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Redox modulation of $GABA_A$ receptors obscured by Zn^{2+} complexation☆

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Abstract

Redox reagents are thought to modulate γ-Aminobutyric acid type A ($GABA_A$) receptors by regulating the redox state of the N-terminal disulphide bridge. Examining the redox sensitivity of recombinant $GABA_A$ receptors in human embryonic kidney cells, using whole-cell patch clamp techniques, revealed that $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ receptors, which are both less sensitive to Zn^{2+} and H^+ modulation, ablated the potentiating effect of the reducing agent, dithiothreitol (DTT) seen for $\alpha1\beta2$ receptors. This effect could result from disruption to the redox signal transduction pathway or be due to DTT chelating Zn^{2+} from its H267 inhibitory binding site, consequently potentiating GABA-activated currents in $\alpha1\beta2$ but not $\alpha1\beta2^{H267A}$ or $\alpha1\beta2\gamma2$ receptors. A Zn^{2+} chelating agent, tricine, potentiated GABA currents for the αβ constructs and vertically displaced GABA dose–response curves, suggesting that these receptors are subject to some inhibition by basal Zn^{2+}. Tricine, did not affect the GABA currents of either $\alpha1\beta2^{H267A}$ or $\alpha1\beta2\gamma2$ receptors but did prevent the potentiation by 2 mM DTT and reduced the potentiation caused by 10 mM DTT on $\alpha1\beta2$ receptors. Thus, at low concentrations of DTT, a substantial component of the potentiation probably occurs via Zn^{2+} chelation from H267 in the ion channel. In contrast, at higher DTT concentrations, it is more likely to be acting as a redox agent, which modulates both αβ and αβγ subunit receptors.

Keywords: $GABA_A$ receptor; Redox; Zn^{2+}; Histidine; Chelation

1. Introduction

γ-Aminobutyric acid type A ($GABA_A$) receptors are the major inhibitory synaptic receptors in the brain and those that are expressed on the neuronal cell surface will be subject to regulation by numerous endogenous processes, including receptor trafficking, endocytosis, as well as modulation of function by phosphorylation, redox reagents and ions normally present at relevant concentrations in vivo, e.g. Zn^{2+} and H^+ (Kaila, 1994; Moss and Smart, 2001; Rabow et al., 1996; Sieghart, 1995; Smart et al., 1994).

$GABA_A$ receptors are presumed to be pentameric proteins (Nayeem et al., 1994) composed of combinations of the following subunits: α(1–6), β(1–3), γ(1–3), δ, ε, π and θ with the $\alpha1\beta2\gamma2$ isoform forming a dominant population in the CNS (Mehta and Ticku, 1999; Rabow et al., 1996). Each individual subunit is composed of four transmembrane domains, a large intracellular loop, an external N- and C-terminus, and two cysteine residues located on the N-terminus that are thought to participate in forming a disulphide bridge. This disulphide bridge has been considered to be prone to oxidation and reduction by redox reagents to cause a potentiation and reduction in the GABA-activated current, respectively (Amato et al., 1999; Pan et al., 1995, 2000). Regulation of $GABA_A$ receptor function by redox reagents is also dependent upon the receptor subunit composition causing potentiation of GABA-activated responses on $\alpha1\beta_i$ (where $i = 1,2$) subunit $GABA_A$ receptors that is largely reduced or ablated by co-expression with the γ2 subunit (Amato et al., 1999; Pan et al., 1995, 2000).

Exactly how these redox agents act on the structure

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of the $GABA_A$ receptor has not been addressed successfully. The reducing agent, dithiothreitol (DTT), which potentiated GABA-activated currents, may act by reducing the disulphide bridge, on the cystine residues, leaving intact sulphydryl groups on two separate cysteines. However, traditional attempts to confirm this structure as the site of action of redox agents have been difficult largely because mutation of individual cysteine residues in this part of the N-terminal cysteine loop domain prevents the mutated subunit from being functionally expressed in the $GABA_A$ receptor complex (Amin et al., 1994). Moreover, if the disulphide bridge can be reduced, then it should be possible to alkylate the free sulphydryls irreversibly preventing further reduction or oxidation. Alkylation has successfully prevented the action of redox agents on neuronal $GABA_A$ receptor function in one study (Pan et al., 1995) but had little effect when using recombinant $GABA_A$ receptors (Amato et al., 1999). So the precise structural components of the $GABA_A$ receptor that contribute to the redox modulation remain unclear.

