Bisphenol A activates BK channels through effects on α and β1 subunits

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We demonstrated previously that BK (K$_\text{Ca}_{1.1}$) channel activity (NP$_o$) increases in response to bisphenol A (BPA). Moreover, BK channels containing regulatory β1 subunits were more sensitive to the stimulatory effect of BPA. How BPA increases BK channel NP$_o$ remains mostly unknown. Estradiol activates BK channels by binding to an extracellular site, but neither the existence nor location of a BPA binding site has been demonstrated. We tested the hypothesis that an extracellular binding site is responsible for activation of BK channels by BPA. We synthesized membrane-impermeant BPA-monosulfate (BPA-MS) and used patch clamp electrophysiology to study channels composed of α or α + β1 subunits in cell-attached (C-A), whole-cell (W-C), and inside-out (I-O) patches. In C-A patches, bath application of BPA-MS (100 μM) had no effect on the NP$_o$ of BK channels, regardless of their subunit composition. Importantly, however, subsequent addition of membrane-permeant BPA (100 μM) increased the NP$_o$ of both α and α + β1 channels in C-A patches. The C-A data indicate that in order to alter BK channel NP$_o$, BPA must interact with the channel itself (or some closely associated partner) and diffusible messengers are not involved. In W-C patches, 100 μM BPA-MS activated current in cells expressing α subunits, whereas cells expressing α + β1 subunits responded similarly to a log-order lower concentration (10 μM). The W-C data suggest that an extracellular activation site exists, but do not eliminate the possibility that an intracellular site may also be present. In I-O patches, where the cytoplasmic face was exposed to the bath, BPA-MS had no effect on the NP$_o$ of BKα subunits, but BPA increased it. BPA-MS increased the NP$_o$ of α + β1 channels in I-O patches, but not as much as BPA. We conclude that BPA activates BK α via an extracellular site and that BPA-sensitivity is increased by the β1 subunit, which may also constitute part of an intracellular binding site.

Introduction

Large conductance Ca$^{2+}$/voltage-activated K$^+$ (BK) channels are expressed in the plasma membranes and intracellular organelles of numerous cell types. BK channels are important physiological regulators whose functions are impacted by and/or underlie disease. These channels are composed of pore-forming α subunits encoded by the KCNMA1 gene. The minimal BK channel is an assembly of 4 α subunits around a central axis. Each α subunit has an extracellular N-terminus, 7 transmembrane segments, and an intracellular C-terminus containing numerous regulatory domains and sites. α-only tetramers are fully functional K$^+$-selective channels with inherent Ca$^{2+}$- and voltage-sensitivity. Variations in α subunit properties result from alternative splicing of KCNMA1. However, a variety of important post-translational modifications also regulate BK channel properties, including their trafficking and anchoring to the membrane. Other differences in BK channels can be due to co-assembly with regulatory β subunits, 4 of which have been identified. β subunits can dramatically change channel properties including gating kinetics, Ca$^{2+}$/voltage-sensitivity, and pharmacology. Interestingly, it has been demonstrated recently that the N-terminus of intermediate conductance Ca$^{2+}$-activated K$^+$ channels reduces BK channel NP$_o$, by the same open channel block underlying β2- and β3-induced inactivation. β subunits can also influence the response of BK channels to pharmacological blockers and openers.

BK channels are activated by estradiol and xenoestrogens. Regulation by estrogenic substances, in multiple cases, involves β subunits. We demonstrated previously that BPA increases the open probability (NP$_o$) of BK channels without affecting single channel conductance. BPA appears to have less of an effect on BK channels lacking the β1 subunit, as a 10-fold higher concentration of BPA was needed to activate BK α subunits alone. Thus, effects of BPA, like those of other xenoestrogens, seem to be modulated by β subunits. However, a particular extracellular or intracellular binding site for the molecule has not been identified; BPA is lipophilic and it would be reasonable to suggest sites on either side of the membrane. It is possible that BPA binds to the α subunit, but this interaction is made much more efficient by addition of the β1 subunit. Another possibility may be that 2 different BPA binding sites exist when
the β1 subunit is present. Whether these sites are intracellular, extracellular, or both remains unclear. In this study, we test the hypothesis that an extracellular binding site is responsible for activation of BK channels by BPA. In order to examine this possibility, we synthesized membrane-impermeant BPA-monosulfate (BPA-MS). This novel reagent allows us to determine whether an intracellular or extracellular binding site exists (under the presumptions that BPA-MS: [1] can bind to the same site(s) as BPA and [2] similarly increase channel activity). W-C, C-A, and I-O recordings were made on cells expressing BK channels composed of α or α + β subunits.

