British Journal of Pharmacology (1996) 118, 2115-2125

Potentiation of chloride responses to glycine by three 5-HT_3 antagonists in rat spinal neurones

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1 Modulations of Cl^- responses to glycine by 5-hydroxytryptamine ligands were studied in cultured spinal neurones, by the whole-cell recording technique.

2 Three 5-HT_3 antagonists were found to potentiate reversibly responses to low concentrations of glycine. Potentiations were induced by micromolar concentrations of LY-278,584 (1-10 μM) and by concentrations of MDL-72222 or ICS-205,930 between 10 nM and 1 μM.

3 Potentiations were observed over the whole voltage range without any change in the reversal potential of the glycine responses and without affecting the resting conductance.

4 The degree of potentiation was variable among cells. It increased with the concentration of the modulator, but only up to 100 nM for MDL-72222 and ICS-205,930.

5 The potentiation appeared to result from an increase in the affinity for glycine of glycine receptors.

6 Neither the blockade of glycine uptake by Na^+ removal, nor the excision of membrane patches prevented the potentiation.

7 At high concentrations (10 μM), both MDL-72222 and ICS-205,930 had, in contrast, a blocking effect on glycine responses.

8 Potentiation by LY-278,584 and a dose-dependent modulation by MDL-72222 were also observed for taurine responses.

9 The effects on glycine responses of various ligands of 5-HT_3 receptors (including agonists) are discussed. The ability of LY-278,584, MDL-72222 and ICS-205,930 to potentiate glycine responses appears to be independent of their known 5-HT_3 receptor antagonist properties. It would be interesting to look for chemically related drugs that would be specific potentiators of glycine responses.

Keywords: Glycine; 5-HT_3 antagonists; 5-HT_3 agonists; anaesthetics

Introduction

Glycine-activated chloride currents are the major inhibitory responses seen in spinal neurones. In comparison with the variety of pharmacological agents (benzodiazepines, barbiturates, steroids, anaesthetics) known to enhance Cl^- responses to γ-aminobutyric acid (GABA) (see Macdonald & Olsen, 1994 for review), there are few examples of positive modulators of glycine responses (see Kuhse *et al.* 1995 and Lambert *et al.* 1995, for reviews; see also Wakamori *et al.*, 1991; Hales & Lambert, 1991; 1992).

Following preliminary experiments that were initially designed to study the possible modulation of glycine responses by 5-hydroxytryptamine (5-HT), it appeared that some drugs previously known as specific antagonists of 5-HT_3 receptors can also act as positive modulators of glycine responses. These unexpected pharmacological results offer new perspectives concerning the regulation of glycine responses.

Methods

The experiments were performed at room temperature (20-23°C) on primary cultures of ventral spinal cord neurones from rat embryos, using the whole-cell configuration of the patch-clamp technique in most cases, and the outside-out configuration in a few cases.

Cell preparation

Female rats (OFA, Iffa Credo) carrying E16 embryos were killed by CO_2-induced anoxia. No anaesthetic was used. The embryos were rapidly removed and decapitated. Their spinal cord was taken out and dissected under a microscope in a phosphate buffer saline (PBS without Ca and Mg, Gibco) supplemented with glucose (33 mM). The meninges were first removed, then the ventral part of the spinal cord was separated from the dorsal parts and cut in small pieces in order to dissociate the neurones as described below.

The tissues were first incubated for 15 min at 37°C in PBS-glucose containing 10% trypsin-EDTA (10 x from Gibco). After three successive washes with PBS-glucose, the tissues were incubated for a further 15 min at 37°C in PBS-glucose containing deoxyribonuclease I (DN-25 from Sigma, 0.03 mg ml^{-1}), $MgCl_2$ (0.1 mg ml^{-1}) and bovine serum albumin (A2058 from Sigma, 1 mg ml^{-1}). This step was followed by gentle mechanical dissociation. The cells were then washed in PBS-glucose by two successive centrifugations (7 min at 700 r.p.m.) and the pellet was resuspended in 1 ml of culture medium (DMEM-F12 from Gibco supplemented with 2 mM glutamine, 10 mM HEPES buffer, 9 mM sodium bicarbonate, 33 mM glucose, 5 iu ml^{-1} penicillin/streptomycin from Specia and 0.25 μg ml^{-1} fungizone from Gibco). The cells were seeded at a density of $1-2 \times 10^5$ cells per dish on the centre of 35 mm dishes which had been precoated first with polyornithine (1.5 μg ml^{-1} for one night at 37°C followed by three washes) and then with 20% foetal calf serum (in culture medium, for 1 h at 37°C followed by two washes). Cells were allowed to settle for 15 to 30 min at 37°C. Then 1 ml of complete culture medium containing 5% foetal calf serum and 5% horse serum (heat-inactivated Gibco) was added to each dish. Between days 5 and 7, the cells were treated with 5-fluoro-2'-deoxyuridine-5'-monophosphate (FUDR) (12 μM) and uridine (40 μM). The cells were used for electrical recording between day 11 and day 15.

Experimental solutions

Before recording, the culture medium was replaced by the external solution to be used during the recording. In most experiments this solution contained (in mM): NaCl 150, KCl 2.5, $CaCl_2$ 1.8, $MgCl_2$ 1, glucose 20 and HEPES-NaOH 10 pH 7.4. Some experiments were also performed with a Na^+-free external solution where Na^+ was replaced by N-methyl-glucamine (NMG). The internal solution used to fill the recording electrode contained (in mM): Cs methanesulphonate 145, CsCl 15, $MgCl_2$ 1, EGTA 0.1, ATP-Mg 3, GTP-Na 0.3 and HEPES-CsOH 10, pH 7.2.

Drugs

Stock solutions of 5-HT oxalate (Sigma), 5-HT hydrochloride (Research Biochemicals International: RBI), ±8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT, Sigma), α-methyl-5-HT maleate (RBI), 5-methoxytryptamine hydrochloride (5-MeOT, RBI), 1-(*m*-chlorophenyl)-biguanide hydrochloride (mCPBG, RBI), 1-phenylbiguanide (PBG, RBI), 2-methyl-5-HT maleate (RBI), 1-methyl-N-(8-methyl-8-azabicyclo[3.2.1]-oct-3-yl)-1H-indazole-3-carboxamide maleate (LY-278,584, RBI), 3-tropanyl-indole-3-carboxylate hydrochloride (ICS-205,930, RBI) were prepared at 10 mM in distilled water.

Stock solutions of 3-tropanyl-3,5-dichlorobenzoate (MDL-72222, RBI) were prepared in dimethylsulphoxide (DMSO), usually at 10 mM, and in some cases at 1 mM. In all the experiments where the modulatory effects of MDL-72222 on glycine responses were tested, all the solutions applied by the fast perfusion system (including the control and glycine solutions without MDL-72222) contained the same amount of DMSO (1/100 000 for tests of concentrations of MDL-72222 up to 100 nM, 1/10 000 for tests of 1 μM and 1/1 000 for tests of 10 μM). A tube identical to that used for the stock MDL-72222 solution, but containing only DMSO, was used, in parallel, after having been vortexed and sonicated exactly as the tube containing the stock MDL-72222. Thus the modulatory effects of MDL-72222 on glycine responses cannot be attributed to DMSO or to any contaminant possibly released by the tubes used for preparing the stock MDL-72222 solution.

(+)-Tubocurarine chloride (Sigma) was dissolved in water at 2 mM. All stock solutions mentioned above were prepared daily. A given stock solution of glycine (Sigma), prepared at 10 mM in water and kept at −20°C, was used for several weeks.

Perfusion system

The culture dish was continuously perfused with the external solution. In addition, a fast perfusion system was used for rapid application of glycine and other drugs. All solutions applied via this system contained tetrodotoxin (TTX) 0.2 μM. The fast perfusion system was made of two glass barrels and lateral movements of the two barrels were controlled by a computer-driven motor in order to apply the solution of the desired barrel to the cell. Each of the barrels could be used to apply two different solutions into the recording chamber (barrel 1 could be used to apply either the control solution or, for example 5-HT, whereas barrel 2 could be used to apply either glycine or, for example, glycine + 5-HT). Two identical teflon valves were used (one for each barrel); each valve had two inputs and two outputs, and either one of the two input solutions could flow either through the output connected to the glass barrel into the recording chamber, or through the second output into another chamber. Thus, all solutions flowed continuously and the lateral movement made in order to apply glycine was always exactly the same whether or not e.g. 5-HT was present, avoiding any possible mechanical artefact. The fast perfusion rate was identical for the two barrels, and for each position of the valves. All tubings were of Teflon, and traces of chemicals previously used were eliminated by extensive washing. The reliability of the fast perfusion system was checked regularly by 'control' experiments, performed either before or after the test of the modulator on the same cell, using the valves in the same way but without adding the modulator.

