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pH-dependent blocking actions of three novel antiarrhythmic compounds on K^+ and Na^+ currents in rat ventricular myocytes

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Abstract

Three novel chemically related compounds were studied for their pH-dependent ion channel blocking actions on the transient outward K^+ current (I_{to}) and the Na^+ current (I_{Na}) in isolated rat ventricular myocytes. The (±)-*trans*-napthylethoxycyclohexylamines, RSD1108, RSD1070 and RSD1067, showed similar potencies in reducing the inactivation time course of I_{to} at pH 7.4. However, RSD1108 (pK_a 6.8) was a more potent blocker of I_{to} at pH 6.4 than the other two compounds (pK_a values near 8.0). The reduction of inactivation times induced by the RSD compounds was consistent with open channel blockade and in consequence an open channel block model was used in order to estimate blocking and unblocking rate constants. This analysis showed no apparent correlation between pK_a and onward blocking rate constants for the compounds. However, the unblocking rate constant for the low pK_a compound RSD1108 at acid pH decreased markedly from that found at normal pH. Both RSD1108 and RSD1070 showed an enhanced potency to block I_{Na} at acid pH relative to pH 7.4. However, RSD1108 showed significantly less inhibition of I_{Na} at both pH values compared to RSD1070 and RSD1067. Differences in channel block were also evident between RSD1070 and RSD1067, which could be attributed to the difference in napthyl groups between their chemical structures. The compounds exhibited use- and frequency-dependent blockade of I_{Na} with the amount of use-dependent blockade greater for RSD1108 and RSD1067 than for RSD1070 at acid pH compared to neutral pH. Greater frequency-dependent inhibition was apparent for RSD1108 as compared to RSD1070 and RSD1067 at both pH 7.4 and 6.4. These results point out the importance of the magnitude of pK_a and chemical structure in ion channel blocking actions of a series of structurally related compounds.

Keywords: Transient outward current (I_{to}); Inward Na^+ current (I_{Na}); Antiarrhythmic; Hydrogen ion concentration; Open channel blockade; Patch clamp

1. Introduction

A continuing aspect of antiarrhythmic therapy is design and development of antiarrhythmic agents which effectively suppress and prevent arrhythmias without causing mortality as a result of proarrhythmic actions (Nattel and Singh, 1999). As a result of the Cardiac Arrhythmia Suppression Trial (CAST), which demonstrated that Na^+ channel blockers administered to post-myocardial infarction patients increased mortality, antiarrhythmic drug development shifted to agents that prolong the cardiac action potential and increase refractoriness. However, such agents have also proven to induce high incidences of arrhythmia in addition to other side effects, which include central nervous system toxicity and cardiovascular depression (Katz, 1998; Roden, 1994; Schlepper, 1989).

Although sodium channel blockers have been shown to be effective in the prevention of ventricular fibrillation due to myocardial ischaemia (Hine et al., 1989), evidence also indicates that sodium channel blockers have a pro-fibrillatory effect in acute ischaemia. The combination of a lack of selectivity for cardiac and ischaemic tissue along with other pharmacological actions has been used to explain the lack of efficacy of antiarrhythmic agents against ischaemia-induced arrhythmias (Barrett et al., 1995; Hondeghem, 1991). It has been hypothesized that in order to develop potential ischaemic-selective antiarrhythmics for ischaemia-induced arrhythmias, agents possessing pK_a values near ischaemic pH would be more selective for ischaemic tissue and decrease the incidence of proarrhythmias (Bain et al., 1997; Yong et al., 1999). This is in part

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due to the increased concentration of charged molecules at ischaemic pH for agents with pK_a values close to the pH of ischaemic tissue estimated to be near 6.0 (Khandoudi et al., 1990; Kida et al., 1991). Selectivity for ischaemic tissue over normal tissue may help prevent the reduction of refractoriness due to ischaemia and through blockade of both sodium and potassium channels would reduce electrical activity in the damaged tissue (Yong et al., 1999). At present, however, the efficacy of antiarrhythmic agents in relation to pK_a is not well tested and requires extensive in vitro studies using agents with common chemical structures but with different properties such as pK_a.

We have studied the pH-dependent actions on the transient outward K^+ current (I_{to}) and inward Na^+ current (I_{Na}) in rat ventricular myocytes of three novel agents with a commonality in chemical structure. The compounds RSD1108, RSD1070 and RSD1067 are (±)-*trans*-napthylethoxycyclohexylamines of similar size and molecular weight but differ in pK_a (values near 8.0 for the latter two agents and 6.8 for RSD1108). In addition, there are small variations in chemical structure between the compounds (RSD1108 and RSD1070 differ in basicity of ionizable nitrogen and RSD1070 and RSD1067 differ in napthyl groups). Previous work has demonstrated, both in vivo and in vitro, the ischaemic efficacy of a member of the napthylalkylcyclohexyl derivatives, RSD1000 (pK_a 6.1) and the three RSD compounds studied presently also exhibit antiarrhythmic actions in vivo (unpublished data). The study with RSD1000 has suggested the importance of low pK_a as a factor in developing ischaemia selective antiarrhythmic agents (Yong et al., 1999). The objective of the study was to investigate the relationships between pK_a, chemical structure and pH on the effects of a chemically related group of RSD compounds on properties of I_{to} and I_{Na}. The actions of the RSD compounds on the state-dependent properties of I_{to} and I_{Na} including channel blockade, voltage dependence of activation and inactivation, and recovery from inactivation were also investigated. The information obtained gives some insight into the influence of structure on antiarrhythmic activity of compounds possessing both Class I and Class III activity and how pK_a may be of value in developing new ischaemic-selective antiarrhythmic compounds.

2. Materials and methods

2.1. Isolation of rat ventricular myocytes

Rat ventricular myocytes were isolated from male Sprague-Dawley rats (250–300 g) using procedures that have been described previously (McLarnon and Xu, 1995; Mitra and Morad, 1985). Approval for this use of rats was obtained from the UBC Ethics Committee. In brief, the isolation of cells involved initial removal of the heart from a pentobarbitone anaesthetized rat. After removal, the heart was immediately hung on a constant flow Langendorff system. The heart was then perfused with a modified Tyrode's solution containing: 133.5 mM NaCl, 4 mM KCl, 1.2 mM $MgCl_2$, 1.2 mM NaH_2PO_4, 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and 11 mM glucose at pH 7.4. This solution was oxygenated and maintained at a temperature of 37 °C. When the heart appeared to have been cleared of blood, the solution was exchanged with modified Tyrode's solution containing 0.07% collagenase (Type II, Worthington Biochemical), 25 μM $CaCl_2$, 1 mg/ml of bovine serum albumin (Boehringer Mannheim). Upon sufficient digestion of the tissue, the heart was cut into small pieces and agitated in the modified Tyrode's solution to further enhance dissociation of cells. The solution was then filtered and centrifuged for 5 s at $1000 \times g$, and the cells were again resuspended in fresh modified Tyrode's solution and left to rest at room temperature between 10 min intervals of wash with successively stepwise increased Ca^{2+} concentrations (100, 250, 500 μM Ca^{2+} and to a final Ca^{2+} concentration of 1 mM).

2.2. Electrophysiology

The procedures used in this study to record whole-cell currents in rat ventricular myocytes have been previously described (McLarnon and Xu, 1995, 1997). In the present study, the currents analyzed were the transient outward potassium current (I_{to}) and inward sodium (Na^+) current (I_{Na}). Micropipettes were fabricated from Corning 7052 capillary glass (A-M Systems, Everett, WA) with resistance values in the range of 2–3 MΩ. An axopatch amplifier was used (Model 200A, Axon Instruments, Foster City, CA) to record whole-cell currents with the low pass filter set at 1 or 2 kHz. Capacitative currents and series resistance were compensated using the analog circuitry of the amplifier with the latter routinely set at 80–85%. Typical values for seal resistances were 41 ± 6 GΩ ($n = 5$) and cell capacitances were 101 ± 9 pF ($n = 5$) for whole-cell studies on rat ventricular myocytes.

I_{to} was activated using a depolarizing step to +60 mV applied from a holding potential of −70 mV. The blocking properties of the RSD compounds on the voltage dependence of I_{to} activation, inactivation and recovery from inactivation at pH 7.4 were also determined. A holding level of −70 mV was also used in the studies of the effects of RSD compounds on I_{Na}. A depolarizing step to −20 mV following an initial prepulse to −140 mV for 30 ms to remove resting inactivation was used in order to activate I_{Na}. The effects of the RSD compounds on the voltage dependence of activation and inactivation of I_{Na} were also investigated at pH 7.4. For use-dependent analysis of I_{Na}, a holding potential of −100 mV was used and a series of depolarizing steps were applied at frequencies of 5 and 20 Hz at both pH 7.4 and 6.4. The voltage clamp protocols were generated by computer (pClamp 6, Axon

Instruments) and data recorded on disk for off-line analysis. A detailed description of the specific protocols applied is given in the Results section. All data were recorded at room temperature (22–24 °C).

