Enhancing effects of salicylate on quinidine-induced block of human wild type and LQT3 related mutant cardiac Na⁺ channels

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ABSTRACT

It is unknown whether salicylate enhances the action of antiarrhythmic agents on human Na⁺ channels with state dependency and tissue specificity. We therefore investigated effects of salicylate on quinidine-induced block of human cardiac and skeletal muscle Na⁺ channels. Human cardiac wild-type (hH1), LQT3-related mutant (Δ KPQ), and skeletal muscle (hSkM1) Na⁺ channel α subunits were expressed in COS7 cells. Effects of salicylate on quinidine-induced tonic and use-dependent block of Na⁺ channel currents were examined by the whole-cell patch-clamp technique. Salicylate enhanced the quinidine-induced tonic and use-dependent block of both hH1 and hSkM1 currents at a holding potential (HP) of -100 mV but not at -140 mV. Salicylate decreased the IC₅₀ value for the quinidine-induced tonic block of hH1 at an HP of -100 mV, and produced a negative shift in the steady-state inactivation curve of hH1 in the presence of quinidine. According to the modulated receptor theory, it is probable that salicylate decreases the dissociation constant for quinidine binding to inactivated-state channels. Furthermore, salicylate significantly enhanced the quinidine-induced tonic and use-dependent block of the peak and steady-state Δ KPQ channel currents. The results suggest that salicylate enhances quinidine-induced block of Na⁺ channels via increasing the affinity of quinidine to inactivated state channels.

Acetylsalicylic acid, the most widely studied antiplatelet drug, suppresses platelet aggregation by inhibiting cyclo-oxygenase. Salicylic acid (C₆H₄(OH)COOH) belongs to the group of aromatic monocarboxylic acids, which by themselves do not have an anesthetic action. However, monocarboxylic acids such as salicylic, benzoic, acetic, propionic and butyric acid have been reported to enhance the action of local anesthetics (7, 13). In particular, monocarboxylic

acids containing a lipophilic moiety such as an aliphatic hydrocarbon chain or aromatic ring can enhance the action of local anesthetics in nerves; indeed, salicylate has been reported to enhance the blocking action of procainamide on sodium (Na⁺) channels in nerves (7). And Katsuki *et al.* (13) demonstrated that salicylate decreased the intracellular pH, which resulted in an increase of the proportion of charged molecules of procaine and the enhancement of its local anesthetic action on nerves.

As for the effect of salicylate on cardiac myocytes, Tanaka *et al.* (23) reported that it enhanced the action of Na⁺ channel blockers with higher liposolubility by increasing their affinity for inactivated state channels. Since Na⁺ channels in individual

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states might have different drug-binding properties based on their state-dependent conformations, salicylate could modify the state-dependent blocking action of Na⁺ channel blockers. Native Na⁺ channels consist of a tissue-specific α subunit and a β_1 and/or β_2 subunit (6, 15). It has been reported that the heart type Na⁺ channel (hH1- β_1) was threefold more sensitive to resting state block by lidocaine than skeletal muscle type Na⁺ channels ($\mu 1$ - β_1), indicating that structural differences within the Na⁺ channel α subunit can determine the tissue-specific sensitivity of cardiac and skeletal muscle Na⁺ channels to blockers. However, it remains unknown whether salicylate can enhance the action of Na⁺ channel blockers on Na⁺ channel α subunits in a state-dependent manner or regardless of the specificity of the subunits.

Upon membrane depolarization, wild-type Na⁺ channels open briefly and are then rapidly inactivated; they fail to reopen during maintained membrane depolarization, *i.e.*, fast inactivation is absorbing. On the other hand, a mutation in SCN5A (official name: sodium channel, voltage-gated, type V, α subunit) gene encoding the α subunit of cardiac Na⁺ channels, Δ KPQ mutation (deletion mutation of nine bases that encode for Lys1505, Pro1506 and Gln1507 in the highly conserved portion of the III-IV linker that plays a central role in inactivation) yields reopening from the inactivated state, giving rise to a steady-state current (17, 26). Thus, Δ KPQ mutant channels are considered to cause a congenital form of long-QT syndrome type 3 (LQT3). Δ KPQ mutant channels have been reported to have higher resting state affinity than wild-type channels for the class I antiarrhythmic agents flecainide and mexiletine (17, 26), indicating the beneficial roles of these agents in preventing the occurrence of life-threatening arrhythmias in LQT3 patients. However, it remains unknown whether salicylate can enhance the actions of Na⁺ channel blockers on Δ KPQ channels.

