Estrogen (17β-estradiol; 17βE) and xenoestrogens, estrogenic compounds that are not steroid hormones, have non-genomic actions at plasma membrane receptors unrelated to the nuclear estrogen receptor. The open probability (Pₒ) of large conductance Ca²⁺/voltage-sensitive K⁺ (BK) channels is increased by 17βE through the regulatory β1 subunit. The pharmacological nature of the putative membrane binding site is unclear. We probed the site by determining whether tamoxifen ((Z)-1-(p-dimethylaminoethoxy-phenyl)-1,2-diphenyl-1-butene; Tx), a chemotherapeutic xenoestrogen, increased Pₒ in clinically relevant concentrations (0.1–10 μM). In whole cell patch clamp recordings on canine colonic myocytes, which express the β1 subunit, Tx activated charybdoxin-sensitive K⁺ current. In single channel experiments, Tx increased the NPo (Pₒ × number channels; N) and decreased the unitary conductance (γ) of BK channels. Tx increased NPo (EC₅₀ = 0.65 μM) in excised membrane patches independent of Ca²⁺ changes. The Tx mechanism of action requires the β1 subunit, as Tx increased the NPo of Slo α expressed in human embryonic kidney cells only in the presence of the β1 subunit. Tx decreased γ of the α subunit expressed alone, without effect on NPo. Our data indicate that Tx increases BK channel activity in therapeutic concentrations and reveal novel pharmacological properties attributable to the α and β1 subunits. These data shed light on BK channel structure and function, non-genomic mechanisms of regulation, and physiologically and therapeutically relevant effects of xenoestrogens.

Estrogens and xenoestrogens have non-genomic effects mediated by plasma membrane receptors unrelated to the nuclear estrogen receptor (1). One of these is to increase the NPo of BK1 channels (2). Ca²⁺-sensitive members of the voltage-gated K⁺ channel superfamily with important functions in many cells (3). BK channels are composed of α and β subunits. The β subunit forms the K⁺-selective pore, while β subunits influence the pharmacology, kinetics, and voltage/Ca²⁺-sensitivity of BK channels. The β1 subunit of smooth muscle BK channels is physiologically important because knockout mice lacking this subunit are hypertensive and demonstrate altered vascular reactivity (4, 5).

Recent studies suggest that BK channels are potential targets for 17βE and xenoestrogens. BK channel NPo, is increased by 17βE, an effect that requires the regulatory β1 subunit (2). 17βE and xenoestrogens reduce coronary vascular tone by inhibiting L-type Ca²⁺ channels and activating BK channels (6). The pharmacological nature of the putative 17βE-binding site on the smooth muscle BK β1 subunit is unknown. The xenoestrogen Tx, a commonly used chemotherapeutic agent, is an antagonist of the nuclear estrogen receptor (7). It is not known, however, if this clinically important drug increases BK channel NPo. We investigated whether Tx increases BK channel NPo in smooth muscle cells and whether the β1 subunit is important for this effect. These findings give insight into BK channel structure and function, non-genomic regulation by xenoestrogens, and Tx-induced side effects.

MATERIALS AND METHODS

Cell Isolation and Preparation—Smooth muscle cells were isolated by enzymatic dispersion described previously (8). Dogs were anesthetized with ketamine, and the colon was removed via a midline incision. Circular muscle of the canine colon and human jejunum was dissected free of mucosa, submucosa, and longitudinal muscle in Ca²⁺-free Hanks solution. Strips of muscle were treated with collagenase (345 units/ml; Worthington Biochemical Corp.; Freehold, NJ) in Ca²⁺-free Hanks at 37 °C to produce suspensions of single cells by gentle stirring. Mouse colon (circular and longitudinal muscle layers) was dissected free of mucosa prior to enzymatic dispersion. Mouse aorta and canine mesenteric vein were enzymatically digested without further dissection.

HEK293 cells (ATCC cell line number CRL-1573; Manassas, VA) were grown in glucose-supplemented RPMI medium (Life Technologies, Inc., Manassas, VA) with 10% heat-inactivated horse serum (Summit Biotechnology; Port Collins, CO) in a humidified atmosphere with 5% CO₂ at 37 °C. cDNA encoding the α and β subunits from canine colonic smooth muscle was cloned into the pژEovS mammalian expression vector (Invitrogen; Carlsbad, CA) as described previously (9). Cells were transiently transfected via electroporation with a total of 40 µg of plasmid DNA encoding either the α subunit alone or with a 1:1 mix of plasmids encoding the α and β subunits. Cells were subcultured on glass coverslips for electrophysiological studies 24–48 h after transfection.

