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Cortisone dissociates voltage-dependent K⁺ channel from its beta subunit

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Abstract

The *Shaker* family voltage-dependent potassium channels (Kv1) are expressed in a wide variety of cells and essential for cellular excitability. In humans, loss-of-function mutations of Kv1 channels lead to hyperexcitability and are directly linked to episodic ataxia and atrial fibrillation. All Kv1 channels assemble with beta subunits (Kvβ) and certain Kvβs, for example Kvβ1, have an N-terminal segment that closes a channel by the N-type inactivation mechanism. In principle dissociation of Kvβ1, although never reported, should eliminate inactivation and thus potentiate Kv1 current. We found that cortisone increases mammalian (rat) Kv1 channel activity by binding to Kvβ1. A crystal structure of the Kvβ-cortisone complex was solved to 1.82 Å resolution and revealed novel cortisone binding sites. Further studies demonstrated that cortisone promotes dissociation of Kvβ. The new mode of channel modulation may be explored by native or synthetic ligands to fine tune cellular excitability.

Voltage-dependent potassium channels (Kv) are tetrameric integral membrane proteins that, upon membrane depolarization, allow potassium ions to flow out of a cell. The outflow of potassium ions brings the membrane potential back to the resting value and therefore controls the timing and duration of an action potential. In humans, loss-of-function mutations in Kv1 channels are directly linked to episodic ataxia and atrial fibrillation^{1,2}. Native Kv1 channels assemble with cytosolic beta subunit (Kvβ), which is also a tetramer, to form a stable (Kv1)₄(Kvβ)₄ complex that is preserved through purification³⁻⁶. The assembly of the Kv1-Kvβ complex occurs in the endoplasmic reticulum⁷, and reciprocal immunoprecipitation experiments showed that all Kv1 channels assemble with Kvβ, and vice versa⁸.

There are three mammalian Kvβ genes, Kvβ1-3, and all have a highly conserved core domain (~330 amino acids) with more than 80% sequence identity. The conserved cores of Kvβ1 and Kvβ2 are functional aldo-keto reductases (AKR) that utilize NADPH (1) as a cofactor^{9,10}. In addition to the AKR core, Kvβ1 has an N-terminal segment that closes an open Kv1 channel by the N-type inactivation mechanism¹¹. In this mechanism, the N-terminal segment, also called the inactivation gate, functions as if it is a tethered channel blocker: it physically occludes an open channel pore within a few milliseconds after the channel is opened by membrane depolarization¹²⁻¹⁶. In principle, releasing Kvβ1 from the channel would potentiate Kv1 current. However, dissociation of the Kv1-Kvβ complex has never been observed.

We have found that cortisone (2) binds to Kvβ and increases Kv1 current. Structural studies revealed two types of cortisone binding sites on Kvβ: one close to the NADPH cofactor and

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another at the interface of the two neighbouring Kv β subunits. Further studies demonstrated that only the interface binding site is required for channel modulation, and that cortisone promotes dissociation of Kv β from the channel.

Results

Cortisone potentiates Kv1.1 channel through Kv β 1

The conserved AKR core of Kv β co-purifies with an NADPH cofactor^{9,10,17}, and we monitored the NADPH fluorescence to identify small-molecule compounds that bind to Kv β . Cortisone was identified by this assay because it induced a reduction ($21 \pm 0.7\%$, $n = 3$) of the fluorescence (Fig. 1a,b). The change in fluorescence occurred immediately after cortisone was mixed with the protein and remained stable for the duration of the experiment (20 minutes). This effect on fluorescence intensity is different from that induced by a substrate, which oxidizes the Kv β -bound NADPH and therefore eliminates the fluorescence^{9,10}.

To test if cortisone modulates channel current, Kv1.1 channel was co-expressed with Kv β 1 in *Xenopus* oocytes and potassium current was recorded on inside-out patches. The presence of fast inactivation indicates that Kv1.1 and Kv β 1 are co-assembled because Kv1.1 expressed alone produces non-inactivating current (compare Fig. 1c,d, black traces). When 500 μ M cortisone was perfused to the intracellular side of the channel, the onset rate of channel inactivation decreased significantly from $302 \pm 7 \text{ s}^{-1}$ to $89 \pm 4 \text{ s}^{-1}$ ($n = 13$, $P < 0.001$ in paired Student's *t* test), and an increase of peak and steady-state currents were observed (Fig. 1c). The increase in current was due to decreased onset rate of inactivation because the rate of recovery from inactivation remained essentially unchanged ($13.9 \pm 1.1 \text{ s}^{-1}$ before and $12.0 \pm 0.8 \text{ s}^{-1}$ after cortisone perfusion, $n = 4$). The current increase reached steady-state in approximately 5 minutes and was not reversed even when perfused extensively with the normal inside solution. To quantify the cortisone effect on current increase, we defined ΔI_{ss} , the increase of the steady-state current normalized to the initial inactivating current. The cortisone effect was highly reproducible with a ΔI_{ss} of $84 \pm 4\%$ ($n = 13$, Fig. 1e). In contrast, the vehicle control (1% dimethyl sulfoxide (DMSO)) induced a significantly smaller response, with a ΔI_{ss} of $10 \pm 0.7\%$ ($n = 8$, $P < 0.001$ in ANOVA test, Fig. 1e,f). To further characterize the cortisone effect, the ΔI_{ss} values were measured at different cortisone concentrations and the data were well-fit by a Hill equation with an EC₅₀ value of $46 \pm 1.1 \text{ }\mu\text{M}$ and a Hill coefficient of 0.97 ± 0.09 (Fig. 1g).