In the present study, we first studied whether salicylate could enhance the quinidine-induced block of human Na⁺ channel α subunits with different tissue-specific structures. Secondly, we compared the enhancing effect of salicylate on the quinidine-induced block of Δ KPQ mutant channels with that on the block of wild-type channels.

MATERIALS AND METHODS

Cell culture and transfection conditions. The vector pRC/CMV (Invitrogen, San Diego, CA, USA) was used as the expression vector for wild skeletal muscle type (hSkM1), wild heart type (hH1) and Δ KPQ

mutant Na⁺ channel α subunit cDNAs, which were provided by Dr. Naomasa Makita. COS7 cells were routinely maintained in Dullbecco's modified Eagle medium (DMEM; GIBCO, Grand Island, NY) containing 10% fetal bovine serum, 2 mmol/L L-glutamine, and 1% penicillin and streptomycin. Cell cultures were kept at 37°C in a 5% CO₂ incubator. Cells grown on glass coverslips in 30 mm dishes were transfected with hSkM1, hH1, or Δ KPQ mutant cDNA along with green fluorescence protein cDNA (Invitrogen) as a marker to identify the transfected cells using lipofectamine method. After 48 h, transiently transfected cells were visualized by the fluorescence light, and subsequently electrophysiological recordings were performed using the cells expressing green fluorescence proteins (8).

Electrophysiological recordings in cultured cells. Whole-cell patch clamp experiments were performed at 22°C as described previously (27). The external solution had the following composition (mmol/L): NaCl 140, CsCl 5.0, MgCl₂ 1.0, CaCl₂ 1.8, HEPES 10 and glucose 10 (pH 7.4 with NaOH). Patch-clamp electrodes were filled with an internal solution of the following composition (mmol/L): CsF 90, CsCl 10, CsEGTA 10, NaF 10, MgCl₂ 2 and HEPES 10 (pH 7.4 with CsOH).

Determination of tonic and use-dependent block of Na⁺ channel by quinidine. Blockade of Na⁺ channel currents by its inhibitors was evaluated in two different ways, *i.e.*, by estimating its tonic block and use-dependent block (UDB). The tonic block was defined as the drug-induced decrease in the current mediated by Na⁺ channel α subunits determined at a low pulse frequency of 0.1 Hz, which was sufficiently low to eliminate UDB of the drug (9, 10). Na⁺ channel currents were elicited by 30 ms depolarization to -20 mV from a holding potential (HP) of -140 or -100 mV. The amount of tonic block was calculated as the % decrease in peak currents during perfusion of a drug with respect to the control currents. The zero current level was obtained after application of 100 μ M tetrodotoxin. To determine the values of IC₅₀ and Hill coefficient, the concentration dependence data for the peak current was fitted with the following Hill equation:

$$\% \text{ tonic block} = 1 / [(IC_{50} / [D])^n + 1], \quad (1)$$

where [D] is the drug concentration, with IC₅₀ and n being the apparent dissociation constant and Hill coefficient, respectively.

To study the drug-induced shift in the voltage-dependent inactivation kinetics of Na⁺ channels, the steady-state inactivation (h_{∞}) curve was determined under control conditions and during exposure to the agent using the following standard two-pulse protocol: A 1000-ms conditioning pulse to a designated level of membrane potential was followed by a 0.1-ms interpulse interval and then a 30-ms test pulse to -20 mV, which was applied every 10 s (at 0.1 Hz). The data points were fitted by the following Boltzmann equation:

$$h_{\infty} = (1 + \exp[(V_m - V_h) / k])^{-1}, \quad (2)$$

where V_m is the prepulse potential, with V_h and k being the half-maximum prepulse potential and slope factor, respectively.

To study the UDB of Na⁺ channel currents, the currents elicited by a train of 20 depolarizing pulses of 30 ms from an HP of -100 or -140 mV to -20 mV were recorded at various frequencies. The peak current amplitude decreased during a pulse train and reached a new steady state. The amount of UDB was calculated as the % decrease of the current in the new steady state with respect to that of the first pulse (9, 10).