**Results**

We recorded BK currents in AD 293 cells expressing α or α + β subunits (Fig. 1). Our goal was to determine whether BPA-MS, a membrane-impermeant derivative of BPA, increased whole-cell BK current. Further, if BPA-MS were to have an effect, we were interested in determining whether the presence of the β1 subunit influenced it. In cells expressing BK α subunits, whole-cell current was unaffected by 10 μM BPA-MS (Fig. 1A and B). Specifically, in cells expressing α subunits alone, current in the presence of BPA-MS was 106 ± 4% of control (n = 5). There was no BK current in non-transfected cells (data not shown) and the current in transfected cells was identified as BK through its sensitivity to 1 μM penitrem A (93 ± 4% block). In cells expressing BK α + β1 subunits, current magnitude was increased by 10 μM BPA-MS (Fig. 1C and D). Specifically, at +100 mV, BPA-MS increased current 34 ± 4% (an increase of 712 ± 103 pA; n = 7) when the β1 subunit was present. These data are very much like what we reported previously for the effect of BPA on whole-cell BK current and the role of the β1 subunit (ref. 19); however, these experiments with BPA-MS further indicate that the stimulatory effect of BPA is probably mediated, at least in part, by an extracellular binding site.

In our previous report, we demonstrated that a 10-fold higher concentration of BPA (100 μM) could activate BK channels composed of α subunits alone. Thus, in the present study, we determined whether a log order higher concentration of membrane-impermeant BPA-MS (100 μM) could also activate BK α subunits (Fig. 2). Bath application of 100 μM BPA-MS to W-C patches increased BK α current (Fig. 2A). Specifically, at +100 mV, 100 μM BPA-MS increased BK α current 36 ± 4% (an increase of 1151 ± 187 pA; n = 6). These data support what we demonstrated previously regarding the role of the β1 subunit in determining sensitivity to BPA. Further, these data lead us to suggest that the lower affinity site for activation of BK α by BPA/BPA-MS is extracellular.

We performed additional W-C experiments to further characterize the concentration-dependence of BPA-MS effects (Fig. 3). In cells expressing BK α + β1 subunits, BPA-MS increased current in a concentration-dependent manner (Fig. 3A). The increase in BK α + β1 current by BPA-MS (Fig. 3B) was qualitatively similar to effects of BPA we reported previously. Quantitatively, in
cells expressing BKα + β1 subunits, the magnitude of BPA-MS-activated current was less than that elicited by BPA (Fig. 3B). However, the current activated by BPA-MS was greater in cells expressing BKα + β1 subunits than cells expressing BKα alone (Fig. 3B). These data may indicate that effects of BPA-MS are less than BPA because intracellular and extracellular binding sites exist, but bath-applied, membrane-impermeant BPA-MS is restricted to acting on only the extracellular site. Further, the data may indicate that BPA-MS is a more potent and efficacious agonist of BK channels when the β1 subunit is present.

Our next set of experiments was designed to demonstrate that BPA-MS is, indeed, membrane-impermeant. Our rationale was that: (1) the W-C data suggest a low affinity extracellular site for BPA exists; (2) BPA is membrane-permeant and should activate channels in C-A patches through extracellular sites and intracellular sites (if any exist); (3) BPA-MS, if membrane-impermeant, should be unable to activate BK channels (α or α + β1) physically isolated from the test solutions by the pipette glass and plasma membrane. Results from C-A patches are shown in Figure 4. In C-A patches on cells expressing BKα + β1, adding 10 μM BPA-MS to the bath had no effect on NPo (Fig. 4A). In contrast, adding 10 μM BPA to the bath increased NPo 695 ± 325% (n = 8; Fig. 4A and B). We performed additional experiments with a higher concentration of BPA-MS (100 μM) to more rigorously test whether it might cross the membrane and activate channels. In C-A patches on cells expressing BKα + β1, adding 100 μM BPA-MS to the bath had no effect on channel activity (NPo was 132 ± 9% of control; n = 4). In contrast, adding 100 μM BPA to bath solution in these experiments increased NPo 521 ± 154% (P < 0.05). These data demonstrating differential effects of BPA and BPA-MS on NPo reassure us that the latter is likely membrane-impermeant (and that our preparation of BPA-MS is not contaminated with significant amounts of BPA). Further, the data also lead us to suggest that AD 293 cells do not metabolize BPA-MS by desulfation to produce BPA, as can be observed in cells expressing estrone sulfatase activity.20