2.3. Data analysis

Data analysis was carried out using pClamp software. A single exponential was found to be a better fit for the data for the time course of decay of I_{to} (time constant, τ) rather than a biphasic, consistent with previous results from this laboratory (McLarnon and Xu, 1995, 1997). In order to construct dose–response curves for the effects of RSD1108, RSD1070 and RSD1067 on the time course of decay of I_{to}, τ were normalized to control and plotted against concentration. In order to determine the potency of inhibition of RSD compounds on I_{Na}, the effects of RSD1108, RSD1070 and RSD1067 on the amplitudes of I_{Na} were used to plot dose–response curves. Dose–response curves of normalized inactivation time constants (τ_n) and normalized amplitudes of I_{Na} (I_n) plotted versus drug concentrations were fitted with a logistic function ($y = 1/[1 + (x/x_{50})^b]$ where x is the concentration, x_{50} is the effective concentration for a 50% response (EC_{50}) and b is the slope factor-Hill coefficient). EC_{50} results are presented as mean ± S.E.M. The effects of pH were evaluated by comparing drug changes in acid to normal pH using Student's paired *t*-test ($P < 0.05$). When comparing the effects of all three compounds at each pH, an ANOVA was performed followed by Tukey's test ($P < 0.05$).

2.4. Experimental compounds and solutions

The compounds RSD1108 [(±)-*trans*-[2-(3′-oxo-pyrollidinyl)-1-(1-napthaleneethoxy)]cyclohexane hydrochloride], RSD1070 [(±)-*trans*-[2-morpholinyl-1-(1-napthaleneethoxy)]cyclohexane hydrochloride], RSD1067 [(±)-*trans*-[2-morpholinyl-1-(2-napthaleneethoxy)]cyclohexane hydrochloride] were synthesized by Nortran Pharmaceuticals, Vancouver, British Columbia. All compounds are (±)-*trans*-napthylethoxycyclohexylamines with similar size and molecular weight. Both RSD1067 and RSD1070 have pK_a values near 8.0 while the pK_a of RSD1108 is 6.8. The chemical structures of the compounds are shown in Fig. 1 with their pK_a values. For each experiment, fresh stock solution of the drug used was prepared and applied using a continuous gravity-flow perfusion system.

Several studies have used a specific Tyrode's solution to mimic ischaemic conditions (Ferrier et al., 1985; Ferrier and Guyette, 1991; Cordeiro et al., 1994); however, this study has focused only on altered pH and thus extracellular solutions used in the study did not include some factors relevant in simulating ischaemic conditions. In the experiments on I_{to}, the bath solution was a modified Tyrode's solution and contained 133.5 mM NaCl, 4 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM TES, 1.2 mM NaH_2PO_4, 11 mM glucose and pH adjusted to 7.4 or 6.4 with NaOH. The patch pipette solution contained: 10 mM NaCl, 140 mM KCl, 10 mM EGTA, 10 mM TES, 5 mM $MgCl_2$, 5 mM Na_2ATP and 10 mM HEPES; pH was adjusted to 7.4 with KOH.

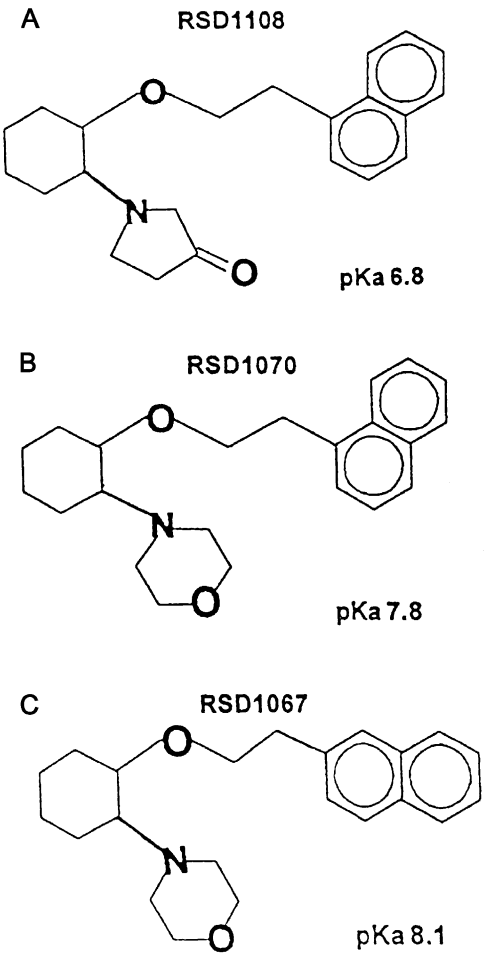

Fig. 1. Chemical structures and pK_a of RSD compounds. (A) RSD1108, pK_a 6.8; (B) RSD1070, pK_a 7.8; (C) RSD1067, pK_a 8.1.

Experiments on I_{Na} used the following bath solution: 50 mM NaCl, 87 mM Tris, 4 mM CsCl, 1 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM TES, 11 mM glucose, 1.2 mM NaH_2PO_4, 11 mM glucose; pH was adjusted to 7.4 or 6.4 with HCl. The pipette solution contained 10 mM NaCl, 120 mM CsCl, 12 mM EGTA, 10 mM TES, 1 mM $MgCl_2$, 5 mM NaATP; pH was adjusted to 7.4 with CsOH.

3. Results

3.1. RSD effects on the inactivation time course (τ) for I_{to} at pH 7.4

The effects of the three compounds RSD1108, RSD1070 and RSD1067 were studied on the inactivation time course

of I_{to}. It was assumed that only the inactivating component, blocked by 4-aminopyridine (4-AP), was I_{to}. The residual steady state component, which may represent a delayed rectifier type of K^+ current, is considered a separate entity (Apkon and Nerbonne, 1991; Castle and Slawsky, 1993; McLarnon and Xu, 1995, 1997). Typical currents elicited in control and in the presence of two concentrations of RSD1108 (10 and 60 μM) and RSD1067 (5 and 50 μM) at pH 7.4 are shown in Fig. 2. The agents, in a dose-dependent manner, visibly decreased the time course of I_{to}. Similar effects of RSD1070 on I_{to} were also obtained (data not shown). Partial recovery of I_{to} was found for all agents following a wash-out for 5 min.

Dose–response curves for actions of RSD compounds to reduce τ (time constant of decay) at pH 7.4 are shown in Fig. 3 (closed circles) fitted to a logistic function. The concentration of RSD1108 which reduced τ to 50% of the control value (EC_{50}) at pH 7.4 was 11 ± 2 μM ($n = 8$). As well, the EC_{50} for the effects of RSD1070 to reduce τ of I_{to} was 2.1 ± 0.2 μM ($n = 10$) and for RSD1067, EC_{50} was 0.94 ± 0.07 μM ($n = 6$). The concentrations of RSD1108, RSD1067 and RSD1070, which reduced τ to 50% of control value (i.e. EC_{50}) at pH 7.4, are summarized in Table 1. The order of potency was 1067 > 1070 > 1108 with significant difference between the EC_{50} values ($P < 0.05$). As evident from Fig. 2, at higher concentra-

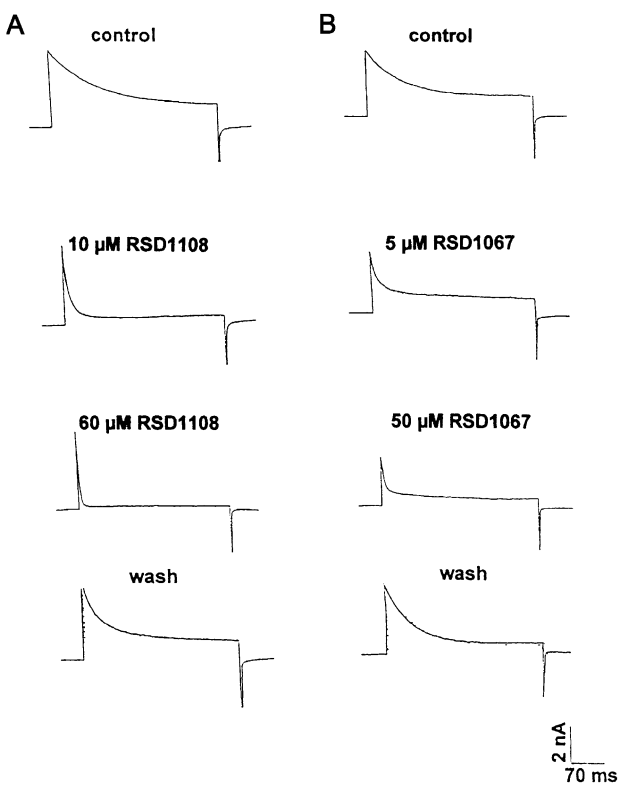

Fig. 2. Dose-dependent actions of RSD compounds on I_{to} at pH 7.4. (A) traces (top to bottom) show I_{to} in control, 10 μM RSD1108, 60 μM RSD1108, partial recovery after prolonged wash-off. (B) Traces (top to bottom) show I_{to} in control, 5 μM RSD1067, 50 μM RSD1067, partial recovery after prolonged wash-off. The I_{to} currents were elicited with a depolarizing step from a holding potential of −70 to +60 mV.