If not otherwise mentioned, the glycine concentration used was 20 μM. At this concentration, successive responses recorded in the same solution were quite stable, even when tested every 40 s. When higher concentrations were used (≥80 μM), a slight decrease in the response over successive applications was frequently observed even when applications were separated by long intervals (80 s). Repetitive measurements of successive glycine responses at a fixed interval have been systematically performed in order to separate true modulatory effects from slow spontaneous changes in the response.

Except when indicated otherwise, the drugs tested as possible modulators of glycine responses were applied 'with pre-incubation', that is before, between and during the successive glycine applications.

Recording

Patch-clamp micropipettes were made from hard glass (Kimax 51); the shank of each pipette was covered with Sylgard and the tip was fire-polished. The resistance of these electrodes filled with the internal solution was between 5 and 10 MΩ. The cells were voltage-clamped by an EPC7 List amplifier, controlled by a TANDON 38620 computer, via a Cambridge Electronic Design (CED) 1401 interface, using CED patch- and voltage-clamp software. The current monitor output of the amplifier was filtered at 0.3 kHz before being sampled on-line at 0.6 kHz. The bath was connected to the ground via an agar bridge. Membrane potentials were corrected for the junction potential of 10 mV amplitude that was measured between the recording pipette and the usual external solution.

The series resistance (*Rs*) was systematically measured several times during each experiment. Particular care was taken to eliminate experiments in which *Rs* changed suddenly. *Rs* was between 10 and 20 MΩ. These values are high enough to introduce a difference of a few mV between the voltage applied to the electrode and that actually applied to the inside of the cell (error of maximum 10 mV for the largest responses). No corrections have been used either during or after the recording to compensate for these errors. However, the current modulations observed occurred without any simultaneous change in *Rs* and thus cannot result from changes in the applied voltage.

The zero indicated on current traces is the absolute zero current level.

All values are expressed as mean ± s.d. (number of observations).

Results

Reduction of glycine responses by 5-HT and by micromolar concentrations of mCPBG

In a previous series of experiments designed to study the effect of 5-HT on NMDA responses (Chesnoy-Marchais & Barthe, 1996), glycine was present in all solutions (at concentrations ≥ 1 μM) in order to saturate the glycine site of NMDA receptors. During this study, we noticed that in the absence of NMDA and in the presence of a concentration of glycine of at least 10 μM, high concentrations of 5-HT (such as 100 μM) could slightly reduce the outward currents recorded at positive membrane potentials. This effect was not observed in the presence of 1 μM strychnine. Thus, it appeared that 5-HT could reduce a sustained Cl^- response to glycine. This result was confirmed in the present study by a series of experiments performed by repetitive application of 20 μM glycine for a few seconds only, either in the absence of 5-HT or in the continuous presence of 5-HT (Figure 1a). The two-barrel perfusion system that was used is described in the Methods section.

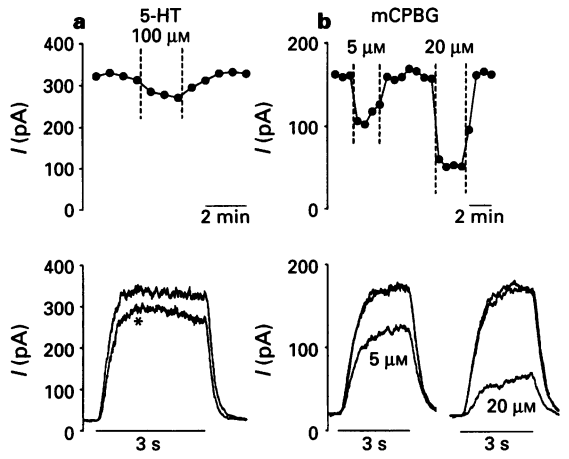

Figure 1 Inhibitory effect of 5-HT and mCPBG on the Cl^- glycine response. (a) Amplitude of successive peak responses to 20 μM glycine (top panel) and mean of two current traces (lower panel) recorded in the absence or in the presence (*) of 100 μM 5-HT. (b) Amplitude of successive peak responses to 20 μM glycine (top panel) and mean of three current traces (lower panels) recorded before mCPBG application, in the presence of mCPBG (5 or 20 μM) and after wash of mCPBG. The peak glycine response was reduced by about 32% by 5 μM mCPBG and by 70% by 20 μM mCPBG. Holding potential −10 mV in (a) and (b). On each set of current traces, glycine applications are indicated by the lower bar. 5-HT and mCPBG were applied 'with preincubation' (see Methods).

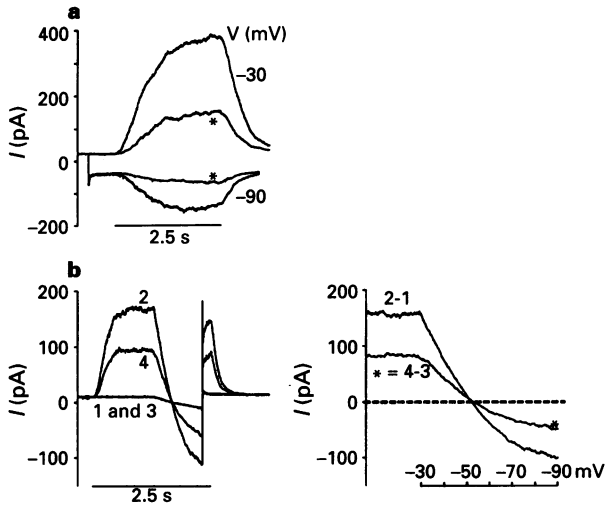

Figure 2 Voltage-independence of the inhibitory effect of mCPBG on glycine responses. (a) Glycine responses alternately recorded at −30 mV and −90 mV in the same cell, in the absence and presence (*) of 20 μM mCPBG. Note that, in the absence of glycine, mCPBG did not affect the cell conductance measured between −30 and −90 mV by the initial voltage jump. (b) Left panel, the reversal potential of glycine responses was measured by applying voltage ramps from −30 mV to −90 mV in the absence (traces 1 and 3) or presence of glycine (traces 2 and 4), without mCPBG (traces 1 and 2) or in the presence of 20 μM mCPBG (traces 3 and 4). Note that traces 1 and 3 are superimposed. All traces are the mean of three individual records. Glycine applications are indicated by the 2.5 s bars. Right panel, *I-V* curves of glycine responses, in the absence (2-1) or in the presence of mCPBG (4-3), presented on an expanded scale. These curves were obtained by subtracting the traces recorded in the absence of glycine from the corresponding traces recorded in the presence of glycine. Note that these two *I-V* curves cross the zero current axis at about the same membrane potential. There is a difference of only a few millivolts (about 5 mV) between the measured reversal potential of glycine responses and the chloride equilibrium value (E_{Cl}, −57 mV) obtained from the Nernst equation and the Cl^- concentrations of the external and internal solutions used. Note that this calculation of E_{Cl} supposes that methanesulphonate ions are totally impermeant across glycine-gated channels, and that the exchange of the intracellular content by the solution of the recording pipette is perfect (even in the finest neurites and in the area the most remote from the cell body). These conditions might not be quite fulfilled, which would explain the slight discrepancy between the measured reversal potential and the calculated E_{Cl} value. (a) and (b) are from two different cells.

It was perfectly reliable as shown by control experiments on those cells where the effect of 5-HT was observed (see Methods). The amplitude of the blocking effect of 5-HT on glycine responses was small. At 100 μM, 5-HT reduced the peak glycine responses by only 13.5% ± 3.5% (9) at −10 or −30 mV (outward Cl^- responses) and by 16% ± 3% (5) at −90 mV (inward Cl^- responses). At 40 μM, a small blocking effect was also detected (of 7.4% ± 4% (5)), whereas at lower concentrations, 5 μM (6 cells), 1 μM (4 cells) or 0.1 μM (4 cells), no effect was observed.

A few other 5-HT agonists were tested in an attempt to detect stronger modulations of glycine responses. The 5-HT$_1$ receptor agonist, 8-OH-DPAT, at 1 μM (6 cells) or 10 μM (12 cells), the 5-HT$_2$receptor agonist, α-methyl-5-HT, at 20 μM (5 cells), and 5-MeOT, at 10 μM (6 cells) or 20 μM (3 cells), were all ineffective.

In contrast, micromolar concentrations of the 5-HT$_3$ agonist, mCPBG, were found to inhibit strongly glycine responses. This is illustrated in Figure 1b. In this experiment, where the response to 20 μM glycine was regularly measured at −10 mV, the effect of mCPBG was tested first at 5 μM, then at 20 μM. The inhibitory effect observed was reversible and dose-dependent. Average values of the percentage reduction of peak responses to 20 μM glycine by 0.1, 1, 2, 5 and 20 μM mCPBG respectively are 0% (4), 7% ± 7% (4), 8% ± 7% (5), 30% ± 7% (5) and 59% ± 15% (8) respectively, at −10 or −30 mV.