Drugs. Salicylate-sodium (Nakarai Chemical, Tokyo, Japan) and quinidine (Nakarai Chemical) were used in this study. Salicylate did not change the pH of the solution even at the highest concentration (3 mM) used in this study ($\text{pH} = 7.35 \pm 0.05$ in control, and 7.34 ± 0.02 in the presence of 3 mM salicylate; $n = 4$ each).

Data analysis. Analysis of the data was performed on a personal computer (Macintosh LC630) using a custom-made software. All curve fittings were done with a non-linear least square algorithm using a Marquardt routine. The results were expressed as the mean $\pm$ SEM. Statistical significance was determined using the one-way analysis of variance (ANOVA) test, and the difference was considered to be significant when the P value was less than 0.05.

Determination of binding constants for quinidine with a state model of Na⁺ channel. To elucidate the kinetic mechanism underlying the enhancement of quinidine-induced Na⁺ channel block by salicylate, we determined the dissociation constants (K_d) for quinidine binding to the resting (K_{dR}) and inactivated (K_{di}) states of the Na⁺ channel (hH1) using a state model (Scheme 1 in Fig. 8A) and tonic block

data. As described previously (3, 12), the voltage-dependent inactivation curve of Na⁺ channels (with quinidine) and concentration dependence of quinidine-induced tonic block were calculated for different K_d values; the K_{di} value was determined for the state model to reproduce the experimental V_h and IC_{50} values, with the K_{dR} value set equal to the experimental IC_{50} at an HP of -140 mV. Rate constants for state transitions were formulated as functions of the Gibbs free energy composed of chemical and potential terms, as described in our previous articles (1, 23).

RESULTS

Effects of salicylate on the tonic block of human Na⁺ channel currents by quinidine

Fig. 1 shows the enhancing effect of salicylate on quinidine-induced tonic block of hH1 currents elicited by 30-ms test pulses to -20 mV at 0.1 Hz with different HPs of -100 and -140 mV. Quinidine at $6 \mu\text{M}$ decreased hH1 currents by 37% at an HP of -100 mV (Fig. 1A, a: control, b: +quinidine). Addition of 1 mM salicylate enhanced quinidine-induced tonic block of hH1 currents, with the peak current being decreased by 60% (Fig. 1A, c). Washout of both quinidine and salicylate restored hH1 currents partially (Fig. 1A, d). With an HP of -140 mV, $6 \mu\text{M}$ quinidine blocked hH1 currents by 24% (Fig. 1B, e: control and f: +quinidine); addition of 1 mM salicylate did not enhance its block of hH1 currents at this HP (Fig. 1B, g).

Fig. 1C and D show the concentration dependence of the tonic block of hH1 currents by quinidine (0.06 to $600 \mu\text{M}$) in the absence and presence of 1 mM salicylate at an HP of -100 or -140 mV. At an HP of -100 mV, 1 mM salicylate significantly enhanced the tonic block of hH1 by quinidine at various concentrations, resulting in a significantly smaller IC_{50} value for the quinidine-induced tonic block ($6.0 \mu\text{M}$) than that without salicylate ($14.56 \mu\text{M}$), as shown in Fig. 1C. In contrast, at an HP of -140 mV, salicylate did not cause a significant shift in the concentration dependence of the quinidine block (control: $IC_{50} = 21.87 \mu\text{M}$, +salicylate: $IC_{50} = 19.46 \mu\text{M}$), as shown in Fig. 1D. These results suggest that salicylate increases the affinity of quinidine to the inactivated state of hH1.

Fig. 2 shows the steady-state inactivation curves of hH1 currents under control conditions, in the presence of $6 \mu\text{M}$ quinidine alone, and in the presence of both $6 \mu\text{M}$ quinidine and 1 mM salicylate. V_h (k) values were -69.4 ± 5.4 mV ($k = 6.4$) under

