoxifen. Chloride channels in S1/1.1 cells were measured in the whole-cell patch clamp configuration. The traces shown are for an individual cell. Similar results were obtained for four separate cells. *Top left*, the voltage protocol and the composition of the pipette solution (which included 10 μM tamoxifen). The times given in *a–f* indicate the time after breaking the seal and initiating the experiment. No currents were detected under isotonic conditions (*a*), but typical chloride currents were observed 1 min after shifting to a hypotonic bathing solution (*b*). The hypotonic bathing solution was then replaced with an identical solution containing 10 μM 4-hydroxy-tamoxifen, and current traces were taken 1 min later (*c*). Next, the bathing solution was replaced by a hypotonic solution lacking 4-hydroxy-tamoxifen, and 2 min later the currents could again be detected (*d*). Tamoxifen (10 μM) was then added, and the currents were recorded after a further minute (*e*). The bathing solution was then replaced with a similar hypotonic solution lacking tamoxifen and, after 1 min of incubation, currents were measured (*f*). Finally, *g* shows currents observed 2 min after returning the bathing solution to isotonicity.

(S1/1.1 cells) or Dulbecco's modified Eagle's medium containing 1 μg ml<sup>-1</sup> colchicine (NIH3T3 *MDR1* cells), supplemented with 10% fetal calf serum, and were used 48 h after subculturing.

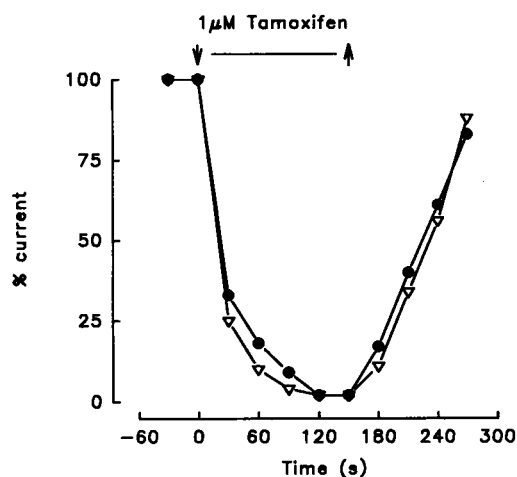
**Lens organ culture.** Lenses were dissected from bovine eyes within 2 h postmortem and incubated at 37°C in a buffered salt solution that resembles the aqueous humor (AH buffer: NaCl, 125 mM; KCl, 4.4 mM; NaHCO<sub>3</sub>, 10 mM; Hepes, 10 mM; CaCl<sub>2</sub>, 2 mM; MgCl<sub>2</sub>, 0.5 mM; glucose, 5 mM; sucrose, 20 mM; adjusted to pH 7.4 with 1 M NaOH).<sup>1</sup> The transparency of the lens was measured by placing it in a glass organ culture pot on the stage of a low-power microscope and measuring the light transmitted with a photoconductive cell (ORP-12; Radiospares, Corby, Northants, United Kingdom). The measured value was expressed as a fraction of the light transmitted by the medium alone. This fraction is termed transmittance; a value of 1.0 indicates 100% light transmittance.

**Electrophysiology.** Chloride currents in individual tissue culture cells were measured by the whole-cell recording mode of the patch-clamp technique as described previously (23, 15). The ionic compositions of the pipette and bath solutions were appropriate for measuring chloride currents. The holding potential was 0 mV, except where indicated, and currents were measured in response to square voltage pulses of 600 ms in duration. The pipette solution contained 140 mM *N*-methyl-D-glucamine chloride (NMDGCl), 1.2 mM MgCl<sub>2</sub>, 1 mM EGTA, 2

mM ATP, and 10 mM Hepes, with a final pH of 7.4. Isotonic bathing solution contained 140 mM NMDGCl, 1.3 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, and 10 mM Hepes with a final pH of 7.4. The hypotonic bathing solution was identical to the isotonic solution except that the NMDGCl concentration was reduced to 105 mM. The tonicities of the isotonic and hypotonic solutions were 300 and 220 mosmol liter<sup>-1</sup>, respectively, as measured by freezing point depression. When required, the extracellular (bathing) solution was changed by directing a small jet of solution at the cell, as described previously (24).