As shown by Figure 2a, the blocking effect of mCPBG on glycine responses was observed on both sides of the reversal potential and was not markedly voltage-dependent. In this experiment, the response to glycine was alternately measured at −30 mV and at −90 mV (using voltage jumps of 4 s duration between −30 and −90 mV), in the absence or presence of 20 μM mCPBG. mCPBG did not modify the resting conductance of the cell (in the absence of glycine) but markedly reduced the glycine response at both membrane potentials. From the 4 cells in which the effect of mCPBG was alternately measured at −30 and −90 mV, the average values of the percentage of reduction of glycine responses were 57% ± 16% (4) at −30 mV and 61% ± 15% (4) at −90 mV.

Figure 2b shows that the reversal potential of the glycine response was not affected by mCPBG. In this type of experiment, the holding potential was −30 mV and a 1 s voltage ramp from −30 to −90 mV was applied in the absence of glycine and at the plateau of the glycine response; the same protocol was used during the period when mCPBG was applied (both in the absence and presence of glycine). The left panel of Figure 2b actually contains 4 current traces, two of them, the control (1) and mCPBG (3) traces, were obtained in the absence of glycine and are superimposed (further illustrating that mCPBG did not affect the resting conductance of the cell); the remaining two traces (2 and 4), show the glycine responses in the absence (2) or presence (4) of mCPBG. The reversal potentials of the glycine response measured in the absence of mCPBG (intersection between traces 1 and 2) and in the presence of mCPBG (intersection between traces 3 and 4) were both close to the calculated E_{Cl} value. This is illustrated more precisely in the right panel of Figure 2b which shows (on an expanded time scale) the *I−V* curves of the glycine responses, derived by subtracting the current traces obtained in the absence of glycine from the corresponding traces obtained in the presence of glycine. These results were confirmed in another similar experiment.

The blocking effect of 20 μM mCPBG was observed even when mCPBG was applied 'without preincubation', that is

only during the glycine applications; in contrast, if mCPBG was applied only between (and not during) glycine applications, no blocking effect was observed (2 cells; data not shown).

In 4 cells, possible effects of 20 μM mCPBG on responses to 80 μM glycine were investigated and no effect was detected (not shown), whereas, as usual, 20 μM mCPBG clearly reduced the responses to 20 μM glycine that were also recorded in 3 of these 4 cells.

Possible modulations of responses to 20 μM glycine by two other 5-HT_3 receptor agonists were investigated. Whatever the concentration (0.1 μM (3), 1 μM (3) or 20 μM (4); same number of cells tested for each agonist), neither PBG nor 2-methyl-5-HT had any effect.

In order to understand the origin of the blocking effect of mCPBG, it might have been interesting to try to impede this effect with some 5-HT_3 antagonist. However, since the blocking effect of mCPBG on glycine responses might result from some structural analogy between glycine receptors and 5-HT_3 receptors, it was necessary to check whether 5-HT_3 antagonists were active on glycine responses. As described below, several 5-HT_3 antagonists affected glycine responses, and, interestingly, potentiations of glycine responses could be observed.

Potentiation of glycine responses by micromolar concentrations of LY-278,584

LY-278,584 is a potent water-soluble 5-HT_3 receptor antagonist. In the micromolar range, this compound was shown to *increase* the response to 20 μM glycine (Figure 3). The positive modulation observed was reversible and dose-dependent, clearly more pronounced at 10 μM than at 1 μM (Figure 3a). This modulation was observed on both sides of E_{Cl} (Figure 3b) and was not related to any change of the reversal potential of the glycine response (Figure 3c). LY-278,584 did not affect the resting conductance of the cells in the absence of glycine. From 3 experiments in which glycine responses were alternately measured on the same cell at −30 mV and −90 mV, average values of the percentage increase of these responses by 10 μM LY-278,584 were 108% ± 4% (3) and 125% ± 7% (3) at −30 and −90 mV respectively.

Some variability in the amplitude of the modulation was observed from one cell to the other. Average values (obtained by pooling the data recorded at −10 and −30 mV) of the percentage increase of peak glycine responses by 0.1, 1, 3 and 10 μM LY-278,584, respectively, are 0% (4), 23% ± 8% (5), 34% ± 14% (4) and 73% ± 30% (11) respectively (see also Figure 6).

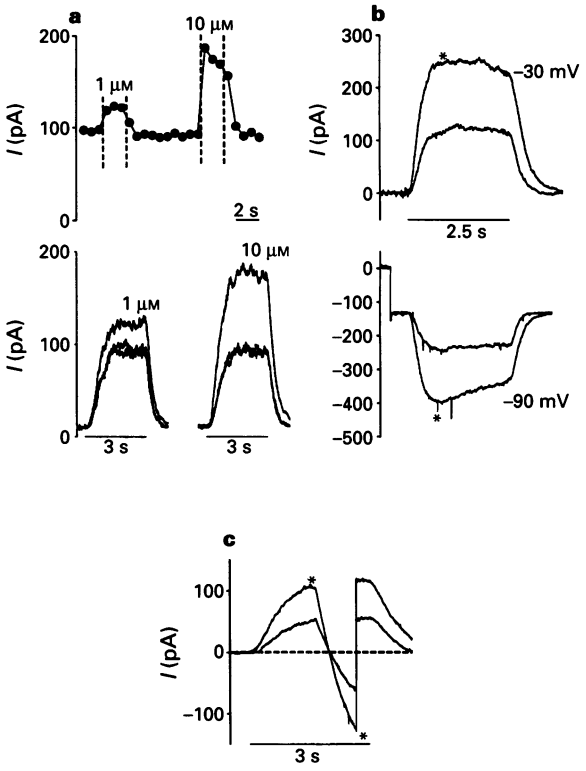

Figure 3 Potentiation of glycine responses by LY-278,584. (a) Dose-dependent reversible potentiation of the response to 20 μM glycine by LY-278,584, successively applied on a same cell, with preincubation, at 1 μM and 10 μM. Upper panel, amplitude of successive peak glycine responses. Lower panels, mean of three current traces recorded before, during and after the LY-278,584 applications. Holding potential, −10 mV. (b) Potentiation by 10 μM LY-278,584 of both the outward glycine response (upper panel) and the inward glycine response (lower panel) alternately recorded in another cell at −30 mV and −90 mV. Note in the lower panel that in the absence of glycine, LY-278,584 did not affect the cell conductance measured between −30 and −90 mV by the initial voltage jump. In this cell, the degree of potentiation of glycine responses by LY-278,584 was 132% at −90 mV and 103% at −30 mV. Traces recorded in the presence of LY-278,584 are indicated by *. (c) *I-V* curves of the glycine responses recorded in a third cell, in the absence and presence of 10 μM LY-278,584 (*), between −30 mV (holding potential) and −80 mV (final level reached during the voltage ramps). Same protocol as in Figure 2b. Each trace illustrated was obtained after subtracting the average of three identical traces recorded in the absence of glycine from the average of three identical traces recorded in the presence of glycine.

Dose-dependent modulation of glycine responses by MDL-72222: potentiation by high nanomolar concentrations, inhibition by micromolar concentrations

MDL-72222 is another 5-HT_3 receptor antagonist having a K_D for 5-HT_3 receptors in the high nanomolar range (between 5 and 50 nM; see Zifa & Fillion, 1992). In this same concentration-range, MDL-72222 likewise behaves as a positive modulator of glycine responses. This is illustrated by Figure 4a, showing the effects of MDL-72222, applied with preincubation, first at 33 nM, then at only 10 nM, then again at 33 nM. The potentiation of the glycine response by these low concentrations of MDL-72222 was dose-dependent (stronger at 33 nM than at 10 nM) and was slowly reversible.

In a few experiments where MDL-72222 was applied only during glycine applications (together with glycine, but without preincubation), some potentiation of the glycine response could be observed, but this potentiation developed slowly. This is illustrated by Figure 4b. This type of result explains why most experiments were performed by applying the modulators with preincubation (even though it was only in the case of MDL-72222 that such an effect of preincubation was observed).