We excised I-O patches from cells expressing BK channels composed of α or α + β1 subunits and determined the effect of bath-applied BPA-MS (Fig. 5). Our goal was to determine whether BPA-MS increased BK channel NPo at the intracellular face of the membrane. Further, if BPA-MS were to activate BK channels, we were interested in determining whether the presence of the β1 subunit influenced this effect. In I-O patches from cells expressing BKα alone, adding 100 μM BPA-MS to the bath had
no significant effect on NP\(_o\) (156 ± 15% of control, n = 8; Fig. 5A and B). However, adding 100 \(\mu\)M BPA to the bath increased NP\(_o\) 462 ± 147% (Fig. 5A and B). The response to intracellular BPA-MS was much different when BK channels contained the \(\beta_1\) subunit (Fig. 5C). In I-O patches from cells expressing BK \(\alpha + \beta_1\) subunits, adding 10 \(\mu\)M BPA-MS to the bath increased NP\(_o\) 403 ± 97% (n = 15; Fig. 5C and D). Replacing BPA-MS with BPA increased NP\(_o\) 910 ± 247% (Fig. 5C and D). Thus, BPA-MS can activate BK channels from the cytoplasmic face of the membrane only if the \(\beta_1\) subunit is present. These data lead us to suggest that the \(\beta_1\) subunit may comprise or complete an intracellular binding site for BPA.

Until this point, all of our single channel experiments had been performed at only 1 voltage, +40 mV on the membrane. We were interested in determining the effect of BPA-MS on BK channel activity at a variety of voltages. Rather than repeat the experiments outlined above at constant holding potentials, we opted to construct activation curves for channels in I-O patches (Fig. 6). Thus, I-O patches were excised from cells expressing BK \(\alpha\) or \(\alpha + \beta_1\) subunits, adding 10 \(\mu\)M BPA-MS to the bath increased NP\(_o\) 403 ± 97% (n = 15; Fig. 5C and D). Replacing BPA-MS with BPA increased NP\(_o\) 910 ± 247% (Fig. 5C and D). Thus, BPA-MS can activate BK channels from the cytoplasmic face of the membrane only if the \(\beta_1\) subunit is present. These data lead us to suggest that the \(\beta_1\) subunit may comprise or complete an intracellular binding site for BPA.

Effects of BPA-MS on NP\(_o\) are reversible (Fig. 7). The results in Figure 7A are from a single I-O patch pulled from a cell expressing BK \(\alpha + \beta_1\). NP\(_o\) at +40 mV is plotted vs. time. BPA-MS increased channel activity, but channel activity returned to baseline upon washout. Thus, effects of BPA-MS mediated at the intracellular face of the membrane are reversible. Data from a representative W-C experiment are shown in Figure 7B and C. These data indicate that effect of BPA-MS to increase BK current from extracellular face of the membrane is also reversible. We reported previously that BPA had no effect on BK channel unitary conductance. It appears that BPA-MS, at least from the cytoplasmic side, does not alter unitary conductance either (see Fig. 5; also a lack of effect can be inferred from Fig. 6, where G is...
However, to determine whether BPA-MS affected unitary conductance from the extracellular side of the membrane, we performed experiments in outside-out patches. Extracellular BPA-MS had no effect on BK channel amplitude, as channels demonstrated conductances of 240 ± 10 and 241 ± 10 pS before and during exposure to 100 μM BPA-MS, respectively (n = 4).

