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The amiodarone derivative 2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl) benzofuran (KB130015) opens large-conductance Ca^{2+}-activated K^+ channels and relaxes vascular smooth muscle

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Abstract

2-Methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran (KB130015) has been developed to retain the antiarrhythmic properties of the parent molecule amiodarone but to eliminate its undesired side effects. In patch-clamp experiments, KB130015 activated large-conductance, Ca^{2+}-activated BK_{Ca} channels formed by hSlo1 (α) subunits in HEK 293 cells. Channels were reversibly activated by shifting the open-probability/voltage (P_o/V) relationship by about −60 mV in 3 μM intracellular free Ca^{2+} ($[Ca^{2+}]_{in}$). No effect on the single-channel conductance was observed. KB130015-mediated activation of BK_{Ca} channels was half-maximal at 20 μM with a Hill coefficient of 2.8. BK_{Ca} activation by KB130015 did not require the presence of Ca^{2+} and still occurred with saturating (100 μM) $[Ca^{2+}]_{in}$. Effects of the prototypic BK_{Ca} activator NS1619 (1,3-dihydro-1-[2-hydroxy-5-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2*H*-benzimidazol-2-one) and those of KB130015 were not additive suggesting that both activators may at least partially share a common mechanism of action. KB130015-mediated activation was observed also for BK_{Ca} channels from insects and for human BK_{Ca} channels with already profoundly left-shifted voltage-dependence. In contrast, human intermediate conductance Ca^{2+}-activated channels were inhibited by KB130015. Using segments of porcine pulmonary arteries, KB130015 induced endothelium-independent vasorelaxation, half-maximal at 43 μM KB130015. Relaxation was inhibited by 1 mM tetraethylammonium, suggesting that KB130015 can activate vascular smooth muscle type BK_{Ca} channels under physiological conditions. Interestingly, the shift in the P_o/V relationship was considerably stronger (−90 mV in 3 μM $[Ca^{2+}]_{in}$) for BK_{Ca} channels containing Slo-β1 subunits. Thus, KB130015 belongs to a novel class of BK_{Ca} channel openers that exert an effect depending on the subunit composition of the channel complex.

Keywords: K^+ channel opener; BK_{Ca} channel; Patch clamp; KB130015; Smooth muscle

1. Introduction

BK_{Ca} channels are activated by depolarization and an increase in the intracellular free calcium concentration ($[Ca^{2+}]_{in}$) (Kaczorowski et al., 1996). Activation of BK_{Ca} channels promotes hyperpolarization, inhibiting Ca^{2+} influx through voltage-gated Ca^{2+} channels and reducing cellular excitability. Thereby, they provide a Ca^{2+}-dependent negative feedback mechanism to regulate e.g. vasorelaxation (Brenner et al., 2000) and neurotransmitter release (Kaczorowski et al., 1996). While four α-subunits encoded by *slo1* (KCNMA1, Tseng-Crank et al., 1994) are sufficient to form functional BK_{Ca} channels, smooth muscle BK_{Ca} channels are composed of 4 Slo1 (α) and up to 4 Slo-β1 subunits. Such channel complexes show an enhanced overall Ca^{2+} sensitivity. Splice variants of Slo1 (α) (Tseng-Crank et al., 1994; Xie and McCobb, 1998) and four different genes for Slo-β subunits (Orio et al., 2002) as well as modulation by phosphorylation, oxidation and additional interaction partners (reviewed in Weiger et al., 2002; Lu et al., 2006) result in a variety of BK_{Ca} channels to fulfill numerous functions in different tissues. The physiological significance of BK_{Ca} channels is highlighted by mutations or functional knockouts of either the β1-subunit that strongly affect blood pressure

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regulation (Brenner et al., 2000; Fernández-Fernández et al., 2004) or of the α-subunit, leading to erectile dysfunction, incontinence, progressive hearing loss, and neuronal disorders (Lu et al., 2006). This increasing number of disorders that could result from BK_{Ca} dysfunction underscores the urgent need for potassium channel openers targeting BK_{Ca} channels. Development of such drugs must consider the almost ubiquitous expression and diverse functions of BK_{Ca} channels; several types of K^+ channel openers are required to activate BK_{Ca} channels in a tissue-specific fashion. Ideally, such openers only activate BK_{Ca} channels with a specific subunit composition under defined, tissue- and disease-related conditions.