To study chloride channels in the lens of the bovine eye, excised inside-out membrane patches were obtained from fiber cell vesicles (25). These vesicles were formed by removing the posterior core of the bovine lens and incubating in a shaking water bath at 37°C for 10–20 min in AH buffer (see above) lacking MgCl<sub>2</sub> and CaCl<sub>2</sub> but containing 0.5% trypsin and 0.2% EDTA. The lens fiber cells, which are up to 1 cm long, break up into vesicles during this treatment. The vesicles were embedded in agar by plating onto the surface of a cooling agar solution (0.6% in AH buffer) on a cover slip. The embedded vesicles were then placed in tissue culture medium (E199) containing 10% fetal calf serum. Patch-clamping was with a pipette solution (the extracellular solution for inside-out patches) consisting of NMDGCl, 130 mM; MgCl<sub>2</sub>, 0.5 mM; Hepes, 10 mM; CaCl<sub>2</sub>, 1 mM; sucrose, 30 mM adjusted to pH 7.4 with Trizma base. After formation of a high-resistance seal between the electrode and the vesicle, the electrode was withdrawn, excising the patch of membrane beneath the electrode. After excision, the bath solution (intracellular for inside-out patches) was changed to a buffer of the same composition as the pipette solution but with 1.1 mM EGTA and 1.05 mM CaCl<sub>2</sub> giving a buffered Ca<sup>2+</sup> concentration of 10<sup>-6</sup> M; 500 μM ATP was added as indicated. Single

1. **Abbreviations used in this paper:** AH, aqueous humor; NMDGCl, *N*-methyl-D-glucamine chloride; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid.



**Figure 2.** Time course of tamoxifen inhibition and its reversibility. Chloride channels in an S1/1.1 cell were activated by a 3-min exposure to hypotonic medium. At appropriate time intervals, currents were monitored during square voltage pulses of +80 mV (●) or -80 mV (▽). At time zero, 1  $\mu$ M tamoxifen was added to the hypotonic bathing solution and was removed 2 min later (arrows). Chloride currents, as a percentage of the current before adding tamoxifen, are plotted as a function of time.

channel activity was monitored at either 3 kHz (for amplitude and kinetic data) or 300 Hz (for blocking experiments). When used to block the lens channels, drugs were added to the extracellular (pipette) solution.

**Chemicals.** 5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), a kind gift of Smith, Kline and Beecham (Betchworth, Surrey, United Kingdom) was made up as a 10 mM stock solution in DMSO. Tamoxifen and its derivatives were made up as a 5 mM stock in ethanol (for experiments with cultured cells) and as a 50 mM stock solution in methanol (for lens experiments). Controls showed that these solvents had no effects on channel activity at the concentrations used (data not shown).

## Results

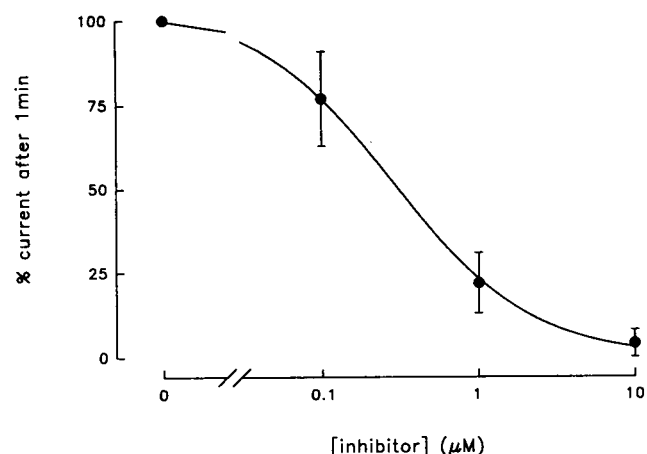
**Tamoxifen reversibly inhibits cell volume-regulated chloride currents.** The effects of tamoxifen on cell volume-regulated chloride currents in an individual S1/1.1 cell were studied by whole-cell patch clamping (Fig. 1). Currents were activated by replacing the extracellular (bathing) solution with a hypotonic solution, as described previously (16). Tamoxifen (10  $\mu$ M) was included in the pipette (intracellular) solution throughout the experiment to ascertain whether the drug influences channel function from the intracellular face of the membrane. No chloride currents were detected when the extracellular (bathing) solution was isotonic (Fig. 1 *a*), as expected. When the isotonic bathing (extracellular) solution was replaced with a hypotonic solution (Fig. 1 *b*), characteristic chloride currents were observed. In four separate experiments, the current evoked by a +80 mV pulse in the presence of 10  $\mu$ M intracellular tamoxifen was  $31 \pm 5$  pA/pF. This compares with  $28 \pm 3$  pA/pF ( $n = 38$ ) in the absence of tamoxifen (means  $\pm$  SEM). Thus, intracellular tamoxifen does not affect channel activity. However, when the hypotonic bathing (extracellular) solution was replaced with an identical solution containing 10  $\mu$ M 4-hydroxy-tamoxifen (a metabolite of tamoxifen generated *in vivo*; 26), the chloride