As in the case of LY-278,584, the positive modulatory effect of MDL-72222 was observed on both sides of E_{Cl}, was not related to any change in the resting conductance of the cells (not shown) nor to any change of the reversal potential of glycine responses (2 experiments performed at 100 nM). From 4 experiments in which glycine responses were alternately measured in the same cells at −30 and −90 mV, the average values of the percentage increase in these responses by 100 nM MDL-72222 were 53% ± 12% (4) and 59% ± 15% (4) at −30 and −90 mV, respectively. Some variability in the amplitude of the positive modulatory effect of MDL-72222 was observed between cells (see large error bars in Figure 6). However, both from mean values of the percentage increase in glycine responses (Figure 6) and from data obtained from individual cells where several MDL-72222 concentrations were tested successively, it was clear that the positive modulatory effect of this compound increased between 10 and 100 nM. However, the use of higher concentrations, such as 10 μM, revealed an opposite, negative modulatory effect of MDL-72222 on glycine responses, that was observed on both sides of E_{Cl} (Figure 4c). During the initial period of wash without MDL-72222, this

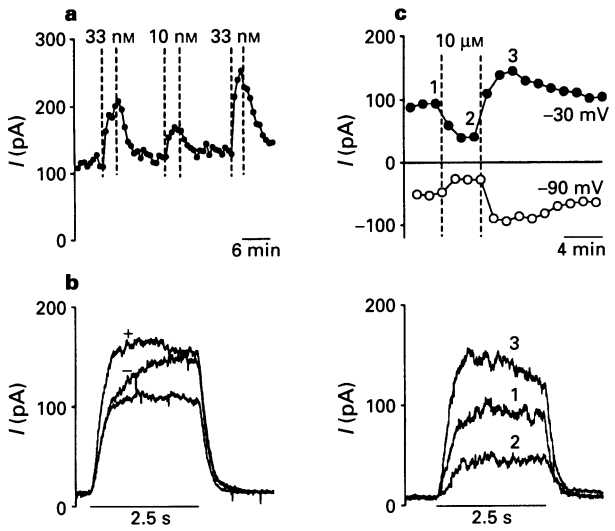

Figure 4 Concentration-dependent change in modulation of glycine responses by MDL-72222. (a) Concentration-dependent and reversible potentiation by low concentrations (10–33 nM) of MDL-72222 of the peak amplitude of the response to 20 μM glycine. Holding potential, −10 mV. In this experiment, MDL-72222 was applied, as usual, using the preincubation protocol. Note that the reversibility of the potentiating effect of MDL-72222 was slow. (b) Glycine responses recorded in another cell, at −30 mV in control and in the presence of 100 nM MDL-72222, both when this modulator was applied without preincubation (−) (only during glycine applications) and when it was applied with preincubation (+). The potentiating effect observed without preincubation developed slowly. (c) Inhibition of responses to 20 μM glycine by 10 μM MDL-72222. Upper panel, peak amplitude of glycine responses alternately measured on a same cell at −30 mV (•) and −90 mV (○) (same voltage-jump protocol as in Figure 2a and 3b). Similar effects were observed at both potentials: an inhibition during the MDL-72222 application and a rebound potentiation after wash of MDL-72222. Lower panel, individual glycine responses (see numbers indicated on both panels).

blocking effect was reversible, and the response became even larger than at the beginning of the recording. A slow recovery towards the initial response was then observed. The mean value of the percentage decrease of responses to 20 μM glycine produced by 10 μM MDL-72222 was 39% ± 17% (9 experiments performed at −10 or −30 mV).

Modulations of glycine responses by ICS-205,930

The potent 5-HT$_3$ receptor antagonist, ICS-205,930, recently became commercially available and its effects on glycine responses were also investigated. The results described below show that, like MDL-72222, this chemical potentiated glycine responses, when used at low concentrations, between 20 nM and 1 μM. It had the advantage over MDL-72222 of being more water-soluble and of inducing a more rapidly reversible potentiation (compare Figure 4a and 5a). Figure 5a illustrates an experiment where the effects of ICS-205,930 were tested successively at 20 nM and 100 nM (with preincubation); the potentiation induced by 100 nM was clearly more pronounced than that induced by 20 nM on the same cell. Glycine responses obtained in the presence of a potentiating concentration of ICS-205,930 were almost superimposed without or with preincubation with ICS-205,930 (Figure 5b), in contrast with the results obtained with MDL-72222 (Figure 4b). As reported above for both LY-278,584 and MDL-72222, the positive modulatory effect of ICS-205,930 (up to 1 μM) was observed on both sides of E_{Cl}, and was not associated with any change of the resting conductance (Figure 5c), or with any change of the reversal potential of glycine responses (Figure 5d; result confirmed in two additional experiments). The percentage increase of the responses to 20 μM glycine by 100 nM ICS-205,930 was variable among cells (between 25% and 90% at −30 mV; mean value: 49% ± 21% (10)). From 4 cells in which the glycine responses were alternately measured at −30 and at −90 mV and which showed similar effects of 100 nM ICS-205,930, the mean percentage increase of the glycine response was 37% ± 7% (4) at −30 mV and 51% ± 3% (4) at −90 mV.

When the effect of ICS-205,930 on glycine responses was tested at a much higher concentration (10 μM), the peak glycine response was little affected during the ICS-205,930 application but was clearly enhanced during the early period of wash (Figure 5e). These results show some similarities with those described above for 10 μM MDL-72222. Concentrations of ICS-205,930 higher than 10 μM were not tested.

Figure 6 pools all the data (obtained from different cells) concerning the effects of various concentrations of LY-278,584, MDL-72222 or ICS-205,930 on the responses to 20 μM glycine at −10 or −30 mV.

Small modulatory effects of (+)-tubocurarine on glycine responses

The three 5-HT$_3$ receptor antagonists used above show some structural similarities, in particular a common tropanyl group. (+)-Tubocurarine (TC) has a different structure and is known to be a 5-HT$_3$ receptor antagonist, at least in mouse and rat neurones (see Newberry *et al.*, 1991 for the species-specificity of the effect of (+)-tubocurarine; see Yang *et al.*, 1992 for its effect in rat superior cervical neurones).

At 10–100 nM, no modulation of glycine response by TC was observed (6 cells). At 1 μM, a small positive modulatory effect was observed in only 3 out of 6 cells (mean percentage increase of the glycine responses: 17% ± 2% (3)). At 10 μM, a small negative modulatory effect was detected in the 4 cells tested (mean percentage decrease: 12% ± 2% (4)). These modulations were much less pronounced than those observed with the other 5-HT$_3$ receptor antagonists tested. However, once again a dose-dependent change in the type of modulation could be detected. On two of the four cells where TC was tested at 10 μM (and had a slight negative modulatory effect) it had been tested first at 1 μM and had a small positive modulatory effect (of 17% and 14% respectively); furthermore, on these two cells as in many cells, control experiments were also performed (see Methods).

Sensitivity of the potentiations to glycine concentration, insensitivity to inhibition of glycine uptake

All the positive modulatory effects described above concerned the Cl$^-$ response to a relatively low concentration of glycine, 20 μM. Further experiments were performed with other concentrations of glycine. When tested with preincubation, at concentrations that induced strong potentiation of responses to 20 μM glycine, none of the three compounds tested (LY-278,584, MDL-72222, ICS-205,930) showed any reliable positive modulatory effect on responses to 80 μM glycine (Figure 7a, b and c). The average percentage increase of the peak response to 80 μM glycine was 6% ± 4% (7) for 10 μM LY-278,584, 0% ± 5% (9) for 100 nM MDL-72222 and 3% ± 1% (4) for 100 nM ICS-205,930. Further, responses to an even higher glycine concentration, 320 μM, were not affected by 100 nM MDL-72222 (3 cells, not illustrated).

These results suggested that LY-278,584, MDL-72222 and ICS-205,930 increased the responses to low concentrations of glycine by increasing the affinity for glycine of glycine receptors. This mechanism was confirmed by experiments comparing the dose-response curves of glycine responses recorded in the same cells in the absence or in the presence of ICS-205,930. ICS-205,930 was chosen for these experiments because of the rapidity of both the onset (Figure 5b) and the decline (Figure 5a) of its effect. The potentiating effect of ICS-205,930 (applied here at 200 nM without preincubation) was

