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Orphan receptor GPR158 serves as a metabotropic glycine receptor: mGlyR

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Abstract

Glycine is a major neurotransmitter involved in several fundamental neuronal processes. The identity of metabotropic receptor mediating slow neuromodulatory effects of glycine is unknown. We identified an orphan G protein Coupled Receptor (GPCR) GPR158 as a metabotropic glycine receptor - mGlyR. Glycine and related modulator taurine directly bind to a Cache domain of GPR158 and this event inhibits the activity of the intracellular signaling complex RGS7/Gβ5 associated with the receptor. Glycine signals *via* mGlyR to inhibit production of the second messenger cAMP. We further show that glycine but not taurine acts via mGlyR to regulate neuronal excitability in cortical neurons. Together, these results identify a major neuromodulatory system involved in mediating metabotropic effects of glycine with implications for understanding cognition and affective states.

Glycine is the simplest amino acid ubiquitously present in all mammalian tissues. Glycine serves as an inhibitory neurotransmitter but it can be excitatory in developing neurons(1,2). Glycinergic neurons are distributed across the brain, however glycine can also be released by glial cells(3). Known receptors for glycine belong to the family of pentameric ligand-gated ion channels(4). Glycine also serves as co-agonist of N-methyl-D-aspartate (NMDA) receptors(5). Metabotropic neuromodulatory effects of glycine have been observed(6,7) but

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Author contributions: T.L. and K.A.M. conceived the project, T.L. performed all functional experiments, S.Z. performed electrophysiology experiments, C.G., D.P., S.F. and M.H. performed and analyzed computational docking and structural modeling; B.A.W and R.N.R. performed and analyzed ITC experiments, T.L. and K.A.M. wrote the manuscript with input from all other authors; K.A.M supervised the project.

Competing interests: T.L and K.A.M. are listed as inventors on a patent application describing methods to study GPR158 activity, K.A.M. is a co-founder of Blueshield Therapeutics Inc, a startup company pursuing GPR158 as a drug target.

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no receptors mediating these actions have been found. Glycine has distinct effects on neural circuits(3) and glycinergic transmission has been implicated in pathological conditions including depression(8–10).

Metabotropic neuromodulation in the nervous system is mainly mediated by G Protein Coupled Receptors (GPCR). GPCRs play essential roles in neuronal physiology, pathology and present targets for the development of drugs(11). Canonically, GPCRs transduce their signals by activating heterotrimeric G proteins(12,13). However, G protein independent modes of signal transduction triggered by the recruitment of β-arrestins and other scaffolds to activated GPCRs have also been described(14–16). G protein signaling is controlled by Regulator of Protein Signaling (RGS) proteins which facilitate their deactivation(17). RGS proteins also interact with several GPCRs(18–22).

Except for glycine and taurine, GPCRs mediate the effects of all major neurotransmitters. However, many GPCRs still have no identified endogenous ligands. Orphan GPCRs may have potential for obtaining insights into physiology and for drug development(23,24).

GPR158 is one of the most abundant orphan GPCRs in the brain that transduces signals by coupling to RGS proteins(25,26). In neurons, it regulates signaling to second messenger cAMP and controls key ion channels, kinases and neurotrophic factors involved in neuronal excitability and synaptic transmission(25,27). Accordingly, GPR158 has been heavily implicated in cognition and affective states(25,28,29). Genetic suppression of GPR158 in mice results in prominent antidepressant phenotype and stress resiliency making GPR158 an attractive target for development of novel anti-depressants(25).

The endogenous ligand for GPR158 remains unknown. Recent structures of GPR158 revealed the presence of an extracellular Cache domain, a putative ligand-binding module(30,31).

RESULTS

Glycine signals via GPR158 to regulate cAMP

Structure of GPR158 revealed the presence of a Cache domain, which serves as a ubiquitous ligand binding module in bacterial chemoreceptors(30). We found that GPR158 Cache domain had a small pocket with similar organization of amino acid binding pocket in other Cache domains (Fig.1A). We hypothesized that GPR158 may have an amino acid ligand. We screened a library of amino acids for their ability to alter GPR158-mediated signaling. Because GPR158 has been linked to regulation of cAMP in the brain(25,27), we used a Bioluminescence Resonance Energy Transfer (BRET)-based cAMP biosensor(32) (Fig.1B). Out of all amino acids tested, only glycine showed significant decrease in cAMP when applied to HEK293 cells expressing GPR158 relative to non-transfected cells (Fig.1C).

To study this effect in more detail we analyzed the individual responses to glycine in a kinetic mode. We found that glycine application to U87 glioblastoma cells expressing GPR158, resulted in cAMP decrease. No glycine-induced changes in cAMP were observed in cells lacking GPR158 (Fig.1D,E). This inhibitory effect of GPR158 was further

potentiated by co-expressing RGS7/Gβ5, suggesting that GPR158 signals via this protein complex to affect cAMP levels (Fig.1D,E).

To further tested the effect of taurine, a compound closely related to glycine, which binds to several common receptors(33) including ionotropic glycine receptors(34). Taurine caused a significant decrease in cAMP levels only in HEK293 cells expressing GPR158 (Fig.S1A,B). Again, this effect was potentiated by co-expressing RGS7/Gβ5 suggesting that these proteins act in complex with GPR158 in mediating the effects of taurine. However, when compared directly, the effect of taurine on GPR158-mediated suppression of cAMP was weaker than the effect of glycine (Fig.S1C).