Patch Clamp Techniques—Currents were recorded from whole cell, inside-out, and outside-out patches. An Axopatch 1D amplifier and CV-4 headstage were used for data acquisition (Axon Instruments; Foster City, CA). Data were acquired in pCLAMP version 5.5.1 (Clampex and Patch; Axon). The digitization rate was 4 kHz, and the cut-off frequency for filtration was 1 kHz. Data were analyzed in pCLAMP (version 6; whole cell I-V curves, Clampfit; Axon) and the Analysis of Single Channel Data program (NPₒ, γ, and histograms; University of Leuven, Belgium).
Tamoxifen Activates Smooth Muscle Maxi-K

**RESULTS**

Voltage steps from a holding potential of −80 mV elicited two types of voltage-dependent current in canine colonic myocytes studied in the whole cell patch clamp configuration with physiological ion gradients (5 mM K⁺ outside; 140 mM K⁺ inside; Fig. 1A). Depolarizations to 0 mV activated delayed rectifier current, while greater depolarizations activated BK current (11). Tx (1 μM) produced two effects on voltage-dependent outward currents. First, in the range of potentials from 0 to +40 mV, Tx inhibited the delayed rectifier component of outward current as reported previously (8). Second, Tx increased outward current at potentials positive to +40 mV.

The outward current activated by Tx was carried by K⁺, as dialysis of cells with NMDG (N-methyl-D-glucamine) or TEA (tetraethylammonium) instead of K⁺ prevented the stimulatory effect of Tx. Qualitatively similar effects of Tx were noted when voltage step protocols were used (holding potential −80 mV; Fig. 1, A and B) or when membrane voltage was ramped from −80 to +80 mV (Fig. 1B, inset). Tx activated K⁺ current whether cells were studied with the amphotericin-perforated or conventional dialyzed patch technique, suggesting preservation of the intracellular signaling milieu was not necessary. Tx increased current when cells were dialyzed with a pipette solution containing 10 mM BAPTA and no added Ca²⁺. The calculated free Ca²⁺ was 2 nM (assuming 50 μM Ca²⁺ contamination from water and reagents) and the free BAPTA concentration was greater than 9.5 mM, providing a large buffer against Ca²⁺ changes. Thus, experiments with BAPTA suggest that the increase in outward current due to Tx was not likely due to changes in Ca²⁺.

Tx-activated current was noisy (Fig. 1A), suggesting it was due to openings of channels with a large unitary conductance. Tx-activated current was blocked by TEA (1 mM). For example, outward current activated by steps to +80 mV averaged 1.77 ± 0.31 nA and was increased to 4.14 ± 0.51 nA by 1 μM Tx (n = 3; p < 0.05 versus control by one-way repeated measures ANOVA). Addition of TEA (1 mM) in the continued presence of Tx reduced outward current to 0.95 ± 0.15 nA (p < 0.05 versus both control and Tx). Charybdotoxin (Ctx; 50 nM), a specific peptide inhibitor of BK channels, also inhibited the current activated by Tx. A summary of five experiments testing the effect of Ctx on Tx-activated current is shown in Fig. 1C. We tested whether the addition of a BK channel blocker abrogated the subsequent effect of 1 μM Tx (n = 3; all comparisons by one-way repeated measures ANOVA). The addition of 1 mM TEA reduced current at +80 mV to 22 ± 9% of control (p < 0.05). Current with the addition of 1 μM Tx, in the continued presence of 1 mM TEA, was 43 ± 8% of control (p < 0.05 versus control, not different from TEA alone). Removal of TEA,
Tamoxifen Activates Smooth Muscle Maxi-K

FIG. 3. Tx activates charybdotoxin-sensitive channels in canine colon smooth muscle. A, representative single channel experiment in an outside-out patch (100 nM free Ca\(^{2+}\)). Patch potential was +40 mV. Currents were measured before and after the addition of 1 μM Tx, which increased NPo. Ctx (50 nM) blocked channel openings in the continued presence of Tx. B, all-points amplitude histograms from 60 s of recording demonstrate the effect of Tx and Ctx. A single open level was observed under control conditions. Tx (1 μM) increased NPo, revealing two more open levels, and decreased γ. The decrease in γ resembles a flicker block, as there was a broadening of the Gaussian peaks and the shoulders between them. Ctx (50 nM) inhibited channel openings in the continued presence of 1 μM Tx.