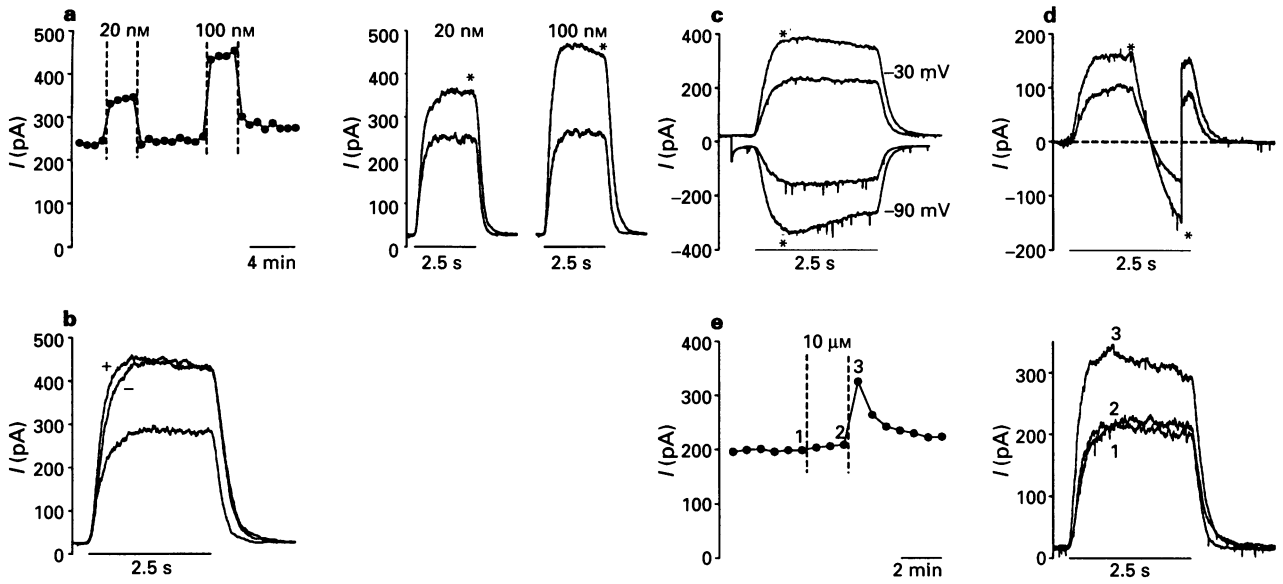

Figure 5 Potentiation of glycine responses by ICS-205,930. (a) Concentration-dependent reversible potentiation of the response to 20 μM glycine by ICS-205,930, successively applied on a same cell at 20 nM and 100 nM. Left panel, amplitude of successive peak glycine responses. Right panel, mean of three traces recorded before and during (*) the ICS-205,930 applications. (b) Potentiation of glycine response induced by 100 nM ICS-205,930 applied either without (−) or with (+) preincubation. Holding potential, −10 mV in both (a) and (b). (c) Potentiation of glycine responses alternately recorded at −30 mV and −90 mV by a continuous application of 1 μM ICS-205,930. (d) *I-V* curves of the glycine responses recorded in the absence and presence (*) of 100 nM ICS-205,930 between −30 mV (holding potential) and −80 mV (final level reached during the voltage ramps). Same protocol as in Figure 2b and 3c. Each trace illustrated was obtained after averaging and subtraction as in Figure 3c. (e) Effect of an application of 10 μM ICS-205,930 on the response to 20 μM glycine. Same cell as in Figure 5c, but the holding potential was kept at −30 mV. The left panel illustrates the successive values of the peak amplitude of the glycine response whereas the right panel shows individual responses (see numbers on both panels). There was almost no modulation of the glycine response during the 10 μM ICS-205,930 application (apart from an apparent slowing of the decline of the response), whereas a clear rebound potentiation was observed after the washout of ICS-205,930.

recorded successively for increasing concentrations of glycine (from 10 μM to 160 μM). The degree of potentiation decreased progressively for increasing concentrations of glycine (Figure 7d). For each cell, the two dose-response curves obtained were fitted independently in order to derive for each the EC_{50} for glycine, the Hill coefficient (n_H) and the maximum response (y_{max}) (see Figure 7e as an example). From 8 such experiments (in which the effect of ICS-205,930 was measured at least for 10 μM, 20 μM and 40 μM glycine), neither the Hill coefficient (2.22 ± 0.33 (8) in control) nor the maximum response was significantly affected by ICS-205,930, whereas the apparent affinity for glycine was increased. The EC_{50} value (34 ± 11 μM (8) in control) was reduced by 25% ± 4% (8) by 200 nM ICS-205,930.

The removal of external Na^+ is known to prevent cellular uptake of glycine (see Guastella *et al.*, 1992; Liu *et al.*, 1993). Thus, experiments were performed in a Na^+-free external solution in order to check that the positive modulations of responses to 20 μM glycine were not due to possible modulations of glycine uptake. This control was performed for the three positive modulators studied. The percentage increase in the peak response to 20 μM glycine was not significantly different in the absence of Na^+ (than in the usual external solution): 79% ± 20% (3) and 73% ± 30% (11) for 10 μM LY-278,584, 68% ± 22% (3) and 60% ± 23% (11) for 100 nM MDL-72222, 50% ± 7% (3) and 49% ± 21% (10) for 100 nM ICS-205,930, in the absence and presence of Na^+, respectively.

Inhibitory effects of 10 μM MDL-72222 or ICS-205,930 on responses to high concentrations of glycine

Unlike the potentiating effect of low concentrations of MDL-72222, the inhibitory effect of high concentrations of MDL-72222 was still detectable when the glycine concentration was 80 μM (Figure 8a). The mean value of the percentage decrease of the responses to 80 μM glycine by 10 μM MDL-72222 was 38% ± 13% (11) at −30 mV. The reversibility of this inhibitory effect was rapid (complete within the 80 s separating two successive glycine applications) and no post wash enhancement (such as that seen in Figure 4c) was observed (in agreement with the absence of a potentiating effect of low concentrations of MDL-72222 on responses to 80 μM glycine). As shown by Figure 8b, the inhibitory effect of 10 μM MDL-72222 on glycine responses decreased with a further increase of the glycine concentration. The peak response to 320 μM glycine was reduced by only 5.6% ± 2.2% (6) at −30 mV.

In 5 experiments, the voltage-sensitivity of the inhibitory effect of 10 μM MDL-72222 was investigated by alternately recording the responses to 80 μM glycine at two different membrane potentials. The inhibitory effect of 10 μM MDL-72222 was observed on both sides of the reversal potential of glycine responses and was systematically slightly more pronounced at −90 or −110 mV than at −30 mV. In 3 cells, the percentage reduction was 41% ± 0.5% at −30 mV and 51% ± 3% at −90 mV. In the 2 remaining cells, it was 43% and 44%, respectively, at −30 mV, and 57% and 56% respectively at −110 mV.

The use of 80 μM glycine, which eliminated potentiating effects of the modulators tested but still allowed detection of the inhibitory effect of 10 μM MDL-72222, was helpful for understanding the surprising results obtained with 10 μM ICS-205,930 on responses to lower concentrations of glycine (Figure 5e; see Discussion). In 4 cells, it was shown that at 10 μM, ICS-205,930, like MDL-72222, was able to reduce responses to 80 μM glycine. This is illustrated in Figure 8c. The inhibitory effect of 10 μM ICS-205,930 was less pronounced than that of MDL-72222; the mean value of the percentage reduction was 14% ± 3% (4) at −30 mV.

The inhibitory effects of 10 μM MDL-72222 or ICS-205,930 were never more pronounced at the end of glycine applications than at the peak of glycine responses. Thus, there is evidence

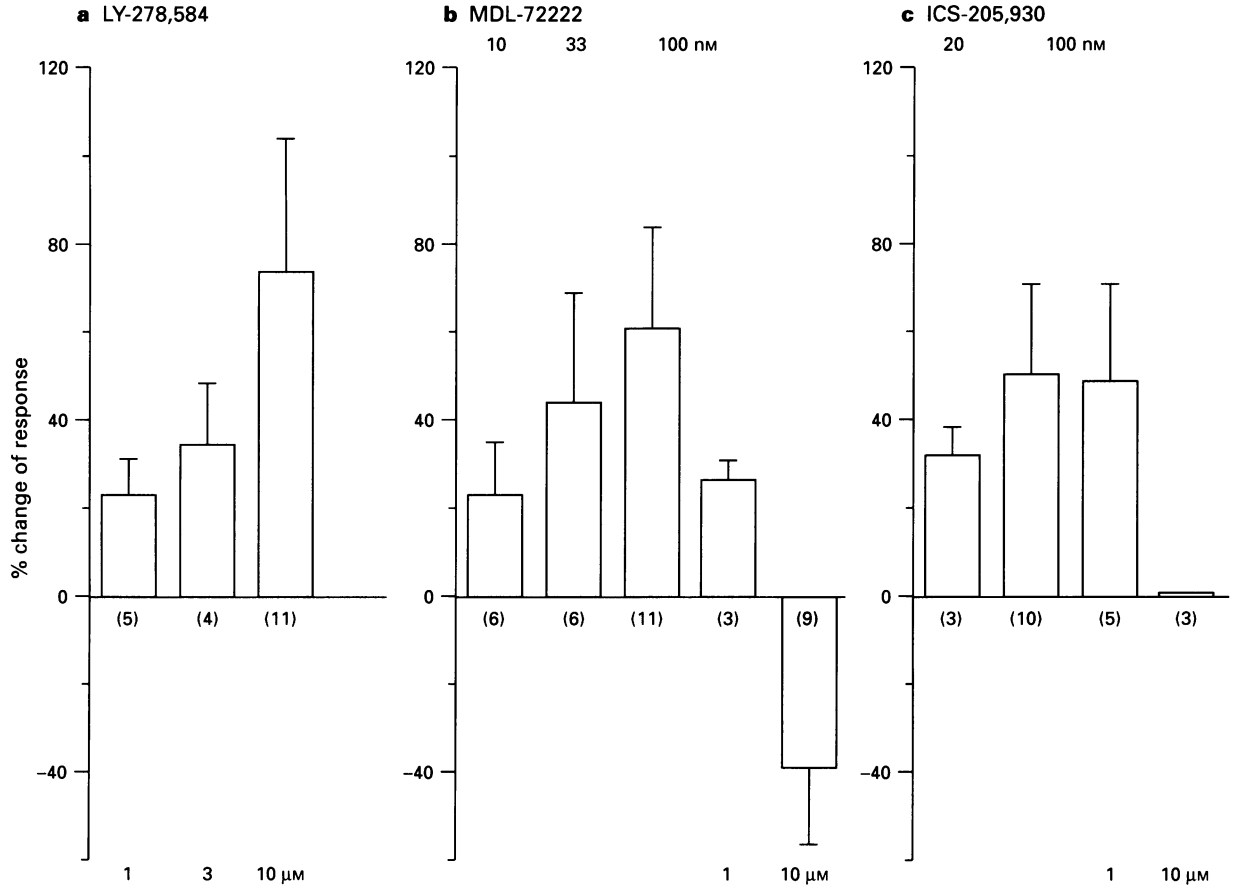

Figure 6 Summary of the modulations of the response to 20 μM glycine by LY-278,584 (a), MDL-72222 (b) and ICS-205,930 (c). Only results obtained in the usual external solution, at -30 or -10 mV, have been averaged. Since the voltage-sensitivity of the modulations described was weak, pooling the data obtained at these two close membrane potentials did not contribute much to the observed variability. This variability results rather from the heterogeneity of the cells. Each column gives the mean value of the percentage change of the glycine response (percentage increase for positive values, percentage decrease for negative values, zero indicating the absence of modulation); standard deviation is shown and number of cells is indicated in parentheses. The concentration of the modulator is indicated either below (in μM) or above (in nM) each column.

neither for a use-dependence of these blocking effects, nor for an increase in the rate or amplitude of the desensitization process occurring during the 2 to 3 s of the glycine applications.