Glycine inhibits modulation of RGS7/Gβ5 by GPR158—To understand how glycine action on GPR158 regulates intracellular cAMP we focused on GPR158 interaction with RGS7/Gβ5, an established GTPase Activating Protein (GAP) for the Gαi/o proteins(35) known to regulate cAMP production(26). We used a cell-based assay to monitor GAP activity by following kinetics of G protein deactivation(36) (Fig.2A). In this assay, activation of G proteins by GPCR stimulation generates the BRET signal upon interaction of liberated Venus-Gβγ subunits with the masGRK3CT-Nluc reporter. This signal is quenched when Gα deactivation is triggered by GPCR antagonism and recombines with Venus-Gβγ to form inactive heterotrimer. As previously reported(22), we found that introduction of RGS7/Gβ5 accelerated deactivation of its substrate, Gαo (Fig.2B,D). Application of glycine had no effect on either baseline Gαo deactivation or RGS7/Gβ5-assisted process (Fig.2B,D). However, when GPR158 was co-expressed together with RGS7/Gβ5, glycine significantly decelerated Gαo deactivation (Fig.2C,D) suggesting that it specifically inhibited the GAP activity of RGS7/Gβ5 via engaging GPR158. Dose response studies showed that the IC_{50} of glycine on GPR158 is ~3 μM (Fig.2E). Taurine displayed similar inhibitory effect on Gαo deactivation only in cells co-expressing and GPR158 with RGS7/Gβ5, but lower IC_{50} of ~6μM (Fig.2B–E).

We further tested whether glycine or taurine could induce GPR158 to activate G proteins as canonical GPCRs do (Fig.S2A). We observed no significant activation of any G proteins tested with either glycine or with taurine (Fig.S2B–I). We also tested if glycine could induce β-arrestin recruitment to GPR158 using BRET assay which produced no significant response (Fig.S3).

GPR158 directly binds glycine—To confirm that GPR158 is indeed a direct target of glycine we used several strategies. First, we devised a flow cytometry-based assay to monitor binding to FITC-conjugated glycine to cells expressing GPR158 (Fig.3A). When HEK293 cells expressing GPR158 were incubated with FITC-glycine we observed labeling of a significant population of cells (Fig.3B). No such labeling was evident when FITC-glycine was incubated with cells not transfected with GPR158. Dose response studies further confirmed the binding and its selectivity across the ranges of glycine used (Fig.3C).

Next, we performed radioligand binding assays examining binding of [^{3}H]-labeled glycine to HEK293 cells expressing GPR158 (Fig.S4A). We detected significant binding of [^{3}H]-glycine to GPR158-expressing cells across concentrations (Fig.S4B). We isolated cellular

membranes and conducted classical radioligand titration experiments (Fig.3D). We detected saturable [^{3}H]-glycine binding to membranes containing GPR158 in substantial excess over linear non-specific binding to membranes devoid of GPR158 (Fig.3E). Scatchard analysis (Fig.3F) estimated K_d of GPR158 for glycine to be ~3μM. Binding competition experiments directly comparing the ability of glycine and taurine to displace [^{3}H]-glycine bound to GPR158 (Fig.S5) confirmed specificity of glycine and taurine binding to GPR158 and also revealed two-fold lower affinity of taurine relative to glycine (IC_{50}: ~3μM vs ~6μM).

Finally, we examined binding of non-labeled glycine directly to purified GPR158 using Isothermal Titration calorimetry (ITC) approach (Fig.3G). Titration experiments showed saturation of the heat released upon glycine addition to GPR158 yielding binding constants (K_D) ranging from 2μM to 16μM across experiments conducted first with a fresh sample (Fig.3H,I) and subsequently rerun following removal of glycine and detergent (Fig.S6). The affinity of glycine obtained in direct binding experiments is in a good agreement with the affinity measured in the functional GAP assays indicating that binding to glycine is responsible for changes in GPR158 activity.

Glycine binds to Cache domain of GPR158 and modulates GAP activity of RGS7-Gβ5 complex—We performed molecular docking experiments fitting glycine into a model of the putative ligand binding pocket in the Cache domain of GPR158, built by supplementing experimental structure(30) with a missing loop taken from the AphaFold2 prediction (Fig.4A;TableS1.). While another structure of GPR158 is available(31), it did not resolve side chain conformations and thus was not considered as a source of alternate receptor conformations for docking. For the best scored glycine pose, glycine could be well accommodated in a pocket where it is stabilized by a network of hydrogen bonding interactions with S172, R173, E271, and D307 sidechains, with the charged sidechains ideally positioned to stabilize the carboxylate and amine moieties of the zwitterion. These residues are embedded in a web of other hydrophilic residues located in a close vicinity (e.g. K264, S266, Y269, T284, and K305) lining the pocket (Fig.4B). Docking studies with taurine found a cluster of poses that overall matched the putative binding mode of glycine, retaining the features described for glycine (Fig.S7). The size of the pocket is spatially constrained, particularly by L282, in such a way that other amino acids cannot be easily accommodated without steric clashes with side chains of residues lining the pocket, providing possible explanation for the selectivity of the recognition (Fig.S8).