To find out if the cortisone effect is mediated by the conserved AKR core of Kv β 1, we tested cortisone on a chimeric channel, Kv1.1-inact, which was constructed by connecting the first 70 amino acid residues of Kv β 1 directly to the N terminus of Kv1.1. Thus, Kv1.1-inact is a Kv1.1 channel that has an N-type inactivation gate from Kv β 1 but does not have the conserved AKR core domain. Kv1.1-inact produced inactivating current similar to that from co-expression of Kv1.1 and Kv β 1 (Fig. 1h), but the current was not potentiated by cortisone: cortisone (500 μ M) induced a ΔI_{ss} of $6 \pm 0.8\%$ ($n = 7$, Fig. 1e,h) on Kv1.1-inact, not significantly different from that of vehicle control and significantly smaller than that from co-expression of Kv1.1 and Kv β 1. In addition, we also examined cortisone on Kv1.1 expressed without Kv β 1, and the ΔI_{ss} is $4.5 \pm 0.5\%$ ($n = 5$, Fig. 1d,e). Thus, we conclude that the large potentiation of current by cortisone is mediated by the conserved core domain of Kv β 1.

Two cortisone molecules bind to each Kv β subunit

A structural approach was taken to determine where cortisone binds on Kv β . The conserved AKR core of Kv β 1 protein can be expressed and purified in large quantity but is prone to aggregation and did not yield crystals of sufficiently high diffraction quality even after extensive crystallization trials. Therefore the AKR core of Kv β 2 was used for co-crystallization

with cortisone. Since the amino acid sequence of the Kv β 1 core is ~80% identical to that of Kv β 2, the structures of the two are likely similar. Kv β 2 was not used in the functional studies in the first place because it does not have the N-type inactivation gate. To test if the conserved core of Kv β 2 also mediates the cortisone effect, we used a chimera, Kv β 12, which was constructed by splicing the inactivation gate of Kv β 1 to the conserved core of Kv β 2^{5,18}. Similar to Kv β 1, Kv β 12 produced fast inactivation when co-expressed with Kv1.1, and more importantly, the inactivation was reduced by cortisone (500 μ M) with a ΔI_{ss} of $89 \pm 4\%$ ($n = 12$, Fig. 2a,b), significantly larger than that of the vehicle control ($\Delta I_{ss} = 10 \pm 0.8\%$, $n = 4$; $P < 0.001$ in ANOVA test, Fig. 2a,b), but not significantly different from that mediated by Kv β 1 ($P > 0.05$ in ANOVA test, Fig. 2b).

Crystals of Kv β 2 core in complex with cortisone grew with a tetragonal symmetry (I422), and the structure was solved by molecular replacement. The structure refined well ($R_{free} = 20.7\%$, Supplementary Table 1 online) using data measured to 1.82 Å Bragg spacing. Simulated-annealing Fo-Fc (observed and calculated structure factor) omit map revealed unambiguously the location and the molecular structure of two types of cortisone binding site for each Kv β subunit. One cortisone molecule binds close to the NADP cofactor and therefore we define the site as the *enzymatic site*, and another cortisone molecule binds in between the two neighbouring Kv β subunits and we define it as the *interface site* (Fig. 2c).

Channel modulation does not require the enzymatic site

To examine which binding site is responsible for the cortisone effect on channel current, we perturbed the binding site by making point mutations and examined cortisone binding by X-ray crystallography. For mutations that eliminate cortisone binding, we then investigated if they still mediate the cortisone effect on channel current.