Discussion

BPA, a component of polycarbonate plastic used in food and beverage containers, is an estrogenic endocrine disruptor found in the urine of > 95% of Americans. We and others have demonstrated that a wide variety of ion channels (GABA receptors, nicotinic receptors, voltage-gated Na⁺ channels, voltage-gated Ca²⁺ channels, and BK channels) are targets of BPA. Specifically, we have shown previously that BPA activates BK channels; however, channels containing regulatory β₁ subunits were approximately 1 log-order more sensitive to BPA. In the present study, we tested the hypothesis that an extracellular binding site is responsible for activation of BK channels by BPA. This hypothesis was based on previous studies indicating that membrane-impermeant estrogen and estrogen receptor modulators activate BK channels by binding to an extracellular site. In order to test the hypothesis, we synthesized membrane-impermeant BPA-MS and used patch clamp electrophysiology to study BK channels composed of α or α + β₁ subunits in C-A, W-C, and I-O patches. Our 4 major findings included: (1) BK α was activated by extracellular 100 μM BPA-MS (Fig. 2); (2) the β₁ subunit made BK channels 10x more sensitive to extracellular BPA-MS (Fig. 1); (3) BK channels containing the β₁ subunit were activated by intracellular BPA-MS, whereas α only BK channels were not (Figs. 5 and 6); and (4) BPA was a more efficacious activator of BK α + β₁ than was BPA-MS (Figs. 3, 5, and 6). These data lead us to conclude that the hypothesis is supported. That is, an extracellular binding site appears to be responsible, at least in part, for the activation of BK channels by BPA (and BPA-MS). Importantly, however, we did not predict that BPA-MS would activate BK α + β₁ channels from the cytoplasmic face of the membrane or that a difference would exist in the magnitude of NPₒ responses to intracellular BPA and BPA-MS. These surprising findings lead us to suggest that the β₁ subunit may also constitute at least a part of an intracellular binding site.

The idea that the β₁ subunit may contribute to an intracellular binding site gains support from a substantial and integrative body of work from Dopico and colleagues on lithocholate, a cholane-derived steroid. Lithocholate activates smooth muscle BK channels from wild type, but not β₁ subunit knockout mice. The second transmembrane domain of β₁ is critical for conferring sensitivity to lithocholate, especially residues T169, L172, and L173. Membrane topology and computational modeling place these 3 residues in an intracellular-facing region where hydrogen bonding and hydrophobic interactions form a binding site. Whether this pocket is responsible for our observed effects of intracellular BPA on BK channels remains
Figure 6. BPA-MS activates BK channels containing the β1 subunit over a wide range of voltages. (A) Shows families of current traces from a representative I-O patch taken from a cell expressing BKα (symmetrical 140 mM K+; pCa 6.3). The voltage template is shown below. (B) Contains the I-V relationship for this single patch. BPA (100 μM), but not BPA-MS (100 μM), appears to affect current. (C) Contains group data (n = 8) showing that BPA-MS does not shift the midpoint (V½) of the activation curve. (D) Shows families of current traces from a representative I-O patch taken from a cell expressing BKα + β1 (symmetrical 140 mM K+; pCa 6.3). A slightly different voltage template is shown below. (E) Contains the I-V relationship for this single patch. Both BPA-MS (100 μM) and BPA (100 μM) appear to affect current. (F) Contains group data (n = 12) showing that BPA-MS and BPA both hyperpolarize the V½, with BPA being more effective. See text for statistical analyses of V½ and k for each set of experiments.
and interfered with estrogen binding proteins in yeast. The relevant mechanisms of BPA began in 1993 with the observation that it was released from polycarbonate plastics during autoclaving. Insight into the biologically significant site for BPA was recombinant GABA<sub>α</sub> receptors in 2001. Complex effects were demonstrated, as low concentrations of BPA potentiated responses to low concentrations of GABA, but high concentrations of BPA inhibited GABA responses. Later, BPA was shown to elicit postsynaptic current in CA3 pyramidal neurons, likely mediated by GABA<sub>α</sub> channels. The activation of GABA<sub>α</sub> channels by BPA may be interestingly related to mechanisms responsible for BK channel activation by BPA, as both channels are also potentiated by ethanol. Effects of BPA on the other identified ion channel targets share a common feature, as BPA uniformly inhibits a variety of voltage-gated Na<sup>+</sup> channels (tetrodotoxin-sensitive and -resistant) and voltage-gated Ca<sup>2+</sup> channels (L-, N-, P/Q- and T-type). In contrast, we show that BPA activates BK channels, which, interestingly, share some structural and regulatory features of voltage-gated Na<sup>+</sup> and Ca<sup>2+</sup> channels. Whether these non-genomic effects of BPA on BK channels (as well as other ion channels) are relevant to human disease remains to be determined. Genomic effects of BPA on ion channel expression are also possible and merit investigation. The present study, however, focused on non-genomic mechanisms and we have shown that 100 μM BPA-MS activated current in cells expressing α subunits, whereas cells expressing α + β1 subunits responded equally to 10 μM BPA-MS. The W-C data suggest that an extracellular site exists for BPA on the α subunit. Our data show that in I-O patches, where the cytoplasmic face was exposed to the bath, BPA-MS had no effect on the NP<sub>o</sub> of channels containing α alone; however, BPA-MS increased the NP<sub>o</sub> of BK channels composed of α + β1 subunits. These I-O data suggest that the β1 subunit completes or comprises an intracellular binding site for BPA. Together, the data lead us to conclude that BPA activates BK channels via an extracellular binding site and via an intracellular binding site that depends on the presence of the β1 subunit. We predict that the intracellular binding site may be the same one identified for interactions with lithocholate and HENA.