An alternative mode of action, at least for the reducing agent, DTT, is that it could reduce the basal levels of Zn^{2+} in solution by forming a complex with the free thiol groups of the DTT molecule. Such an action would effectively reduce free Zn^{2+} which has been established as a potent inhibitor on αβ subunit containing $GABA_A$ receptors (Smart et al., 1994), thereby causing an apparent potentiation of GABA-activated currents. This study was designed to address this question and concludes that at least some of the actions ascribed to the reducing agent, DTT, are likely to be caused by the complexation of basal levels of Zn^{2+} and that a residual component of DTT is possibly due to a reduction of the $GABA_A$ receptor.

2. Methods

2.1. cDNA constructs and site-specific mutagenesis

The murine $GABA_A$ receptor α1 and β2 and γ2s subunit cDNAs were cloned into the vector pRK5. Site-specific mutagenesis was undertaken using oligonucleotides in conjunction with a primer-directed polymerase chain reaction method (Quickchange, Stratagene). Purified DNAs were then prepared using the Plasmid Maxi Kit (QIAGEN). The entire coding region of the β2 subunit mutant was sequenced using the BigDye ready reaction mix (Perkin–Elmer/Applied Biosystems) and an ABI 310 automated DNA sequencer (Applied Biosystems).

2.2. Cell culture and electroporation

Human embryonic kidney (HEK) cells were cultured as previously described (Wooltorton et al., 1997b). The cells were transfected in the presence of equal ratios of cDNAs for the $GABA_A$ receptor subunits (12 μg total) and 3 μg of green fluorescent protein (GFP) using electroporation (Gene Electropulser II). Electroporated cells were plated onto poly-L-lysine coated glass cover slips, which were used for electrophysiological recording 18–72 h after transfection.

2.3. Patch clamp electrophysiology

Membrane currents were recorded using whole-cell patch clamp technique from single GFP fluorescing HEK cells with an Axopatch 1-C amplifier (Axon Instruments). Patch pipettes (resistance 3–5 MΩ) were filled with a solution containing (mM): 120 KCl, 1 $MgCl_2$, 11 EGTA, 30 KOH, 10 HEPES, 1 $CaCl_2$, 2 adenosine triphosphate and 12 creatine phosphate; pH 7.11. The cells were continuously perfused with Krebs solution containing (mM): 140 NaCl, 4.7 KCl, 1.2 $MgCl_2$, 2.52 $CaCl_2$, 11 glucose and 5 HEPES. The Krebs pH was adjusted to 7.4 with 10 N NaOH. Membrane currents were filtered at 5 kHz (−3 dB, 6th pole Bessel, 36 dB/octave) and analysed with Clampex 8 (Axon instruments). Any change of more than 10% in the membrane conductance/series resistance resulted in the cessation of recording. Drugs and solutions were rapidly applied to the cells using a modified Y-tube positioned approximately 300 μm from the recorded cell. The response rise-times were within 20–30 ms (Wooltorton et al., 1997b).

2.4. Drugs and solutions

Tricine was dissolved in a Krebs solution to give a final concentration of 10 mM; this will lower the free Zn^{2+} concentration to less than 1 nM. The pH was returned to pH 7.4 using 10 N NaOH. DTT was dissolved in distilled water to give a concentration of 0.5 M and then added directly to the Krebs solution and to solutions containing GABA (1 and 10 μM) to give a final concentration of 2 and 10 mM.

2.5. Analysis of whole-cell current data

Peak amplitude membrane currents activated by GABA (I) were determined at −50 mV holding potential. GABA equilibrium concentration–response relationships were constructed by measuring the peak GABA currents, which were normalised to the response induced by 10 μM GABA in control Krebs at pH 7.4 (I_{10}) and subsequently fitted with the Hill equation

$$I/I_{10} = [1/1 + (EC_{50}/[A]^n],$$

where EC_{50} represents the concentration of GABA ([A]) inducing 50% of the maximal current evoked by a saturating concentration of GABA and n is the Hill coef-

ficient.The GABA concentration–response curve data were analysed using analysis of variance with a Bonferroni post hoc test. Significance was determined at the $P < 0.05$ level.