FIG. 4. Rapid and reversible effects of Tx and 17βE on NPo. A, NPo versus time from an inside-out patch in symmetrical 140 mM K\(^{+}\) (pCa 7) at +80 mV. Tx (1 μM) and 17βE (10 μM) increased NPo in a rapid and reversible manner. B, time control demonstrating that NPo was relatively stable over time and that vehicle (Me\(_2\)SO) had no effect (n = 6).

in the continued presence of 1 μM Tx, increased current to 211 ± 42% of control (p < 0.05 versus control, TEA alone, and TEA plus Tx). When all drugs were washed out, current at +80 mV returned to within 13 ± 11% of control (not different). The pharmacological profile of the current activated by Tx is consistent with BK channels. Experiments were performed to determine the time course and reversibility of the effects of 1 μM Tx on whole-cell current at +80 mV (Fig. 1D). Tx increased current 183 ± 40% (n = 6; p < 0.05) in 143 ± 35 s. Current returned to within 19 ± 13% of the control value within 3–5 min of Tx removal (not different from control; all comparisons made by one-way repeated measures ANOVA).

Excised patch recordings were performed to identify the conductance activated by Tx. In inside-out or outside-out patches from canine colonic myocytes, large conductance (>200 pS), Ca\(^{2+}\) - and voltage-dependent channel openings were observed (12). The channels were highly selective for K\(^{+}\) over Na\(^{+}\). In outside-out patches, submillimolar concentrations of TEA caused a flicker block of these channels. These are properties of BK channels, which have been identified and characterized previously in canine colonic myocytes (12). Tx (1 μM) increased NPo of BK channels in excised patches (Fig. 2). Additionally, Tx decreased γ. For example, in the records of Fig. 2, γ was 269 pS and was reduced to 243 pS by 1 μM Tx. Similar to the results in whole cell patches with TEA and Ctx (Fig. 1), the single channel currents activated by Tx were blocked by Ctx (50 nM; Fig. 3A). To determine this, outside-out patches were studied. Tx had the same effect in outside-out patches as it did in inside-out patches (and in cell-attached patches; not shown). As can be seen in the all-points amplitude histogram from 1 min recordings from an outside-out patch, Tx increased NPo and decreased the γ of BK channels (Fig. 3B). In the control record, the Gaussian mean for the single channel openings at +40 mV was centered at 10.4 pA. In contrast, the unitary currents in the presence of Tx were centered at 8.9 pA.

Single channel currents were recorded for long durations to accurately assess the effect of Tx on NPo. As the recording in Fig. 4A shows, NPo was stable after patch excision and Tx and 17βE increased it in a reversible manner. Me\(_2\)SO, the vehicle used for Tx and 17βE, had no effect on NPo (Fig. 4B). Tx increased BK NPo in a concentration-dependent manner (0.1–10 μM) with an EC\(_{50}\) of 0.65 μM (Fig. 5A; n = 15–22). NPo was allowed to reach a steady-state value for ~5 min before the addition of Tx. Current was then recorded for 1–10 min under control conditions, and then Tx was added. NPo was measured in the presence of Tx for 1–10 min and the mean change was calculated. These data demonstrate that Tx increases BK chan-
nel NP; in vivo, however, most Tx is bound to plasma proteins. Thus, it is uncertain whether the presence of albumin (the major Tx-binding protein), for example, would alter the efficacy and potency of Tx in these in vitro measurements. We performed experiments to determine the Tx concentration-NPo response relationship in the presence of 20 mg/ml albumin. The effects of Tx were still apparent in the presence of protein; however, albumin decreased the efficacy of Tx ~60% and caused a slight right shift in the EC_{50} (2.0 μM; Fig. 5A).