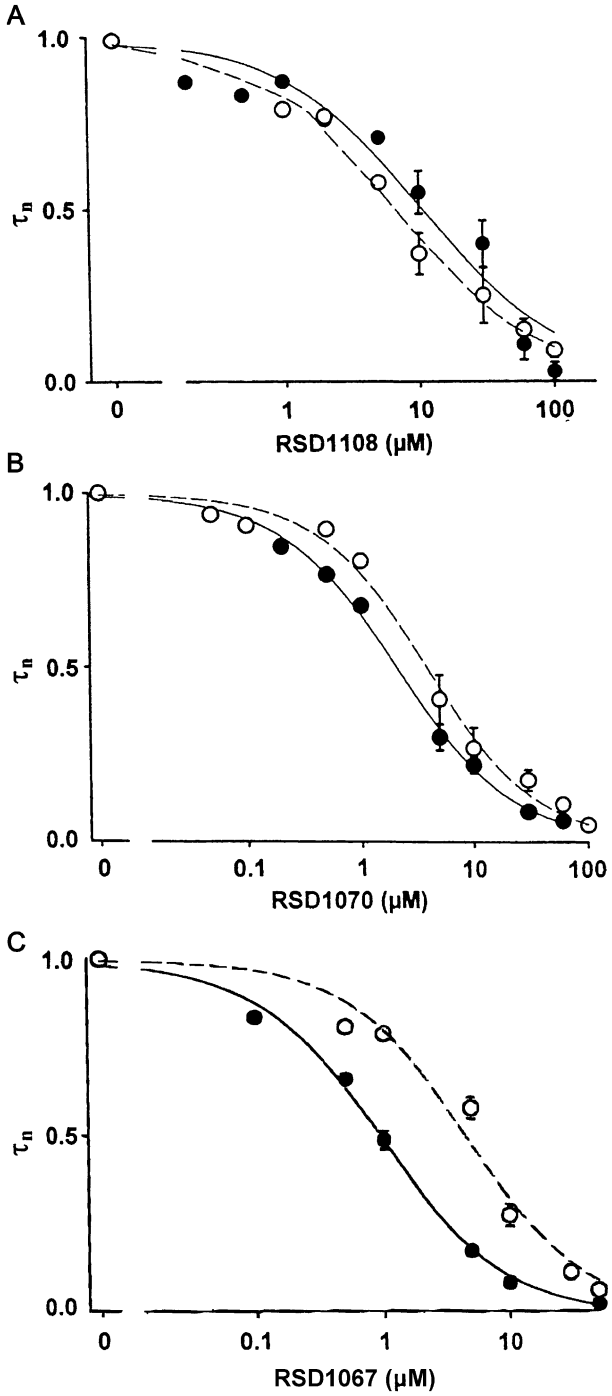

Fig. 3. Dose–response curves for the effect of RSD compounds at pH 7.4 and 6.4 on I_{to}. (A) RSD1108 at pH 7.4 (closed circles) ($n = 8$) and pH 6.4 (open circles) ($n = 7$). (B) RSD1070 at pH 7.4 (closed circles) ($n = 10$) and pH 6.4 (open circles) ($n = 7$). (C) RSD1067 at pH 7.4 (closed circles) ($n = 6$) and pH 6.4 (open circles) ($n = 7$) on time constant τ. Data of normalized time constant (τ_n) versus concentration of RSD compound were fit using a logistic function represented as a solid line for pH 7.4 and dashed line for pH 6.4. Typical mean ± S.E.M. are presented for higher concentrations of RSD compounds.

Table 1
The EC_{50} values for the effects of the RSD compounds on the inactivation time course of I_{to} at pH 7.4 and 6.4

pH	EC_{50} (μM)		
	RSD1108	RSD1070	RSD1067
7.4	11 ± 2^a ($n=8$)	2.1 ± 0.2^a ($n=10$)	0.94 ± 0.07^a ($n=6$)
6.4	6.0 ± 0.6^b ($n=7$)	3.9 ± 0.6 ($n=7$)	5.5 ± 0.8 ($n=7$)

The inactivation time course of I_{to} (τ) was measured at different concentrations of RSD1108, RSD1070 and RSD1067 and normalized to control values; dose–response curves were constructed and EC_{50}s were determined. Values are mean ± S.E.M.; n are presented in the table.

[a] $P < 0.05$ versus other two RSD compounds at same pH (ANOVA and Tukey's test).

[b] $P < 0.05$ versus compound's effect at normal pH.

tions, the compounds also reduced the amplitude of I_{to}; this is discussed later.

3.2. RSD effects on the inactivation time constant (τ) for I_{to} at pH 6.4

The effects of the three compounds on the inactivation time course of I_{to} were also studied at pH 6.4 (examples not shown) with I_{to} activated as described in the previous section. Dose-dependent plots were then constructed from the data and are shown in Fig. 3 (open circles) fitted to a logistic function. The EC_{50} for the effects of RSD1108 on τ for I_{to} at pH 6.4 was 6.0 ± 0.6 μM ($n=7$). Similarly, the EC_{50} for RSD1070 and RSD1067 to reduce τ was 3.9 ± 0.6 μM ($n=7$) and 5.5 ± 0.8 μM ($n=7$).

The EC_{50}s for each of the compound's effects on I_{to} at pH 6.4 (Fig. 3) are summarized in Table 1. The most potent agent was RSD1070 with an EC_{50} of 3.9 ± 0.6 μM ($n=7$) and the least potent RSD1108, EC_{50} of 6.0 ± 0.6 μm ($n=7$). The two compounds with pK_a values near 8.0 (RSD1070 and RSD1067) had reduced potency at pH 6.4 whereas RSD1108 was more potent at acid pH. Overall, the blocking effects of RSD1108 on the inactivation time course of I_{to} were significantly enhanced at pH 6.4 as compared with pH 7.4 ($P < 0.05$).

3.3. Determination of channel blocking and unblocking rate constants

The data presented above showed that the RSD compounds RSD1108, RSD1070 and RSD1067 decreased the inactivation time course of I_{to} in a concentration-dependent manner. These results would be consistent with blockade of the open I_{to} channel as previously found for other RSD compounds, RSD1000 (Yong et al., 1999) and RSD921 (Pugsley and Goldin, 1999). Open channel blockade of I_{to} has also been demonstrated for a diversity of agents including tedisamil (Dukes et al., 1990), KC8851, a structural analogue of tedisamil (McLarnon and Xu, 1997), terikalant (McLarnon and Xu, 1995), bupivicaine (Castle, 1990) and 4-AP (Castle and Slawsky, 1993). The following open channel block model was used,

$$\mathrm{C} \rightleftharpoons \mathrm{O} \overset{k_1}{\rightleftharpoons} \mathrm{B}$$
$$\underset{\mathrm{I}}{\overset{k_1}{\rightleftharpoons}}$$

where C, O, B and I represent the closed, open, blocked and inactivated state of the channel and k_1 (M^{-1} s^{-1}) and k_{-1} (s^{-1}) are respective channel block and unblock rate constants. The inverse of the decay time constant in the presence of drug is $k_1[D] + k_{-1}$, where D is the concentration of RSD compound (McLarnon and Xu, 1995, 1997). In order to quantify the magnitude of k_1 and k_{-1}, the time course of decay of I_{to} in the presence of two concentrations of RSD compound were each subtracted from the time course of decay of I_{to} in control, and by using two simultaneous equations, k_1, k_{-1} and k_d ($k_d = k_{-1}/k_1$) were determined as described previously (Castle, 1990; McLarnon and Xu, 1997). The analysis is shown for RSD1108 block of I_{to} at pH 7.4 where typical I_{to} in control and after addition of two concentrations of the compound are shown (Fig. 4A). The results of subtracting the two-time courses measured in the presence of RSD1108