To test the role of the residues forming the putative glycine pocket in the GPR158 Cache domain, we performed site directed mutagenesis. In radioligand binding assays R173A, E271A, and Y269A mutants showed near complete loss of [^{3}H]-glycine binding confirming the essential role of these residues in ligand coordination (Fig.4B). We then tested each of the mutants in functional assays (Fig.4C,D). Each of the mutants defective in glycine binding also lost an ability to inhibit the GAP-activity of RGS7/Gβ5 (Fig.4E). The activity of S266A mutant which normally binds glycine was not regulated by it suggesting that some of the residues in the binding pocket are involved in conformational transitions triggered by ligand interaction(37). The mechanism by which mutating E271 residue resulted in loss of glycine responsiveness also deviated for that of other mutations. This mutant exhibited

a much slower deactivation kinetics in the absence of glycine, generating a constitutively inhibited receptor.

Glycine modulates neuronal excitability via GPR158—We finally assessed the impact of glycine modulation of GPR158 on neuronal activity. We examined the intrinsic properties of layer II-III neurons in the prelimbic cortex, where GPR158 is prominently expressed(25) and regulates neuronal excitability(27). The metabotropic effects of glycine are not well characterized across the nervous system. Therefore, we started by defining the effects of glycine on layer II-III neurons. To isolate metabotropic actions, we antagonized excitatory and inhibitory synaptic drive with pharmacological blockade and measured the I-V relationship in response to a depolarizing current ramp. Application of glycine significantly increased the number of action potentials while decreasing the amount of current necessary to elicit the first action potential (Fig.5A–C) without changes in the resting membrane potential (Fig.S9). This excitatory effect of glycine is distinct from its canonical inhibitory action mediated by GlyR ion channels. Interestingly, glycine application did not produce any changes on the intrinsic excitability of layer V neurons (Fig.S9) which do not express GPR158(27).

To confirm the involvement of GPR158 in the effects of glycine we studied *Gpr158* knockout (*Gpr158 KO*) mice. Glycine application failed to alter excitability of layer II-III neurons in prefrontal cortex of *Gpr158 KO* mice (Fig.5D–F). We also tested the effect of taurine on the excitability of layer II-III neurons (Fig.S11). These experiments revealed small effects on neuronal firing in the same direction as glycine. However, it did not reach the criteria for statistical significance, possibly due to lower efficacy of taurine.

DISCUSSION

In this study we demonstrate that GPR158 serves as a metabotropic receptor for glycine. We also report that GPR158 can be modulated by taurine which acts as a partial agonist for this receptor. This finding was enabled by recently obtained high-resolution structure of the receptor which revealed the presence of a ligand binding module - the Cache domain. Cache domains are well known receptors for amino acids and other related small molecules ubiquitously used by bacterial chemoreceptors. Only two GPCRs contain them including GPR158 and night-blindness associated receptor GPR179(38) whose ligand remains to be established. We present evidence that glycine acts as a *bona fide* ligand on GPR158 including direct binding and resultant change in receptor activity eliciting cellular response. This puts GPR158 in line with other class C GPCRs many of which are amino acid sensors such as the glutamate receptors, mGluR and receptors for GABA- GABAB. Thus, we propose a generic name for GPR158 to be metabotropic Glycine Receptor or mGlyR. We did not observe significant GPR158-mediated neuronal responses to taurine in cortical neurons, consistent with weaker effects of taurine on GPR158 relative to glycine. However, it remains possible that GPR158 may still mediate the effects of taurine in other neuronal populations or under certain conditions possibly making GPR158 a receptor for both glycine and taurine.

The mechanism by which mGlyR (GPR158) signals upon glycine or taurine binding deviates from canonical actions of GPCRs. Instead of activating G proteins, mGlyR recruits a RGS7/Gβ5 complex docking it into the intracellular pocket which canonical GPCRs use for interacting with G proteins and relaying changes in 7TM architecture upon ligand binding into conformational changes in Gα triggering nucleotide exchange. Thus, in the model we propose (Fig.5H) glycine binding to the Cache domain of mGlyR changes the conformation of the intracellular surface which in turn affects conformation of RGS7/Gβ5. This change reduces the ability of RGS7/Gβ5 to stimulate Gα GTPase, likely by disfavoring its orientation toward the membrane. In this sense, glycine serves as an antagonist of the GPR158-RGS7-Gβ5 complex by reducing its activity. Because RGS7/Gβ5 is a selective GAP for the inhibitory Gi/o proteins which regulate cAMP production(39,40), inhibition of RGS7/Gβ5 activity via GPR158 influences cAMP levels. The direction of the effect on the cAMP production is likely determined by the identity of the adenylyl cyclases present in a particular cell as they are known to be differentially regulated by Gαi and Gαo (via Gβγ) (41). Thus, glycine signals via mGlyR by inhibiting inhibitory G protein regulation thereby generating an excitatory influence. This regulation endows the metabotropic glycinergic system with a distinct feature that makes the degree of its influence scale with the extent of Gi/o activation by other GPCR cascades, with its influence increasing upon the increase in Gi/o inputs.

The discovery of mGlyR also opens many interesting avenues for exploring metabotropic influence of glycine and its role in the nervous system physiology. Indeed, metabotropic effects of glycine have been anecdotally noted(6,7,42) but molecular and circuit dissection of this influence have been limited. The relatively high affinity of mGlyR for glycine (~3μM) should allow it to signal without concomitant engagement of GlyRs which have an order of magnitude lower affinity for glycine creating an independent neuromodulatory channel(6). The mGlyR effects on neurons that we observe are also excitatory in contrast to largely inhibitory influence of ionotropic GlyR receptors(9,43). The two systems likely overlap and are involved in autotuning and homeostatic feedback as has been noted for other pairs of ionotropic and metabotropic system. Thus, in the context of intact neural circuitry, glycine likely triggers more complex responses which may involve interplay between ionotropic and metabotropic systems.