Fig. 1 Effects of salicylate on concentration dependence of the quinidine-induced tonic block of hH1 currents at different HPs. **A, B:** Original current traces. **a** and **e:** hH1 currents elicited by the test pulses to -30 mV at 0.1 Hz from an HP of either -100 (**a**) or -140 (**e**) mV. **b** and **f:** hH1 currents recorded in the presence of 6μ M quinidine with a HP of either -100 (**b**) or -140 (**f**) mV. **c** and **g:** hH1 currents recorded in the presence of both 6μ M quinidine and 1 mM salicylate with a HP of either -100 (**c**) or -140 (**g**) mV. **d** and **h:** hH1 currents recorded after washout of quinidine and salicylate. The vertical and horizontal bars indicate 1 nA and 10 ms, respectively. **C, D:** The concentration-dependent tonic block of hH1 currents by quinidine in the absence and presence of 1 mM salicylate, determined at HP = -100 mV (**C**) or -140 mV (**D**). The ordinate indicates % tonic block of hH1 and the abscissa indicates the log value of quinidine concentrations (0.06 – 600μ M). Open circles and closed circles indicate the data for quinidine alone and for quinidine plus 1 mM salicylate, respectively ($n = 8$ for each concentration).

Fig. 2 Effects of salicylate on the quinidine-induced shift in the inactivation curve of hH1 currents. Circles, control conditions; triangles, 6μ M quinidine; diamonds, 6μ M quinidine plus 1 mM salicylate; squares, washout. The ordinate indicates the availability of hH1 and the abscissa the conditioning potentials.

the control conditions, -80.0 ± 4.0 mV ($k = 6.0$) in the presence of quinidine alone, -91.8 ± 1.3 mV ($k = 6.1$) in the presence of both the agents, and -78.0 ± 3.7 mV ($k = 6.0$) after the washout. These results indicate that salicylate also produced a significant shift of voltage-dependent hH1 inactivation toward the hyperpolarized direction, while the slope factor k remained unchanged.

Fig. 3 shows the summary of the enhancing effects of salicylate on quinidine-induced tonic block of hH1 in comparison with those on quinidine-induced tonic block of hSkM1 at an HP of -100 or -140 mV. Salicylate significantly enhanced the quinidine-induced tonic block of wild-type hH1 and hSkM1 at HP = -100 mV but not at HP = -140 mV.

As shown in Fig. 4, the salicylate-induced enhancement of the quinidine block was well reproduced by the state model for Na^+ channels (Scheme 1 in Fig. 8) when salicylate was assumed to increase the affinity of quinidine to the inactivated state (*i.e.*, reduce the K_{di}). Decreasing the K_{di} yielded apparent reduction in the IC_{50} at HP = -100 mV and a negative shift in the V_h . The state model with the K_{di} values of 0.70 and 0.11 μM ($K_{diR} = 19.46$ μM) predicted the V_h of -82.1 and -93.2 mV, respectively

($k = 6.4$); and the IC_{50} of 16.2 and 8.3 μM , respectively. These predicted values of the V_h and IC_{50} are very close to the experimentally determined ones. Thus, salicylate was suggested to yield more than 6-fold increase in the affinity of quinidine to inactivated hH1 channels. Taken together, salicylate appears to increase the binding affinity of quinidine to inactivated Na^+ channel α subunits and enhance its tonic block without tissue specificity.

Effects of salicylate on UDB of human Na^+ channel currents by quinidine

Fig. 5A shows the enhancing effect of salicylate on the quinidine-induced UDB of hH1 currents elicited by a train of 30-ms depolarizing pulses to -20 mV from an HP of either -100 or -140 mV at 2 Hz. The currents elicited by the first depolarizing pulse (open circles) and by the 20th pulse (closed circles) in the absence or presence of 1 mM salicylate are illustrated. At an HP of -100 mV, 6 μM quinidine produced a UDB of peak hH1 currents by 7% (Fig. 5A, a). The addition of 1 mM salicylate enhanced the UDB of hH1 by 6 μM quinidine, which reduced the peak current by 19% (Fig. 5A, b). In contrast, at an HP of -140 mV, 6 μM quinidine produced a UDB of

Fig. 3 Enhancing effects of salicylate on the tonic block of hH1 and hSkM1 currents by quinidine at the different HPs. **A, B:** The summary of the % tonic block of peak hH1 currents by quinidine in the absence and presence of 1 mM salicylate at HP = -100 mV(A) and -140 mV(B). $n = 6$ each, $**P < 0.01$. **C, D:** The summary of the % tonic block of peak hSkM1 currents by quinidine in the absence and presence of 1 mM salicylate at HP = -100 mV(C) and -140 mV(D). $n = 6$ each, $**P < 0.01$.