The effect of Tx on increasing BK NPo was similar to the effect of 17βE described previously (2). In our experiments, however, Tx was approximately 4-fold more potent than the previously reported EC_{50} of 2.6 μM for 17βE (2). These pharmacological concentrations of 17βE are substantially higher than the 100–600 μM physiological concentration range (13), but the EC_{50} for Tx was well within the therapeutic plasma concentration range measured in plasma of human cancer patients (0.1–10 μM; Refs. 14–17). To compare the potency of 17βE and Tx to increase NPo of BK channels, we tested the two agents in the same excised patches (Fig. 5B). 17βE (10 μM) was applied first and NPo was allowed to reach a steady state. 17βE was then washed out, and 1 μM Tx was added, which increased NPo to a higher level. These studies indicated that Tx is at least one log order more potent than 17βE, as Tx had a greater potentiating effect than a 10-fold higher concentration of 17βE.

We also compared the reduction in γ caused by Tx to 17βE. I-V curves for BK channels in inside-out patches (asymmetrical K+ gradient; 5 mM pipette; 140 mM bath) are shown in Fig. 5C. Conductance at +80 mV was calculated to be 247 ± 3 pS (n = 6) under control conditions, and γ was reduced to 204 ± 4 pS in the presence of 1 μM Tx (18 ± 2% decrease). A similar, but less potent, effect was observed with 17βE (Fig. 5D). In these experiments, BK channel γ averaged 261 ± 5 pS at +80 mV under control conditions (inside-out patches, asymmetrical 140 mM K+; n = 10), and γ was reduced to 243 ± 6 pS by 10 μM 17βE (8 ± 1% decrease; p < 0.05). 17βE was washed out, then Tx was added. Tx (1 μM) further reduced γ to 207 ± 4 pS (21 ± 6% decrease; p < 0.05).

Tx shifted the voltage dependence of steady-state activation to more negative potentials. Inside-out patches were studied in symmetrical 140 mM K+ and varying Ca^{2+} concentrations. The patch potential was held at 0 mV and stepped to different potentials to determine steady-state activation before and after the addition of 1 μM Tx. The patch potential was more positive until conductance was maximal and then activation curves (i.e. normalized conductance versus voltage) were generated. Tx (1 μM) decreased current (Fig. 6A) and shifted the voltage of half-activation (V_{1/2}) to more negative values at the same free Ca^{2+} concentration (Fig. 6B). Tx had no effect on BK channel activation kinetics. The time constant of activation at +150 mV in 100 nM free Ca^{2+} was 23 ± 2 and 24 ± 3 ms before and after the addition of 1 μM Tx, respectively (n = 18). Experiments were performed in Ca^{2+} concentrations ranging from pCa 7 to 3. Increasing the free Ca^{2+} concentration made V_{1/2} more negative; however, Tx (1 μM) caused the same hyperpolarizing shift in V_{1/2} independent of the free Ca^{2+} concentration (Fig. 6C).

The effect of Tx to increase BK NPo was not limited to canine colonic smooth muscle. We tested the effect of Tx on BK channels in 4 additional smooth muscle cell types from both visceral and vascular tissues. All of the same effects of Tx observed in canine colonic myocytes were noted in smooth muscle cells from the human jejunum, mouse colon, mouse aorta, and canine mesenteric vein. Tx increased BK current in whole cell recordings; however, delayed rectifier current was inhibited, as in canine colon (Fig. 7A). Tx increased the NPo of BK channels in excised patches in a concentration-dependent manner (Fig. 7B) and γ was decreased (Fig. 7C). Representative data from these experiments are shown in Fig. 7 and summarized in Table I.

Experiments were performed to determine whether the effects of Tx are due to interactions with the α or β subunits of BK channels. We expressed the cloned canine Slo α subunit in HEK293 cells, which do not express an endogenous β1 subunit (18). Inside-out patches from these cells contained K+ channels with a slope conductance of 264 ± 13 pS (n = 7). Tx had no effect on the NPo of single channels in cells expressing Slo α, but Tx did reduce γ (233 ± 11 pS; p < 0.05, paired Student’s t test; 12 ± 4% reduction; Fig. 8A). Expression of Slo α and β1 subunits together produced channels with an average γ of 266 ± 8 pS (n = 9). This conductance was not significantly different from the γ of channels in cells transfected with α subunits alone (p > 0.05; unpaired Student’s t test). Tx increased NPo of channels expressing both α and β1 subunits (Fig. 8B) and decreased γ (230 ± 9 pS; p < 0.05, paired Student’s t test; 13 ± 2% reduction). These data suggest that the Tx-induced increase in NPo requires the presence of the regulatory β1 subunit, however the attenuation of γ does not.