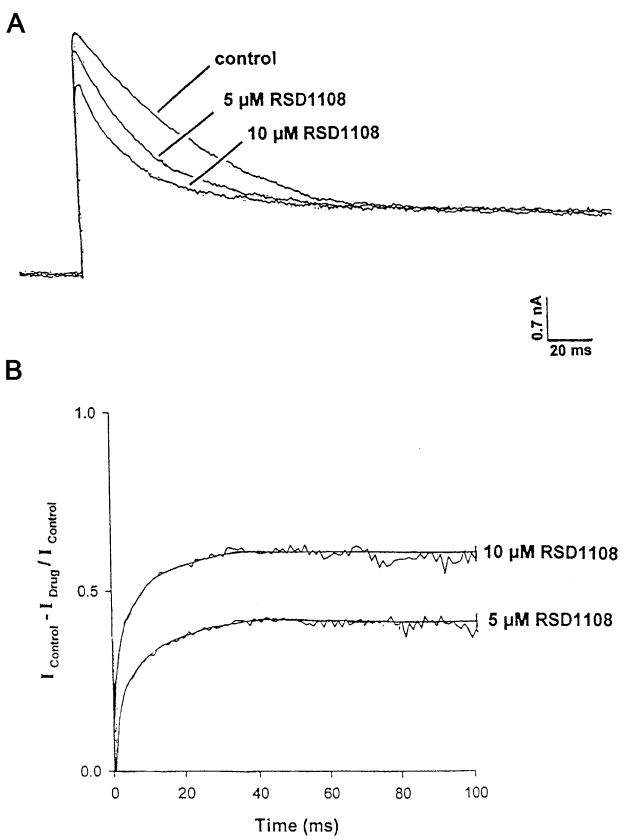

Fig. 4. Analysis of RSD1108 block of I_{to} at pH 7.4. (A) The traces shown are I_{to} in control and after addition of RSD1108 at 5 and 10 μM. (B) The inactivation time course in (A) 5 and 10 μM RSD1108 were subtracted from control and then normalized to control. The fits shown are fits to the data using the equation in the text.

from the time course of I_{to} inactivation in control are presented in Fig. 4B. The results are normalized using the expression $(I_{control} - I_{drug})/I_{control}$. The data shown in Fig. 4B were fitted according to the following equation (Castle, 1990; McLarnon and Xu, 1997):

$$I(t) = I_{max}(1 - e^{-(k_1[D]+k_{-1})t})$$

where I_{max} equals maximum block at drug concentration $[D]$; $I(t)$ refers to the amount of inhibition at any time t; and k_1 and k_{-1} are as defined above. The single exponential fits extrapolated to zero at the beginning of the depolarization steps (Fig. 4B) indicate that there was little block of I_{to} before activation.

Mean values for on and off-rate constants were also determined for RSD1070 and RSD1067. In these experiments, concentrations that approximated EC_{50} values of each of the compounds (Table 1) were used. The results of mean on and off-rate constants in the presence of the three compounds at pH 7.4 are summarized in Table 2. Onward blocking rate constants for RSD1070, $k_1 = 21 \pm 3.0 \times 10^6$ M^{-1} s^{-1} ($n = 3$) and RSD1067, $k_1 = 15 \pm 2.0 \times 10^6$ M^{-1} s^{-1} ($n = 3$) were not significantly different ($P > 0.05$). However, the onward rate constant for RSD1108, $k_1 = 6.7 \pm 1.1 \times 10^6$ M^{-1} s^{-1} ($n = 4$) was significantly less than the k_1 for both RSD1070 and RSD1067 ($P < 0.05$).

The rank order for onward blocking rate constants were RSD1070 ≥ RSD1067 > RSD1108, where ≥ represents insignificantly greater than ($P > 0.05$) and > represents significantly greater than ($P < 0.05$). Off-rate constants for RSD1108, $k_{-1} = 43 \pm 4.8$ s^{-1} ($n = 4$) and RSD1070, $k_{-1} = 51 \pm 6.5$ s^{-1} ($n = 3$) were not significantly different ($P > 0.05$). The off-rate constant for RSD1067, 20 ± 4.1 s^{-1} ($n = 3$) was significantly different from both the k_{-1} of RSD1108 and RSD1070 ($P < 0.05$). The ranked order for off-rate constants for the compounds from least to greatest was RSD1067 < RSD1108 ≤ RSD1070 where ≤ represents insignificantly less than ($P > 0.05$) and < represents significantly less than ($P < 0.05$). The larger off-rate constants for RSD1108 and RSD1070 were not significantly different whereas RSD1067 had a significantly lower k_{-1} ($P < 0.05$). A lower k_{-1} could indicate increased effectiveness of channel blockade by RSD1067. The k_d values of channel block for each of the compounds approximated their EC_{50} values on I_{to} at pH 7.4 (Table 1).

Blocking properties of the three compounds were also studied at pH 6.4. The onward blocking rate constants, k_1, for RSD1108, $10 \pm 1.6 \times 10^6$ M^{-1} s^{-1} ($n = 4$) and RSD1067, $7.7 \pm 1.7 \times 10^6$ M^{-1} s^{-1} ($n = 3$) were not significantly different ($P > 0.05$). The onward blocking rate constant for RSD1070, $32 \pm 7.9 \times 10^6$ M^{-1} s^{-1} ($n = 4$) was significantly different from the onward rate constants of both RSD1067 and RSD1108 ($P < 0.05$). The rank order for onward blocking rate constants was RSD1070 > RSD1108 ≥ RSD1067, where > and ≥ are as defined above. The off-rate constants for RSD1070, 38 ± 10. The off-rate constants for RSD1070, 38 ± 10 s^{-1} ($n = 4$) and RSD1067, 35 ± 2.5 s^{-1} ($n = 3$) were not significantly different ($P > 0.05$). The rank order for off-rate constants for the compounds was RSD1108 < RSD1067 ≤ RSD1070 where < and ≤ are as defined above. The k_d values for RSD1070 and RSD1067 were similar to their EC_{50} values on I_{to} at pH 6.4. The k_d of RSD1108 was lower than its EC_{50} on I_{to} at pH 6.4 which may reflect its lower k_{-1} and increased effectiveness of channel blockade at acid pH.

The onward rate constants for each of the compounds at acid pH were not significantly different ($P > 0.05$) from the values estimated at normal pH. However, the off-rate constants estimated for RSD1108 and RSD1067 at pH 6.4 were significantly different ($P < 0.05$) from the values found at pH 7.4, whereas the off-rate constant for RSD1070 was not altered with the change in pH ($P > 0.05$). The differences between the off-rate constants at acid pH compared to normal pH for RSD1108 and RSD1067 is reflective of their potencies in reducing the τ of I_{to}, i.e. RSD1108 being more potent at acid pH is correlated with a decreased off-rate constant and RSD1067 demonstrating less potency at acid pH as compared to normal pH is associated with an increased off-rate constant. Table 2 summarizes the results of the blocking properties of the RSD compounds and pH dependence of k_1 and k_{-1}.

3.4. Other state-dependent interactions of RSD compounds with I_{to}

The preceding data show that the actions of RSD compounds were consistent with open channel blockade.

Table 2
The values of I_{to} open channel blockade of RSD1108, RSD1070 and RSD1067

Rate constants	RSD1108		RSD1070		RSD1067	
	pH 7.4 ($n = 4$)	pH 6.4 ($n = 4$)	pH 7.4 ($n = 3$)	pH 6.4 ($n = 4$)	pH 7.4 ($n = 3$)	pH 6.4 ($n = 3$)
k_1 ($\times 10^6$) (M^{-1} s^{-1})	6.7 ± 1.1[a]	10 ± 1.6	21 ± 3.0	32 ± 7.9[a]	15 ± 2.0	7.7 ± 1.7
k_{-1} (s^{-1})	43 ± 4.8	16 ± 2.9[b]	51 ± 6.5	38 ± 10	20 ± 4.1[a]	35 ± 2.5[b]
k_d (μM)	6.4	1.6	2.4	1.2	1.3	4.5

On-rate constants (k_1), off-rate constants (k_{-1}) and k_d (k_{-1}/k_1) were determined at both pH 7.4 and pH 6.4 for RSD1108, RSD1070 and RSD1067 using the solution of two simultaneous equations. Data are presented as mean ± S.E.M.; n are presented in the table.

[a] $P < 0.05$ versus other two RSD compounds at same pH (ANOVA and Tukey's test).

[b] $P < 0.05$ versus compound's effect at normal pH.