The enzymatic site cortisone has hydrophobic interactions with the side chain of residue Trp121: the A and B rings of cortisone stack against the flat surface of the indole ring (Fig. 3a). Therefore Trp121 was mutated to an alanine, and the W121A mutant was co-crystallized with cortisone and the structure was solved to 2.0 Å resolution ($R_{free} = 24.0\%$, Supplementary Table 1 online). In the mutant structure, although cortisone density was clearly resolved at the interface site (Supplementary Fig. 1 online), no cortisone density was present at the enzymatic site (Fig. 3b). In addition, the Arg189 side chain conformation provides further indication that a cortisone molecule is not present at the enzymatic site: when a cortisone molecule is present at the enzymatic site, the Arg189 side chain adopts a bend conformation as seen in the structure of Kv β 2-cortisone (Fig. 3a). However, at the absence of cortisone, the Arg189 side chain adopts an extended conformation, as seen in the structure of Kv β 2 (W121A)-cortisone (Fig. 3b) as well as in that of the wild type Kv β 2 crystallized without cortisone (Supplementary Fig. 2 online). The overall structure of cortisone in complex with the W121A mutant Kv β 2 is similar to that of the wild type (PDB ID 1EXB⁵), with a root-mean-square deviation (RMSD) of 0.22 Å for all the main chain atoms.

The equivalent position of Trp121 on Kv β 1 is Trp155, and we mutated it to an alanine. When Kv1.1 was co-expressed with the W155A Kv β 1, inactivating current was observed, indicating that the mutant Kv β 1 had assembled with the channel (Fig. 3c). Cortisone (500 μ M) induced a large increase in channel current, with a ΔI_{ss} of $93 \pm 8\%$ ($n = 6$, Fig. 3d), significantly higher than the vehicle control ($\Delta I_{ss} = 13 \pm 0.7\%$, $n = 4$; $P < 0.001$ in ANOVA test), but not significantly different from that mediated by the wild type Kv β 1 ($P > 0.05$ in ANOVA test). To further investigate the cortisone effect mediated by the W155A mutant Kv β 1, the ΔI_{ss} was measured at different cortisone concentrations and the data were well-fit by a Hill equation with an EC_{50} of $33 \pm 1.1 \mu$ M and a Hill coefficient of 0.96 ± 0.07 , both similar to these of the wild type Kv β 1 (Fig. 3e). Therefore binding of cortisone at the enzymatic site is not required for channel modulation.

The interface site is required for channel modulation

The interface site cortisone fits into a deep pocket formed by two neighbouring Kv β subunits (Fig. 4a,b). The A, B, and C rings of cortisone are immersed in the binding pocket, and make extensive contacts with residues lining the pocket (Fig. 4b). Compared with cortisone-Kv β interactions observed at the enzymatic site, more residues participate in coordinating cortisone and some of them, for example, Arg203 and Glu167, are part of the interface between the two neighbouring Kv β s. Using the structure as a guide, we analyzed, *in silico*, mutations to the residues lining the binding pocket, and found that mutating residue Ile211 to one with a bulkier side chain, such as an arginine or a tryptophan, may interfere with cortisone binding and at the same time have a minimum impact on Kv β tetramerization. We therefore produced the I211R and I211W mutant Kv β 2, co-crystallized the mutants with 2 mM cortisone, and solved the structures to 1.95 Å and 1.9 Å, respectively. Since both mutations have the same effect on cortisone binding, the I211R structure ($R_{\text{free}} = 23.4\%$, Supplementary Table 1 online) is presented. In the wild type structure the isoleucine side chain packs against the hydrophobic core of the protein (Fig. 4b), while in the mutant structure the arginine side chain protrudes into the binding pocket and forms a steric hindrance to prevent cortisone from entering the binding pocket (Fig. 4c). As a result, although cortisone density was observed at the enzymatic site (Supplementary Fig. 3 online), no cortisone density was present at the interface site. The loss of cortisone binding at the interface site is due to local perturbations at the binding site, because the structure of cortisone in complex with the I211R Kv β 2 aligns well with that of the wild type Kv β 2 with a RMSD of 0.22 Å.

The equivalent of the Ile211 on Kv β 1 is a valine at position 245, and we mutated it to an arginine and co-expressed the V245R Kv β 1 with Kv1.1. Cortisone, even at 500 μ M, only induced a small increase of current with a ΔI_{ss} of $13 \pm 1\%$ ($n = 13$, Fig. 4d,e), not significantly different from the vehicle control ($\Delta I_{\text{ss}} = 10 \pm 1\%$, $n = 4$; $P > 0.05$ in ANOVA test) but is significantly smaller than that of the wild type Kv β 1. Thus, we conclude that cortisone binding at the interface site is required for channel modulation.

Cortisone compromises Kv1-Kv β assembly

Except for small adjustments at the cortisone binding sites, the overall structure of the Kv β 2-cortisone is almost identical to that of Kv β 2, with a RMSD of 0.21 Å for all the main chain atoms. To further understand how cortisone binding induces increase of current, we aligned the Kv β 2-cortisone onto the structure of the Kv1.2-Kv β 2 complex (PDB ID 2A79³).