2.6. Estimation of total Zn^{2+} content in Krebs

Atomic absorption spectroscopy was used to calculate the concentration of total Zn^{2+} present in the Krebs solution. An Analyst 100 (Perkin–Elmer instruments, Bucks, UK) with a Zinc Hollow cathode lamp (Perkin–Elmer) was used. Solutions were aspirated into the flame and absorbance was measured at a wavelength of 213.9 nm. A calibration curve was constructed using solutions containing 0.1, 0.08, 0.06, 0.04 and 0.02 μg/ml $ZnCl_2$, these samples were measured nine times for the construction of a calibration curve. Samples of Krebs were measured nine times and the concentration of total Zn^{2+} calculated.

3. Results

3.1. Modulation of recombinant receptors by DTT

In HEK cells expressing α1β2 receptor constructs, the response to 10 μM GABA was potentiated by 124 ± 3%of control following exposure to 2 mM DTT in accord with previous studies (Amato et al., 1999; Pan et al., 2000). Moreover, GABA-activated responses evoked on the α1β2γ2 receptor subunit combination were relatively insensitive to a 2 mM application of DTT (Fig. 1). This reliance of redox modulation on subunit composition was reminiscent of the sensitivity of $GABA_A$ receptors to Zn^{2+} and H^+, where the γ2 subunit confers a reduced sensitivity against inhibition by Zn^{2+} and potentiation by H^+ relative to an αβ subunit containing receptor (Krishek et al., 1996; Wooltorton et al., 1997a). Given the similarity in subunit dependence, we assessed whether mutating a histidine (H) residue in the ion channel lining, H267, in the mutant receptor construct, $\alpha1\beta2^{H267A}$, which markedly reduced the sensitivity of the receptor to inhibition by Zn^{2+} and potentiation by H^+ (Wooltorton et al., 1997a; Horenstein and Akabas, 1998; Wilkins et al., 2002), would similarly influence the action of redox agents. Indeed, membrane currents activated by 10 μM GABA on $\alpha1\beta2^{H267A}$ receptors, were insensitive to 2 mM DTT indicating that H267 may play a pivotal role in the modulation of $GABA_A$ receptor function by redox and other endogenous agents, or that the effect of DTT is dependent on the removal of Zn^{2+} from H267 in the ion channel of the $GABA_A$ receptor.

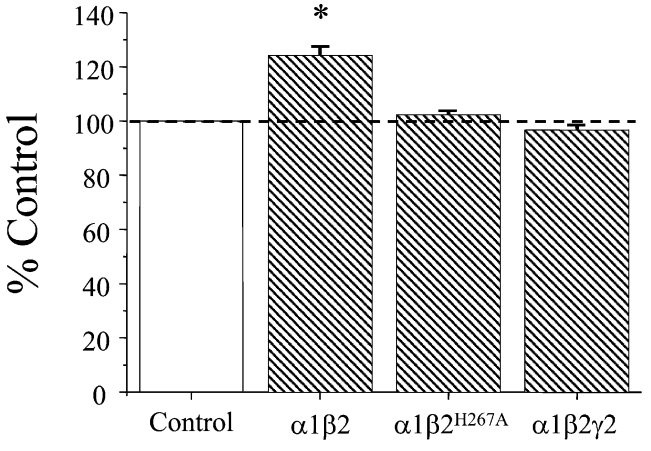

Fig. 1. DTT modulation of GABA-activated currents on α1β2, $\alpha1\beta2^{H276A}$ and α1β2γ2 receptors. Bargraph of GABA current activated by 10 μM GABA in the absence and presence of 2 mM DTT. All currents were normalised with respect to the control GABA-activated currents (= 100%) with each receptor construct and were altered in the presence of DTT by: 24 ± 3,2 ± 1 and −3 ± 2% for α1β2, $\alpha1\beta2^{H267A}$ and α1β2γ2 $GABA_A$ receptors, respectively. All bars, other than control, represent the mean ± s.e. from n = 3to8 cells. The ∗ indicates significance from control at $P < 0.05$.