Fig. 4 Predicted voltage-dependent inactivation curve of the hH1 channel (with 6 μ M quinidine) and concentration dependence of quinidine-induced tonic block (at HP = -100 mV) for the K_{dl} value of 0.70 and 0.11 μ M ($K_{dR} = 21.87$ μ M). Steady-state probability of a channel to be in the drug-free resting state (state R in Scheme 1) was calculated and normalized as described previously (23). The rate constants for the transition between the resting and inactivated states of hH1 channels are the same as used in Miake *et al.* (16); the Gibbs free energy peak for the quinidine unbinding (dissociation from the channel) was assumed to be 34 (in RT unit). The open circles and lines indicate the predicted probabilities of state R (normalized to the maximum) and their fits with Eq. 1 or 2, respectively. The numbers at the bottom represent the V_h and IC_{50} values determined by the fittings.

Fig. 5 Effects of salicylate on the use-dependent block (UDB) of hH1 and hSkM1 currents by quinidine at different HPs. **A:** hH1 currents elicited by a train of 30 ms depolarizing pulses to -20 mV at 2 Hz from a HP of -100 (a, b) or -140 (c, d) mV. Open and closed circles indicate the peak hH1 currents elicited by the first depolarizing pulse and by the 20th pulse, respectively. Currents were evoked in the presence of 6 μ M quinidine alone (a, c) or in the presence of both 6 μ M quinidine and 1 mM salicylate (b, d). **B, C:** The summary of the salicylate effects on UDB of hH1 (B) and hSkM1 (C) currents by quinidine at HP = -100 mV and -140 mV. UDB was tested in the presence of 6 μ M quinidine alone (closed column) or in the presence of both 6 μ M quinidine and 1 mM salicylate (shaded column). $n = 6$ each; * $P < 0.05$, ** $P < 0.01$.

6% (Fig. 5A, c), with the addition of 1 mM salicylate not enhancing the UDB of hH1 by quinidine (Fig. 5A, d). Fig. 5B shows the summary of the en-

hancing effects of salicylate on the UDB ($n = 6$ for each group). Salicylate at 1 mM could significantly enhance the UDB by 6 μ M quinidine at an HP of

Fig. 6 Enhancing effects of salicylate on quinidine-induced tonic block of Δ KPQ peak and steady-state currents. **A, B:** Peak (A) and steady-state (B) Δ KPQ currents elicited by 30 ms depolarizing pulses to -20 mV from a HP of -100 mV at 0.1 Hz in control (circles), in the presence of $6\mu\text{M}$ quinidine alone (triangles), in the presence of $6\mu\text{M}$ quinidine plus 1mM salicylate (diamonds), and washout (squares). **C, D:** The summary of the quinidine block of peak (C) and steady-state (D) Δ KPQ currents in the absence and presence of 1mM salicylate ($n = 8$ each). * $P < 0.05$, ** $P < 0.001$ (vs. quinidine alone).

-100 mV ($7 \pm 3\%$ without salicylate vs. $23 \pm 3\%$ with 1mM salicylate; $P < 0.05$), but not at an HP of -140 mV ($7 \pm 2\%$ without salicylate vs. $6 \pm 2\%$ with 1mM salicylate). Salicylate also enhanced the quinidine-induced UDB of hSkM1 (Fig. 5C). Salicylate at 1mM could significantly enhance the UDB of hSkM1 by $6\mu\text{M}$ quinidine at HP = -100 mV ($10 \pm 2\%$ without salicylate vs. $24 \pm 10\%$ with 1mM salicylate; $P < 0.05$), but not at HP = -140 mV ($8 \pm 2\%$ without salicylate vs. $7 \pm 3\%$ with 1mM salicylate). These results indicate that salicylate enhances the UDB by quinidine of Na^+ channel α subunits without isoform specificity.