The structures of Kv β 2 in complex with either an entire Kv1 channel³ or the intracellular tetramerization domain (T1) of the channel⁵ both showed that Kv β docks onto the channel by interacting with four highly conserved loops from the T1 domain. When the Kv β 2-cortisone structure was aligned with that of Kv1.2-Kv β 2, it became clear that the interface site cortisone is close to where the T1 loops contact Kv β (Fig. 5a,b). The 16-position carbon, 17-position hydroxyl oxygen and the 21-position carbon of cortisone are 2.9-3.7 Å away from the side chains of Pro75 and Leu76 of the T1 loop (Fig. 5b). The proximity of cortisone to the T1 loops naturally led to the hypothesis that cortisone may destabilize the Kv1-Kv β complex. We examined this hypothesis with the following two experiments.

First, we co-expressed and purified the T1-Kv β 2 complex, and used a solid phase binding assay to examine if cortisone affects the association of the two. When the complex was incubated with cortisone, dissociation occurred with an EC₅₀ value of $68 \pm 1.1 \mu\text{M}$ (Fig. 5c,d). As a control, we expressed the T1 domain in complex with the I211R mutant Kv β 2 and we found that the mutant Kv β 2 did not dissociate from the T1 domain at the presence of 2 mM cortisone (Fig. 5c).

Second, we constructed a chimera, Kv β 1-Kv1.1, by covalently linking the C terminus of Kv β 1 to the N terminus of Kv1.1 so that Kv β 1 will not be able to diffuse away even when it falls off the T1 domain. The Kv β 1-Kv1.1 chimera expressed well, and cortisone at 500 μ M induced a small change in channel current, with a ΔI_{ss} of $13 \pm 1\%$ ($n = 9$, Fig. 5e,f), not significantly different from the vehicle control ($\Delta I_{ss} = 10 \pm 0.7\%$, $n = 4$; $P > 0.05$ in ANOVA test). Results from both experiments support the hypothesis that dissociation of Kv β 1 from the channel is required for potentiation of channel current by cortisone.

Discussion

In conclusion, we have identified a binding site on Kv β through which the assembly of the Kv1-Kv β complex can be tuned. Dissociation of Kv β provides a novel mechanism for modulation of Kv1 channel functions, and has the following three implications on K channel physiology and pharmacology.

First, acute dissociation of Kv β provides a novel method to examine Kv β 's physiological functions. The conserved core of Kv β is a functional aldo-keto reductase, and that oxidation of the Kv β -bound NADPH modulates channel activity^{9,10}. Based on these observations, it has been proposed that Kv β is a redox sensor that couples intracellular redox chemistry to the excitability of a cell. However, this hypothesis has not been tested in an organism. In addition to being a functional aldo-keto reductase, Kv β increases surface expression level of Kv1 channels^{19,20} and is required for axonal targeting of Kv1 channels in mammalian neurons²¹. Thus, eliminating Kv β in an organism affects channel distribution and localization, making it difficult to pinpoint the exact physiological functions of Kv β as an enzyme. The dissociation of Kv β “*in situ*” after the Kv1-Kv β complex has already been delivered to its proper cellular location will assist the understanding of Kv β 's physiological role.

Second, that Kv β can be competed off the channel with a small-molecule compound suggests that the Kv1-Kv β complex may not be permanent. As a recent review has pointed out²², it is in general very difficult to obtain small-molecule compounds that interfere with protein-protein interactions because the interfaces are usually large and extensive, and do not have grooves and pockets for small ligands. In contrast, Kv1-Kv β interface lacks an extensive interaction surface^{3,5} (Fig. 5b) and, as this study has shown, has in fact a deep pocket suitable for a small ligand. These properties suggest that association of Kv β to the channel is tuneable under physiological conditions. The dynamic modulation of Kv1 channel activities by association of Kv β may be important for proper cellular responses to different redox environments that mammals have to experience during development.

Third, dissociation of Kv β 1 potentiates channel current due to loss of the N-type inactivation. Cortisone is therefore a rare Kv1 channel opener and the interface cortisone binding site could be exploited by small-molecule compounds to modulate Kv1 channels. Since Kv β assembles exclusively with Kv1 family channels^{5,23,24}, compounds targeting Kv β will be highly specific to only the Kv1 channels.

Discovery of cortisone as a channel modulator was a coincidence: Cortisone was identified from a small collection of ~120 known aldo-keto reductase substrates by a “low-throughput” manual screen, and the readout for the screen was Kv β -bound NADPH fluorescence (Fig. 1a). Although cortisone changes the fluorescence, it is not a Kv β substrate and the interface binding site is far away from the active site. The micromolar range EC₅₀ value indicates that cortisone does not modulate channel activity under normal physiological conditions. The accidental discovery of cortisone as a channel modulator, however, provides proof-of-concept for employing a high-throughput screen to obtain small-molecule compounds that modulate association of Kv β to the channel. Furthermore, the interface binding site is largely

hydrophobic and recognizes the A, B and C rings of cortisone. It is likely that other members of the corticosteroid family, or their synthetic derivatives, may bind to the interface site as well. The combination of unbiased high-throughput screens and structure-based modification of cortisone promises higher affinity probes for achieving pharmacological control of Kv1-Kv β assembly.