currents were abolished (Fig. 1 *c*). This inhibition was reversed when the bathing solution was replaced with an equivalent hypotonic solution lacking 4-hydroxy-tamoxifen (Fig. 1 *d*). Tamoxifen blocked the currents in a similar manner to 4-hydroxy-tamoxifen (Fig. 1, *e* and *f*). Finally, at the end of the experiment, the cell was returned to isotonic conditions; the currents returned to baseline, showing that the cell had remained intact throughout the manipulations (Fig. 1 *g*). Similar results were obtained for four independent cells. Thus, when added to the extracellular face of the membrane, tamoxifen reversibly blocked cell volume-regulated chloride currents.

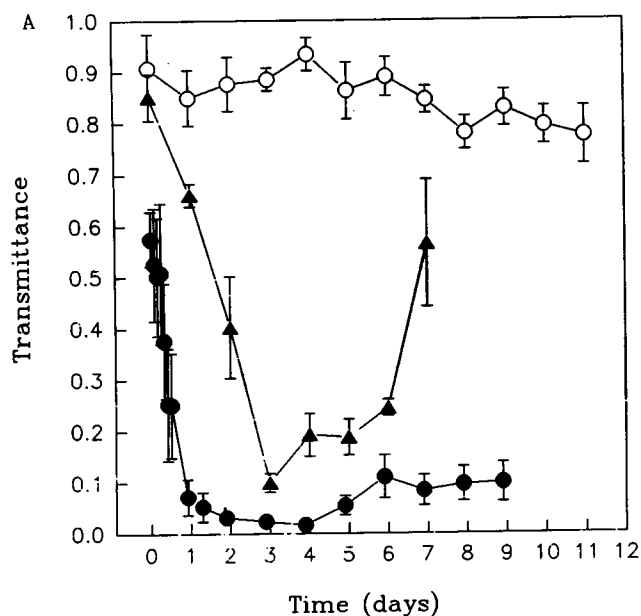
Extracellular tamoxifen inhibited the chloride currents when ATP was replaced with the nonhydrolyzable analogue AMP-PNP ( $n = 4$ ; data not shown). Tamoxifen also blocked cell volume-regulated chloride currents in other cell lines. The cell volume-regulated chloride currents in NIH3T3 *MDR1* cells are indistinguishable from those in S1/1.1 cells (15–17), and the effects of tamoxifen on currents in this cell line were indistinguishable from those on S1/1.1 cells (5  $\mu$ M tamoxifen inhibited the currents by  $75 \pm 7\%$ ;  $n = 9$ ). Volume-activated chloride currents in MCF7<sup>Adr</sup> cells were similarly inhibited by tamoxifen (data not shown).

Fig. 2 shows a time course for inhibition of these chloride currents by tamoxifen. Cells were exposed to hypotonic bathing solution to activate the channels, and currents were monitored at the indicated time intervals after adding 1  $\mu$ M tamoxifen. Within 30 s of adding tamoxifen, the chloride currents were reduced by > 70%, and inhibition was essentially complete by 120 s. Inhibition was fully reversed within 2 min of removing extracellular tamoxifen. Inhibition by tamoxifen was similar at both +80 and -80 mV pulses, indicating that channel blocking was not voltage dependent.