Modulation of taurine responses by LY-278,584 and MDL-72222

Cl^- responses similar to those evoked by glycine are known to be evoked by another agonist, taurine, which has a lower affinity for glycine receptors (Lewis *et al.*, 1991; Schmieden *et al.*, 1992; Vandenberg *et al.*, 1992). In 6 cells the effect of 10 μM LY-278,584 on the Cl^- responses to 100 μM taurine was studied. This concentration of taurine activated Cl^- responses of small amplitude (between 50 and 120 pA at -10 mV in these cells). As illustrated by Figure 9a, a potentiation of these responses was observed in the presence of LY-278,584. The average value of the percentage increase of the responses was $32\% \pm 4\%$ (6). Possible effects of MDL-72222 on responses to taurine were also investigated. As illustrated by Figure 9b, 100 nM MDL-72222 potentiated responses to 100 μM taurine by $27\% \pm 8\%$ (4). As previously described for glycine responses, increasing the concentration of MDL-72222 to 10 μM revealed a second opposite modulatory effect. MDL-72222 10 μM decreased responses to 100 μM taurine by $38\% \pm 16\%$ (5). Furthermore, during wash-out of this high concentration of MDL-72222, a slight rebound of the taurine response (above its control value) was observed (Figure 9c).

Persistence of the positive modulatory effect of ICS-205,930 in excised outside-out patches

A few experiments were performed in the outside-out configuration using the same solutions as in whole-cell experiments. ICS-205,930 was tested since in whole-cell experiments its positive modulatory effect was observed at low concentrations and was rapidly reversible. The method used was identical to that used in whole-cell experiments; brief applications of 20 μM glycine were regularly repeated (every 40 s), first in control, then in the continuous presence of ICS-205,930, then again in the absence of ICS-205,930. However, in these outside-out experiments, in order to obtain a reliable mean glycine response, it was necessary to average many successive traces recorded during identical glycine applications. Figure 10 illustrates the result of such an experiment. The mean glycine response (only 3 pA in the control) was almost doubled in the presence of 100 nM ICS-205,930 and recovered its control value after wash of ICS-205,930. The persistence of the positive modulatory effect of ICS-205,930 on outside-out patches was confirmed in 3 other experiments where a stable and large enough mean glycine response ($\geqslant$ 4 pA at 0 mV) was recorded before test of ICS-205,930. In other patches where the glycine response decreased spontaneously with successive glycine applications, the effect of ICS-205,930 was less easy to quantify but was still detectable.

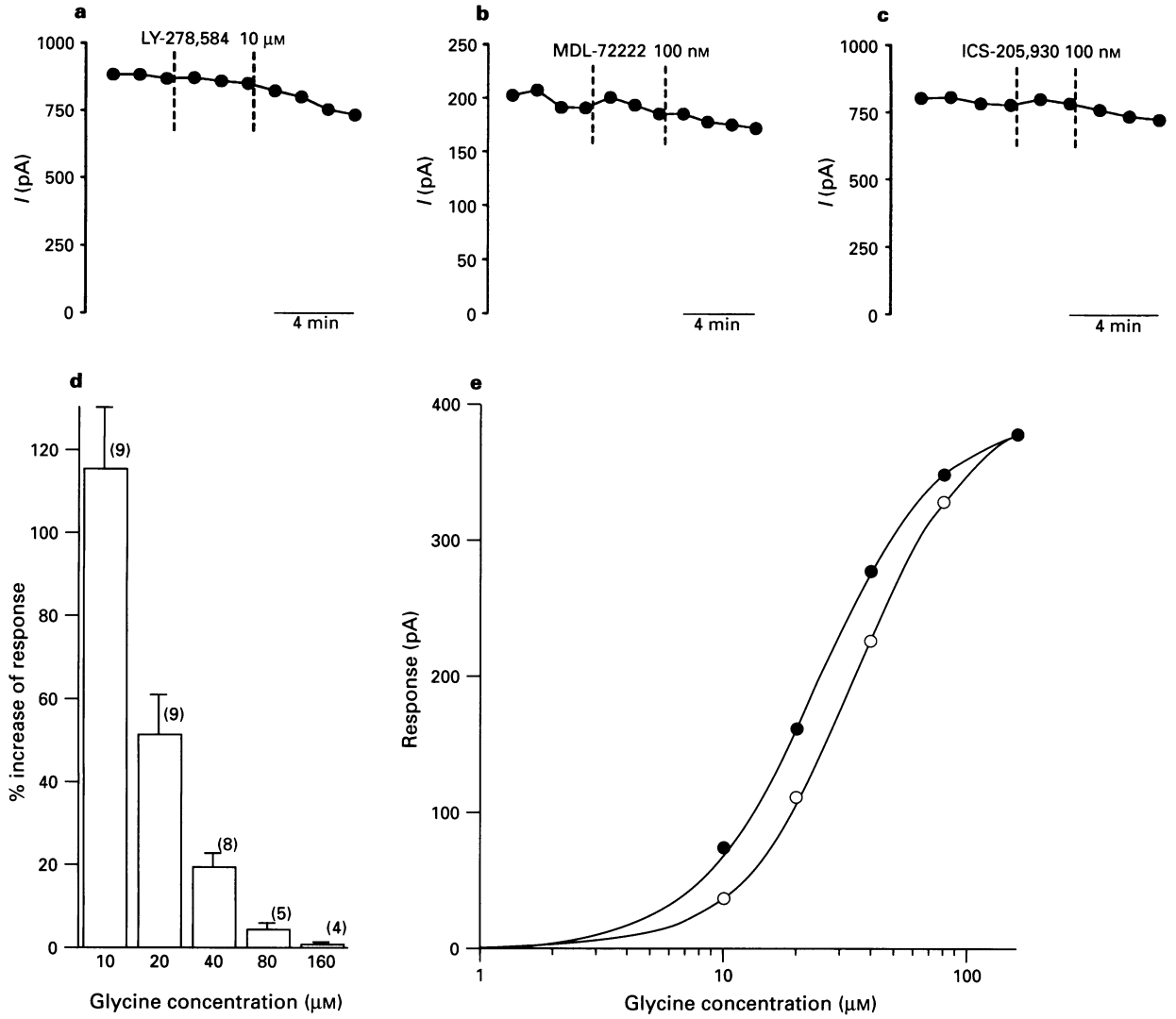

Figure 7 Sensitivity of the positive modulatory effects of LY-278,584, MDL-72222 or ICS-205,930 to the concentration of glycine. (a), (b) and (c) Absence of potentiation of responses to high concentrations of glycine. Successive values of the peak amplitude of the response to 80 μM glycine of three different cells exposed to 10 μM LY-278,584 (a), 100 nM MDL-72222 (b) or 100 nM ICS-205,930 (c). These compounds were applied, as usual, with preincubation. (d) and (e) An increase in affinity for glycine accounts for the potentiation of responses to low concentrations of glycine by ICS-205,930. Results from experiments to establish dose-response curves in the absence and presence of 200 nM ICS-205,930 (applied without preincubation). For each glycine concentration, the response was measured successively without, with, and again without ICS-205,930. This was done first with the lowest glycine concentration tested (10 μM), then with increasing concentrations of glycine (20, 40, 80 and 160 μM). When the responses recorded before and after test of ICS-205,930 differed, the experiment was eliminated. (d) Mean value of the percentage increase by ICS-205,930 of the peak response to various concentrations of glycine (indicated below each column). (e) Example of the dose-response curves derived from a single cell in the absence (○) and in the presence (●) of ICS-205,930. Each curve has been fitted independently by the following equation

$$y = \frac{y_{max}}{1 + \left(\frac{EC_{50}}{x}\right)^{n_H}}$$

The values derived from this cell were very similar in the absence and presence of ICS-205,930 both for y_{max} (402±4 pA and 393±4 pA, respectively) and n_H (1.80±0.05 and 1.72±0.05, respectively). In contrast, EC_{50} was reduced by about 30% by ICS-205,930, being 34.9±0.7 μM in control and 24.5±0.5 μM in ICS-205,930 (errors given by the fitting programme). Glycine was applied every 80 s and the holding potential was −30 mV in all the experiments described here.