Methods

Fluorescence measurement of cortisone binding

Kv β 2 conserved core (residues 36 to 367) was expressed and purified as described⁹. The Kv β 2-bound NADPH fluorescence spectrum was recorded at room temperature (20–22 °C) with an excitation wave length of 360 nm at 1 nm slit size. 150 μ l of purified Kv β 2 core (2 μ M) in buffer A was used in each experiment. Buffer A contains 20 mM Tris (pH 8.0) and 150 mM KCl. After a spectrum was recorded, 3 μ l of cortisone stock solution (100 mM in DMSO) was pipetted into the Kv β 2 solution and quickly mixed. A spectrum was recorded immediately after mixture (time 0) and then at different time points afterwards. As a control experiment, Kv β 2 was substituted with free NADPH and cortisone did not induce a significant change in fluorescence.

Kv β 2 crystallization

Purified Kv β 2 protein was loaded onto a Superdex 200 column (GE Healthcare) for final purification. Kv β 2 protein was concentrated to $\sim$ 12 mg ml⁻¹ and then mixed with 2 mM cortisone for co-crystallization. Crystals were grown by the sitting-drop vapor diffusion method at 20 °C by mixing equal volumes of protein with a reservoir solution containing 6–15% glycerol, 1.5 M ammonium sulfate, 0.1 M Tris (pH 7.9–8.8). Glycerol concentration in reservoir solution was gradually raised to 25% for cryo-protection and crystals were flash-frozen in liquid nitrogen-cooled liquid propane. Two crystals forms were obtained under similar conditions, one with a P2₁2₁2 symmetry and another one with an I422 symmetry. Since the I422 crystal form consistently gave better resolution it was used for structural solution.

X-ray diffraction data collection and crystallography

For Kv β 2-cortisone complex, X-ray diffraction data were collected from a frozen crystal on Brookhaven National Laboratory (BNL) beamline X29. The intensity data from a single crystal were integrated and scaled using the HKL2000 program suite²⁵. Molecular replacement solution was obtained using the program MOLREP²⁶ starting with a model of Kv β 2 (extracted from PDB ID 1EXB⁵), from which all solvent molecules and the cofactor NADP were removed. The initial calculations, including rigid-body refinement and simulated annealing procedures were performed using the program suite CNS²⁷. Initial electron density maps were calculated and a difference map showed clear density for the cofactor NADP and two cortisone molecules. NADP and cortisone were then placed into the density. Cortisone was built with PRODRG²⁸.

The model was adjusted in program O²⁹ against the 2Fo-Fc and Fo-Fc maps, where Fo and Fc are the observed and calculated structure factors, respectively. Water molecules were added depending on their location in relation to significant electron density and the presence of satisfactory hydrogen bonding interactions. The resultant models were then subjected to cycles of refinement in which simulated annealing, coordinate minimization and B-factor refinement were conducted in CNS. Model validation was conducted using PROCHECK³⁰ and structure alignment was performed with LSQKAB³¹.

Data sets for Kv β 2 (W121A)-cortisone and Kv β 2 (I211R)-cortisone were collected at BNL beamlines X4A and X4C, respectively. Since both mutations have the same I422 symmetry as

the wild type, the refined wild type Kv β 2 model with only the protein atoms was used to calculate the initial electron density map. In each case, the mutant side chain, the NADP cofactor, and one cortisone molecule were identified unambiguously by the electron density map. The models were then further refined following the same procedure for the wild type.

Structures shown in Figs. 2–5 were all drawn in PyMOL (www.pymol.org).

Dissociation assay for the T1-Kv β 2 complex

Kv1.1T1-Kv β 2 complex was produced following a published strategy⁵. Briefly, the cDNA for the T1 domain of Kv1.1 (residues 2 to 135) was cloned (between the *NotI* and *SaI* sites) into a modified pET-SUMO vector (Invitrogen Inc.) which has a kanamycin resistance gene. The expressed fusion protein has an N terminus 6-histidine tag, a SUMO protein, and the T1 domain. The histidine tag and the SUMO protein are cleaved when the fusion protein is treated with SUMO protease, which was produced in the lab. The conserved core of rat Kv β 2 (residues 36 to 367) was cloned (between the *NdeI* and *XhoI* sites) into a modified pET31 vector which has an ampicillin resistance gene, and the expressed Kv β 2 core does not have an affinity tag. Kv1.1T1-Kv β 2 complex protein was purified by cobalt affinity resin. 20 μ l cobalt resin, which absorbed $\sim$ 40 μ g T1-Kv β 2 complex, was re-suspended in 1 ml of Buffer B that contains the desired concentrations of cortisone. After gentle mixing at room temperature for 30 minutes, the resin was spun down and washed 4 times, each time with 1 ml of Buffer B. The resin was then re-suspended in 200 μ l of Buffer B with 10 μ g of SUMO protease and incubated at room temperature for 30 minutes with gentle mixing. The supernatant was collected and 15 μ l of which was loaded onto an SDS-PAGE for further analysis. Each sample was loaded in duplicates as an internal control for consistency of sample loading.