Fig. 3 shows the dose dependence of tamoxifen inhibition. The concentration of tamoxifen required to achieve half-maximum inhibition of chloride currents (measured 1 min after adding the inhibitor) was  $\sim 0.3$   $\mu$ M. The currents were inhibited by > 90% with 10  $\mu$ M tamoxifen.



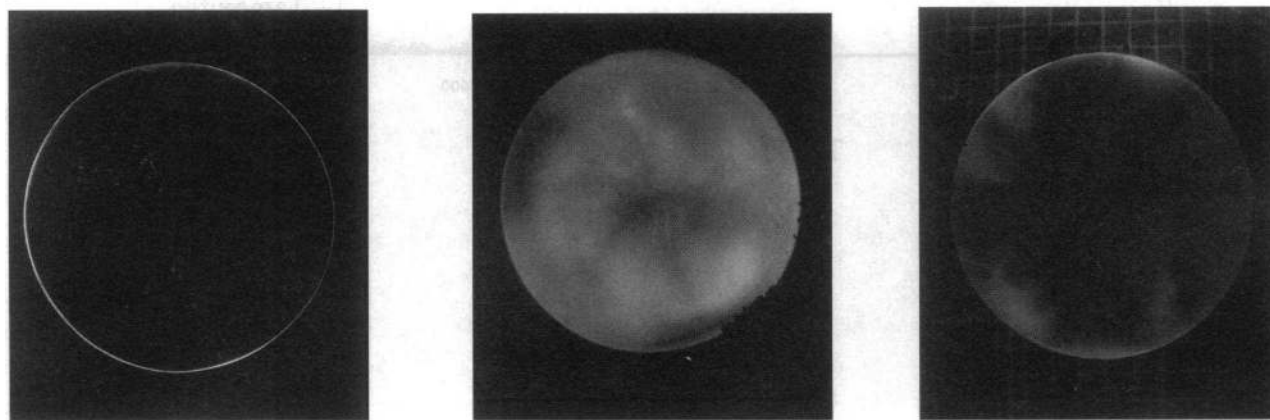
**Figure 3.** Dose-response curve for tamoxifen. Chloride currents were activated by hypotonicity and inhibition measured 1 min after adding tamoxifen to the bathing solution. Current is plotted as a percentage of the current elicited in the same cell before tamoxifen addition. The data are for S1/1.1 cells and are expressed as the mean ( $\pm$  SEM) of measurements on at least three separate cells. The line is a best-fit to a rectangular hyperbola.