Discussion

Blocking effect of mCPBG on glycine responses

The initial observation of the present paper was the blocking effect of micromolar concentrations of mCPBG on the Cl$^-$ response to a low concentration of glycine (20 μM). Even though mCPBG is regarded as a potent and selective agonist of 5-HT$_3$ receptors (see Hoyer *et al.*, 1994), this blocking effect appears to be unrelated to the 5-HT$_3$ agonist properties of mCPBG for two main reasons. First, the blocking effect of mCPBG was observed without simultaneous effect on the resting conductance of the cells (whereas activation of 5-HT$_3$ receptors should have induced an increase in cationic conductance). Secondly, other 5-HT$_3$ agonists (PBG and 2-methyl-5-HT, up to 20 μM) did not mimic the blocking effect of mCPBG.

The mCPBG concentrations inducing a strong reduction of glycine responses are close to those which maximally activate the cationic response of recombinant 5-HT$_3$ receptors expressed in *Xenopus* oocytes (see Figure 3b in Maricq *et al.*, 1991) or the 5-HT$_3$ response of N1E-115 cells (Sepulveda *et al.*, 1991; EC_{50} of

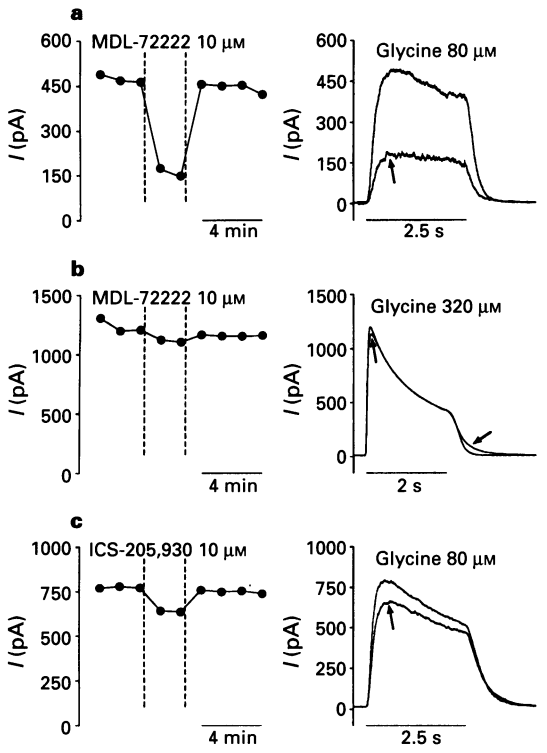

Figure 8 Effects of 10 μM MDL-72222 (a and b) or ICS-205,930 (c) on the responses to high concentrations of glycine. Responses to 80 μM glycine (a and c) were reversibly reduced by both compounds, and no rebound was observed during wash of the modulators. The response to 320 μM glycine (b) was almost unaffected by 10 μM MDL-72222. Holding potential −30 mV. Arrows indicate traces in the presence of MDL-72222 (a, b) and ICS-205,930 (c).

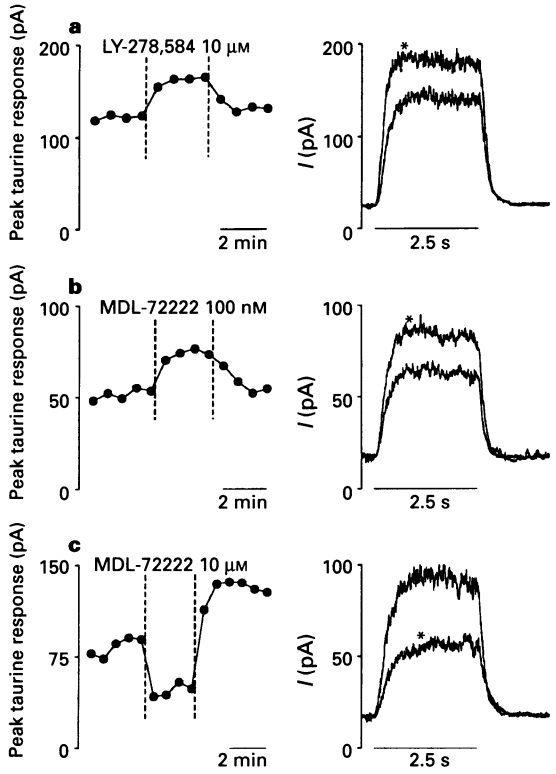

Figure 9 Modulation of taurine responses by LY-278,584 and MDL-72222. Left panels, amplitude of successive peak responses to 100 μM taurine. Right panels, mean of 3 (a) or 4 (b, c) taurine responses recorded before and during (*) the application of the modulator tested. (a) LY-278,584 10 μM; (b) MDL-72222 100 nM; (c) MDL-72222 10 μM. Holding potential −10 mV in (a) and (c), −30 mV in (b). (a), (b) and (c) derived from three different cells.

mCPBG of 1.4 μM) and NG 108-15 cells (Boess *et al.*, 1992; EC_{50} of mCPBG of 3.1 μM). However, from radioligand binding data, it has been reported that the affinity of mCPBG for 5-HT_3 receptors is in the nanomolar range (see references in Hoyer *et al.*, 1994). This strong discrepancy between the affinity values derived from electrophysiological experiments and binding experiments has been interpreted as a preferential binding of mCPBG to some high-affinity desensitized state of 5-HT_3 receptors (Sepulveda *et al.*, 1991; Boess *et al.*, 1992).

The blocking effect of mCPBG on glycine responses was not markedly voltage-dependent. It was sensitive to the glycine concentration and no longer detectable for a high glycine concentration (80 μM), which suggests some competition between mCPBG and glycine on the glycine receptors.

Potentiating effects of LY-278,584, MDL-72222 and ICS-205,930 on glycine response

The main result of the present paper is the demonstration that glycine responses can be potentiated by three related chemicals: LY-278,584, in the 1–10 μM concentration-range, MDL-72222 and ICS-205,930 in a remarkably low concentration-range, between 10 and 100 nM. Even though these three compounds are well-known 5-HT_3 receptor antagonists, their potentiating effects on glycine response appear to be unrelated to their 5-HT_3 antagonist properties. None of them induced any change of the resting conductance of the cells in the concentration-ranges of their potentiating effects. In the case of LY-278,584, the concentrations inducing a potentiation of glycine responses (micromolar) are much higher than the K_D of this antagonist for 5-HT_3 receptors (values in the nanomolar range: Zifa & Fillion, 1992). The order of potency of the three compounds is different for their potentiating effect on glycine responses (MDL-72222 ~ ICS-205,930 >> LY-278,584) and for their antagonist effects on 5-HT_3 receptors (ICS-205,930 ~

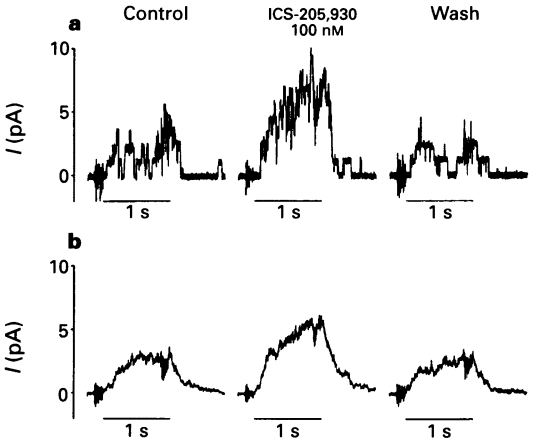

Figure 10 Persistence of the potentiation of glycine responses by ICS-205,930 after excision of a membrane patch. Outside-out patch held at −10 mV. Individual responses (a) to 1 s applications of glycine (indicated by the lower bars) and mean of 10 successive individual traces (b) recorded in control, in the presence of 100 nM ICS-205,930 and after wash of this modulator. The noise visible at the beginning and at the end of each glycine application results from the movement of the perfusion system.

LY-278,584 > MDL-72222; see Zifa & Fillion (1992) for binding data; see also Maricq *et al.* (1991) for a physiological assay showing that ICS-205,930 is a more potent 5-HT_3 antagonist than MDL-72222). Furthermore, the potentiating effect of ICS-205,930 on glycine responses was very rapidly and completely reversible (see e.g. Figure 5a), whereas the antagonism of 5-HT_3 responses by nanomolar concentrations of

ICS-205,930 is known to be very slowly and often incompletely reversible (Neijt *et al.*, 1988; Yakel & Jackson, 1988; Lambert *et al.*, 1989; Maricq *et al.*, 1991).