SDS-PAGE gels were stained with Coomassie Blue and digitized by the FluorChem 8900 system (Alpha Innotech Co.) for quantification. The intensities from the duplicates were averaged for each experiment. The intensity of the T1 band, which varied $< 10\%$ from lane to lane on each gel, serves as a standard for the amount of protein loaded. The intensity of the Kv β 2 band was then normalized to its accompanying T1 band, plotted versus cortisone concentration, and the data points fit with a dose response equation.

Additional methods

The following methodologies can be found in Supplementary Methods online: molecular biology; electrophysiological recordings; and co-expression of Kv1.1T1-Kv β 2 complex.

Data statistics

The Origin 7.5 software package was used for statistical analysis of the data. The results are expressed as mean $\pm$ standard error of mean (s.e.m.). Student's t test and one-way analysis of variance (ANOVA) were used to assess changes of a mean value.

Accession codes

Protein Data Bank (PDB) identifiers: coordinates and structure factors for Kv β 2-cortisone, Kv β 2 (W121A)-cortisone, and Kv β 2 (I211R)-cortisone have been deposited under accession code 3EAU, 3EB3, and 3EB4, respectively. Coordinates of T1-Kv β 2 complex⁵ (1EXB) and Kv1.2-Kv β 2 complex³ (2A79) were downloaded from PDB. GenBank identifiers: *Rattus* Kv1.1, NM_173095; *Rattus* Kv β 2, CAA54142; *Rattus* Kv β 1, NM_017303.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Adelman JP, Bond CT, Pessia M, Maylie J. Episodic ataxia results from voltage-dependent potassium channels with altered functions. *Neuron* 1995;15:1449–1454. [PubMed: 8845167]
- Olson TM, et al. Kv1.5 channelopathy due to KCNA5 loss-of-function mutation causes human atrial fibrillation. *Hum Mol Genet* 2006;15:2185–2191. [PubMed: 16772329]
- Long SB, Campbell EB, MacKinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K⁺ channel. *Science* 2005;309:897–903. [PubMed: 16002581]
- Scott VE, et al. Primary structure of a beta subunit of alpha-dendrotoxin-sensitive K⁺ channels from bovine brain. *Proc Natl Acad Sci U S A* 1994;91:1637–1641. [PubMed: 8127858]
- Gulbis JM, Zhou M, Mann S, MacKinnon R. Structure of the cytoplasmic beta subunit-T1 assembly of voltage-dependent K⁺ channels. *Science* 2000;289:123–127. [PubMed: 10884227]
- Sokolova O, et al. Conformational changes in the C terminus of Shaker K⁺ channel bound to the rat Kvbeta2-subunit. *Proc Natl Acad Sci U S A* 2003;100:12607–12612. [PubMed: 14569011]
- Nagaya N, Papazian DM. Potassium channel alpha and beta subunits assemble in the endoplasmic reticulum. *J Biol Chem* 1997;272:3022–3027. [PubMed: 9006951]
- Rhodes KJ, et al. Voltage-gated K⁺ channel beta subunits: expression and distribution of Kv beta 1 and Kv beta 2 in adult rat brain. *J Neurosci* 1996;16:4846–4860. [PubMed: 8756417]
- Weng J, Cao Y, Moss N, Zhou M. Modulation of voltage-dependent shaker family potassium channels by an aldo-keto reductase. *J Biol Chem* 2006;281:15194–15200. [PubMed: 16569641]
- Pan Y, Weng J, Cao Y, Bhosle R, Zhou M. Functional coupling between the Kv1.1 channel and an aldo-keto reductase Kvbeta1. *J Biol Chem* 2008;283:8634–8642. [PubMed: 18222921]
- Heinemann S, et al. The inactivation behaviour of voltage-gated K-channels may be determined by association of alpha- and beta-subunits. *J Physiol Paris* 1994;88:173–180. [PubMed: 7833860]
- Zagotta WN, Hoshi T, Aldrich RW. Restoration of inactivation in mutants of Shaker potassium channels by a peptide derived from ShB. *Science* 1990;250:568–571. [PubMed: 2122520]
- Hoshi T, Zagotta WN, Aldrich RW. Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* 1990;250:533–538. [PubMed: 2122519]
- Zhou M, Morais-Cabral JH, Mann S, MacKinnon R. Potassium channel receptor site for the inactivation gate and quaternary amine inhibitors. *Nature* 2001;411:657–661. [PubMed: 11395760]
- del Camino D, Holmgren M, Liu Y, Yellen G. Blocker protection in the pore of a voltage-gated K⁺ channel and its structural implications. *Nature* 2000;403:321–325. [PubMed: 10659852]
- Isacoff EY, Jan YN, Jan LY. Putative receptor for the cytoplasmic inactivation gate in the Shaker K⁺ channel. *Nature* 1991;353:86–90. [PubMed: 1881453]
- Gulbis JM, Mann S, MacKinnon R. Structure of a voltage-dependent K⁺ channel beta subunit. *Cell* 1999;97:943–952. [PubMed: 10399921]
- Heinemann SH, Rettig J, Graack HR, Pongs O. Functional characterization of Kv channel beta-subunits from rat brain. *J Physiol (Lond)* 1996;493(Pt 3):625–633. [PubMed: 8799886]
- Shi G, et al. Beta subunits promote K⁺ channel surface expression through effects early in biosynthesis. *Neuron* 1996;16:843–852. [PubMed: 8608002]
- Accili EA, Kuryshv YA, Wible BA, Brown AM. Separable effects of human Kvbeta1.2 N- and C-termini on inactivation and expression of human Kv1.4. *J Physiol* 1998;512(Pt 2):325–336. [PubMed: 9763623]