Additional experiments were carried out at pH 7.4 to determine if the RSD compounds also had any effects on the inactivated state of the I_{to} channel or on the voltage dependence of activation and recovery from inactivation as has been previously described (McLarnon and Xu, 1997). Briefly, the protocol for investigating the effects of the RSD compounds on the voltage dependence of activation of I_{to} consisted of applying depolarizing steps from a holding potential of −70 to +60 mV in 10 mV increments. The peak currents at different test depolarizing potentials were measured and compared to activation thresholds in control and in the presence of RSD1108 ($n = 4$), RSD1070 ($n = 3$) and RSD1067 ($n = 4$). In order to investigate the effects of the RSD compounds on the voltage dependence of inactivation of I_{to}, a two-pulse protocol was applied which consisted of applying conditioning pulses from −90 to −30 mV for 300 ms followed by a single test pulse to +60 mV for 200 ms from a holding potential of −70 mV. The peak amplitudes of the I_{to} associated with the test pulse were normalized to the I_{to} amplitude at a conditioning pulse of −90 mV in both control and in the presence of RSD1108 ($n = 5$), RSD1070 ($n = 3$) and RSD1067 ($n = 3$), and normalized data were plotted versus conditioning voltages. The resulting curves were fit by the Boltzmann equation from which $V_{1/2}$, the potential at which normalized current is 0.5, and k, the slope factor, were determined. In order to investigate the effects of the compounds on recovery from inactivation of I_{to}, a depolarizing conditioning pulse to +60 mV for 200 ms was applied to ensure inactivation of I_{to} followed by a variable recovery time which varied from 5 to 1000 ms at a holding potential of −70 mV and was followed by a test pulse to +60 mV to examine recovery in control and in the presence of RSD1108 ($n = 5$), RSD1070 ($n = 3$) and RSD1067 ($n = 3$). The extent of recovery from inactivation was determined as the change in I_{to}, i.e. test pulse peak I_{to} divided by conditioning pulse peak amplitude at each episode. The normalized currents were plotted vs. recovery times, and time courses of recovery were determined from an exponential fit to the data. The voltage dependence of activation and inactivation of I_{to} as well as the recovery from inactivation of I_{to} were not altered in the presence of each of the compounds (data not shown).

3.5. Actions of RSD compounds on the amplitude of I_{Na} at pH 7.4

Representative data are shown in Fig. 5 for effects of (A) RSD1070 and (B) RSD1067 on I_{Na}. I_{Na} was progressively decreased in amplitude with increasing concentrations of RSD compounds. After prolonged wash-out of the agents for periods of up to 10 min, I_{Na} showed partial recovery to control levels. Similar results were obtained for RSD1108 (data not shown). In these experiments, I_{to} was not blocked with 4-AP so currents in Fig. 5 show activation of this current following inactivation of I_{Na}.

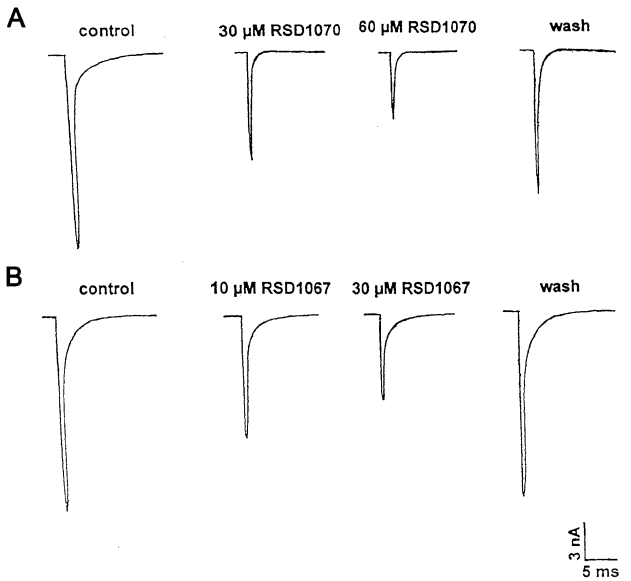

Fig. 5. Dose-dependent actions of RSD compounds on I_{Na} at pH 7.4. (A) Traces (left to right) show I_{Na} in control, 30 μM RSD1070, 60 μM RSD1070, partial recovery after prolonged wash-off. (B) Traces (left to right) show I_{Na} in control, 10 μM RSD1067, 30 μM RSD1067, partial recovery after prolonged wash-off. The I_{Na} currents were elicited with an initial hyperpolarizing step to −140 mV for 30 ms from a holding potential of −70 mV followed by a depolarizing step to −20 mV.

Previous studies have shown that the absence or presence of 4-AP had no significant effect of agents blocking actions on I_{Na} (McLarnon and Xu, 1995, 1997).

The dose–response curves for the effects of RSD1067, RSD1070 and RSD1108 on I_{Na} at pH 7.4 are shown in Fig. 6 (closed circles), with data fitted to a logistic function. The EC_{50} for RSD1108 at pH 7.4 was 45 ± 3 μM ($n = 8$). The corresponding EC_{50}s for RSD1070 and RSD1067 were 6.9 ± 1 μM ($n = 8$) and 4.4 ± 0.9 μM ($n = 8$). When comparing the effects of the three compounds on I_{Na}, RSD1070 and RSD1067 were more potent than RSD1108 in reducing the amplitude of I_{Na} at pH 7.4.

3.6. Actions of RSD compounds on the amplitude of I_{Na} at pH 6.4

The effects of the three compounds in reducing I_{Na} amplitude at pH 6.4 were also investigated. All compounds reduced I_{Na} at pH 6.4 in a dose-dependent manner as shown in Fig. 6 (open circles). The mean EC_{50} for RSD1108 at pH 6.4 was 34 ± 3 μM ($n = 8$) and the corresponding mean EC_{50}s for RSD1070 and RSD1067 were 0.36 ± 0.05 μM ($n = 7$) and 3.9 ± 1.0 μM ($n = 5$). The mean EC_{50}s for the effects of the three RSD compounds on I_{Na} at pH 7.4 and 6.4 are summarized in Table 3. The results show that RSD1108 and RSD1070 were more potent at pH 6.4 as compared to pH 7.4 ($P < 0.05$). However, the blocking effects of RSD1070 were more enhanced than RSD1108 at pH 6.4 ($P < 0.05$).

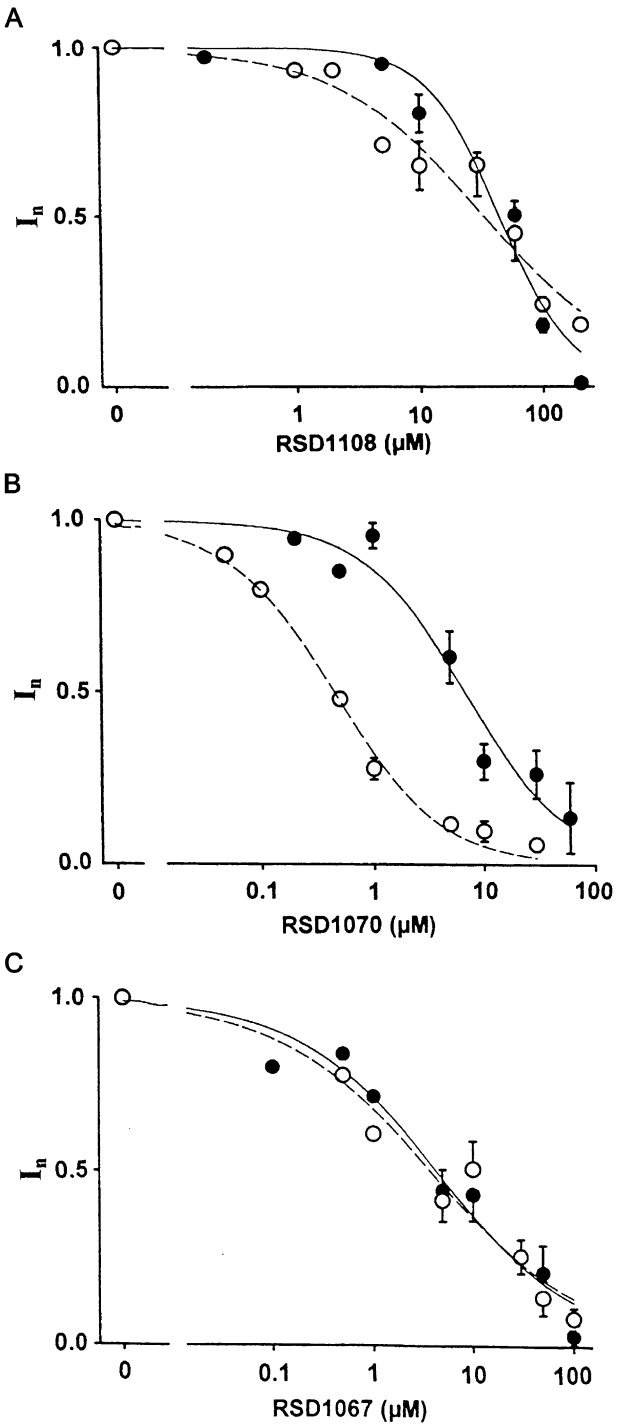

Fig. 6. Dose–response curves for the effect of RSD compounds at pH 7.4 and 6.4 on amplitude of I_{Na}. (A) RSD1108 at pH 7.4 (closed circles) ($n=8$) and pH 6.4 (open circles) ($n=8$). (B) RSD1070 at pH 7.4 (closed circles) ($n=8$) and pH 6.4 (open circles) ($n=7$). (C) RSD1067 at pH 7.4 (closed circles) ($n=8$) and pH 6.4 (open circles) ($n=5$) on amplitude of I_{Na}. Data of normalized current (I_n) versus concentration of RSD compound were fit using a logistic function represented as a solid line for pH 7.4 and dotted line for pH 6.4. Typical mean ± S.E.M. are presented for higher concentrations of RSD compounds.