**Methods**

**Cell culture and transfection**

The techniques used here were similar to those we have used previously. AD 293 cells (Agilent Technologies) were grown in
Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Culture flasks were incubated in a 5% CO₂ incubator, humidified, and kept at 37 °C. Plasmids encoding hSlo α and hSlo α + β1 were kindly provided by Dr Jonathan Lippiat (University of Leeds). 2–3 Cells were transiently transfected with pIRES-hSlo or pIRES-hSloβ1 and pmaxGFP (AMAXA) using Lipofectamine LTX with PLUS reagent (Invitrogen). Cells at 50–70% confluence in 35-mm dishes were transfected with 0.5–2.5 μg of DNA. Transfected cells were selected in DMEM supplemented with 0.5 mg/ml G418, 1% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. Currents were recorded from cells expressing green fluorescent protein (GFP) 1–3 d later.

**Electrophysiology**

BK channel currents were recorded at room temperature from inside-out (I-O), cell-attached (C-A), and whole-cell (W-C) patches as described previously. 2–5 The bath flowed at a rate of approximately 2–3 ml/min into a chamber with a volume of approximately 0.2–0.3 ml throughout the recordings. For W-C recordings, bath solution contained (mM) 135 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 glucose, 10 HEPES, and 5 Tris; pH 7.4. Chemicals were purchased from Fisher Scientific or Sigma-Aldrich. For W-C recordings, pipette solution contained (mM) 140 KCl, 1 MgCl₂, 1 EGTA, 0.281 CaCl₂, 1 Mg-ATP, 0.1 Na-GTP, 10 HEPES, and 5 Tris; pH 7.1; pCa 7. I-O and C-A recordings were made in symmetrical (mM) 140 KCl, 1 MgCl₂, 1 EGTA, 10 HEPES, and 5 Tris; pH 7.1. CaCl₂ was added to these solutions to achieve free Ca²⁺ concentrations of 100 nM (pCa 7; calculated using Maxchelator; http://www.stanford.edu/~cpatton/maxc.html) or 500 nM (pCa 6.3; calculated in ref. 42) as indicated for individual experiments. Because Cl⁻ was symmetrical in all recording conditions, no adjustments existed. When only 2 values were compared, a paired Student t test was used. In all tests, P < 0.05 was considered significant.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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**References**

1. Singh H, Stefani E, Toro L, Intracellular BK. Intracellular BK(Ca) (iBK(Ca)) channels. J Physiol 2012; 590:5937-47; PMID:22930268; http://dx.doi.org/10.1111/jphysiol.2011.215533
2. Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Koragal P, Lüders HO, Shi J, Cui J, et al. Calcium-sensitive potassium channelopathy in vascular function. J Pharmacol Exp Ther 2013; 342:453-60; PMID:22580348; http://dx.doi.org/10.1124/jpet.112.191072
3. McGahon MK, Zhang X, Scholfield CN, Curtis SA, Wang L, Kotagal P, Lüders HO, Shi J, Cui J, et al. Calcium-sensitive potassium channelopathy in vascular function. J Physiol 2012; 590:5937-47; PMID:22930268; http://dx.doi.org/10.1111/jphysiol.2011.215533
4. Hill MA, Yang Y, Ella SR, Davis MJ, Braun AP. Large conductance, Ca²⁺-activated K⁺ channels (BKCa) and arteriolar myogenic signaling. FEBs Lett 2010; 584:2033-42; PMID:20178789; http://dx.doi.org/10.1016/j.febslet.2010.02.045
5. No potential conflicts of interest were disclosed.

