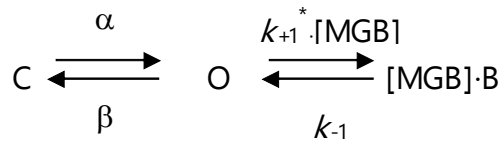


Supplementary Information

Kinetic evaluation for MGB-induced block of $I_{Na(T)}$ evoked by short step depolarization in pituitary GH₃ cells

During cell exposure to MGB, apart from the reduction in peak I_{Na} (i.e., $I_{Na(T)}$), the inactivation rate of the current elicited by brief depolarizing pulse from -100 to -10 mV was noticed to become elevated. Hence, we extended our investigations to evaluate the inactivation kinetics of MGB-mediated suppression of $I_{Na(T)}$ in situations where GH₃ cells were exposed to different MGB concentrations. The concentration dependence of I_{Na} inactivation rate (i.e., $1/\tau_{inact(S)}$) caused by adding this drug was constructed and is hence illustrated in **Figure 2B**. The observations indicated that its effects on $I_{Na(T)}$ resulted in a concentration-dependent increase in the slow component in rate of current inactivation, since the inactivation trajectories of the current were well fitted at various MGB concentrations to two-exponential process. As a result, the inhibitory effect on $I_{Na(T)}$ inherently in GH₃ cells can be reasonably explained by a state-dependent blocking mechanism through which the MGB molecule can predominantly bind to the open or open-inactivated state residing in the Nav channels. The first-order binding scheme for this perspective is thus simplified as follow.



where α and β are called the rate constants for the opening and closing of the Nav channel, respectively; k_{+1}^* and k_{-1} are those for blocking (i.e., depending on the MGB concentration) or unblocking by adding MGB; $[MGB]$ is the MGB concentration used. C, O, and $[MGB] \cdot B$ indicate the closed (or resting), open, and blocked states of the Nav channel, respectively.

The blocking (forward, k_{+1}^*) and unblocking (backward, k_{-1}) rate constants were estimated based on the slow component of the inactivation time constant ($\tau_{inact(S)}$) of $I_{Na(T)}$ during cell exposure to different MGB concentrations (**Figure 2B**). The relationship can be satisfied by

$$1/\tau_{inact(S)} = k_{+1}^* \times [MGB] + k_{-1}$$

where k_{+1}^* and k_{-1} were respectively derived from the slope (i.e., $\Delta(1/\tau_{inact(S)})/\Delta([MGB])$) and from the y-axis intercept at $[MGB]=0$ (i.e., the point at which the line crosses the y-axis) of the linear regression at which the reciprocal time constants of the current (i.e., $1/\tau_{inact(S)}$) versus the MGB concentration was interpolated. The blocking and unblocking rate constants were therefore estimated to yield $0.124 \text{ msec}^{-1} \mu\text{M}^{-1}$ and 0.102 msec^{-1} , respectively. According to the evolving rate constants, dividing k_{-1} by k_{+1}^* gave a dissociation constant (K_D) of $8.2 \mu\text{M}$ during exposure to MGB, a value that was noticed to be close to effective IC_{50} ($7.3 \mu\text{M}$) of the drug needed for inhibition of late I_{Na} (**Figure 1B**).