B (I) Control

(ii) Low Chloride

(iii) NPPB

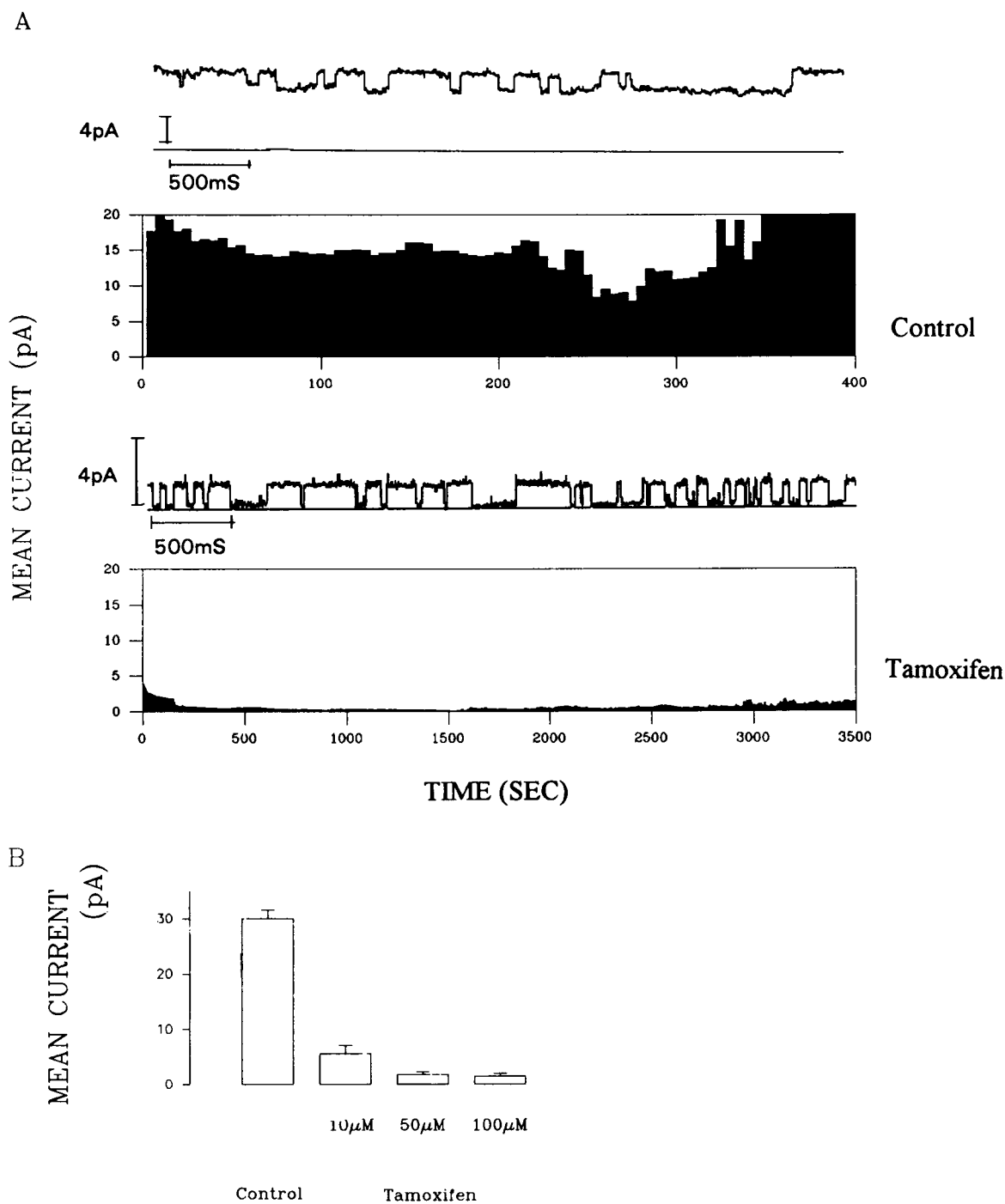


**Figure 4.** Light transmittance of the bovine lens in organ culture. (A) Bovine lenses were incubated at 37°C in a medium resembling aqueous humor (AH buffer). Transparency over a 12-d period was measured as described in Methods. ○, data for control lenses in AH buffer. ●, data for lenses incubated in AH buffer in which chloride had been replaced with gluconate. ▲, data for lenses incubated in AH buffer containing the chloride channel blocker NPPB (100 μM). The regional nature of the opacities in NPPB (see below) meant that transmittance data were more variable. The data are the mean (±standard error) for three lenses in each case. (B) Changes in lens opacity are illustrated by photographic record, viewed from above after 24 h of incubation in organ culture. (I) Control lens; (ii) lens incubated in reduced chloride; (iii) lens incubated with 100 μM NPPB.

We also examined the effects of various tamoxifen derivatives on channel function. 4-Hydroxy-tamoxifen, a metabolite of tamoxifen generated *in vivo*, inhibited the chloride currents in S1/1.1 cells slightly less than tamoxifen (65±13% [*n* = 3] and 88±8% [*n* = 5] inhibition after 1 min at 5 and 10 μM 4-OH tamoxifen, respectively). The related antiestrogen, toremifene (27), as well as its 4-hydroxy metabolite, at 5 μM, each inhibited the chloride currents (in NIH3T3 *MDR1* cells) by 75±2% (*n* = 7) and 74±7% (*n* = 4), respectively (means±SEM).