**Statistics**

Data are presented as the mean ± standard error of n number of patches. Current-voltage relationships were analyzed by 2-way repeated measures analysis of variance (2RM-ANOVA). NP values under control conditions and with BPA-MS and BPA stimulation (i.e., 3 values) in C-A and I-O patches were compared by 1-way repeated measures analysis of variance (1RM-ANOVA). Bonferroni post hoc tests followed 1RM-ANOVA and 2RM-ANOVA when appropriate to determine where differences existed. When only 2 values were compared, a paired Student t test was used. In all tests, P < 0.05 was considered significant.

2. Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Koragal P, Lüders HO, Shi J, Cui J, et al. Calcium-sensitive potassium channelopathy in vascular function. J Pharmacol Exp Ther 2013; 342:453-60; PMID:22580348; http://dx.doi.org/10.1124/jpet.112.191072
3. Thompson J, Begenisich T. Mechanistic details of BK channel inhibition by the beta 1 subunit. J Biol Chem 2001; 276:34594-9; PMID:11454866; http://dx.doi.org/10.1074/jbc.M111.335547
4. Milton AM, Zhang X, Scholfield CN, Curtis SA, Wang L, Kotagal P, Lüders HO, Shi J, Cui J, et al. Calcium-sensitive potassium channelopathy in vascular function. J Pharmacol Exp Ther 2012; 342:453-60; PMID:22580348; http://dx.doi.org/10.1124/jpet.112.191072
5. Dick GM, Rossow CF, Smirnov S, Horowitz B, Sanders KM. Tamoxifen activates smooth muscle BK channels through the regulatory beta 1 subunit. J Biol Chem 2001; 276:34594-9; PMID:11454866; http://dx.doi.org/10.1074/jbc.M1046809200
6. Soom M, Gesnzer G, Heuer H, Hoshi T, Heinemann SH. A mutually exclusive alternative exon of slo1 codes for a neuronal BK channel with altered function. Channels (Austin) 2008; 2:278-82; PMID:18719396; http://dx.doi.org/10.4161/chan.2.4.6571
7. Kyle BD, Hurst S, Swayze RD, Sheng J, Braun AP. Specific phosphorylation sites underlie the stimulation of a large conductance, Ca(2+)-activated K(+) channel by cGMP-dependent protein kinase. FASEB J 2013; 27:2027-38; PMID:23407708; http://dx.doi.org/10.1096/fj.12-223669
8. Tian L, McClafferty H, Knaus HG, Ruth P, Shipston M111.335547
9. Sun X, Yazzman MA, Cui J. Regulation of Voltage-Activated K⁺ channels by Transmembrane β Subunits. Front Pharmacol 2012; 3:63; PMID:22529812; http://dx.doi.org/10.3389/fphar.2012.00063
10. Sweet TB, Cox DH. Measuring the influence of the BKCa beta subunit on Ca2+ binding to the BKCa channel. J Gen Physiol 2009; 133:139-50; PMID:19193175; http://dx.doi.org/10.1101/jgp.200810129
11. Thompson J, Begenisich T. Mechanistic details of BK channel inhibition by the beta 1 subunit. J Biol Chem 2001; 276:34594-9; PMID:11454866; http://dx.doi.org/10.1074/jbc.M111.335547
12. Asano S, Bratz IN, Berwick ZC, Fancher IS, Tune JD, Dick GM. Penitrem A as a tool for understanding the role of large conductance Ca(2+)-voltage-sensitive K(+) channels in vascular function. J Pharmacol Exp Ther 2012; 342:453-60; PMID:22580348; http://dx.doi.org/10.1124/jpet.111.191072
13. Dick GM, Rossow CF, Smirnov S, Horowitz B, Sanders KM. Tamoxifen activates smooth muscle BK channels through the regulatory beta 1 subunit. J Biol Chem 2001; 276:34594-9; PMID:11454866; http://dx.doi.org/10.1074/jbc.M1046809200
14. Bukiya AN, Liu J, Toro L, Dopico AM. Beta1 (KCNMB1) subunits mediate lithocholate activation of large-conductance Ca2+-activated K+ channels and dilation in small, resistance-size arteries. Mol Pharmacol 2002; 61:1105-13; PMID:11961128; http://dx.doi.org/10.1124/jm.1.1105

