Supporting Information

Mechanism of Inhibition of the GluA2 AMPA Receptor Channel Opening by Talampanel and its Enantiomer: The stereochemistry of the 4-Methyl Group on the Diazepine Ring of 2,3-Benzodiazepine Derivatives

Congzhou Wang and Li Niu*

Department of Chemistry and Center for Neuroscience Research, University at Albany, Albany, NY 12222, USA

1. Equations

The equations used to calculate the inhibition constants for BDZ-*d* or BDZ-*e* are described below. It should be noted that all of the equations are generally labeled by English alphabet; however, we specifically single out those that are used directly for calculating inhibition constants by labeling them with numerical numbers here and in the text. First, we present the equations derived from a channel-opening mechanism in the absence of any inhibitor.

(i) A General Mechanism of Channel Opening for AMPA Receptors and Equations. A general mechanism of channel opening for an AMPA receptor is shown below.

$$A + L \underset{}{\overset{K_1}{\rightleftharpoons}} AL_n \overset{\Phi}{\rightleftharpoons} \underset{\text{(open)}}{\overline{AL_n}}$$

A represents the active, unliganded form of the receptor, AL_n are the closed-channel form of the receptor, and $(\overline{AL_n})$ represents the open-channel form. K_1 is the intrinsic dissociation constant of glutamate; Φ, the $AL / \overline{AL_n}$; I_A, the current amplitude corresponding to a certain concentration of ligand or in this case, glutamate; I_m, the current per mole of receptor sites; and R_m, the moles of receptor sites.

$$I_A = I_M R_M \frac{L^n}{L^n + \Phi(L + K_1)^n} = I_M R_M \left(\overline{AL}_n\right)_o \qquad \text{eq a}$$

The number of glutamate molecules to bind to the receptor and to open its channel, n, can be from 1 to 4, assuming that a receptor is a tetrameric complex and each subunit has one glutamate binding site. However, our study of the channel-opening process of AMPA receptors supports the assumption that a minimum of binding of two glutamate molecules per receptor complex is sufficient to open an AMPA receptor channel.[1-5] For simplicity and without contrary evidence, it is further assumed that glutamate binds with equal affinity or K_1 at all binding steps.

Using $n = 2$, we can then define that $\left(\overline{AL_n}\right)_0$ or $\left(\overline{AL_2}\right)_0$ represents the fraction of the open-channel form, and is proportional to the current amplitude, as shown in eq a. Furthermore, $\left(\overline{AL_2}\right)_0$ can be further expressed as a function of the fraction of all receptor forms.

$$(\overline{AL}_2)_O = \frac{\overline{AL}_2}{A + AL + AL_2 + AL_2} = \frac{L^2}{L^2(1+\Phi) + 2K_1 L\Phi + K_1^2\Phi} \qquad \text{eq b}$$

The channel-opening kinetic process, observed in a laser-pulse photolysis measurement, followed a single exponential rate expression, in eq c, for ~95% of the rise time, as shown in Figure 4a in the text.

$$I_t = I_{\max}(1 - e^{-k_{obs}t}) \qquad \text{eq c}$$

In eq c, I_t represents the current amplitude at time t and I_{max} the maximum current amplitude. From eq c, an observed rate constant, k_{obs}, can be calculated. By using the scheme for channel opening described above, we can express k_{obs} in eq d.

$$k_{obs} = k_{cl} + k_{op}\left(\frac{L}{L + K_1}\right)^2 \qquad \text{eq d}$$

In deriving eq d, it is assumed that the ligand-binding rate is fast relative to the channel-opening rate and $n = 2$.[1-5] Here k_{op} and k_{cl} are the rate constant of channel opening and closing, and $k_{cl}/k_{op} = \Phi$ as in the general mechanism of channel opening.

(ii) Use of whole-cell current amplitude to determine an inhibition constant. The mechanism of inhibition of AMPA receptors or precisely the GluA2Q$_{flip}$ receptor channel by BDZ-*d* as in this study is shown in Figure 5 in the text.

From this mechanism of inhibition, we can derive eq 1, shown below, and use it to determine an inhibition constant from the effect of BDZ-*d* on the amplitude of whole-cell current:

$$\frac{A}{A_I} = 1 + I\frac{(\overline{AL_2})_O}{K_I} \qquad \text{eq 1}$$

A and A_I are the whole-cell current amplitude in the absence and presence of an inhibitor; I represents inhibitor. $\left(\overline{AL_2}\right)_0$ is expressed in eq b above. More importantly, the magnitude of $\left(\overline{AL_2}\right)_0$ depends on glutamate concentration. As explained in the text, for GluA2Q$_{flip}$, we chose 100 μM and 3 mM, which correspond to ~4% and ~95% of the fraction of the open-channel form in a receptor population, respectively.[2] Consequently, the inhibition constant of BDZ-*d*, for example, for both the closed-channel and the open-channel state were determined (in Figure 2).

