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SCIENTIFIC CORRESPONDENCE

Reply

Dr Sheridan makes a number of important points concerning the complications of trying to compare the affinities of RSD 921 for the different states of the sodium channel. The most straightforward way to address these points is by examining the affinity of the drug for each state of the channel, as Dr Sheridan has done so carefully in his letter.

(1) *Affinity of RSD 921 for the resting state*. This parameter is presented in the form of concentration-response curves (Figure 1) and in tabular form (Table 1) by Pugsley & Goldin (1999). These values were determined by measuring current amplitudes during an infrequent pulsing protocol, which provides an estimate of RSD 921 affinity for the resting state of the channel. These values are more reliable than the estimates determined by Dr Sheridan from Figure 5 in Pugsley & Goldin (1999), in that they were determined from the complete range of concentrations in the curves presented in Figure 1, rather than being estimated from block by a single concentration of RSD 921. The values for resting state block are presented under the column labelled K_R in Table 1 of this letter.

(2) *Affinity of RSD 921 for the inactivated state*. Dr Sheridan correctly points out that the affinity for the inactivated state can be determined by shifts in the steady-state inactivation curves only if certain assumptions are valid. The first assumption is that the test depolarizations must be of sufficient length to allow for equilibrium binding. The data that were presented by Pugsley & Goldin (1999) were obtained using depolarization intervals of 500 ms. We presented those data because we did not observe any significant shifts with longer depolarizations. The values for $V_{½}$ and slope factor obtained using 5 s depolarizations are shown in Table 2 of this letter. As can be seen, there are still no significant shifts in the $V_{½}$ for inactivation for any of the channels. The fact that the results were comparable for depolarization intervals of 500 ms and 5 s suggests that these intervals are sufficient for equilibrium binding. The second assumption is that the drug does not change the slope factor of the curve. Although there was a slight decrease in the slope factor for the heart channel using the 500 ms depolarization (see Table 4 in Pugsley & Goldin, 1999), no significant changes in slope factor were observed using the 5 s depolarization for any of the channels (Table 2 of this letter). Since these two assumptions are valid, then the absence of shifts in the inactivation curves implies that the affinities for the inactivated state are equivalent to the affinities for the resting state, as pointed out by Dr Sheridan. The values in Table 1 under the heading K_I represent these calculated values for inactivated state affinities.

(3) *Affinity of RSD 921 during use-dependence*. Although the affinities for the resting and inactivated states must be comparable, we observed a significant amount of use-dependent block in the presence of RSD 921, as shown in Figure 4 in Pugsley & Goldin (1999). The use-dependence was most pronounced for the heart channel and the IFMQ3 mutant channel. The data in that figure were normalized to the current during the first depolarization in the presence of drug to emphasize the use-dependent (phasic) block by RSD 921. The fractional block resulting from both tonic and phasic components was not shown in that figure, but the approximate values are 0.82 for heart, 0.78 for skeletal muscle, 0.74 for neuronal and 0.82 for IFMQ3 channels. These values can be used to calculate affinities during the final depolarizations of the use-dependent protocols (K_{UD}), according to the logistic equation that was presented by Dr Sheridan for K_R:

$$K_{UD}[(1/a) - 1] * [\text{RSD } 921],$$

in which a is the fractional tonic and phasic block of sodium current. These calculated values are shown under the heading K_{UD} in Table 1 of this letter.

(4) *Affinity of RSD 921 for the open state*. There is no direct way to calculate the affinity of RSD 921 for the open state of the wild-type channels, because inactivation occurs too rapidly for equilibrium to be reached. However, as described in Pugsley & Goldin (1999), this affinity can be calculated for the IFMQ3 channel, which lacks inactivation. This value is shown in Table 1 of this letter.

Table 1 Affinity values for RSD 921 with different states of the sodium channel

	K_R (μM)	K_I (μM)	K_{UD} (μM)	K_O (μM)
Heart	47	47	22	ND
Skeletal muscle	35	35	29	ND
Neuronal	37	37	35	ND
IFMQ3	110	ND	23	117

From the affinity values presented in Table 1, it can be seen that there is a higher affinity of RSD 921 for the heart and skeletal muscle channels during repetitive depolarizations. This increase in affinity cannot be explained by an increased affinity for the inactivated state because of the lack of shifts in the $V_{½}$ of inactivation. We therefore believe that this increased affinity is due to the channel being in the open state. It should be pointed out that these results do not demonstrate that RSD

Table 2 Effect of RSD 921 on the voltage-dependence of inactivation with 5 s prepulses

	Heart		*Skeletal muscle*		*Neuronal*	
	k	$V_{½}$	k	$V_{½}$	k	$V_{½}$
Control	5.6±1.2	−76±2.0	4.8±1.0	−53±1.0	5.1±0.8	−54±3.0
100 μM	5.5±1.0	−79±1.3	4.5±1.1	−54±1.9	5.8±1.0	−57±1.7

921 directly binds to the open state with greater affinity. For example, an alternative explanation is that the increased affinity results from an increased concentration of RSD 921 inside the cell because the drug passes through the open channel. Since RSD 921 is a basic drug with a tertiary nitrogen moiety with a p*K*a of 9, the drug would be present almost exclusively in the charged form at physiological pH

The results with the neuronal channel indicate that there are comparable affinities for all three states of this isoform, consistent with the fact that very little use-dependent block of the neuronal isoform was observed. The results with the IFMQ3 mutant channel are more difficult to interpret. The affinities for the resting and open states appear to be comparable, although the mutation decreases the affinity for the resting state approximately 3 fold compared to the wild-type channel. However, a significant extent of use-dependent block of the IFMQ3 channel was observed, despite the fact that this channel does not demonstrate any significant extent of fast inactivation. One possible explanation for this result is that RSD 921 binds with higher affinity to an intermediate state of the channel, which would be present during repeated depolarizations but not during one sustained depolarization.

In summary, we believe that the data indicate that RSD 921 has comparable affinities for the resting and inactivated states of all three isoforms of the sodium channel, and that there is a higher affinity block of the heart isoform and to a lesser extent of the skeletal muscle isoform when the channels open.

Reference

PUGSLEY, M.K. & GOLDIN, A.L. (1999). Molecular analysis of the Na^+ channel blocking actions of the novel class I anti-arrhythmic agent RSD 921. *Br. J. Pharmacol.,* **127,** 9–18.

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