Finally, we think that glycinergic signaling *via* mGlyR has implications for understanding mood disorders and development of new pharmacological strategies. mGlyR is prominently expressed in the mPFC(25), a region critically involved in depression(44). Glycine and its transporters are also co-localized in the mPFC(45–47). Both taurine and glycine have been heavily implicated in pathophysiology of depression(8–10,48) and are dysregulated in plasma of humans diagnosed with major depressive disorder(10). Furthermore, taurine has an antidepressant effect on stress-induced depressive rats(49). Because these amino acids inhibit mGlyR and because knockout of mGlyR in mice also results in anti-depressive phenotype and stress resilience(25) it seems possible that anti-depressant properties of glycine and taurine may be mediated by mGlyR. The ubiquitous nature and multitude of the effects limit potential of glycine and taurine to be used as medications. However, identification of mGlyR presents a new target for the development of anti-depressants which

we postulate to be small molecules inhibiting this receptor with selectivity to avoid possibly related receptors, such as GPR179 in the eye.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data and material availability:

All data that support the findings are available at Zenodo (50). All plasmids generated during this study are freely available upon request.

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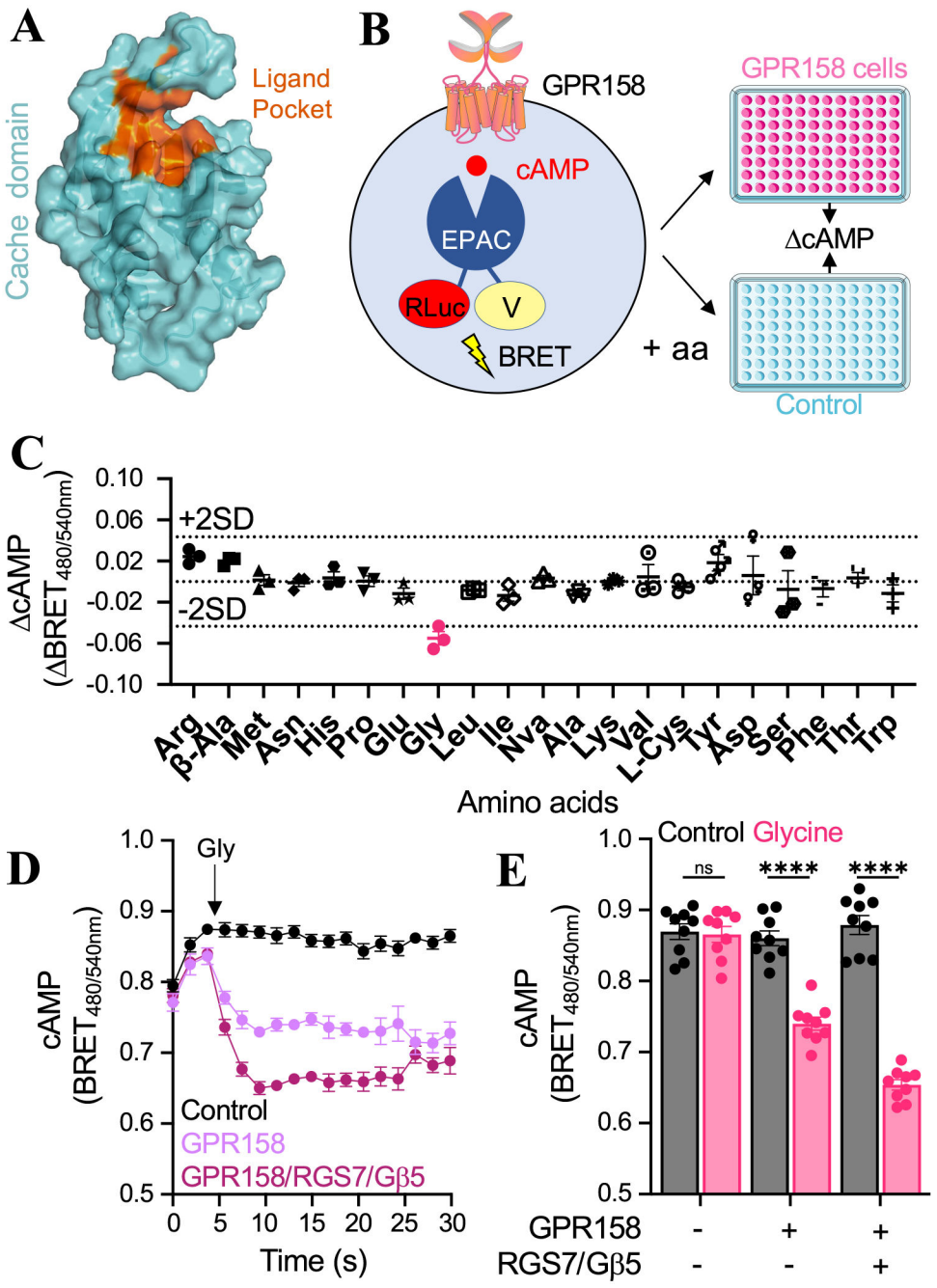