(iii) Use of the Rate Constant of the Channel Opening to Determine an Inhibition Constant and to Characterize the Mechanism of Inhibition. When the channel-opening rate was inhibited noncompetitively (as in Figure 5), the expression for the observed first-order rate constant or k_{obs} was given by eq e, where only one rate was observable (this rate is assigned to the first step, corresponding to the formation of the initial inhibitor-receptor intermediate). As such, the effect of an inhibitor on the channel-closing rate constant, k_{cl}, was determined by using eq 2, where the inhibition constant associated with the open-channel state ($\overline{K_I}$) could be further estimated (at low ligand concentration: see text for further explanation). At a higher ligand concentration, the difference between k_{obs} and k_{cl}' was determined, giving rise to the effect of an inhibitor on k_{op}, as shown in eq 3.

$$k_{obs} = k_{cl}\left(\frac{\bar{K}_I}{\bar{K}_I + I}\right) + k_{op}\left(\frac{L}{L + K_1}\right)^2\left(\frac{K_I}{K_I + I}\right) \qquad \text{eq e}$$

$$\frac{1}{k_{obs}} = \frac{1}{k_{cl}} + \frac{1}{k_{cl}} \frac{I}{\bar{K}_I} \qquad \text{eq 2}$$

$$(k_{obs} - k_{cl}^{'})^{-1} = [k_{op} L/(L + K_1)^2]^{-1}(1 + I/K_I) \qquad \text{eq 3}$$

Therefore, as a noncompetitive inhibitor, BDZ-*d* is expected to inhibit both k_{cl} *and* k_{op}. Furthermore, at the low and high glutamate concentrations, the inhibition constants pertinent to the closed-channel and open-channel states can be further measured (see Figures 4b and 4c) by the use of eqs 2 and 3. If BDZ-*d* is a competitive inhibitor, it only inhibits k_{op} but not k_{cl}. Conversely, if BDZ-*d* is an uncompetitive inhibitor or open-channel blocker, it will inhibit only k_{cl} nut not k_{op}.

(iv) Use of a Double-Inhibitor Experiment to Determine Whether the Two Inhibitors Bind to the Same Site or Two Different Sites. To determine whether BDZ-*d* and GYKI 52466 bound to the same site or two different sites (i.e., two mutually exclusive sites), we used the two inhibitors simultaneously to inhibit the GluA2Q$_{flip}$ channel activity. Specifically, the amplitude was used, similar to eq 1, to plot $A/A_{I,P}$ vs. one inhibitor concentration. Here, one inhibitor was represented as *I* in molar concentration while the other was *P.* Based on the assumption that one inhibitor bound per receptor and binding of inhibitor excluded the binding of the other (i.e., one-site model or A_I or A_P are allowed but not $A_{I,P}$), the ratio of the current amplitude was given in eq 4.

$$\text{One-Site Model: } \frac{A}{A_{I,P}} = (1 + \frac{P}{K_P}) + \frac{I}{K_I} \qquad \text{eq 4}$$

On the other hand, for a two-site model in which there are two sites for *I* and *P* separately (i.e., both A_I and A_P and $A_{I,P}$ are all allowed), the ratio of the current amplitude is therefore given in eq 5.

$$\text{Two-Site Model: } \frac{A}{A_{I,P}} = (1 + \frac{P}{K_P}) + (1 + \frac{P}{K_P})\frac{I}{K_I} \qquad \text{eq 5}$$

The data are shown in Figure 6. The dashed-line is the simulated inhibition ratios assuming the two inhibitors bound to two separate sites on GluA2Q$_{flip}$. To simplify the term, we continued to use A/A_I in Figure 6 to express the ratio of whole-cell current amplitude in the absence and presence, in this case, two inhibitors.