**DISCUSSION**

Tx, a commonly used chemotherapeutic agent for the treatment and prevention of estrogen receptor-dependent cancers, increased NPo of BK channels. Activation of BK channels by Tx was observed in tonic and phasic smooth muscle myocytes from mice, dogs, and humans, and it may be a common phenomenon for cells that express the regulatory β1 subunit (as do the tissues used here, Ref. 9). The effect of Tx to increase BK NPo required the presence of the regulatory β1 subunit, but the exact site of action is unknown. In the absence of the regulatory β1 subunit, Tx decreased γ without affecting NPo. The activating effect of Tx is similar to that reported for 17βE on BK channels containing the β1 subunit (2) and novel β subunits.
Tamoxifen Activates Smooth Muscle Maxi-K

Fig. 7. Tx activates BK channels in various smooth muscle cell types. A, representative whole cell currents in a human jejunal myocyte were recorded before and after the addition of 1 μM Tx. Delayed rectifier current was inhibited by 1 μM Tx. B, single channel records in an inside-out patch taken from a mouse colonic myocyte. The holding potential was +60 mV and the bath contained 100 nM Ca2+. Tx increased NPo in a concentration-dependent manner. C, single channel records from an inside-out patch of canine mesenteric vein smooth muscle. The holding potential was +80 mV, and the bath contained 100 nM Ca2+. Tx increased NPo and decreased the γ (asymmetrical K+; 5 mM pipette, 140 mM bath).

Table I

| Tissue                      | NPo, I(μM)    | Conductance (pS) |
|-----------------------------|--------------|------------------|
| Human jejunum              |              |                  |
| control                     | 0.16 ± 0.04  |                  |
| Tx                          | 0.79 ± 0.16* | 254 ± 6          |
| n                           | 4            |                  |
| Mouse colon                 |              |                  |
| control                     | 0.08 ± 0.02  |                  |
| Tx                          | 0.20 ± 0.07* | 250 ± 8          |
| n                           | 3            |                  |
| Mouse aorta                 |              |                  |
| control                     | 0.24 ± 0.13  |                  |
| Tx                          | 0.70 ± 0.21* | 243 ± 9          |
| n                           | 3            |                  |
| Canine mesenteric vein      |              |                  |
| control                     | 0.14 ± 0.08  |                  |
| Tx                          | 0.40 ± 0.15* | 269 ± 7          |
| n                           | 5            | 227 ± 8*         |

*p < 0.05 by paired Student’s t test.

(19). However, Tx was at least 10 times more potent than 17βE. Further, the EC50 for Tx (0.65 μM) falls within the plasma concentration range measured during chemotherapy (0.1–10 μM; Ref. 14). This contrasts with the peak plasma concentrations of 17βE (<1 nM; Ref. 13) and the reported EC50 (2.6 μM) for 17βE effects on BK channels (2). Both Tx and 17βE decreased the γ of BK channels, a novel effect seen in addition to other signaling mechanisms such as nitric oxide production or phosphorylation (well recognized mechanisms that affect BK NPo). The effects of Tx and 17βE are likely direct and do not involve other signaling mechanisms such as nitric oxide production or phosphorylation (well recognized mechanisms that affect BK NPo). The effects of 17βE on BK NPo, conductance were observed in whole cell, cell-attached, outside-out, and inside-out patches. However, Tx and 17βE also acts non-genomically in patches of excised membrane. The actual site of action could be at the extracellular surface, at the cytoplasmic face, or within the plasma membrane itself. The exact site is presently unknown and might be resolved in the future by using site-directed mutagenesis. What can be determined from the work of Valverde et al. (2) is that a membrane-impermeant form of 17βE conjugated to albumin elicits effects only when applied to the extracellular side of the membrane. The lack of effect from the cytoplasmic face for albumin-conjugated 17βE may be due to some steric hindrance and not indicative of a known position for the putative binding site. Regardless, the β subunit is an integral, requisite part of the signaling mechanism. Further, the effects of Tx and 17βE are likely direct and do not involve other signaling mechanisms such as nitric oxide production or phosphorylation (well recognized mechanisms that affect BK NPo), the effects are observed in cell-free patches in the absence of appropriate substrates for those signal transduction cascades. Additional strength for a direct effect of Tx and 17βE is that they have no effect on the NPo of the α subunit alone, which possesses a stimulatory protein kinase G phosphorylation site (18).