Effects of salicylate on quinidine-induced tonic block and UDB of Δ KPQ channel currents

LQT3-related Δ KPQ mutant current is composed of peak current (Fig. 6A) and steady-state current (Fig. 6B); the former represents the transition to the absorbing inactivated state and the latter represents the reopening of Na^+ channels in the burst mode (Scheme 2 in Fig. 8). Studying the interaction of local anesthetics with Δ KPQ clarified their state-

dependent block of the mutant Na^+ channel (2, 12, 17, 25, 26). As shown in Fig. 6A, $6\mu\text{M}$ quinidine reduced the peak current by 10%, and salicylate produced the further block of 23%. Quinidine at $6\mu\text{M}$ also reduced the steady-state current by 38%, with salicylate producing the further block of 78% (Fig. 6B). Washout of both the agents restored the peak and steady-state current almost completely. Fig. 6C and D show the summary of the effects of salicylate on the quinidine-induced tonic block of Δ KPQ. Salicylate significantly enhanced the quinidine block of peak currents from 13 ± 2 to $28 \pm 0.5\%$ ($n = 6$) and also the quinidine block of steady-state currents from 22 ± 10 to $68 \pm 5\%$ ($n = 6$).

Fig. 7 shows the summary of the enhancing effects of salicylate on quinidine-induced UDB of Δ KPQ peak and steady-state currents. Salicylate slightly increased the UDB of peak currents from 25 ± 7 to $41 \pm 5\%$, while it more dramatically increased the UDB of steady-state currents from 27 ± 7 to $65 \pm 9\%$.

DISCUSSION

The enhancing effect of salicylate on quinidine-induced block of Na⁺ channels does not depend on the tissue-specific structures of Na⁺ channels

In a previous study we found that salicylate selectively increased the quinidine-bound inactivated Na⁺ channels, enhancing thereby both tonic block and UDB by quinidine in guinea-pig ventricular myocytes (23). The structural differences among channel proteins may account for tissue-specific sensitivity

Fig. 7 Enhancing effects of salicylate on quinidine-induced UDB of peak and steady-state Δ KPQ currents. Currents were elicited at 2 Hz in the absence and presence of 1 mM salicylate ($n = 8$ each). ** $P < 0.001$ (vs. quinidine alone).

of cardiac and skeletal muscle Na⁺ channels to drugs; *e.g.*, the time course of recovery from inactivation in the presence of lidocaine was much slower in heart type Na⁺ channel α subunit (hH1- β_1) than in skeletal muscle type Na⁺ channel α subunit (μ 1- β_1) (18). The III-IV linker region that mediates the inactivation process is the most conserved region in various Na⁺ channel isoforms (3, 5, 20, 21, 24), while the transmembrane segment S6 of domain IV is important for the resting state block by the local anesthetic etidocaine (11). In the present study, the salicylate-induced enhancement of the tonic block and UDB by quinidine showed voltage dependency but not isoform specificity, indicating that salicylate would increase the binding affinity of quinidine for inactivated Na⁺ channel α subunits through the conserved region of the Na⁺ channel.

The preferential blockade of open/inactivated Na⁺ channels without tissue specificity by charged local anesthetics is quite reasonable, because the binding of charged local anesthetics to different subtypes of Na⁺ channels is known to be highly conserved. It might also be relevant that flecainide (mostly charged) preferentially suppresses late Na⁺ currents in the LQT3 Δ KPQ mutant (17). In the present study, salicylate at the concentration of ~ 3 mM did not lower the pH of the external solution. However, the neutral form of salicylate can diffuse into the cell and lower the intracellular pH (13); inside the

Fig. 8 Markovian state schemes for the gating kinetics of the wild-type and Δ KPQ mutant Na⁺ channels. **A** (Scheme1): A simplified scheme showing the transitions between the rested and inactivated states of the channel and binding of quinidine molecules to each state. D stands for the drug molecule. R and RD are the drug-free and drug-bound resting states, respectively; I and ID are the drug-free and drug-bound inactivated states, respectively. k_r and k_i are the drug binding rate constants for the resting and inactivated states; l_r and l_i are the corresponding dissociation rate constants. α is the forward rate constant for a transition from state I to R (α_c) or from ID to RD (α_d); β is the backward rate constant from R to I (β_c) or from RD to ID (β_d). K_{dR} and K_{dI} are the equilibrium dissociation constants for the resting and inactivated states, respectively ($K_{dR} = l_r/k_r$, $K_{dI} = l_i/k_i$). **B** (Scheme2): A schematic diagram illustrating two modes of Δ KPQ channel gating. Mode A represents the steady-state current of Δ KPQ. In Mode A, the mutant channel open briefly and then inactivate without reopening as the wild-type hH1 channel, where the backward rate constant (β_1) is much smaller than the forward rate constant (α_1). In Mode B, the mutant channel repeatedly transits between an inactivated state (I') and open state (O'), where the backward rate constant (β_2) is significantly larger than that in Mode A (β_1).

cell, therefore, salicylate may increase the proportion of charged quinidine with a pKa of 8.56 enhancing thereby the blocking effect of quinidine.