Fig. 1. Identification of glycine as GPR158 ligand

A) 3D model of the GPR158 cache domain (cyan) with putative ligand-binding pocket (orange). **B**) Schematic of the screening assay design. **C**) Quantification of cAMP changes mediated by GPR158. BRET signal in control cells is subtracted from the signal from cells expressing GPR158 and the difference is plotted. Dotted lines denote 2X SD confidence interval. Data shown represent mean ± s.e.m. determined from 3 independent experiments performed in triplicate. **D**) Time course of cAMP change induced with 10μM of forskolin in U87 MG glioblastoma cells in response to glycine (100μM) application (arrow) **E**) Quantification of maximal amplitudes of responses to glycine in panel D. Data shown

represent mean ± s.e.m. determined from 3 independent experiments performed in triplicate. **p<0.01 (one-way ANOVA).

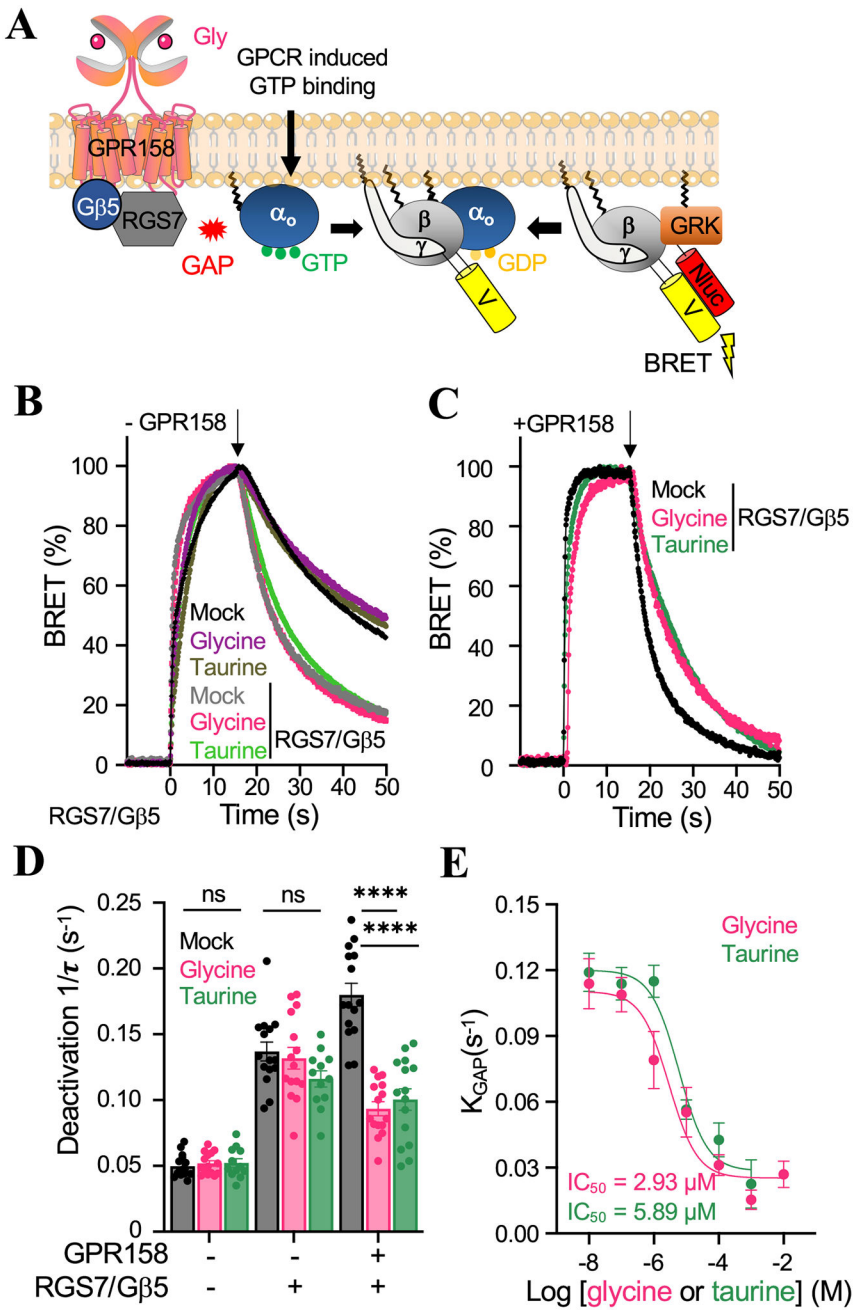

Fig. 2. Glycine and taurine slow deactivation of Gαo by GPR158-RGS7/Gβ5 complex

A) Schematics of the BRET-based GAP assay. G proteins are activated at t=0s by stimulating GPCR (D2R, 0.1mM). After reaching steady state the GPCR activity is terminated by injection of haloperidol (0.1 mM) at t=15s (arrow). G protein deactivation is then monitored by following quenching of the BRET signal. **B,C**) Traces of BRET signal showing Gαo activation and deactivation time course with or without glycine or taurine (100μM) treatment in cells without GPR158 (B) or cells transfected with GPR158 (C). **D**) Quantification of deactivation time constant of the reactions presented in panels B and C. 1/τ is calculated from deactivation curves of n=5 independent experiments in triplicate

from each cell transfection group. Data shown represent mean ± s.e.m. ****p<0.0001, ns, p>0.05 (two-way ANOVA). **e**) Dose response profile of changes in GAP activity (K_{GAP}) calculated by subtracting the baseline deactivation rate ($1/\tau$) from the rate of the reaction in the presence of the GPR158/RGS7/Gβ5. Data shown represent mean ± s.e.m of n=4 independent experiments each conduced in triplicate.