3.2. Tricine potentiates GABA-activated currents on α1β2 receptors

To assess whether basal contaminating levels of Zn^{2+} were causing a partial, persistent inhibition of the α1β2 subunit containing $GABA_A$ receptors, the heavy metal chelating agent, tricine (*N*-tris[hydroxymethyl] methylglycine), that complexes with free Zn^{2+}, was added to the Krebs solution at a final concentration of 10 mM. At this concentration of tricine, and assuming levels of contamination of Zn^{2+} in the region of several hundred nanomoles, we expected free Zn^{2+} to be reduced to a level approaching 1 nM, a concentration that would not cause any inhibition to the function of αβ subunit $GABA_A$ receptors (Wooltorton et al., 1997a). GABA concentration–response relationships were constructed in the absence and presence of 10 mM tricine for α1β2 $GABA_A$ receptors (Fig. 2(A)). The curve in the presence of tricine was vertically displaced at each GABA concentration causing an increase in the maximum response to GABA when compared with the control maximum GABA response in the absence of tricine. The GABA EC_{50} was slightly reduced from 2.4 ± 0.4μM(control) to 1.1 ± 0.1μM(+tricine). This potentiation of the GABA-activated currents was probably due to the complexation of free Zn^{2+} from the Krebs solution thereby removing the basal inhibition of the $GABA_A$ receptor. At a GABA concentration of 10 μM, the addition of 10 mM tricine caused a 37 ± 8% increase in the amplitude of the GABA-activated current for α1β2 $GABA_A$ receptors (Table 1, Fig. 2(A)). The GABA concentration curve displacement by tricine with little change in the GABA EC_{50} was to be expected since Zn^{2+} is a non-competitive inhibitor on the $GABA_A$ receptor (Smart and Constanti, 1990). Similarly, the effect of DTT on the GABA con-