Tx also affects other ion channels. Tx inhibits volume-sensitive Cl- and delayed rectifier K+ currents in canine colonic smooth muscle (8). Tx also inhibits voltage-gated Na+ (21),

Fig. 8. Tx activates BK channels only in the presence of the regulatory β subunit. A, representative current traces of inside-out patches from HEK 293 expressing either the α subunit alone, or the α and β subunit together. Currents were recorded at +80 mV before and after the addition of 1 μM Tx. Note free Ca2+ concentration between the two groups is different; due to the reduced Ca2+-sensitivity of the α subunit expressed alone, a higher Ca2+ concentration was required to elicit basal activity. B, group data showing the effect of Tx (1 μM) on BK channels composed of α subunits alone (n = 7) or α and β subunits together (n = 9). Tx activates BK channels only in the presence of the regulatory β subunit. The asterisk indicates a significant difference between control and Tx for the α + β group (paired Student’s t test), while the cross indicates a difference in normalized NPo, between α and α + β groups in the presence of Tx (unpaired Student’s t test).

TABLE I

Effect of Tx on smooth muscle BK channels +80 mV (pCa 7)

| Tissue                      | NPo, I(μM)    | Conductance (pS) |
|-----------------------------|--------------|------------------|
| Human jejunum              |              |                  |
| control                     | 0.16 ± 0.04  |                  |
| Tx                          | 0.79 ± 0.16* | 254 ± 6          |
| n                           | 4            |                  |
| Mouse colon                 |              |                  |
| control                     | 0.08 ± 0.02  |                  |
| Tx                          | 0.20 ± 0.07* | 250 ± 8          |
| n                           | 3            |                  |
| Mouse aorta                 |              |                  |
| control                     | 0.24 ± 0.13  |                  |
| Tx                          | 0.70 ± 0.21* | 243 ± 9          |
| n                           | 3            |                  |
| Canine mesenteric vein      |              |                  |
| control                     | 0.14 ± 0.08  |                  |
| Tx                          | 0.40 ± 0.15* | 269 ± 7          |
| n                           | 5            | 227 ± 8*         |

*p < 0.05 by paired Student’s t test.

(19). However, Tx was at least 10 times more potent than 17βE. Further, the EC50 for Tx (0.65 μM) falls within the plasma concentration range measured during chemotherapy (0.1–10 μM; Ref. 14). This contrasts with the peak plasma concentrations of 17βE (<1 nM; Ref. 13) and the reported EC50 (2.6 μM) for 17βE effects on BK channels (2). Both Tx and 17βE decreased the γ of BK channels, a novel effect seen in addition to changes in NPo. Effects of Tx on BK channel NPo were observable at concentrations as low as 0.1 μM, apparent in the presence of 20 mg/ml albumin (a Tx-binding protein), and the effects were rapid and reversible.

The speed and reversibility of the Tx effects on BK channels suggest they are due to a non-genomic mechanism at the plasma membrane (20) (e.g. it has been proposed that there is an extracellular binding site on the regulatory β subunit; Ref. 2). It is unknown whether Tx or 17βE binds directly to β subunits or to an accessory protein, the influence of which is conveyed to BK channels via the β1 subunit. However, the similarities between the effect of Tx and 17βE on BK NPo suggest both agonists may affect the channels via the same binding site, the location of which has not been determined conclusively. The data of Valverde et al. (2) indicate that 17βE activates BK channels in inside-out patches independent of cellular signaling mechanisms. Our data confirm and further show that Tx has a similar ability to activate BK channels in inside-out patches. Tx and 17βE are very lipophilic and cross membranes readily. The intended therapeutic action of Tx is to cross the cell membrane and antagonize the nuclear estrogen receptor. This explains why the same effects on BK NPo and conductance were observed in whole cell, cell-attached, outside-out, and inside-out patches. However, Tx (and 17βE) also acts non-genomically in patches of excised membrane. The actual site of action could be at the extracellular surface, at the cytoplasmic face, or within the plasma membrane itself. The exact site is presently unknown and might be resolved in the future by using site-directed mutagenesis. What can be determined from the work of Valverde et al. (2) is that a membrane-impermeant form of 17βE conjugated to albumin elicits effects only when applied to the extracellular side of the membrane. The lack of effect from the cytoplasmic face for albumin-conjugated 17βE may be due to some steric hindrance and not indicative of a known position for the putative binding site. Regardless, the β1 subunit is an integral, requisite part of the signaling mechanism. Further, the effects of Tx and 17βE are likely direct and do not involve other signaling mechanisms such as nitric oxide production or phosphorylation (well recognized mechanisms that affect BK NPo), the effects are observed in cell-free patches in the absence of appropriate substrates for those signal transduction cascades. Additional strength for a direct effect of Tx and 17βE is that they have no effect on the NPo of the α subunit alone, which possesses a stimulatory protein kinase G phosphorylation site (18).