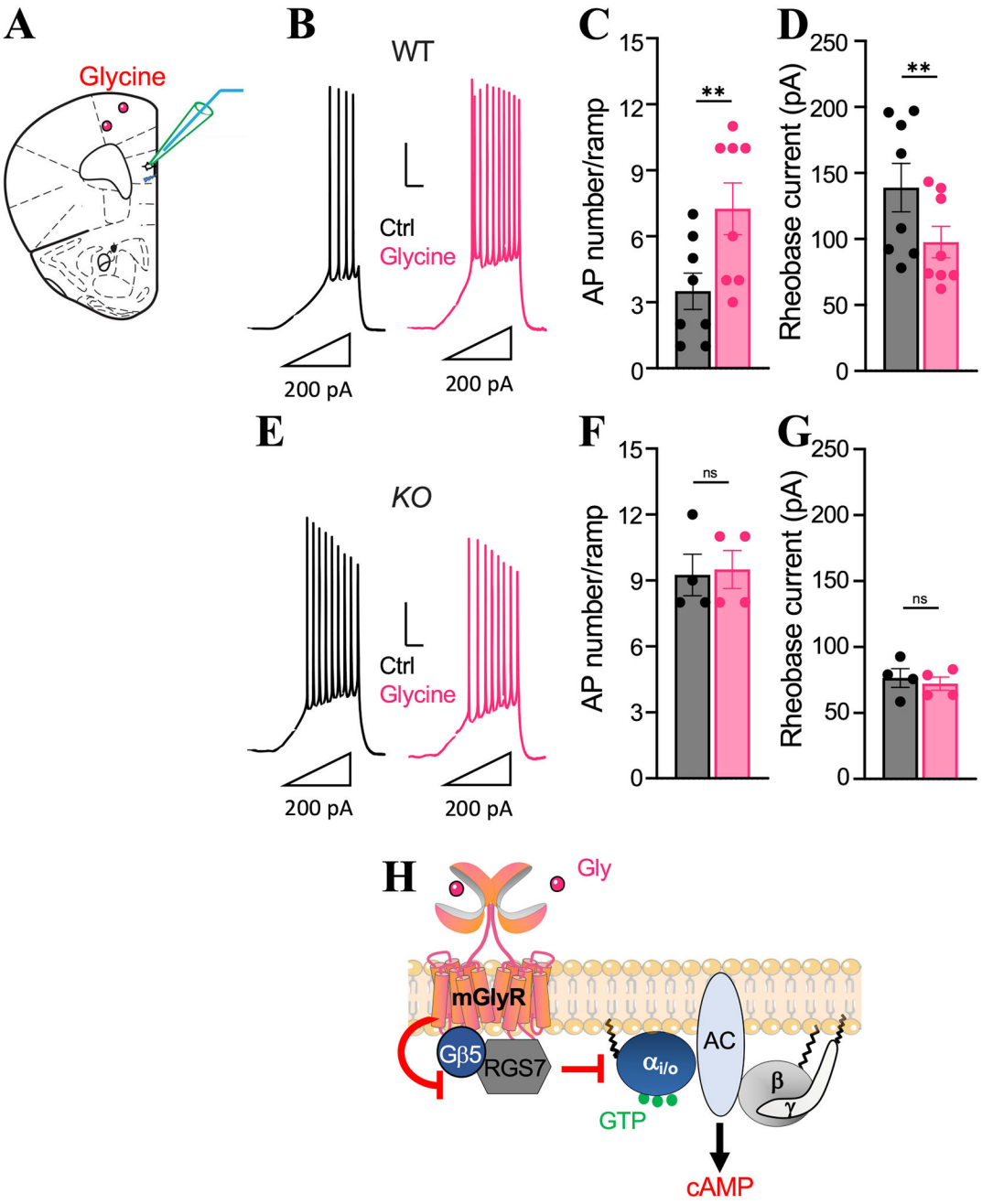

Fig. 3. Direct interaction of glycine with GPR158

A) Schematics of assay design for detecting glycine binding to GPR158 by flow cytometry. **B**) Flow cytometry histogram showing distribution of cellular populations after sorting. **C**) Quantification of FITC-glycine binding detected in flow cytometry experiments. The median of fluorescence (MFI) is quantified and plotted. Error bars are s.e.m. values, n=3, **p<0.01, ****p<0.0001, two-way ANOVA. **D**) Schematics of the radioligand binding assay. **E**) Quantification of ^{3}H-glycine binding to membrane expressing GPR158. Data shown mean of 4 independent experiments error bars are s.e.m. values, n=4. **F**) Scatchard plot of the ^{3}H-glycine radioligand binding assay. Data shown mean of 4 independent experiments

error bars are s.e.m. values, n=4. **G**) Schematics of the assay design detecting glycine binding to GPR158 by Isothermal Titration Calorimetry (ITC) with purified protein. **H**) ITC binding profile showing glycine binding to GPR158 in the initial run with fresh sample. **I**) Quantification of binding determined by fitting the integrated isotherm to an independent binding model. Data shows mean of 4 experimental runs, error bars are s.e.m. values.