21. Gu C, Jan YN, Jan LY. A conserved domain in axonal targeting of Kv1 (Shaker) voltage-gated potassium channels. *Science* 2003;301:646–649. [PubMed: 12893943]
22. Wells JA, McClendon CL. Reaching for high-hanging fruit in drug discovery at protein-protein interfaces. *Nature* 2007;450:1001–1009. [PubMed: 18075579]
23. Rhodes KJ, et al. Association and colocalization of the Kvbeta1 and Kvbeta2 beta-subunits with Kv1 alpha-subunits in mammalian brain K⁺ channel complexes. *J Neurosci* 1997;17:8246–8258. [PubMed: 9334400]
24. Sewing S, Roeper J, Pongs O. Kv beta 1 subunit binding specific for shaker-related potassium channel alpha subunits. *Neuron* 1996;16:455–463. [PubMed: 8789960]
25. Otwinowski Z, Minor W. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol* 1997;276:307–326.
26. Vagin A, Teplyakov A. An approach to multi-copy search in molecular replacement. *Acta Crystallogr D Biol Crystallogr* 2000;56:1622–1624. [PubMed: 11092928]
27. Brunger AT, et al. Crystallography & NMR system: A new software suite for macromolecular structure determination. *Acta Crystallogr sect D* 1998;54(Pt 5):905–921.
28. Schuttelkopf AW, van Aalten DM. PRODRG: a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr D Biol Crystallogr* 2004;60:1355–1363. [PubMed: 15272157]
29. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr sect A* 1991;47(Pt 2):110–119. [PubMed: 2025413]
30. Laskowski RA, MacArthur MW, Thornton JM. Validation of protein models derived from experiment. *Curr Opin Struct Biol* 1998;8:631–639. [PubMed: 9818269]
31. Kabsch W. A solution for the best rotation to relate two sets of vectors. *Acta Crystallogr sect A* 1976;32:922–923.

Figure 1.

Cortisone (**2**) potentiates Kv1.1 current via the AKR core of Kvβ. (a) Fluorescence spectra of Kvβ2 protein before (0 min, black) and 1 and 5 minutes after mixing with 500 μM cortisone (red). (b) Chemical structure of cortisone. The rings and carbon atoms are individually labelled. (c,d) Current traces recorded on inside-out patches from oocytes injected with both Kv1.1 and Kvβ1 (c) and with Kv1.1 (d) before (black) and after (red) perfusion of 500 μM cortisone. (e) ΔI_{ss} after perfusion of cortisone or vehicle control. NS indicates not significantly different and *** for $P < 0.001$, both by ANOVA. (f) Current traces recorded on inside-out patches from oocytes injected with both Kv1.1 and Kvβ1 before (black) and after (red) perfusion of vehicle control (1% DMSO). (g) Normalized ΔI_{ss} versus cortisone concentrations. The mean values of 3 to 13 patches are shown for each concentration. The solid curve is a Hill equation fit to the data points. All error bars are s.e.m. (h) Current traces recorded on inside-out patches from oocytes injected with Kv1.1-inact before (black) and after (red) perfusion of 500 μM cortisone.

Figure 2.

Each Kvβ2 has two cortisone molecules. **(a)** Current traces of Kv1.1 co-expressed with Kvβ12 before (black) and after (red) perfusion of either cortisone (500 μM, left panel) or vehicle control (1% DMSO, right panel). **(b)** ΔI_{ss} after perfusion of cortisone or vehicle control. NS indicates not significantly different and *** for $P < 0.001$, both by ANOVA. Error bars are s.e.m. **(c)** Stereo view of tetrameric Kvβ2 core in complex with cortisone. The four-fold axis is perpendicular to the plane of the paper and its position is marked (+). One pair of diagonally opposed Kvβ subunits are coloured in light green, and another pair in dark green. NADP is shown as cyan stick. Cortisone is shown as a space-filled model with carbon atoms coloured in yellow and oxygen atoms in red. The N and C termini are labelled as N and C respectively.