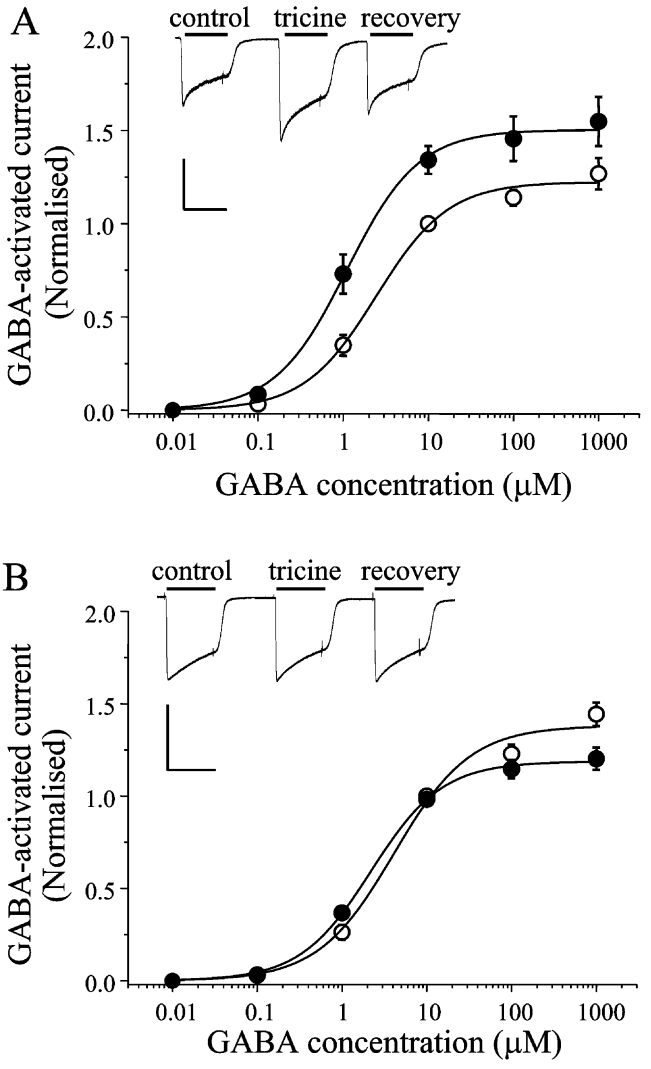

Fig. 2. Tricine potentiates the GABA current on α1β2 but not on $\alpha1\beta2^{H267A}$ $GABA_A$ receptors. GABA concentration–response relationships were constructed in the presence (●) and absence (○) of 10 mM tricine for (A) α1β2 and (B) $\alpha1\beta2^{H267A}$ $GABA_A$ receptors. The GABA-activated currents are normalised in each plot by the control response to 10 μM GABA in the absence of tricine (= 1). The curves were generated from the Hill equation (Section 2) and the points are means ± s.e. from n = 3to7cells. The insets illustrate 10 μM GABA-activated currents in control, +10 mM tricine and following recovery. Calibration bars represent 1000 pA and 2 s, respectively.

centration–response curve also resulted in a vertical displacement with virtually no change in the GABA EC_{50} (Amato et al., 1999).

3.3. Effect of tricine on $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ $GABA_A$ receptors

To ascertain whether the effect of tricine on the GABA concentration–response curve was due to the complexation of Zn^{2+}, we assessed the sensitivity to tricine of the mutant $\alpha1\beta2^{H267A}$ $GABA_A$ receptor which is markedly less sensitive to Zn^{2+} inhibition (Wooltorton et al., 1997a; Horenstein and Akabas, 1998). Application of tricine (10 mM) to these receptors failed to significantly affect the GABA concentration–response curve, which retained similar GABA EC_{50}s (from 4.1 ± 0.1μM (control) to 2.2 ± 0.1μM (+tricine); Fig. 2(B)), from the controls in the absence of tricine. Consequently, the 10 μM GABA-activated current amplitude was not potentiated over control GABA responses by tricine, attaining, 100 ± 1.4% for $\alpha1\beta2^{H267A}$ $GABA_A$ receptors (Table 1). This demonstrated that 10 mM tricine was not directly modulating $GABA_A$ receptor function in a non-specific manner, a feature that the $GABA_A$ receptor seems prone to with other modulatory agents, and unlikely to be chemically affecting other modulators of the $GABA_A$ receptor. The most parsimonious explanation was that tricine chelated basal levels of Zn^{2+}, thereby causing the displacement of Zn^{2+} from the presumed binding site formed, in part, by H267 in the ion channel lumen and consequently alleviated a persistent inhibition of the receptor. As an additional check on the specificity of tricine, similar experiments were conducted on $\alpha1\beta2\gamma2$ $GABA_A$ receptors that are also markedly less sensitive to Zn^{2+}. Tricine (10 mM) failed to affect the amplitude of the 10 μM GABA-activated current again (98.8 ± 3.1% of the control), suggesting that it does not have a direct effect on $GABA_A$ receptor function (Table 1).

Table 1
Effect of 10 mM tricine on α1β2, $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ $GABA_A$ receptors

$GABA_A$ receptor	Control (%)	GABA + tricine	Recovery
α1β2	100	137 ± 8∗	103 ± 2
$\alpha1\beta2^{H267A}$	100	100 ± 1	95 ± 5
$\alpha1\beta2\gamma2$	100	99 ± 3	102 ± 4

Using a control GABA concentration of 10 μM, only the α1β2 $GABA_A$ receptor is potentiated by 10 mM tricine. Both $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ are unaffected by 10 mM tricine. (mean ± s.e.;n = 4to12). The values represent normalised current amplitudes compared to their respective controls. The ∗ indicates significance at $P < 0.05$ from control.

We analysed the level of Zn^{2+} contamination in our solutions using the method of atomic absorption spectroscopy. Using a calibration range of $ZnCl_2$ solutions between 0.02 and 0.1 μg/ml, we estimated the level of total Zn^{2+} to be 0.06 μg/ml corresponding to concentration of approximately 440 nM in Krebs solution. This is in close agreement to other determinations ranging from 275 to 338 nM (Zheng et al., 1998).