Table 3
The EC_{50} values for the effects of RSD compounds on the amplitude of I_{Na} at pH 7.4 and 6.4

pH	EC_{50} (μM)		
	RSD1108	RSD1070	RSD1067
7.4	45 ± 3^{a} ($n=8$)	6.9 ± 1 ($n=8$)	4.4 ± 0.9 ($n=8$)
6.4	$34 \pm 3^{a,b}$ ($n=8$)	0.36 ± 0.05^{b} ($n=7$)	3.9 ± 1 ($n=5$)

The amplitude of I_{Na} was measured at different concentrations of RSD1108, RSD1070 and RSD1067 and normalized to control values; dose–response curves were constructed and EC_{50}s were determined. Values are mean ± S.E.M.; n are presented in the table.

[a] $P < 0.05$ versus other two RSD compounds at same pH (ANOVA and Tukey's test).

[b] $P < 0.05$ versus compound's effect at normal pH.

3.7. Use-dependent inhibition of I_{Na} at pH 7.4 and 6.4

Use-dependent inhibition of I_{Na} by RSD compounds was studied at both pH 7.4 and 6.4 using a series of 20 depolarizing pulses to −20 mV (applied at a frequency of 20 Hz) from a holding potential of −100 mV. It was first ascertained that in the absence of RSD compounds, the amplitude of I_{Na} remained unchanged following the protocol; representative traces are shown in Fig. 7A for pH 7.4. RSD compounds were then applied at levels near the EC_{50} values established from the dose–response data (see Table 3). In Fig. 7B, the effects of RSD1108 (at 30 μM) are shown and the amplitudes of I_{Na} decreased in magnitude with successive depolarizations to a new steady-state level from that in control. The same protocol was carried out in other cells at pH 7.4 using a stimulating frequency of 20 Hz at pH 7.4 (Fig. 7C). Representative traces are shown for RSD1108 (30 μM), RSD1070 (10 μM) and RSD1067 (5 μM) in Fig. 7C, where the current responses to the 20 depolarizing pulses have been superimposed. All compounds showed use-dependent activity with decreases in I_{Na} to new steady-state levels. Overall (see Table 4, where data are represented as amplitude of I_{Na} at 20th pulse divided by amplitude of I_{Na} at first pulse ×100%), the respective % inhibitions (calculated as 1 minus amplitude of I_{Na} at 20th pulse divided by amplitude of I_{Na} at first pulse ×100%) were 36% (30 μM, RSD 1108), 65% (5 μM, RSD 1070) and 23% (5 μM, RSD 1067) with these concentrations close to EC_{50} values (refer to Fig. 6 for EC_{50} values).

The use-dependence protocol was also applied at pH 6.4 in the presence of the compounds. The same concentration(s) of RSD1108 as employed at pH 7.4 was applied at pH 6.4 (refer to Fig. 6 for EC_{50} values). RSD1070 was applied at a concentration of 1 μM in pH 6.4 solution (compared with 10 μM at pH 7.4) since this compound showed a considerably enhanced potency in acid pH (Fig. 6). A preliminary experiment with 5 μM RSD1067 showed little effect on use-dependent inhibition of I_{Na} at pH 6.4 (data not shown). RSD1067 at a concentration of 20 μM showed enhanced use-dependent inhibition in pH 6.4 solution. Representative traces of use-dependent block of

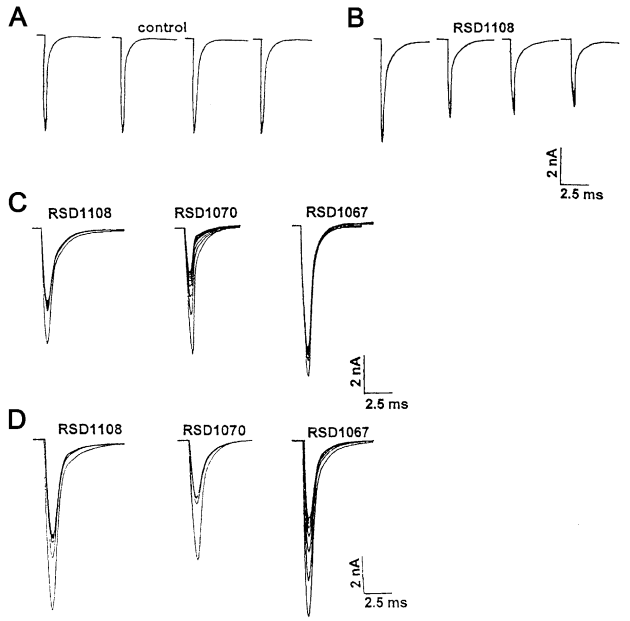

Fig. 7. Use-dependent inhibition of I_{Na}. (A) Typical traces of I_{Na} elicited for the pulses 1, 2, 6, 20 of a 20 pulse sequence of depolarizing steps to −10 mV at a frequency of 20 Hz from a holding potential of −100 mV at pH 7.4. (B) Pulses 1, 2, 6, 20 of a 20 pulse sequence of depolarizing steps to −10 mV at a frequency of 20 Hz from a holding potential of −100 mV in the presence of 30 μM RSD1108 at pH 7.4. (C) Use-dependent inhibition of I_{Na} in the presence of 30 μM RSD1108, 10 μM RSD1070 and 5 μM RSD1067 showing the decreasing amplitudes of I_{Na} to a steady-state level after 20 sequential depolarizing pulses at 20 Hz and at pH 7.4. Each of the 20 traces shown for each compound are superimposed, i.e. traces 2 to 20 in the presence of 30 μM RSD1108 at pH 7.4 are represented by the thicker trace. (D) Use-dependent inhibition of I_{Na} in the presence of 30 μM RSD1108, 5 μM RSD1070 and 20 μM RSD1067 showing the decreasing amplitudes of I_{Na} to steady-state level after 20 sequential depolarizing pulses at 20 Hz and at pH 6.4. Each of the 20 traces for each compound have been superimposed, i.e. traces 6 to 20 in the presence of RSD1108 at pH 6.4 are represented as the thicker trace.

I_{Na} at pH 6.4 are shown in Fig. 7D. Overall, at a stimulation frequency of 20 Hz, the % inhibitions were 56% (RSD1108) (see Table 4), 12% (RSD1070) and 51% (RSD1067). RSD1108 showed an increase in use-dependent inhibition at pH 6.4 from that at 7.4 but the difference was not significant ($P > 0.05$). The use-dependent inhibition of RSD1067 at pH 6.4 was significantly greater from the use-dependent effects of RSD1067 at pH 7.4 ($P < 0.05$) and may be a result of the concentration of compound used. The use-dependent inhibition produced by RSD1070 at pH 6.4 was significantly less than the use-dependent inhibition of the compound at pH 7.4 ($P < 0.05$). However, this result reflects the use of a much lower concentration of RSD1070 at acid pH (1 μM) since the EC_{50} values were markedly different at the two pH values (see Table 3). As shown in Fig. 7D, use-dependent block of I_{Na} by RSD1070 (at 5 μM) yields a considerably higher use-dependent blockade (approximately 50% inhibition).

Use-dependent blockade of I_{Na} was also studied at different stimulation frequencies. The results from a representative experiment using RSD1108 (30 μM at pH 7.4) are shown in Fig. 8A where a plot of the normalized current (measured as the 20th response as a percentage amplitude of the first response) was plotted at stimulation frequencies of 5 and 20 Hz. There was only a small reduction in I_{Na} at 5 Hz with increased frequency-dependent inhibition at the higher frequencies. As shown in Fig. 8C, 5 μM RSD1067 showed some use-dependent block of I_{Na} but with minimal frequency-dependent effects at stimulating frequencies of 5 and 20 Hz. A similar result was found with 5 μM of RSD1070 at pH 7.4 (data not shown). The amount of use-dependent inhibition at pH 7.4 by all three compounds at 5 and 20 Hz is presented in Table 4.