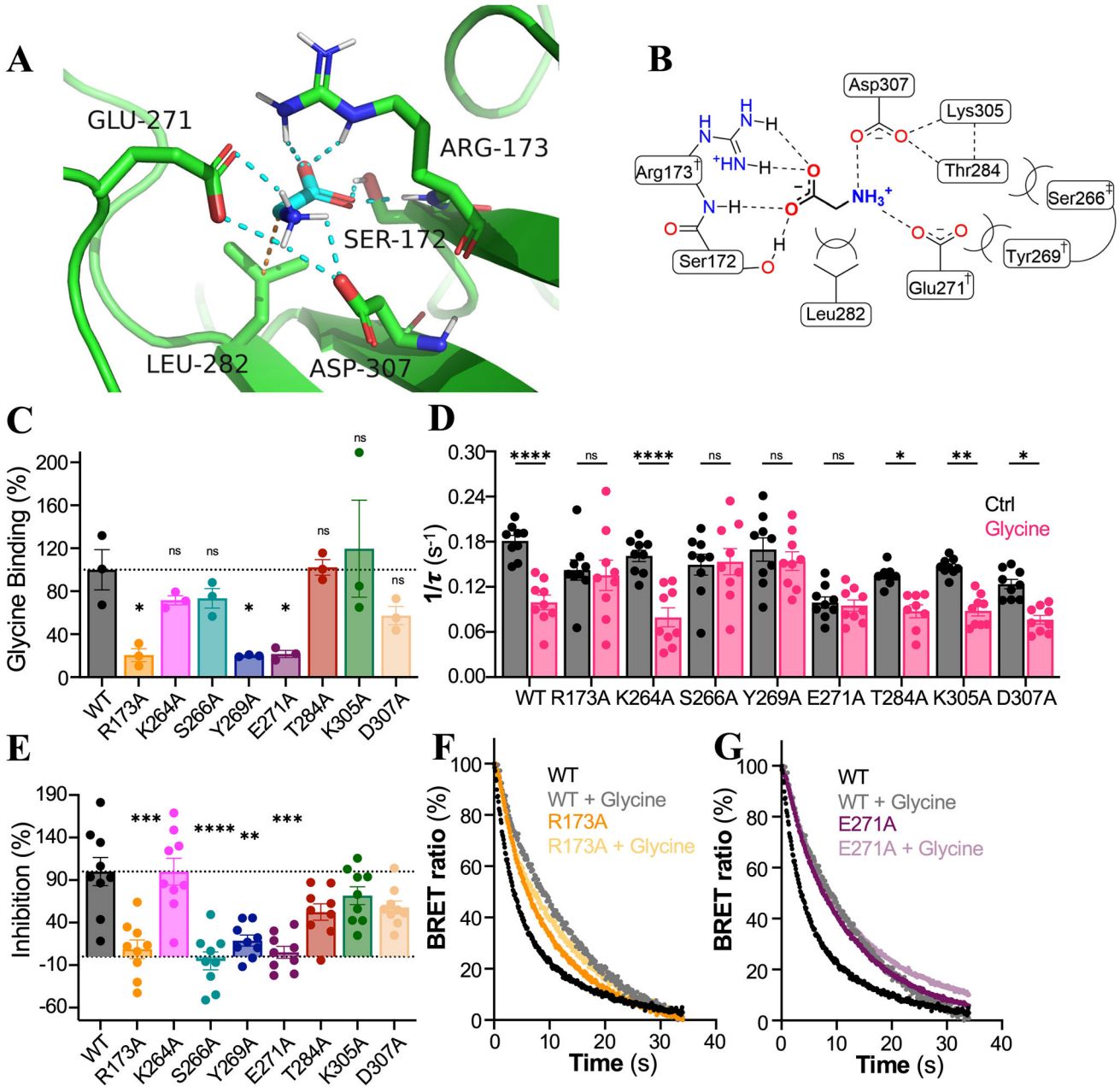

Fig. 4. Probing Cache domain of GPR158 as a ligand binding site

A) Computational docking of glycine (teal) into putative ligand binding pocket on GPR158 Cache domain (green). Glycine and directly interacting residues are shown as sticks. Hydrogen bonds (teal) and van der Waals interactions (orange) are shown as dotted lines. **B)** Diagram showing interactions in the docked model of glycine against GPR158 Cache domain. Hydrogen bonds are shown as dashed lines, van der Waals interactions are shown as intersecting semicircles, and the secondary structural context of Ser266 and Tyr269 is shown as an arc. Dagger indicates a residue implicated in glycine binding in panel **A**. **C**) Radioligand binding assay of ^{3}H-glycine in cells expressing GPR158 mutants. Data shown mean ± s.e.m. of 3 independent experiments, *p<0.05, ns=p>0.05 one-way

ANOVA. **D**) Functional evaluation of GPR158 mutants in GAP BRET assay. Error bars are mean ± s.e.m. of 3 independent experiments in triplicate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=p>0.05, two-way ANOVA **E**) Quantification of glycine inhibitory effect on k_{GAP} normalized to the effect seen with WT receptor. Error bars are mean ± s.e.m. of 3 independent experiments in triplicate. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns=p>0.05, one-way ANOVA. **F,G**) Traces of Gαo deactivation time course upon glycine addition.

