**Figure 1-source data 1**

**(Figure 1-source data 1, *Panel* *B)*. Monomeric status of the purified M2 receptor after chemical cross-linking.** The area under the densitometric trace from each lane of western blots such as those illustrated in Figure 1B was estimated in three segments corresponding roughly to monomers (*a*, 40–75 kDa), dimers (*b*, 75–170 kDa), and tetramers or larger oligomers (*c*, 170–360 kDa). Each value was expressed as a percentage of the total area for that lane, and the values from three different blots were averaged to obtain the means (± S.E.M.) listed in the table.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Intensity (*%*) | | |
| Cross-linking | *a* | *b* | *c* |
| None | 97 ± 1 | 2.6 ± 1.3 |  |
| BS3 | 83 ± 5 | 14 ± 7 | 3.5 ± 1.8 |

**(Figure 1-source data 1, *Panel* *C*). Parametric values for the effect of gallamine on the rate of dissociation of [3H]QNB.** Monomers of the M2 receptor were purified from *Sf*9 cells, and the rate constant for the dissociation of [3H]QNB was measured at 30 ºC in the absence of an allosteric ligand (*k*0) and at graded concentrations of gallamine (G) (*k*obsd). The dose-dependence of the ratio *k*obsd/*k*0 was analyzed in terms of Equation 2 (*n* = 1), and the parametric values are listed in the table. The data and the fitted curve are illustrated in Figure 1C. Also listed in the table are the parametric values from a previous study of the M2 receptor in *Sf*9 membranes (Equation 2, *n* = 2) (Shivnaraine et al., 2012); the corresponding fitted curve is shown by the dashed line in Figure 1C. The number of experiments is shown in parentheses.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| [3H]QNB |  |  |  |  | *k*obsd/*k*0 | | | |
| (nM)*a* | log *K*1 | log *K*2­ | *n*H(1) | *n*H(2) | *F*1 | *F*2 | [G] = 0 | [G]→∞ |
|  |  |  |  |  |  |  |  |  |
| *Purified receptor* | | |  |  |  |  |  |  |
| 1.0 (5) | −4.12 ± 0.13 | — | 1.28 ± 0.42*b* | — | 1.00 | *c* | 0.99 ± 0.01 | 0.11 ± 0.08 |
|  | | |  |  |  |  |  |  |
| *Receptor in membranes* | | |  |  |  |  |  |  |
| 0.20 (4) | −5.99 ± 0.31 | −4.35 ± 0.03 | 0.97 ± 0.30 | 0.80 ± 0.14 | −1.51 | 2.51 ± 0.82 | 1.02 ± 0.04 | 0.00 ± 0.00 |
|  |  |  |  |  |  |  |  |  |

*a* The mean concentration of the probe in the experiments represented in the analysis (S.E.M./*μ* < 0.047).

*b* The sum of squares is not significantly larger with *n*H fixed at 1 (*P* > 0.3).

*c* One class of sites is sufficient for Equation 2 to describe the data (*P* = 0.8).

**(Figure 1-source data 1, *Panel* *D*). Parametric values for the effect of gallamine on the binding of [3H]NMS at equilibrium.** Monomers of the M2 receptor were purified from *Sf*9 cells, and the binding of [3H]NMS was measured at graded concentrations of gallamine after equilibration of the reaction mixture for 21 h at 30 ºC. The data from 3 experiments were analyzed simultaneously in terms of Equation 2 (*n* = 1) to obtain the parametric values listed in the table. The data and the fitted curve are illustrated in Figure 1D. Also listed in the table are the parametric values from a previous study of the M2 receptor in detergent-solubilized extracts from *Sf*9 membranes (Equation 2, *n* = 3) (Shivnaraine et al., 2012); the corresponding fitted curve is shown by the dashed line in Figure 1D. The mean concentration of [3H]NMS in all such experiments with gallamine or strychnine was 10.1 ± 0.2 nM (*i.e.*, Figs. 1C, 1D, 1E-G, and 3F).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| log *K*1 | log *K*2­ | log *K*3­ | *n*H(1) | *n*H(2) | *n*H(3) | *F*1 | *F*2 | *F*3 |
|  | | |  |  |  |  |  |  |
| *Purified receptor* | | |  |  |  |  |  |  |
| −3.54 ± 0.03 | — | — | 1.00 ± 0.06 | — | — | 1.00 | *a* | *a* |
|  | | |  |  |  |  |  |  |
| *Receptor in unprocessed extracts* | | |  |  |  |  |  |  |
| −5.69 ± 0.26 | −4.59 ± 0.11 | −3.36 ± 0.21 | 1.06 ± 0.21 | 1.82 ± 0.49 | 1.25 ± 0.09 | 0.56 | −0.56 ± 0.18 | 1 |
|  |  |  |  |  |  |  |  |  |

*a* One class of sites is sufficient to describe the data.

**(Figure 1-source data 1, *Panels* *E–G*). Parametric values for the effect of strychnine on the binding of [3H]NMS to oligomers and monomers.** Strychnine and [3H]NMS were added either simultaneously or sequentially to preparations of the M2 receptor, as described in the legend to Figure S2. Binding of the radioligand at graded concentrations of strychnine was analyzed in terms of Equation 2 (*n* = 1 or 2) to obtain the parametric values listed in the table. Bell-shaped effects were described with two terms (*n* = 2), one for the ascending limb and one for the descending limb; strictly inhibitory effects were described with one term (*n* = 1). The number of experiments included in each analysis is shown in parentheses, and the data are shown in Figure 1E–G.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Order of mixing | log *K*1 | log *K*2­ | | | *n*H(1) | | *n*H(2) | | *F*1 | | | *F*2 |
|  |  |  | | |  | |  | |  | | |  |
| *Receptor extracted from porcine atria* | |  | | |  | |  | |  | | |  |
| Simultaneous (3) | −5.95 ± 0.10 | −3.70 ± 0.07 | | | 0.81 ± 0.06 | | 1.36 ± 0.39 | | −0.57 ± 0.09 | | | 1.57 |
| Strychnine, then NMS (3) | −6.03 ± 0.10 | −4.58 ± 0.08 | | | 0.70 ± 0.06 | | 1.21 ± 0.24 | | −1.00 | | | 2 |
| NMS, then strychnine (3) | −6.89 ± 0.09 | −4.34 ± 0.14 | | | 1.82 ± 0.89 | | 1*a* | | — | | | *—* |
|  | |  | | |  | |  | |  | | |  |
| *Receptor purified from Sf9 cells* | |  | | |  | |  | |  | | |  |
| Simultaneous (3) | −3.62 ± 0.03 | — | | | 1.23 ± 0.07 | | — | | 1.00 | | |  |
| Strychnine, then NMS (3) | −4.46 ± 0.09 | — | | | 0.62 ± 0.07 | | — | | 1.00 | | |  |
| NMS, then strychnine (3) | −3.32 ± 0.05 | — | | | *b* | | — | | 1.00 | | |  |
|  | | |  |  | |  | |  | |  |  | |

*a*The value is defined by a shallow minimum in the sum of squares and was fixed accordingly.

*b* Not defined.

**References**

Shivnaraine, R. V., Huang, X. P., Seidenberg, M., Ellis, J., and Wells, J. W. 2012. Heterotropic cooperativity within and between protomers of an oligomeric M2 muscarinic receptor. *Biochemistry* **51:** 4518-4540.