*Chloride channels are essential for maintaining lens clarity.* One of the clinical side effects of tamoxifen is impaired vision. To ascertain whether the ability of tamoxifen to block chloride

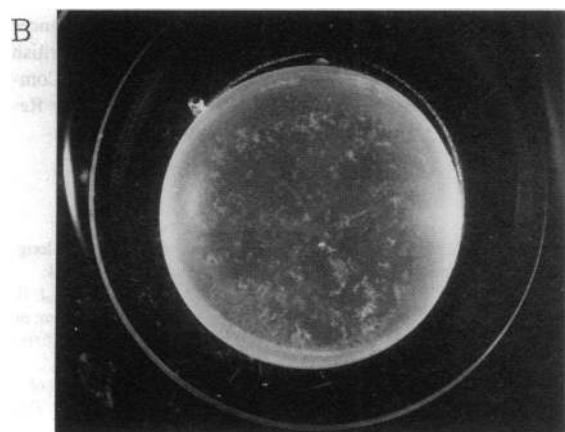
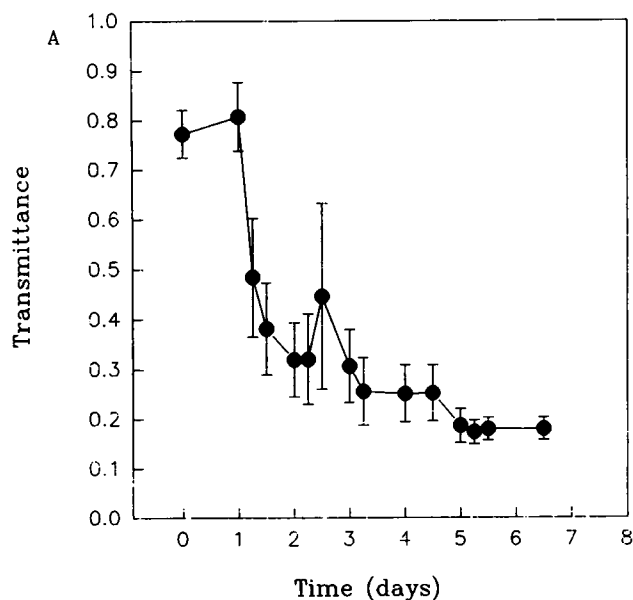
channels might potentiate cataract formation, the role of chloride in lens clarity was investigated. The bovine lens could be maintained in organ culture for up to 12 d without a significant loss of transparency; its transmittance (an index of transparency) remained within 15% of the initial value during the entire culture period (Fig. 4 A, *open circles*). The organ culture medium included 125 mM NaCl. When the chloride concentration was reduced to 10 mM, by replacing 115 mM NaCl with Na gluconate, a rapid opacification of the lens was observed with a time constant of 0.3 d (Fig. 4 A, *filled circles*). The transmittance fell immediately upon placing the lenses into the reduced chloride solution and reached a minimum of 0.016 at 3.9 d.



**Figure 5.** Effect of tamoxifen on chloride channels in lens fiber cells. (A) Single channels in lens fiber cells were studied in excised inside-out patches in the absence (control) or presence of tamoxifen in the pipette (extracellular) solution. Examples (5 s) of single-channel activity under each condition are shown. Below the single-channel traces are shown histograms of the mean current (leak subtracted) calculated for successive 5-s intervals while the patch was held at +100 mV in symmetrical NMDG-Cl solutions. Note the different baselines for the channels in the presence and absence of tamoxifen. (B) The mean patch current (leak subtracted) was calculated for the first 30 s after excision into NMDG-Cl solution containing 500  $\mu\text{M}$  ATP, while holding at +80 mV. The data are from control patches ( $n = 5$ ) and patches exposed to 10  $\mu\text{M}$  ( $n = 5$ ), 50  $\mu\text{M}$  ( $n = 4$ ), or 100  $\mu\text{M}$  ( $n = 3$ ) tamoxifen. The mean current ( $\pm\text{SEM}$ ) in the control was  $30.0 \pm 1.7$  pA, and in 10, 50, and 100  $\mu\text{M}$  tamoxifen was  $5.6 \pm 0.2$ ,  $1.80 \pm 0.06$ , and  $1.50 \pm 0.03$  pA, respectively.