3.4. Modulation of the $\alpha1\beta2$ $GABA_A$ receptor by DTT is affected by Zn^{2+} contamination

Given the likelihood of basal levels of Zn^{2+} present in Krebs, persistently inhibiting the function of αβ subunit

containing $GABA_A$ receptors, it was potentially significant that some or all of the action of DTT may be caused simply by complexation of Zn^{2+}. This would have the effect of removing Zn^{2+} from the presumed ion channel binding site, including H267, allowing an apparent potentiation in the GABA-activated response. This scenario would explain why the substitution of H267 abolished redox modulation since the potent inhibitory effect of Zn^{2+} would have been significantly reduced. This potential interaction between DTT and Zn^{2+} was analysed by measuring DTT-induced potentiation in the presence of tricine. In HEK cells expressing $\alpha1\beta2$ subunit receptors, GABA (10 μM)-activated currents were potentiated by 2 mM DTT in the absence of tricine (124 ± 3%of control, $n = 5$); however, co-application of 2 mM DTT with 10 mM tricine caused no further potentiation of the GABA response over that elicited by tricine and GABA alone (Fig. 3(A)). Increasing the DTT concentration to 10 mM caused a larger potentiation in the response to GABA in control Krebs (164 ± 9%) that was substantially reduced in the presence of tricine (Fig. 3(A)). Lowering the concentration of GABA to 1 μM enabled larger potentiations of the GABA current in the presence of 2 mM DTT to be observed, attaining 172 ± 6% of control (Fig. 3(B)). Tricine again abolished the potentiation induced by 2 mM DTT using 1 μM GABA (109 ± 6%; Fig. 3(B)); however, raising DTT concentration to 10 mM elicited larger potentiations to the GABA current (236 ± 15%) that were not completely abolished by tricine (147 ± 20%;$P < 0.05$; Fig. 3(B)). Taken together, these results suggested that at lower concentrations of DTT (2 mM), most of the potentiation induced in the GABA response appears largely due to DTT complexing with basal levels of Zn^{2+}; however, at higher DTT concentrations (10 mM), the inability of tricine to ablate the DTT-induced potentiation would be in accord with DTT acting as a redox agent on the $GABA_A$ receptor.

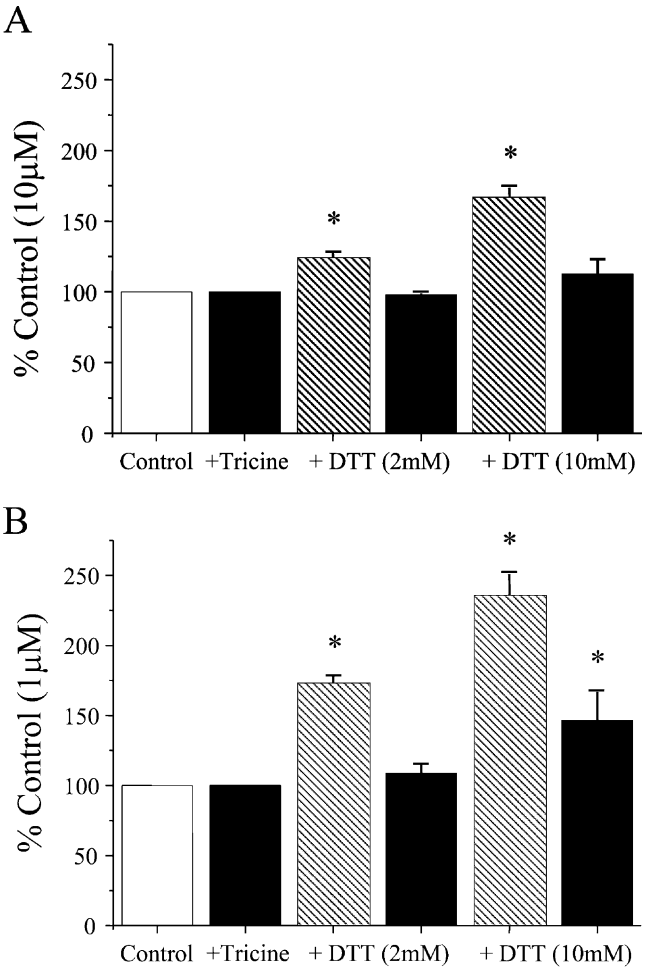

Fig. 3. Effect of DTT on $\alpha1\beta2$ $GABA_A$ receptors following Zn^{2+} chelation. The bargraphs represent GABA current amplitudes activated by (A) 10 μM and (B) 1 μM GABA in the absence and presence of 2 mM DTT and/or 10 mM tricine. All current amplitudes are normalised to the control GABA currents activated in normal Krebs or in 10 mM tricine (= 100%). The data were accrued from $n = 4$to8cells. The ∗ indicates significance from the controls in tricine at $P <$ 0.05.