Figure 3.

Binding to the enzymatic site is not required for channel modulation. **(a,b)** Stereo view of the enzymatic site, for cortisone co-crystallized with the wild type **(a)** or the W121A mutant **(b)** Kvβ2. The Fo-Fc omit maps (blue mesh), calculated with cortisone and the side chains of residues 189 and 121 omitted, were contoured at 2σ level. The backbones are shown as green cartoon, NADP as cyan stick, amino acid side chains as stick, and cortisone as ball-and-stick. For amino acid side chains and cortisone, carbon is shown in yellow, nitrogen in blue, and oxygen in red. **(c)** Current traces of Kv1.1 co-expressed with the W155A mutant Kvβ1 before (black) and after (red) perfusion of either cortisone (500 μM, left panel) or vehicle control (1% DMSO, right panel). **(d)** ΔI_{ss} after perfusion of cortisone or vehicle control. NS indicates not significantly different and *** for $P < 0.001$, both by ANOVA. **(e)** Normalized ΔI_{ss} for different cortisone concentrations in Kv1.1 co-expressed with the W155A Kvβ1 (□) or Kv1.1 co-expressed with Kvβ1 wild type (○). The mean values of 3 to 13 patches are shown for each concentration. All error bars are s.e.m.

Figure 4.

Binding to the interface site is required for channel modulation. **(a)** Stereo view of an interface binding site for cortisone co-crystallized with the wild type Kvβ2 when the four-fold axis is perpendicular to the plane of the paper. Part of the two neighbouring Kvβ2 subunits is shown as surface representation and each coloured in light and dark green. Cortisone is shown as ball-and-stick, with carbon in yellow and oxygen in red. **(b,c)** Stereo view of the interface binding site, for cortisone co-crystallized with the wild type **(b)** or the I211R mutant **(c)** Kvβ2, both viewed with the four-fold axis in parallel to the plane of the paper. The Fo-Fc omit map (blue mesh), calculated with cortisone and the side-chain of residue 211 omitted, was contoured at 2σ level. The backbones of the two neighbouring Kvβ subunits are shown as cartoon and coloured in light and dark greens. Select side chains are shown as stick and cortisone is shown as ball-and-stick, with the same colour coding as that of Figure 3. **(d)** Current traces of Kv1.1 co-expressed with the V245R mutant Kvβ1 before (black) and after (red) perfusion of cortisone (500 μM, left panel) or vehicle control (1% DMSO, right panel). **(e)** ΔI_{ss} after perfusion of cortisone or vehicle control. NS indicates not significantly different and *** for $P < 0.001$, both by ANOVA. Error bars are s.e.m.

Figure 5.

Cortisone promotes dissociation of Kvβ. **(a)** The structure of Kv1.2 (magenta) in complex with Kvβ2 (cyan) presented as cartoon (PDB ID 2A79). Cortisone, shown in a space-fill model, was placed by aligning the Kvβ2-cortisone structure with that of the Kvβ2 in 2A79. Dotted square box demarcates the region magnified in **b**. **(b)** Stereo view of the Kv1-Kvβ interface shown with side chains as stick and cortisone as ball-and-stick representations. Distances (in Å) between the atoms connected by the dotted lines are marked. In the Kvβ2-cortisone complex, two neighbouring Kvβ subunits are shown as dark and light greens, and in the Kv1-Kvβ2 complex, the channel is shown as magenta and Kvβ2 as cyan. **(c)** SDS-PAGE of the Kv1.1-T1 in complex with Kvβ2 wild type (left panel) or Kvβ2 (I211R) (right panel). On each gel, the molecular weight standard is the leftmost lane and labelled. For other lanes, the higher molecular weight band is Kvβ2 and the lower one is the T1 domain. Cortisone concentrations are marked on the top of each lane. Ctr, 1% DMSO vehicle control. **(d)** Normalized Kvβ2 intensity plotted versus cortisone concentrations. Error bars are s.e.m ($n = 4$). The solid curve is a Hill function fit to the data points with an EC_{50} of $68 \pm 1.1 \mu$ M, and a Hill coefficient of 2.9 ± 0.4 . **(e)** Current traces of Kvβ1-Kv1.1 connected chimera before (black) and after (red) perfusion of cortisone (500 μ M, left panel) or vehicle control (1% DMSO, right panel). **(f)**

ΔI_{ss} after perfusion of cortisone or vehicle control. NS indicates not significantly different and *** for $P < 0.001$, both by ANOVA. Error bars are s.e.m.