Finally, when lenses were incubated in the presence of chloride, NPPB, a well characterized blocker of chloride channels (28, 29), caused a severe reduction in transmittance although the regional nature of the opacities meant that the transmittance

data were somewhat variable (Fig. 4 A, *filled triangles*). These changes in opacity are also documented photographically (Fig. 4 B). The data illustrate the regional nature of the opacities in NPPB, posteriorly located and wedge shaped. Together these

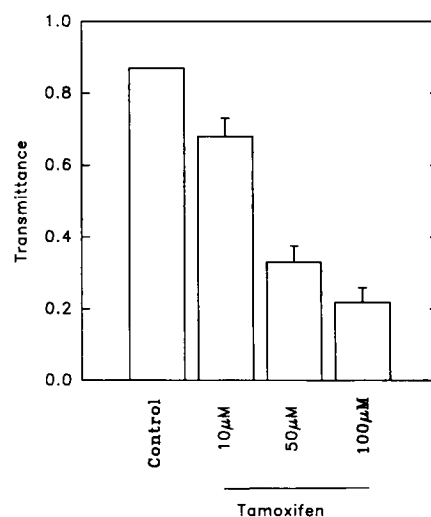


TAMOXIFEN 100 $\mu$ M

**Figure 6.** Effects of tamoxifen on lens opacity. (A) Lenses were cultured in AH buffer containing tamoxifen (100  $\mu$ M), and transmittance was measured as described in the legend to Fig. 4. The data are the mean ( $\pm$ standard error) for three lenses. (B) Lens opacity is illustrated in photographic form, viewed from above after 36 h in AH buffer containing tamoxifen (compare with controls in Fig. 4 B).

data demonstrate that chloride ions and functional chloride channels are required to maintain lens clarity.

**Tamoxifen blocks chloride channels in the lens.** We have recently characterized a chloride channel in the lens of the eye (25). This channel has characteristics in common with the cell volume-regulated chloride channels in S1/1.1 cells; it requires ATP for activation and its pharmacology is similar. We therefore asked whether tamoxifen blocks this lens channel. Single channel activity was recorded in excised inside-out patches of lens fiber cell membranes in symmetrical NMDG-Cl solutions. Stepping to +100 mV induced channel activity. Hypotonic activation is not required in these cells, presumably because the process of vesicle formation preactivates the channel (25). Patches were obtained in the absence (control) or presence of



**Figure 7.** Effects of tamoxifen concentration on lens opacity. Lens transmittance was measured as described above. After 4 d of treatment, transmittance reached a minimum and remained at that level for several further days. The graph presents the mean transmittance ( $\pm$ SEM) at 110 h ( $n = 3$ ). The responses observed at 10, 50, and 100  $\mu$ M tamoxifen were statistically significant (Student's *t* test),  $P < 0.05$ ,  $P < 0.001$ ,  $P < 0.001$ , respectively.

100  $\mu$ M tamoxifen in the pipette (extracellular) solution, as indicated. Fig. 5 A shows examples of single channel recordings and of the mean currents (in picoamperes) per 5 s epoch from a single recording, expressed as a function of time. Clearly, the channels were strongly inhibited when the patch was excised into tamoxifen. Note the different time bases in the experiments illustrated.

The effects of different tamoxifen concentrations on channel activity are shown in Fig. 5 B. Patches were excised into ATP-containing solutions (500  $\mu$ M), held at +80 mV, and the mean chloride currents were measured for the first 300 s after excision. For five control patches, the mean currents ( $\pm$ SEM) were  $30 \pm 1.7$  pA. This current was reduced by 81% in 10  $\mu$ M tamoxifen and by 94 and 95% in 50 and 100  $\mu$ M tamoxifen, respectively.

**Tamoxifen induces lens opacity in organ culture.** The effect of tamoxifen on lens opacity was examined in organ culture. Bovine lenses incubated with tamoxifen (100  $\mu$ M) showed a rapid and significant decrease in transmittance after a slight delay (Fig. 6 A). Just visible beneath the anterior epithelium was a region of discontinuity; this zone appeared not to have opacified and may indicate lens swelling in this region (Fig. 6 B). Similar opacification was observed with lower doses of tamoxifen (Fig. 7), which more accurately reflect serum levels of this drug achieved during tamoxifen therapy (3–10  $\mu$ M; 30). The concentrations of tamoxifen that generated lens opacity were similar to those required to block the lens chloride channels.