References

1. Li, G., and Niu, L. (2004) How fast does the GluR1Qflip channel open?, *J Biol Chem 279*, 3990-3997. Epub 2003 Nov 3910.
2. Li, G., Pei, W., and Niu, L. (2003) Channel-opening kinetics of GluR2Q(flip) AMPA receptor: a laser-pulse photolysis study, *Biochemistry 42*, 12358-12366.
3. Pei, W., Huang, Z., and Niu, L. (2007) GluR3 Flip and Flop: Differences in Channel-Opening Kinetics, *Biochemistry In press*.
4. Li, G., Sheng, Z., Huang, Z., and Niu, L. (2005) Kinetic mechanism of channel opening of the GluRDflip AMPA receptor, *Biochemistry 44*, 5835-5841.
5. Pei, W., Ritz, M., McCarthy, M., Huang, Z., and Niu, L. (2007) Receptor occupancy and channel-opening kinetics: a study of GLUR1 L497Y AMPA receptor, *J Biol Chem 282*, 22731-22736.

2. Supporting Information Figures

Figure 1.

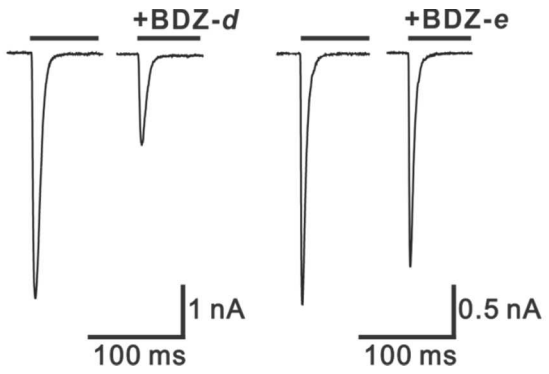

Figure 1. Representative whole-cell currents mediated by the open-channel state of $GluA2Q_{flop}$ receptors expressed in HEK-293S cells in the absence and presence of BDZ-*d* or BDZ-*e* obtained using solution flow measurement. The pair of the two traces on the left represent the currents in the absence (left) and presence (right) of 40 μM BDZ-*d*. The inhibition ratio (A/A_I) was determined to be ~3.3. The pair on the right side were the control or in the absence (left) and presence (right) of 40 μM BDZ-*e*. The inhibition ratio (A/A_I) was determined to be ~1.2. The bar above each current trace represents a pulse of 3 mM glutamate for evoking the whole-cell current. The whole-cell recording was carried out at -60 mV, pH 7.4, and 22 °C.

Figure 2.

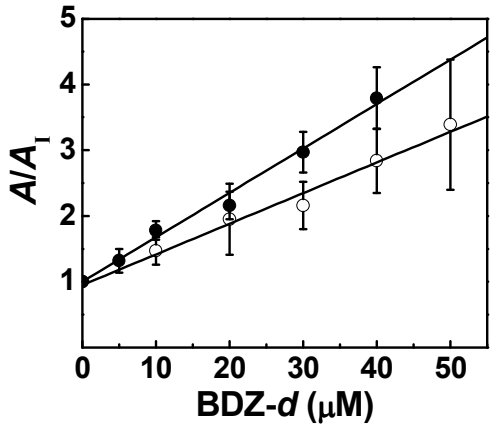

Figure 2. Effect of BDZ-*d* on the whole-cell current amplitude of $GluA2Q_{flop}$ receptors obtained from the flow measurement. A K_I of 15 ± 1 μM was determined the closed-channel state (100 μM glutamate, ●); a $\overline{K_I}$ of 22 ± 1 μM was obtained for the open-channel state (3 mM glutamate, ○).

Figure 3.

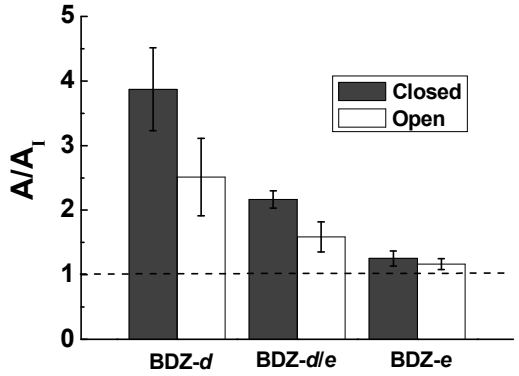

Figure 3. The inhibition ratios (A/A_I) of the mixture containing 20 μM BDZ-*d* and 20 μM BDZ-*e* were determined for both the closed-channel (100 μM glutamate, filled column) and open-channel (3 mM glutamate, open column) states of the homomeric $GluA2Q_{flip}$ receptor. These ratios are compared with those measured from 40 μM BDZ-*d* and 40 μM BDZ-*e*.