15. Dick GM, Sanders KM. (Xeno)estrogen sensitivity of ethylbromide for sulfation-desulfation in the uptake of bisphenol for membrane-impermeant antiestrogen. Br J Pharmacol 2010; 160:160-70; PMID:20353695; http://dx.doi.org/10.10111/j.1476-5381.2010.00687.x

16. Meera P, Wallner M, Toro L. A neuronal beta subunit (KCNMB4) makes the large conductance, voltage- and Ca2+-activated K+ channel resistant to charybdoxin and ibotenic acid. Proc Natl Acad Sci U S A 2000; 97:5562-7; PMID:10792958; http://dx.doi.org/10.1073/pnas.10118597

17. Verdu J, Tune JD, Dick GM. Bisphenol A activates Maxi-K (K(Ca)1.1) channels in coronary smooth muscle. Br J Pharmaco 2010; 160:160-70; PMID:20353695; http://dx.doi.org/10.10111/j.1476-5381.2010.00687.x

18. Dick GM, Sanders KM. (Xeno)estrogen sensitivity of smooth muscle BK channels conferred by the regulatory betal-subunit: a study of betal knockout mice. J Biol Chem 2001; 276:44835-40; PMID:11590153; http://dx.doi.org/10.1074/jbc.M10851200

19. Asano S, Tune JD, Dick GM. Bisphenol A activates Maxi-K (K(Ca)1.1) channels in coronary smooth muscle. Br J Pharmaco 2010; 160:160-70; PMID:20353695; http://dx.doi.org/10.10111/j.1476-5381.2010.00687.x

20. Claye SM, Carvajal R, Hughes DJ, James A. Bisphenol A and xenoestrogens of recombinant human neuronal nicotinic receptors. J Neurochem 2000; 74:263-9; PMID:1079678; http://dx.doi.org/10.1111/j.1476-5381.1999.00038.x

21. Nakazawa K, Ohno Y. Modulation by estrogens and xenoestrogens of recombinant human neuronal nicotinic receptors. Eur J Pharmaco 2001; 430:175-83; PMID:11711029; http://dx.doi.org/10.1016/S0047-2014(00)01389-9

22. O'Reilly AO, Eberhardt E, Weidner C, Altheimer C, Wallace BA, Lampert A. Bisphenol A binds to the local anesthetic receptor site to block the human cardiac sodium channel. PLoS One 2012; 7:e41667; PMID:22848561; http://dx.doi.org/10.1371/journal.pone.0041667

23. Wang Q, Cao J, Zhu Q, Luan C, Chen X, Yi X, Ding H, Chen J, Cheng J, Xiao H. Inhibition of voltage-gated sodium channels by bisphenol A in mouse dorsal root ganglion neurons. Brain Res 2011; 1378:1-8; PMID:21241682; http://dx.doi.org/10.1016/j.brainres.2011.01.022

24. Bukiya AN, McMillan JE, Parrill AL, Dopico AM. The second transmembrane domain of the large conductance, voltage- and Ca2+-gated potassium (BK) channel accessory beta1 subunit. Proc Natl Acad Sci U S A 2011; 108:20207-12; PMID:22139699; http://dx.doi.org/10.1073/pnas.1112901108

25. Bukiya AN, Singh AK, Parrill AL, Dopico AM. Beta1 in sensitizing BK channels to lithocholate. Biochem Pharmacol 2008; 71:141-8; PMID:18799442; http://dx.doi.org/10.1016/j.bcp.2008.04.026

26. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge MH, Wallace BB, Melzer D. Association of urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults. JAMA 2008; 300:1303-10; PMID:18799442; http://dx.doi.org/10.1001/jama.300.11.1303

27. Liu J, Bukiya AN, Kuntamallappanavar G, Singh AK, Dopico AM. Distinct sensitivity of Slo1 channel proteins to ethanol. Mol Pharmacol 2013; 83:235-44; PMID:23093494; http://dx.doi.org/10.1124/mol.112.083519

28. Lippat JD, Standen NB, Harrow ID, Phillips SC, Davies NW. Properties of BK(Ca) channels formed by bistricsteric expression of hisSloalpha and beta-1 subunits in HEK293 cells. J Membr Biol 2003; 192:141-8; PMID:12682801; http://dx.doi.org/10.1007/s00232-002-0170-0

29. Bers DM. A simple method for the accurate determination of free [Ca] in Ca-EGTA solutions. Am J Physiol 1982; 242:C404-8; PMID:6805332