## Discussion

Tamoxifen is an antiestrogen used to treat breast cancer and is being assessed as a prophylactic for those at high risk of developing tumors (1). The pharmacological effects of tamoxifen are believed to be mediated through binding of tamoxifen to

the estrogen receptor, thereby antagonizing receptor function (3–5). We have identified an additional activity: tamoxifen, the related antiestrogen toremifene, and their 4-hydroxy metabolites were all found to be high-potency and relatively specific blockers of chloride channels. Several lines of evidence suggest that this effect is not mediated via the estrogen receptor. First, chloride channels are blocked in fibroblasts that lack the estrogen receptor. Second, chloride currents were inhibited by extracellular but not intracellular tamoxifen, yet the estrogen receptor is located in the cytoplasm. Third, 4-hydroxy-tamoxifen appeared to be a slightly less potent blocker of the channel than is tamoxifen, yet 4-hydroxy-tamoxifen has a 100-fold greater affinity for the estrogen receptor (31).

The finding that tamoxifen is a potent blocker of chloride channels suggests a mechanism whereby it might elicit specific side effects when administered therapeutically. One of the reported side effects of tamoxifen therapy is cataract (7–9). We therefore examined the role of chloride channels in lens transmittance. Functional chloride channels were found to be necessary for normal lens clarity, and these channels were blocked by tamoxifen. Tamoxifen induced lens opacity in organ culture at similar concentrations to those required to block lens chloride channels in isolated patches. This is consistent with the hypothesis that tamoxifen induces opacity and cataract formation through its effects on channel function. Importantly, the concentrations at which tamoxifen blocks lens chloride channels and causes lens opacification in organ culture were not dissimilar to serum levels of 3–10  $\mu\text{M}$  (30) that accrue after normal treatment regimes. This suggests that these *in vitro* effects can be responsible for the *in vivo* side effects of tamoxifen. As the side effects of tamoxifen appear to be mediated by a mechanism distinct from its therapeutic target (the estrogen receptor), it might be possible to identify tamoxifen derivatives that retain antiestrogen activity but lack the ability to block chloride channels.

The tamoxifen-sensitive chloride channels in S1/1.1 and NIH3T3 *MDR1* cells are associated with expression of the multidrug resistant P-glycoprotein (15, 16). We have previously defined four classes of compound based on their ability to block this channel (17). The effects of tamoxifen are similar to those of class II compounds, which include reversers of multidrug resistance such as verapamil and 1,9-dideoxyforskolin. Class II compounds block the channels from the outside of the cell but not from the intracellular face of the membrane, they block active channels rather than simply inhibiting channel activation, and they block the channels when ATP is replaced by nonhydrolyzable derivatives. However, it is not yet known whether all class II compounds block channel activity by the same mechanism. It is important to note that it is not yet known whether the action of these compounds (including tamoxifen) on channel activity is mediated through, or independent of, any interaction with P-glycoprotein. Furthermore, although the characteristics of the lens chloride channels are similar to those of the P-glycoprotein-associated volume-regulated chloride channels (25), it is not yet possible to ascertain whether they are formed by the same or different channel proteins.

The  $\text{IC}_{50}$  of 0.3  $\mu\text{M}$  for tamoxifen is lower than for any other blocker of volume-regulated chloride channels yet identified (the  $\text{IC}_{50}$ s for verapamil, 1,9-dideoxyforskolin, DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid), and NPPB are ~ 70, 40, 100, and 30  $\mu\text{M}$ , respectively). Thus, tamoxifen is the most potent blocker of this channel identified to date and

is one of the most potent chloride channel blockers known. Trinitrophenyl-ATP has been shown recently to block outwardly rectifying chloride channels from colonic epithelium with high affinity (0.27  $\mu\text{M}$ ; 32); this compound, however, does not block volume-regulated chloride channels (our unpublished data). Tamoxifen also appears to be more specific than many other chloride channel blockers; at similar concentrations it does not inhibit the cAMP-regulated chloride channel associated with the cystic fibrosis protein, the  $\text{Ca}^{2+}$ -regulated chloride channel present in many epithelial cells (33), or the volume-activated channels regulated by  $\text{pI}_{\text{Cl}_n}$  expression (Clapham, D. E., personal communication). The relationship between epithelial chloride channels and their respective physiological roles has been the subject of considerable debate (34). Tamoxifen promises to be an important tool in investigating this relationship and for studying cell volume regulation.

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