Tx also affects other ion channels. Tx inhibits volume-sensitive Cl- and delayed rectifier K+ currents in canine colonic smooth muscle (8). Tx also inhibits voltage-gated Na+ (21),
L-type Ca\(^{2+}\) (8, 22), and nonselective cation channels (23, 24). Tx inhibits protein kinase C (25) and calmodulin (26) and antagonizes histamine receptors (27). Thus, the effects of Tx in vivo or in vitro are likely to be multifaceted and are difficult to identify specifically. However, none of these other actions explain the effects of Tx to increase BK channel NP. Tx has a number of effects on smooth muscle tissues. Tx relaxes myometrial arteries and increases uterine blood flow (28). Tx inhibits spontaneous and agonist-induced myometrial contractions (29) and hyperpolarizes and relaxes cerebral arteries (30). These effects are consistent with the action of Tx on BK channels. High extracellular K\(^+\), which limits the degree to which openings of BK channels can hyperpolarize smooth muscle, abrogates the relaxing effect of Tx on myometrium (29). BK channels comprise one element of a negative feedback loop controlling the tone of cerebral arteries. Specifically, release of Ca\(^{2+}\) from the sarcoplasmic reticulum and subsequent activation of BK channels relaxes cerebral arteries (31). It is likely that the hyperpolarization of cerebral arteries (30) and relaxation of myometrium and myometrial arteries (28, 29) by Tx is due, in part, to the activation of BK channels. BK channel activation would drive membrane potential toward E\(K_0\), reducing the P_o of L-type Ca\(^{2+}\) channels independent of (and in addition to) possible direct inhibitory effects on voltage-gated Ca\(^{2+}\) entry. It is possible that Ctx might antagonize Tx-induced relaxation and hyperpolarization reported previously (28–30).

The effects of Tx on ion channels may be responsible for some therapeutic side effects. While Tx chemotherapy is generally well tolerated, side effects include vasomotor (facial flushing; Ref. 32), cardiac (Q-T interval prolongation; Ref. 17) and neurological (tremor and seizure; Ref. 17) symptoms. It is likely that the effect of Tx on ion channels (e.g. BK) plays at least some role in producing these effects. However, our findings also suggest that novel pharmacological targeting of the \(\beta_1\) subunit with xenoestrogens may be useful for treatment of diseases that have a smooth muscle component, such as hypertension, myocardial ischemia, asthma, impotence, and constipation. These findings give insight into BK channel structure and function, signaling roles, and pharmacological properties of the \(\beta_1\) subunit, the non-genomic regulation of BK channels by estrogen and xenoestrogens, as well as Tx-induced side effects.

Acknowledgments—We thank Dr. James L. Kenyon for all his advice and Nancy Horowitz, M.S for the preparation of cells. Tissue was kindly provided Drs. Kathy Keef and Neal Fleming (human jejunum) and Dr. Violeta Mutafova (canine mesenteric vein).