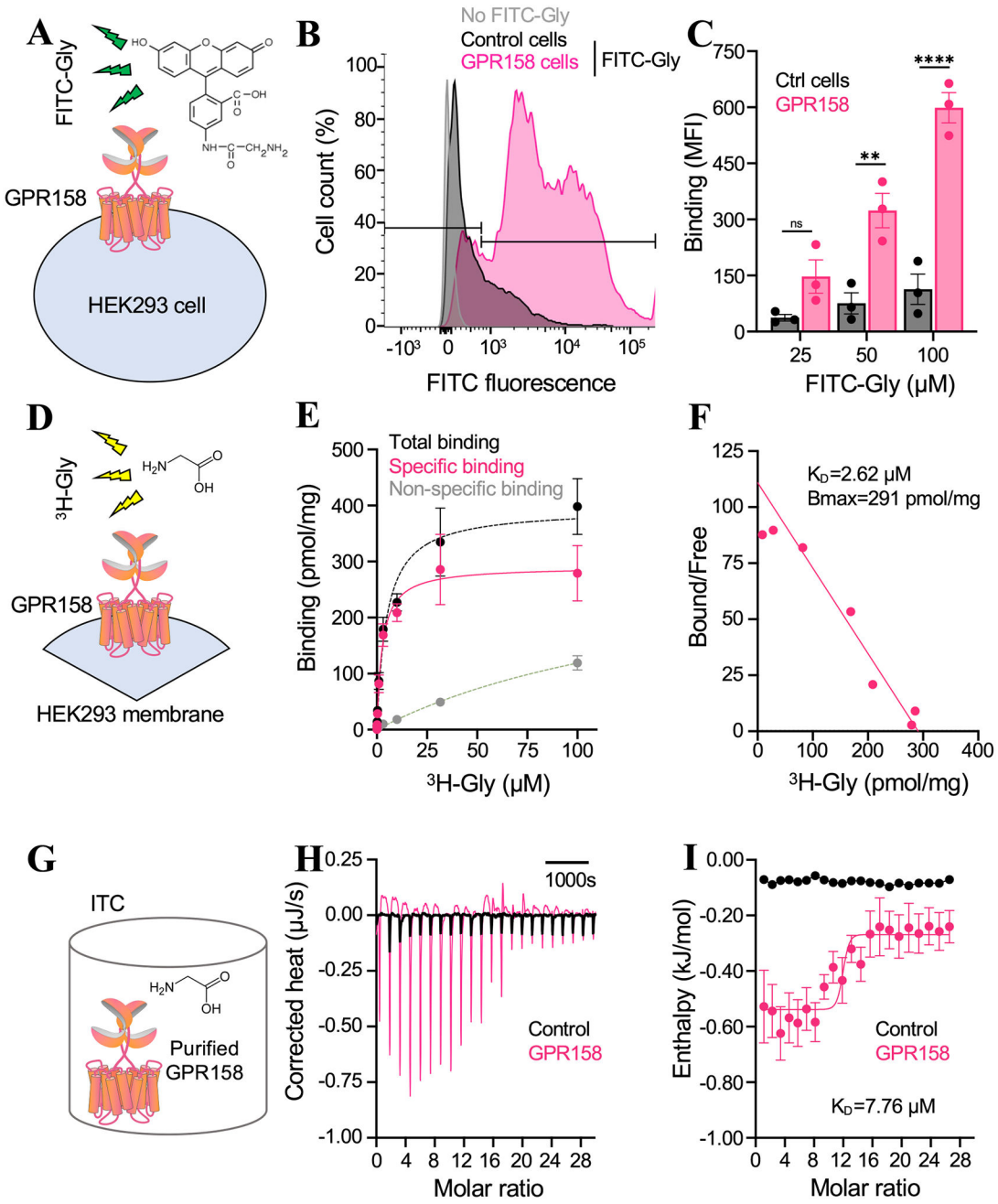

Fig. 5. Effect of glycine on neuronal excitability.

A) Schematic of the electrophysiological recordings in slice preparation targeting mPFC neurons of layer II-III. Experiments were conducted with: picrotoxin (100μM) (blockade of $GABA_A$ receptors), strychnine (1μM) (antagonist of glycine and acetylcholine receptors), CNQX (20μM) (AMPA receptor antagonist), and APV (50μM) (NMDAR antagonist). **b**) Traces of voltage responses to a 200pA current ramp injection under control conditions and after bath application of glycine (1mM). **C**) Quantification of changes in excitability by number of action potentials fired in response to 200pA current ramp (n=8 neurons/5 mice). **D**) Quantification of changes in excitability by rheobase current under control condition

and glycine application (n=8 neurons/5 mice). **e**) Traces of voltage responses to a 200pA current ramp injection obtained from layer II-III pyramidal neurons in GPR158 KO mice under control conditions and during bath application of glycine (1mM). **F**) Quantification of changes in excitability by number of action potentials fired in response to 200pA current ramp (n=4 neurons/3 mice). **G**) Quantification of changes in excitability by rheobase current under control condition and glycine application (n=4 neurons/3 mice). **h**) Schematic representation of the proposed mechanism of glycine effects on mGlyR. In all graphs nonparametric t-test; Wilcoxon test was used for statistical analysis, ns= $p>0.05$, **$p<0.01$.