A state model based on the modulated receptor theory (Scheme 1 in Fig. 8) serves to estimate the binding affinity of drugs to Na⁺ channels in different states. According to this modulated receptor theory (22), 1) drugs bind to a receptor site on or very close to Na⁺ channels; 2) the affinity of drugs to the receptor depends on the channel states, *i.e.*, whether the channel is in the resting, activated, or inactivated state; 3) drug-associated channels differ from drug-free channels in that they are not conductive, and their voltage-dependent ability to be activated is shifted to more negative potentials. It is well known that the tonic block is composed of resting-state block and inactivated-state block (22). We previously reported that salicylate selectively reduced the K_{di} (K_d value of the inactivated state) for quinidine block, increasing thereby the number of drug-bound nonconducting inactivated Na⁺ channels in guinea-pig ventricular myocytes, because salicylate decreased the IC₅₀ for quinidine block at the depolarized membrane potential but not at the hyperpolarized membrane potential. In the present study, salicylate decreased the IC₅₀ for the quinidine block of hH1 channels at the depolarized membrane potential (−100 mV) without changes in the IC₅₀ for the blockade at the hyperpolarized potential (−140 mV). Therefore, we concluded that salicylate enhanced the quinidine-induced tonic block of hH1 channels by decreasing the K_{di} for quinidine block without changing K_{dr} (K_d value in the resting state). This would explain the enhancement of the quinidine-induced UDB of hH1 channels by salicylate as well. As the UDB is composed of the activated and/or the inactivated state block, the salicylate-induced increase in the affinity of quinidine for inactivated hH1 channels would lead to an accumulation of quinidine-bound nonconducting hH1 channels during trains of stimuli, resulting in an enhanced UDB by quinidine.

Salicylate strongly enhanced quinidine block of LQT3 related mutant cardiac Na⁺ channels

It is well-known that the inactivation process of Na⁺ channels has two components, *i.e.*, fast inactivation and slow inactivation. Most wild-type Na⁺ channels in the inactivated state do not reopen during maintained membrane depolarization, *i.e.*, fast inactivation is absorbing. In ventricular myocytes and Purkinje fibers (4, 14, 19), Na⁺ channels reopen from the inactivated states, produce bursts of channel openings

during maintained depolarization, leading to slow inactivation. Salicylate is suggested to selectively decrease the K_{di} for quinidine binding and increase the number of quinidine-bound inactivated channels (State ID in Scheme 1). The channels reopening from the inactivated state frequently transit between the inactivated state and the open state (Mode B in Scheme 2 as shown in Fig. 8B). Salicylate may facilitate quinidine binding to the inactivated state of reopening Na⁺ channels (*i.e.*, State I' in Mode B), as well as its binding to the inactivated state of brief-opening Na⁺ channels (State I in Mode A). This facilitation of quinidine binding yields an increase of quinidine-bound inactivated channels (State ID and ID'), leading to enhancement of the tonic block and UDB of both reopening and brief-opening Na⁺ channels. As predicted, in the present study, salicylate significantly enhanced the quinidine-induced tonic block and UDB of both peak and steady-state ΔKPQ currents. Interestingly, salicylate predominantly enhanced the quinidine-induced tonic block and UDB of steady-state ΔKPQ currents and to a lesser extent those of peak ΔKPQ currents. Since the peak current of ΔKPQ reflects fast inactivation (Mode A), salicylate may only slightly increase quinidine-bound inactivated ΔKPQ at the current peak to decrease the peak amplitude of ΔKPQ currents. On the other hand, the steady-state current of ΔKPQ reflects channel reopening from the inactivated state (Mode B); thus, salicylate may dramatically increase quinidine-bound inactivated ΔKPQ in the steady state, decreasing thereby the steady-state ΔKPQ current.

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