REFERENCES
1. Nadal, A., Ropero, A. B., Laribi, O., Maillet, M., Fuentes, E., and Soria, B. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 11603–11608
2. Valverde, M. A., Rojas, P., Amigo, J., Cosmelli, D., Oriol, P., Bahamonde, M. I., Mann, G. E., Vergara, C., and Latorre, R. (1999) Science 285, 1929–1931
3. Jan, L. Y., and Jan, Y. N. (1997) Annu. Rev. Neurosci. 20, 91–123
4. Brenner, R., Perez, G. J., Bonev, A. D., Eckman, D. M., Kosek, J. C., Wiler, S. W., Patterson, A. J., Nelson, M. T., and Aldrich, R. W. (2000) Nature 407, 870–876
5. Plüger, S., Faulhaber, J., Farstensen, M., Lohn, M., Waldechutt, K., Gollasch, M., Haller, H., Laft, F. C., Ehmke, H., and Pongs, O. (2000) Circ. Res. 87, E55–E60
6. Ruehlmann, D. O., Steinert, J. R., Valverde, M. A., Jacob, R., and Mann, G. E. (1998) FASEB J. 12, 613–619
7. Jordan, V. C. (1976) Cancer Treat. Rep. 60, 1409–1419
8. Dick, G. M., Kong, I. D., and Sanders, K. M. (1999) Br. J. Pharmacol. 127, 1819–1831
9. Vogalis, F., Vincent, T., Qureshi, I., Schmalz, F., Ward, M. W., Sanders, K. M., and Horowitz, B. (1996) Am. J. Physiol. 271, G629–G639
10. Bers, D. M. (1992) Am. J. Physiol. 242, C404–C408
11. Cole, W. C., and Sanders, K. M. (1989) Am. J. Physiol. 257, C461–C469
12. Carl, A., and Sanders, K. M. (1989) Am. J. Physiol. 257, C470–C480
13. Thorneycroft, I. H., Mishell, D. R., Stone, S. C., Kharma, K. M., and Horowitz, B. (1996) Am. J. Obstet. Gynecol. 175, 1819–1831
14. Bergan, R. C., Reed, E., Myers, C. E., Headlee, D., Brawley, O., Cho, H. K., Figg, W. D., Tompkins, A., Linehan, W. M., Kohler, D., Steinberg, S. M., and Blagosklonny, M. V. (1999) Clin. Cancer Res. 5, 2566–2573
15. Berman, E., McBride, M., Lin, S., Menedez-Botet, C., and Tong, W. (1995) Leukemia 9, 1631–1637
16. Deveci, A., Gandini, S., Guerrieri-Gonzaga, A., Johansson, H., Manetti, L., Bonanni, B., Sandri, M. T., Barreca, A., Costa, A., Robertson, C., and Liem, E. A. (1999) J. Clin. Oncol. 17, 2633–2638
17. Trump, D. L., Smith, D. C., Ellis, P. G., Rogers, M. P., Schohl, S. C., Winer, E. P., Panella, T. J., Jordan, V. C., and Fine, R. L. (1999) J. Natl. Cancer Inst. 84, 1811–1816
18. Fukao, M., Mason, H. S., Kenyon, J. L., Horowitz, B., and Keef, K. D. (2001) Mol. Pharmacol. 59, 16–23
19. Behrens, R., Nolting, A., Reimann, F., Schwarz, M., Waldechutt, R., and Pongs, O. (2000) FEBS Lett. 474, 99–106
20. Wehling, M. (1997) Annu. Rev. Physiol. 59, 385–393
21. Hardy, S. P., de Felipe, C., and Valverde, M. A. (1998) FEBS Lett. 434, 236–240
22. Doughty, J. M., Miller, A. L., and Langton, P. D. (1998) J. Physiol. 507, 2, 433–439
23. Allen, M. C., Newland, C., Valverde, M. A., and Hardy, S. P. (1998) Eur. J. Pharmacol. 354, 261–269
24. Welsh, D. G., Nelson, M. T., Conway, M. A., Knot, H. J., and Brayden, J. E. (2000) J. Physiol. 527 Pt 1, 139–148
25. O’Brien, C. A., Liskamp, R. M., Solomon, D. H., and Weinstein, I. B. (1985) Cancer Res. 45, 2462–2465
26. Lam, H. Y. (1984) Biochem. Biophys. Res. Commun. 118, 27–32
27. Kroeger, E. A., and Brandes, L. J. (1985) Biochem. Biophys. Res. Commun. 131, 750–755
28. Marshall, K., and Senior, J. (1987) Br. J. Pharmacol. 92, 429–435
29. Kostrzewska, A., Laudanski, T., and Batra, S. (1997) Am. J. Obstet. Gynecol. 176, 381–386
30. Nelson, M. T., Conway, M. A., Knot, H. J., and Brayden, J. E. (1997) J. Physiol (Lond) 502, 259–264
31. Nelson, M. T., Cheng, H., Ruhart, M., Santana, L. F., Bonev, A. D., Knot, H. J., and Lederer, W. J. (1995) Science 270, 633–637
32. Love, R. R., Cameron, L., Connell, B. L., and Leventhal, H. (1991) Arch. Intern. Med. 151, 1842–1847