3.5. Redox modulation of $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ subunit $GABA_A$ receptors

The redox effect of DTT was re-assessed on the mutant $\alpha1\beta2^{H267A}$ receptor, which would not be susceptible to inhibition by basal levels of Zn^{2+}. With this receptor, GABA-activated responses, using EC_{20} concentrations of GABA, were not potentiated by 2 mM DTT in the absence or presence of 10 mM tricine (Fig. 4). In contrast, 10 mM DTT induced a significant potentiation in the GABA-activated current to 161 ± 14% of control in the absence of tricine. This level of potentiation by DTT was unaffected by 10 mM tricine (163 ± 19% of control). These results suggest that on the mutant receptor, DTT is most probably acting as a redox agent rather than chelating background Zn^{2+}.

Similar to the $\alpha1\beta2^{H267A}$ receptor, the $\alpha1\beta2\gamma2$ subunit $GABA_A$ receptor also has a much lower sensitivity to Zn^{2+} compared to the $\alpha1\beta2$ receptor. We therefore investigated the effect of the higher concentration of DTT (10 mM) on the γ2 subunit containing receptor only in the absence of tricine since the Zn^{2+} chelating agent had no effect on this particular receptor subunit composition (Table 1). Using an EC_{20} concentration of GABA, exposure of $\alpha1\beta2\gamma2$ receptors to 2 mM DTT failed to produce any potentiation as previously reported (Amato et al., 1999). Increasing the DTT concentration to 10 mM, however, did potentiate the response to GABA (138 ± 14%;$P < 0.05$; Fig. 5), which would not have involved the chelation of Zn^{2+} by DTT.

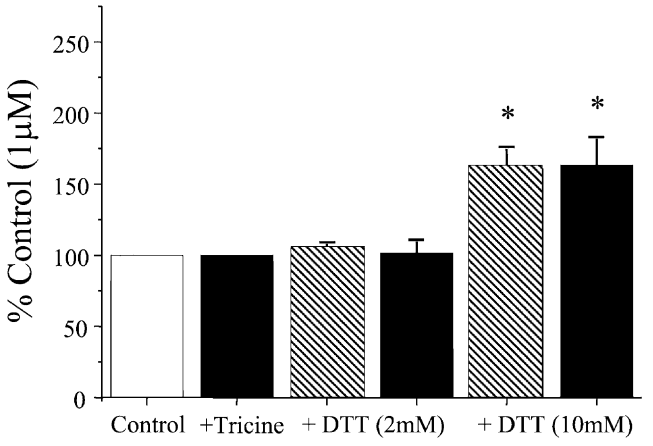

Fig. 4. Effect of DTT and tricine on $\alpha1\beta2^{H267A}$ $GABA_A$ receptors. The potentiations by 2 and 10 mM DTT of membrane currents activated by 1 μM GABA were examined in the presence and absence of 10 mM tricine. Bars are means ± s.e. from $n = 4$to7. The ∗ indicates significance from the controls at $P < 0.05$.