Frequency-dependent inhibition at 5 and 20 Hz was also investigated for the compounds at pH 6.4. As shown in Fig. 8B, 30 μM RSD1108 demonstrated increased use-dependent inhibition and maintained its frequency-dependent inhibition at 5 and 20 Hz (see Table 4). In a preliminary experiment, RSD1067, at a concentration close to EC_{50}, did not significantly reduce I_{Na} at acid pH as compared to effects found at pH 7.4 and demonstrated little frequency-dependent inhibition with increasing frequency (data not shown). On the other hand, 20 μM RSD1067, which exhibited enhanced use-dependent inhibition as compared to effects at normal pH ($P < 0.05$), demonstrated little frequency-dependent inhibition when frequency was increased to 20 Hz (Fig. 8D) as was found at normal pH. Similar results were found with RSD1070 (data not shown) with no evident effects of pH on frequency-dependent block.

Table 4
Use-dependent blockade of RSD1108, RSD1070 and RSD1067 at different stimulation frequencies at both pH 7.4 and 6.4

Frequency (Hz)	Use-dependent blockade (%)					
	RSD1108		RSD1070		RSD1067	
	30 μM, pH 7.4 ($n = 4$)	30 μM, pH 6.4 ($n = 4$)	10 μM, pH 7.4 ($n = 4$)	1 μM, pH 6.4 ($n = 3$)	5 μM, pH 7.4 ($n = 3$)	20 μM, pH 6.4 ($n = 4$)
5	79 ± 6.8	69 ± 2.9	43 ± 0.89[a]	93 ± 0.60[a,b]	82 ± 3.1	54 ± 6.1[b]
20	64 ± 4.9	44 ± 12	35 ± 4.6[a]	88 ± 1.3[a,b]	77 ± 3.1	49 ± 2.9[b]

The amount of use-dependent blockade is calculated by 20th pulse as a percentage of the 1st pulse ×100%. Values are presented as mean ± S.E.M.; n are presented in the table.

[a] $P < 0.05$ versus other two compounds' effects at same pH (ANOVA and Tukey's test).

[b] $P < 0.05$ versus compound's effect at normal pH.

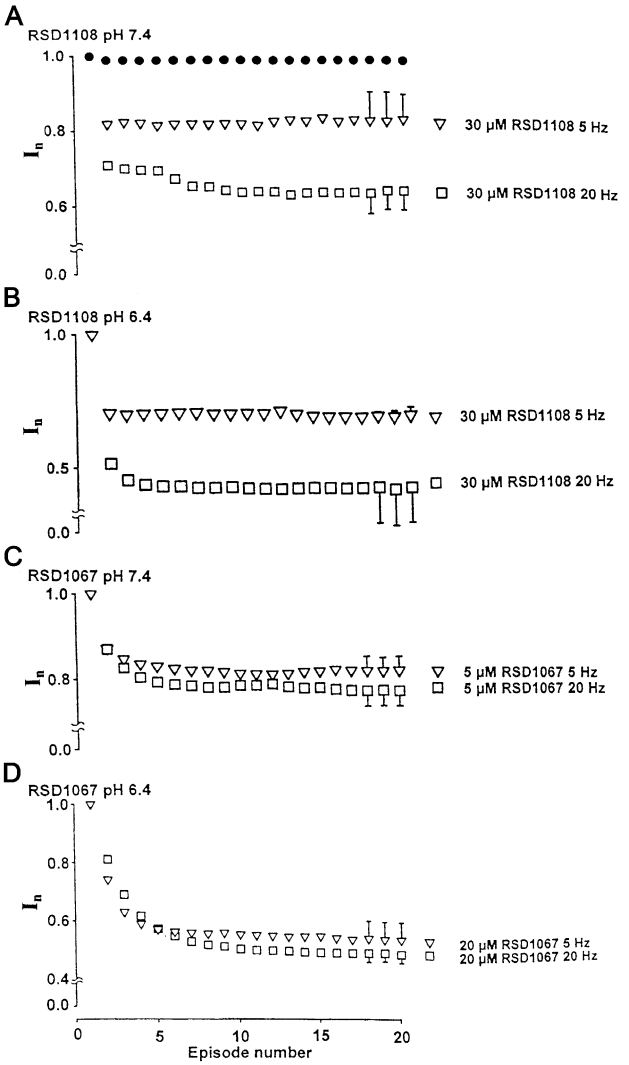

Fig. 8. Frequency-dependent inhibition of I_{Na} with RSD compounds. (A) Use-dependent inhibition in control (closed circles) and in the presence of 30 μM RSD1108 at 5 (open triangles), 20 Hz (open squares) stimulation frequencies ($n = 4$). (B) Use-dependent inhibition in the presence of 30 μM RSD1108 at 5 (open triangles) and 20 Hz (open squares) at pH 6.4 ($n = 4$). (C) Use-dependent inhibition in the presence of 5 μM RSD1067 at 5 (open triangles) and 20 Hz (open squares) at pH 7.4 ($n = 3$). (D) Use-dependent inhibition in the presence of a higher concentration of RSD1067 (20 μM) ($n = 4$) at 5 (open triangles) and 20 Hz (open squares) at pH 6.4. Data at each frequency were normalized to the I_{Na} in the first pulse of the 20-pulse protocol (I_n). Data are presented as means and the typical S.E.M are presented for the 18th–20th pulse.

3.8. Other state-dependent interactions with I_{Na}

The preceding section shows the RSD compounds to dose-dependently decrease I_{Na} and to exhibit a degree of use-dependent blockade. The possible actions of RSD compounds on the voltage dependence of Na^+ activation and inactivation was studied at the single pH of 7.4. The effect of the three compounds on the current–voltage relationship of I_{Na} was first studied. The protocol consisted of a hyperpolarizing step to −130 mV for 50 ms

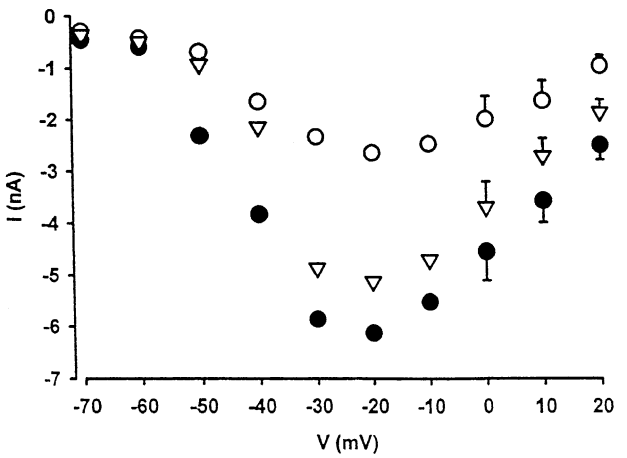

Fig. 9. Effect of RSD1067 on the current–voltage relationship of I_{Na}. I–V relation in control (closed circles), in the presence of 5 μM RSD1067 (open circles) and after wash-off (open triangles) ($n = 4$). RSD1067 did not affect the voltage dependence of I_{Na} activation. Typical mean ± S.E.M. are presented at more positive potentials.

from a holding potential of −70 mV followed by a series of depolarizing potentials (+10 mV steps) from −70 to +20 mV. Typical I–V plots were then constructed. The voltage corresponding to peak Na^+ current was near −20 mV for control, in the presence of 5 μM RSD1067 and after wash-off ($n = 4$) (Fig. 9). On the other hand, 5 μM RSD1070 caused a 7 mV hyperpolarizing shift in peak Na^+ current from that in control ($n = 3$) (data not shown). The shift was significant ($P < 0.05$). Similarly, 50 μM RSD1108 caused a significant ($P < 0.05$) 12 mV hyperpolarizing shift from that in control ($n = 3$) (data not shown).

