 


## SCIENTIFIC CORRESPONDENCE

# Quantification of state-dependent drug interactions with the sodium channel

In their recent paper, Pugsley & Goldin (1999) characterized the interaction of the class I antiarrhythmic and local anaesthetic agent, RSD 921, with rat $Na^+$ channels derived from heart (rH1), skeletal muscle (rSkM1) and brain (rBIIA). Additionally, they describe the actions of RSD 921 on an rBIIA mutant channel lacking fast inactivation (the IFMQ3 mutation). The authors concluded that RSD 921 preferentially interacted with the open state of the $Na^+$ channel (affinity estimated to be 117 $\mu$M). However, an alternative interpretation of their data is that RSD 921 has its greatest affinity for the inactivated and resting states of the $Na^+$ channel. The reasoning is as follows.

*Affinity of RSD 921 for the resting state*. This parameter is readily derived from the steady-state inactivation data presented in Figure 5 of the paper. At very negative holding potentials most channels reside in their resting conformation, with the result that $Na^+$ currents generated by a depolarizing step (say, to 0 mV) from these potentials are at their maximum amplitude. As the holding potential is made less negative so an increasing proportion of channels move into their inactivated conformation until, at holding potentials more positive than about $-20$ mV, no current is generated in response to a step depolarization. Therefore, the effect of a blocker of resting $Na^+$ channels is to depress the current developed at the most negative end of the *non-normalized* steady-state inactivation curve. Assuming a 1 : 1 stoichiometry of block of resting channels by RSD 921, then the affinity of the drug for resting channels ($K_R$) may be derived from the following rearrangement of the logistic equation:

$$K_R = \{(1/a) - 1\} \times [\text{RSD 921}]$$

where $a$ is the normalized inhibition of the $Na^+$ current at the most negative end of the steady-state inactivation curve. From Figure 5 of Pugsley & Goldin (1999), the values of $a$ in the presence of 100 $\mu$M RSD 921 are about 0.52, 0.58 and 0.64 for rH1, rSkM1 and rBIIA, respectively, resulting in corresponding $K_R$ estimates of 92 $\mu$M (rH1), 72.4 $\mu$M (rSkM1) and 56.2 $\mu$M (rBIIA).

*Affinity of RSD 921 for the inactivated state*. The method for deriving affinity for the inactivated state ($K_I$) is based on shifts induced in the steady-state inactivation function by $Na^+$ channel blocking compounds (Bean *et al*., 1983):

$$exp(\Delta V_{half}/k_2) = \{1 + ([\text{RSD 921}]/K_R)\}/\{1 + ([\text{RSD 921}]/K_I)\}$$

where $\Delta V_{half}$ is the shift in the midpoint of the inactivation curve induced by RSD 921 and $k$ is the slope factor of the curve. The above relation predicts that pure resting state blockers will induce concentration-related *rightward* (depolarizing) shifts of the steady-state inactivation curve while pure inactivated state blockers will induce *leftward* (hyperpolarizing) shifts. Compounds blocking both resting and inactivated channels will induce shifts commensurate with their applied concentrations and their relative affinities for these two states. In the exceptional case that a compound has equal affinities for both resting and inactivated channels, no shift in the inactivation curve will arise (irrespective of concentration). Although Pugsley & Goldin failed to detect statistically significant hyperpolarizing shifts of the inactivation curves in the presence of 100 $\mu$M RSD 921 (Figure 5 and Table 4), this presumably reflects the interaction of the compound with the resting state (inducing an opposing depolarizing shift). The lack of observed shift in the presence of RSD 921 implies that the affinity of the compound for the inactivated state is equal to its affinity for the resting state (that is, $K_I = K_R$ for each of the channel subtypes).

Therefore, the affinity profile of RSD 921 for rH1, rSkM1 and rBIIA $Na^+$ channels is: inactivated = resting > open.

Finally, it is well established that in the experimental construction of steady-state inactivation curves it is vital to demonstrate that the test depolarizations from each holding potential are of sufficient length to allow for equilibrium binding of drug to the inactivated state of the $Na^+$ channel. In the case of phenytoin (Kuo & Bean, 1994) test depolarizations of 16 s duration are required for equilibrium, while lifarizine (McGivern *et al*., 1995) requires up to 60 s. Pugsley & Goldin (1999) used test depolarizations of 0.5 s duration and presented no data to show that equilibrium conditions had been achieved. It is therefore conceivable that the hyperpolarizing shifts of their steady-state inactivation curves are incompletely expressed and that the $K_I$s of RSD 921 estimated above are actually underestimates of the true $K_I$s.

### References

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