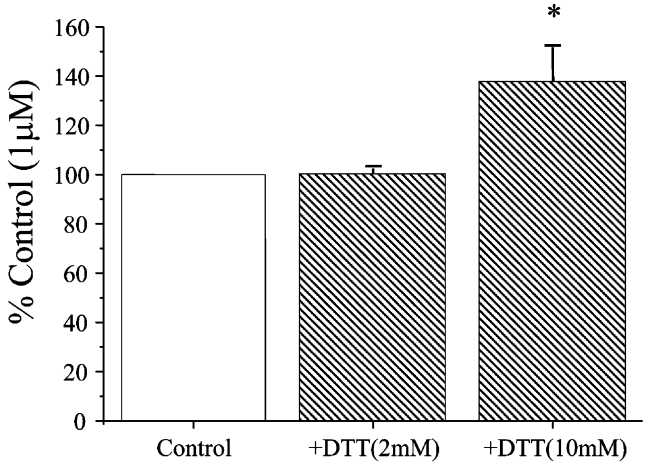

Fig. 5. Effect of DTT on the $\alpha1\beta2\gamma2$ $GABA_A$ receptors. Bargraph depicting the effect of 2 and 10 mM DTT on the 1 μM GABA-activated response recorded from $\alpha1\beta2\gamma2$ $GABA_A$ receptors in the absence of tricine. Data obtained from $n = 5$to6 cells. The ∗ indicates significance from control at $P < 0.05$.

4. Discussion

Redox modulation of $GABA_A$ receptor function has been presumed to rely on the oxidation/reduction state of the disulphide bridge in the N-terminal domain (Amato et al., 1999; Pan et al., 2000) that then affects the probability of GABA ion channel opening (Amato et al., 1999). However, it was a surprise to find that the ion channel histidine 267, in the β subunit, played such a significant role in the redox modulation of ion channel function. While this could have been interpreted as H267 being an important residue relaying the state of the disulphide loop domain to the ion channel, an alternative explanation was that DTT and Zn^{2+} chemically interacted in solution, which would serve to remove Zn^{2+} from its presumed binding site at H267 and thus alleviate a standing inhibition of the αβ subunit containing $GABA_A$ receptor. The lack of effect of DTT on the $\alpha1\beta2$ receptor in the presence of tricine, or on the $\alpha1\beta2^{H267A}$ receptor in control Krebs, was in accord with DTT complexing Zn^{2+}.

At higher concentrations of DTT, the responses to GABA were further potentiated on $\alpha1\beta2$ receptors. This may have been due to further 'stripping' of Zn^{2+} from other Zn^{2+} binding sites on the $GABA_A$ receptor, thereby removing any residual inhibition; however, in the presence of tricine, the higher concentration of DTT still potentiated to a similar extent to that in the absence of tricine, suggesting that Zn^{2+} stripping of the $GABA_A$ receptor was probably not relevant or minimal after the removal of Zn^{2+} from H267. Furthermore, the higher concentrations of DTT (10 mM) also potentiated on the relatively Zn^{2+} resistant receptors of $\alpha1\beta2^{H267A}$ and $\alpha1\beta2\gamma2$ subunit $GABA_A$ receptors. Overall, these results indicate that at the higher concentrations of DTT, the potentiation of GABA-activated currents becomes less reliant on complexation of Zn^{2+} and the removal of a standing inhibition of the receptor, and potentially more reliant on a direct effect on $GABA_A$ receptor function, presumably as a redox reagent.

A recent study addressing the effect of redox reagents on the $\alpha1\beta2$ subunit $GABA_A$ receptors also concluded that the potentiating effect of DTT might be partly explained by Zn^{2+} complexation (Pan et al., 2000). Of interest, DTT failed to affect the function of homomeric $GABA_C$ ρ1 subunits and these are also susceptible to block by Zn^{2+} (Wang et al., 1996), suggesting that the complexation of Zn^{2+} by DTT for these receptors must be quite weak (Pan et al., 2000) However, ρ1 subunits are considerably less sensitive to Zn^{2+} (IC_{50} ~20 μM, unpublished observations) compared to their αβ $GABA_A$ receptor counterparts, and thus basal contaminating levels of Zn^{2+} would be predicted to have little effect on the function of ρ1 subunit GABA receptors. Thus under these conditions, application of DTT, acting as a Zn^{2+} chelator would be expected to have virtually no effect.

Overall, we conclude that if redox reagents modulate $GABA_A$ receptor function in vivo, then part of their effect may be caused by Zn^{2+} complexation, which will be most pertinent for αβ receptor constructs; and secondly, to observe a direct effect of redox agents on the $GABA_A$ receptor that may be ascribed to redox modulation, will require higher concentrations of redox agents than previously envisaged (Amato et al., 1999).

Acknowledgements

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