However, in the case of MDL-72222, the concentrations inducing a potentiation of glycine responses are in the range of the affinity values reported for 5-HT$_3$ receptors (see Hoyer *et al.*, 1994 for data derived from binding assays; see e.g. Yang *et al.*, 1992 for a physiological assay on rat neurones).

The percentage potentiation of the response to 20 μM glycine by each of the modulators tested was variable from one neurone to the other. This variability might result from the heterogeneity of the glycine receptors expressed in the culture.

LY-278,584, MDL-72222 and ICS-205,930 were all able to potentiate the responses to low concentrations of glycine (10 or 20 μM) but could not potentiate responses to higher concentrations of glycine (80, 160 or 320 μM). Furthermore, it has been shown by fitting dose-response curves (such as those of Figure 7e) that ICS-205,930 reduces the EC_{50} for glycine without affecting the Hill coefficient or the maximum response. These results suggest that all three chemicals induce an allosteric potentiation of glycine responses by increasing the affinity of glycine receptors for glycine. Except for Zn^{2+} ions (see below), the only compounds that have been described with similar properties are some volatile anaesthetics, like halothane, enflurane or isoflurane. However, these compounds, which also potentiate GABA$_A$ responses (see Wakamori *et al.*, 1991; Jones *et al.*, 1992, and included references), are active on glycine responses only at very high concentrations (K_D of 370 μM and 630 μM for halothane and enflurane, respectively; Wakamori *et al.*, 1991). Furthermore, these anaesthetics also have direct effects on the resting conductance recorded in cultured neurones, without any addition of inhibitory amino acids. Thus, the 5-HT$_3$ antagonists used in the present study seem to be interesting as a starting point for neurochemists wishing to develop a new family of potent and ideally, selective, modulators of glycine responses (see below for discussion concerning the effects of these 5-HT$_3$ receptor antagonists on GABA$_A$ responses).

Dose-dependent change in the modulatory effects of MDL-72222 and ICS-205,930, and non-specific effects of 5-HT$_3$ antagonists on GABA$_A$ responses

The positive modulatory effect of MDL-72222 on responses to 20 μM glycine was shown to increase with the MDL-72222 concentration between 10 and 100 nM but to decrease with a further increase of the MDL-72222 concentration (between 100 nM and 1 μM); a clear negative modulatory effect was detected with 10 μM MDL-72222 (Figure 4c). When using 80 μM (instead of 20 μM) glycine, no positive modulatory effect was detected but the negative modulation induced by 10 μM MDL-72222 was still detectable (Figure 8a). With an even higher glycine concentration (320 μM), the only effects induced by 10 μM MDL-72222 were a very small inhibitory effect and a slowing of the decline of the response. The mechanism of the negative modulatory effect of MDL-72222 is not clear. This effect was slightly voltage-dependent but was not use-dependent. It was rapidly reversible (Figure 8a), much faster than the positive modulatory effect of low concentrations of MDL-72222 (Figure 4a); this difference in the kinetics of reversibility of the two opposite modulatory effects of MDL-72222 explains the rebound potentiation of responses to 20 μM glycine during washout of high concentrations of MDL-72222 (Figure 4c).

The dose-response curve of the modulatory effect of ICS-205,930 was also found to be non-monotonic. However, in the case of ICS-205,930, it was necessary to use a high concentration of glycine (80 μM) to reveal clearly the small negative modulatory effect of high concentrations (10 μM) of this compound (Figure 8c). As in the case of MDL-72222, this inhibitory effect was rapidly reversible (Figure 8c). The apparent absence of effect of 10 μM ICS-205,930 on responses to 20 μM glycine and the rebound observed during wash (Figure 5e) can be explained by the superposition of two opposite effects cancelling each other during the ICS-205,930 application, a rapidly reversible inhibitory effect and a potentiation which was more slowly reversible during washout of ICS-205,930 from its initial high concentration (10 μM, three orders of magnitude higher than the threshold concentrations able to potentiate glycine responses).

Some non-specific effects of high concentations of 5-HT$_3$ receptor antagonists have been described and one of them is similar to the negative modulatory effect of 10 μM MDL-72222 on glycine Cl$^-$ responses. MLD-72222 10 μM is known to reduce some GABA$_A$ Cl$^-$ responses and all three 5-HT$_3$ receptor antagonists, LY-278,584, MDL-72222 and ICS-205,930, when used at 10 μM, were shown to reduce both muscimol-stimulated Cl$^-$ uptake and the binding of [^{35}S]-TBPS (a marker of the Cl$^-$ channel associated with GABA$_A$ receptors) in mouse cortical vesicles (Klein *et al.*, 1994).

Interestingly, Klein *et al.* (1994) also showed that concentrations of ICS-205,930 close to 1 μM can increase the Cl$^-$ response of some recombinant GABA$_A$ receptors expressed in *Xenopus* oocytes. However, no similar positive modulatory effect was detected with MDL-72222 or LY-278,584, whatever their concentration. Some of the homologies between the GABA$_A$ and glycine receptors might be responsible for the similarities of their modulations by ICS-205,930. In agreement with the results of Klein *et al.* (1994), a few experiments (unpublished observations) confirmed that, in the neurones that I used, neither MDL-72222 (100 nM) nor LY-278,584 (10 μM) could potentiate responses to a low concentration of GABA (2 μM). Thus, MDL-72222 appears to be particularly interesting, being inactive on GABA$_A$ responses but active on glycine responses, at concentrations which are much lower than those of other known modulators of glycine responses. Furthermore, unlike volatile anaesthetics, propofol and chlormethiazole (Wakamori *et al.*, 1991; Hales & Lambert, 1991; 1992; Jones *et al.*, 1992). MDL-72222 did not affect the resting conductance.

Other recent examples of modulations of glycine responses

Recently, potentiations of Cl$^-$ responses to glycine by zinc ions (100 nM and 1 μM) have been reported (Akagi *et al.*, 1993; Bloomenthal *et al.*, 1994; Laube *et al.*, 1995). These potentiations became weaker when the Zn^{2+} concentration was increased above 1–10 μM to 0.1–1 mM, and in this high concentration-range inhibitions of glycine responses were observed (Bloomenthal *et al.*, 1994; Laube *et al.*, 1995). In the case of recombinant α_1 glycine receptors expressed in *Xenopus* oocytes, the potentiation of glycine responses by Zn^{2+} was shown to result from an increase in affinity for glycine (Laube *et al.*, 1995); note however that the control affinity of these receptors for glycine was low compared to the affinity of the same receptors expressed in mammalian cells (see e.g. Rundström *et al.*, 1994; Rajendra *et al.*, 1995) or to the affinity of the native receptors of spinal cord cultures (see Lewis *et al.*, 1991; Laube *et al.*, 1995). An amino acid sequence that is fully conserved in all the α subunits of glycine receptors has been shown to be implicated in the Zn^{2+}-induced potentiation (Laube *et al.*, 1995). It will be interesting to see whether the potentiations described in the present paper (which also result from an increase in glycine affinity) are additive with the potentiation induced by Zn^{2+}. This could help understanding of the underlying mechanisms.

Another recent report (Lynch *et al.*, 1995) describes surprising results which show some analogies with those of the present paper, even though the glycine receptors studied, the mutated human α_1 subunits R271L and R271Q responsible for 'startle disease', are obviously different from those studied here in spinal cord cultures. On these mutated α_1 receptors, which have a very low affinity for glycine (5–10 mM) (Langosch *et al.*, 1994), the usual agonists β-alanine and taurine are competitive antagonists (Rajendra *et al.*, 1995) and the usual antagonist, picrotoxin, is a biphasic modulator, being an allosteric potentiator at low concentrations (10 nM–3 μM) and

an antagonist at higher concentrations ($\geqslant 3$ μM); furthermore the potentiation results from an increase in glycine affinity, as suggested here in the case of LY-278,584, MDL-72222 and ICS-205,930 on native glycine receptors of cultured neurones. The residue 271 of α_1 was shown to be critical for transduction processes following agonist and antagonist binding (Rajendra *et al.*, 1995; Lynch *et al.*, 1995). It would be interesting to see whether this residue (or the analogous residue of other subunits of glycine receptors) is also important for the action of positive allosteric modulators of native glycine receptors.

In conclusion, the present paper has shown that, at concentrations close to 100 nM, two 5-HT$_3$ receptor antagonists, MDL-72222 and ICS-205,930, behave as positive allosteric modulators of glycine receptors, this effect being independent of their action on 5-HT$_3$ receptors. From the chemical structure of these molecules, and from the present knowledge of structure-activity relationships for 5-HT$_3$ antagonists, it should be possible to find potent and selective modulators of glycine receptors. Such drugs would be therapeutic candidates for example for 'startle disease'.

This work was supported by CNRS (URA 1257) and by a grant from the Association Française contre les Myopathies. I thank M. Helms for his contribution to a few experiments. I also thank P. Ascher for useful advice and J. Kehoe for helpful comments on the manuscript. I am very grateful to D. Levy for culturing the cells and to J. Pons for typing the manuscript.

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(Received March 6, 1996
Revised May 16, 1996
Accepted May 21, 1996)