Amiodarone is one of the most effective antiarrhythmic drugs in clinical use (Carlsson et al., 2002; Kodama et al., 1999; Mubagwa et al., 2003). Its clinical benefits, however, are compromised on the one hand by numerous side effects, including vasodilation, pulmonary toxicity, ocular and skin changes as well as thyroid effects, and on the other hand by a slow elimination rate. To circumvent the negative side effects and to improve clearance, a derivative of amiodarone, 2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran (KB130015), has been developed (Carlsson et al., 2002; Mubagwa et al., 2003). KB130015 has already been shown to have unexpected novel effects, e.g. on sodium channel inactivation, (Mubagwa et al., 2003) not shared by the parent drug amiodarone. Using the patch-clamp technique we show that KB130015 activates BK_{Ca} channels and characterize this effect with respect to Ca^{2+}-dependence, requirement of Slo-β1 subunit coexpression and specificity. Employing porcine lung artery ring preparations, we observed that KB130015 relaxes vascular smooth muscle cells presumably via activation of BK_{Ca} channels. KB130015 is a novel BK_{Ca} activator, whose efficacy depends on the subunit composition of the channel complex.

2. Materials and methods

2.1. Chemicals and solutions

KB130015 (kindly provided by Karo Bio AB, Huddinge, Sweden) was stored at −20 °C as DMSO (dimethyl sulfoxide) stock solution (50 mM) and diluted to the final concentration immediately before use. Paxilline and penitrem A were from Alomone (Jerusalem, Israel). Bradykinin, prostaglandin F2α, and *N*-nitro-L-arginine methylester (L-NAME) were obtained from Alexis (Läufelfingen, Switzerland). All other chemicals used were of high grade, obtained from Sigma (Taufkirchen, Germany).

2.2. Lung artery smooth muscle studies

Pig lungs were obtained from a local slaughterhouse. Small branches (1.5–2.0 mm diameter) of pulmonary arteries were removed, carefully cleaned of parenchyma and connective tissues and cut into ~2-mm rings. Force measurements were carried out as described previously (Glusa and Adam, 2001). Artery segments were placed in 10-ml organ bath chambers filled with modified Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM D-glucose) and equilibrated with 5% CO_2 and 95% O_2 at 37 °C. Each artery ring was horizontally suspended between two L-shaped platinum hooks and connected to an isometric force transducer (Hugo Sachs Elektronik, March, Germany). Preparations were equilibrated under constant resting tension of 20 mN for at least 60 min before the assay. Artery rings were contracted at intervals of 45 min once via 45 mM KCl and three times with 3 μM prostaglandin F2α. The response to single or cumulative doses of KB130015 or DMSO (vehicle) was studied after stabilization of the third prostaglandin F2α-induced contraction. To functionally inhibit the endothelium-dependent relaxation response, the NO-synthase inhibitor L-NAME (1 mM) was added 10 min before the artery rings were contracted with prostaglandin F2α. This treatment led to a ≥90% inhibition of the relaxation induced with 10 nM bradykinin (data not shown). The relaxant response to KB130015 was monitored for 25 min and expressed as a percentage of the maximal prostaglandin F2α-induced contraction. To assay the concentration dependence, relative contraction (RC) was plotted against the KB130015 concentration and fitted with a Hill equation:

$$RC = RC_{min} + \frac{RC_{max} - RC_{min}}{1 + \left(\frac{[KB130015]}{EC_{50}}\right)^{n_H}} \quad (1)$$

with the half-maximally relaxing concentration EC_{50} and the Hill coefficient n_H.

2.3. Cell culture and molecular biology

HEK 293 cells were grown in 45% Dulbecco's modified Eagles's medium, 45% F-12 medium and 10% fetal calf serum (Invitrogen, Karlsruhe, Germany). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and subcultured every 3–4 days. Cells were plated on 35-mm petri dishes 1–6 days prior to electrophysiological experiments and transiently transfected with expression plasmids encoding hSlo1 (α) (*U11058*, Gessner et al., 2006), pSlo1 (α) (*AF452164*, Derst et al., 2003), dSlo1 (α) (splice form A1/C2/E1/G3/I0, Derst et al., 2003), hIK and hSlo-β1 (Gessner et al., 2006; in 5-fold excess, if added) using the Rotifect transfection kit (Roth, Karlsruhe, Germany). Marker plasmids encoding either EGFP or CD8 were used for cotransfection (20% of total DNA) to allow identification of transfected cells via fluorescence or specific binding of Dynabeads (Deutsche Dynal GmbH, Hamburg, Germany). Handling of lymph node carcinoma of the prostate (LNCaP) cells was as described previously (Gessner et al., 2006).

2.4. Electrophysiological recordings

Experiments were performed at room temperature (20–23 °C) using an EPC 9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Patch pipettes were fabricated from borosilicate glass (Kimble Glass, Vineland, NJ,