The interaction of the three compounds with the inactivated state of the Na^+ channel was also investigated at pH

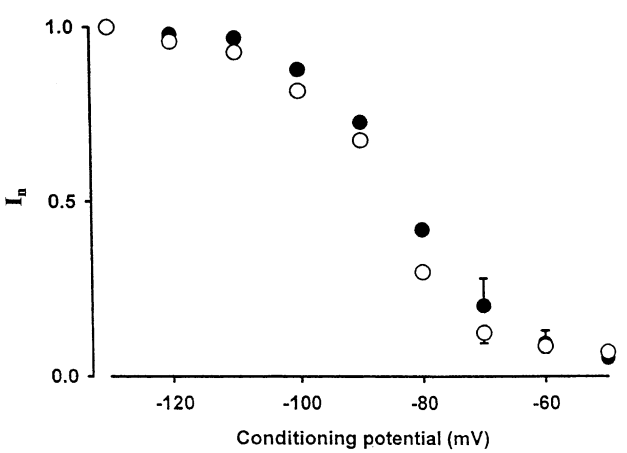

Fig. 10. Effect of RSD1067 on the voltage dependence of I_{Na} inactivation. Normalized current of I_{Na} (I_n) were plotted versus conditioning potential. Control data are presented as closed circles and the effect of 5 μM RSD1067 as open circles ($n = 4$). No alteration of the voltage dependence of inactivation of I_{Na} was observed in the presence of RSD1067. Typical mean ± S.E.M. are presented at more positive potentials.

7.4. The protocol consisted of 600-ms prepulses from −150 to −50 mV from a holding potential of −70 mV followed by a test potential to −20 mV for 20 ms. A typical plot of the effects of 5 μM RSD1067 on the inactivated state of the Na^+ channel is shown in Fig. 10. There was no significant shift in the inactivation curve ($P > 0.05$) ($n = 4$). As well, there was no significant effect of 5 μM RSD1070 ($n = 4$) and 50 μM RSD1108 ($n = 3$) to shift the voltage dependence of inactivation (data not shown).

4. Discussion

The novel availability of a series of chemically related compounds has been used to study the pH-dependent effects of structure and pK_a on properties of I_{to} and I_{Na} in rat ventricular myocytes. The results of this work demonstrate that all three (±)-*trans*-napthylethoxycyclohexylamines, RSD1108, RSD1070 and RSD1067 exhibit dose-dependent blockade of I_{Na} and the main cardiac repolarizing K^+ current in rat ventricle, I_{to}. RSD1000, a napthylalkylcyclohexyl derivative, demonstrated efficacious antiarrhythmic properties both in vivo and in vitro (Yong et al., 1999), and the three RSD compounds studied presently also exhibit selective antiarrhythmic activity in isolated rat heart and rat models of ischaemia at concentrations close to those described in this study (unpublished data). Overall, the EC_{50} values (Tables 1 and 3) established that RSD1067 and RSD1070 are mixed blockers of both currents, whereas RSD1108 exhibits primary actions on I_{to} with a considerably lower potency for actions on I_{Na}.

Although RSD1067 and RSD1070 were potent blockers of I_{to} at pH 7.4, neither agent demonstrated ischaemic selectivity since their potencies were decreased at acid pH. On the other hand, RSD1108, although not as potent I_{to} blocker at pH 7.4 as RSD1070 and RSD1067, demonstrated an enhanced channel blockade at acid pH. This result would suggest that RSD1108 possesses selectivity for block of I_{to} in ischaemic tissue. The differences in pK_a values between RSD1108 (pK_a 6.8) and RSD1070 or RSD1067 (pK_a values 8.0) may underlie the increased potency of RSD1108 at acid pH.

The dose-dependent increases in the rates of decay of I_{to} exhibited by all compounds (Fig. 2) indicate primary actions on the open state of I_{to}. This conclusion is further supported by the observation that the blocking effects of the agents increased with time following the initial 20 ms after channel activation as illustrated for RSD1108 in Fig. 4B. None of the three compounds altered the voltage dependence of activation, steady-state inactivation or recovery from inactivation of I_{to}. An open channel model of blockade was used to estimate onward (k_1) and off-rate (k_{-1}) constants. The data demonstrated diminished values of k_1 for RSD1067 and increased values of k_1 for RSD1108 and RSD1070 at acid pH; however, none of these changes were significant. These results are consistent with diffusion limited onward (blocking) rate constants with little or no dependence on pH. The relatively high values of k_1 (see Table 2) could underlie the diminished amplitudes of I_{to} at higher concentrations of the RSD compounds. For example, taking a k_1 of 10×10^6 M^{-1} s^{-1} and a concentration of 20 μM yields an effective blocking rate constant of 200 s^{-1}. Inverting this value gives a time of 5 ms, which could be considered an estimate for equilibration of an RSD compound to block the open channel. However, the time for peak I_{to} to be reached is about 3 ms (Jahnel et al., 1994) so a portion of the channels would be expected to be blocked during activation. This process would then lead to a diminished amplitude of current with higher concentrations of the RSD compounds, as observed.

An interesting result from the channel block analysis was the significant decrease in the channel unblocking (off) rate constant (k_{-1}) for RSD1108 at acid pH. Neither RSD1067 nor RSD1070 showed any significant pH dependence for this rate constant. The lower k_{-1} associated with RSD1108 blockade at acid pH is likely the basis for the enhanced potency of the compound at pH 6.4. In essence, a pH-mediated decrease of this rate constant prolongs RSD1108 interactions at the blocking site of action. This effect presumably reflects the lower pK_a value of RSD1108 compared with the pK_a values of the other two compounds. As shown in Table 2, the k_d (k_{-1}/k_1) associated with RSD block is decreased by a factor of 4 at pH 6.4 compared with pH 7.4. Interestingly, although RSD1067 and RSD1070 have the same pK_a, their k_d values are differentially modulated by pH suggesting the involvement of particular aspects of chemical structure, specifically the difference in naphthyl groups between the two compounds, in block of I_{to}. The magnitude of k_d for RSD1108 is similar to estimated k_d values for other potent blockers of I_{to} such as tedisamil (Dukes et al., 1990), the tedisamil analogue KC8851 (McLarnon and Xu, 1997) and octylcaine (Castle, 1990). However, an important result in the present work is the enhanced blocking actions of RSD1108 at acid pH. This finding would suggest the antiarrhythmic utility of the compound in prolonging the durations of action potentials shortened during ischaemia-induced arrhythmic activity. Although I_{to} is not the main contributor to repolarization in human ventricle, the presence of this current has been documented in these cells (Shibata et al., 1989; Wettwer et al., 1993; Escande et al., 1987) which would permit extrapolation of these results to humans.

All three RSD compounds inhibited I_{Na} in a dose-dependent manner with RSD1108 and RSD1070 demonstrating enhanced potency at pH 6.4 (Table 3). These results suggest that RSD1108 and RSD1070 would show selectivity for actions on I_{Na} in ischaemic tissue. The considerably higher EC_{50} values at neutral and acid pH for actions of

RSD1108, relative to the values for the other two compounds, could reflect a dependence of I_{Na} block on pK_a. Thus, a higher pK_a value is associated with enhanced I_{Na} block under ischemic conditions. Both RSD1067 and RSD1070, which possess a high degree of commonality in chemical structure with the same pK_a values, showed similar trends in potency with pH (Table 3). The difference in napthyl groups between the two compounds could underlie the significantly greater potency of RSD1070, relative to RSD1067, for blocking actions at acid pH.

All compounds inhibited I_{Na} in a use-dependent manner with stimulation frequencies of 5 and 20 Hz (Table 4). Although RSD1070 exhibited the largest use dependence at both activating frequencies, the use-dependent inhibition was not dependent on pH. RSD1108 was unique in that with acid pH, use-dependent inhibition of I_{Na} was increased from that measured at pH 7.4. Another novel property of RSD1108, not exhibited by the other two RSD compounds, was a significant increase in use-dependent block with the stimulating frequency raised to 20 Hz, which could reflect the lower pK_a of RSD1108. This result is interesting in that RSD1000, with a pK_a of 6.1, showed a pH-dependent enhancement of use- and frequency-dependent block of I_{Na} (Yong et al., 1999). The marked use and frequency dependence for RSD1108 inhibition of I_{Na} at acid pH would suggest the applicability of this compound in ischaemic tissue.

The importance of pK_a in developing ischaemic-selective antiarrhythmics is emphasized in this study and was particularly evident with RSD1108 (pK_a 6.8), which showed increased potency for inhibition of both I_{to} and I_{Na} relative to RSD1070 and RSD1067 at acid pH. In addition, RSD1108 exhibited enhanced use- and frequency-dependent blockade of I_{Na} at acid pH, which may be attributable to its lower pK_a compared to RSD1070 and RSD1067. As well, the contribution of structure in selectivity for both I_{to} and I_{Na} blockade was demonstrated by differences in blocking actions of RSD1070 and RSD1067 since the compounds had similar pK_a values but differed in napthyl groups. The results from this study together with previous work on RSD1000 (Yong et al., 1999) would suggest that pK_a values which approximate the pH of ischaemic myocardium are optimally suited as a criteria in the development of selective antiarrhythmics for ischaemic myocardium.

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