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Modulation of γ-aminobutyric acid responses in the rat optic nerve

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Abstract

Depolarising $GABA_A$ receptor-mediated responses recorded from the optic nerve using a grease gap technique were modulated by classical potentiators of $GABA_A$ receptors. The benzodiazepine, chlordiazepoxide, the barbiturate, pentobarbitone and the widely used anaesthetic, propofol, all potentiated γ-aminobutyric acid (GABA) responses. They did so with different maximal efficacies, propofol > pentobarbitone > chlordiazepoxide, and potencies on the basis of EC_{50} estimates, chlordiazepoxide > propofol > pentobarbitone. The greater than expected GABA potentiating properties of propofol were explained by a direct hyperpolarising action that occurred in the same concentration range as its action at the $GABA_A$ receptor but that was unlikely to be mediated by $GABA_A$ receptors.

Keywords: GABA (γ-aminobutyric acid); Anaesthetic; Benzodiazepine; Optic nerve; Propofol

1. Introduction

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the visual system with a number of actions (Sandell, 1998). GABA immunopositive axons are present in the optic nerve (Wilson et al., 1996) and GABA acts physiologically to depolarise the optic nerve, most likely mediated by movement of Cl^- through the $GABA_A$ receptor channel (Simmonds, 1983; Green and Halliwell, 1997). GABA also alters the electrically recorded compound action potential in the optic nerve and increases extracellular K^+ levels (Sakatani et al., 1994). All these actions of GABA are sensitive to both known and novel $GABA_A$ receptor antagonists (Green and Halliwell, 1997). Here, we examined the effects of three positive modulators of the $GABA_A$ receptor, chlordiazepoxide, a benzodiazepine, the sedative anaesthetic pentobarbitone and propofol, a widely used anaesthetic. These modulators all act at different sites on the $GABA_A$ receptor (Belelli et al., 1996; Fukami et al., 1999) and for the purposes of establishing the optic nerve preparation as a model GABA system, it was important to compare their distinct actions. Chlordiazepoxide and pentobarbitone potentiated optic nerve depolarising GABA responses, to different but predictable extents. In contrast, propofol potentiated GABA responses to a greater than expected level, and to a greater extent than pentobarbitone. A possible explanation lies in the additional direct hyperpolarising action of propofol observed on this preparation.

2. Materials and methods

Two optic nerves were rapidly dissected from male Sprague–Dawley rats (age range was 4–6 weeks) (Harlan Olac, UK) killed by a Home Office approved decapitation method without anaesthesia. The optic nerves were placed in ice-cold, oxygenated (95% O_2/5% CO_2), artificial cerebrospinal fluid (aCSF) of the following composition: NaCl, 135 mM; KCl, 3 mM; NaH_2PO_4, 1.25 mM; $MgCl_2$, 1 mM; $CaCl_2$, 2 mM; glucose, 5 mM; $NaHCO_3$, 26 mM. The two nerves were separated at the optic chiasma and trimmed at one end. The nerves were placed across a greased barrier (vacuum grease, BDH, UK) with the trimmed nerve end exposed to a flow of oxygenated aCSF through the bath while the other uncut end remained in a static aCSF bath. The flow of aCSF was approximately 2.5 ml/min and we maintained the temperature of the bath at 24°C. Electrical recordings from the static bath and the perfused bath were made with Ag/AgCl pellets (Clark

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Electromedical, UK) and the differential signal output to a chart recorder (Kipp and Zoenen, SEMAT, UK). All chemicals were from Sigma, UK, except propofol which was obtained from RBI, UK and Aldrich, UK and pentobarbitone which was obtained as an 816 mM stock solution (Rhone Merrieux, France). Propofol was dissolved with 100% dimethyl sulphoxide and was diluted to less than 0.1% in the final solution. GABA (300 μM) was applied in the superfusate for 1 min every 25 min until a stable response was achieved (usually within three successive applications). A modulator was then applied to the preparation in the superfusate, 15 min before the next test GABA application. After the test GABA application, the modulator was washed out and recovery of the amplitude of the GABA responses was reliably observed.

The modulatory effects are expressed as a mean percentage increase in the amplitude of the GABA response before and after drug application. The concentration of GABA used in these studies was 300 μM, a concentration that gave a sub-maximal response, obtained from four concentration–response curves, shown in Fig. 1b. Concentration–response curves of the mean percentage increase in the amplitude of the GABA responses by different concentrations of the modulators were plotted (GraphPad Prism version 2.01, USA) and EC_{50} values were obtained from curve fits with this program using non-linear regression. Concentrations of modulators, which induced a 20% potentiation of the GABA response, were determined from individual experiments by linear interpolation.

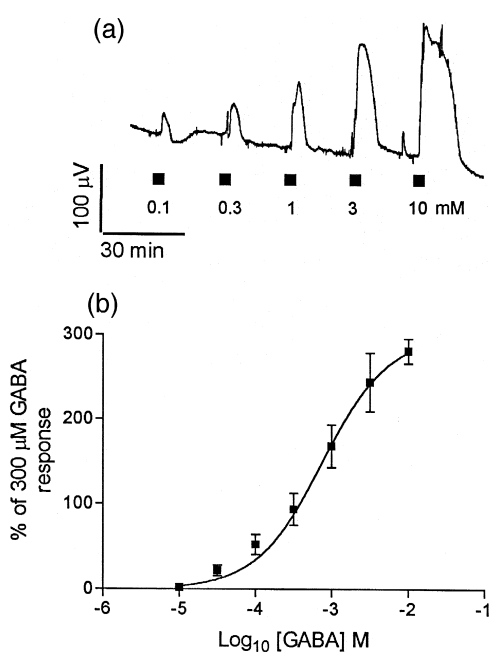

Fig. 1. (a) Increasing concentrations of GABA applied to the optic nerve preparation for 1 min through the superfusate lead to depolarising, upward, responses that reached a plateau in their amplitude. (b) Shows the mean concentration–response curves from four different optic nerve preparations from which an EC_{50} was estimated using the curve fit as shown, values and error bars are mean ± S.E.M.

3. Results

The optic nerve responded to GABA with a large depolarising response that returned to baseline as the GABA was washed off the preparation, shown in Fig. 1a, and as previously reported by Simmonds (1983). The concentration–response curve of the optic nerve preparation to GABA shows the EC_{50} to GABA to be approximately 770 μM (Fig. 1b). We used a concentration of GABA below this level, 300 μM, in all subsequent studies, to assess the alteration of the GABA response by the modulators. The responses to GABA were sensitive to the $GABA_A$ receptor antagonist, *N*-methyl bicuculline, IC_{50} was 2 μM (data not shown, $n = 8$) thereby confirming that the responses were $GABA_A$ receptor-mediated (Green and Halliwell, 1997).

We studied the action of three GABA modulators by expressing the percentage increase in the response to 300 μM GABA evoked by varying concentrations of the modulators. The concentration–response curve (Fig. 1) showed that 3 mM GABA gave a maximal response that was approximately 300% of the response to 300 μM GABA (see Fig. 1). This meant that the maximum observable potentiation by a modulator in this preparation was 200% above the stable 300 μM GABA control response. An example of the potentiation of a control GABA response by 300 μM of the barbiturate pentobarbitone is shown in Fig. 2a. Pentobarbitone potentiated the GABA responses in a concentration-dependent manner and to the maximal observable extent (Fig. 2b, open triangles); EC_{50} was 96 μM, $n = 8$. At concentrations of 1 mM, pentobarbitone had a large, direct slow depolarising effect on the optic nerve that was sensitive to *N*-methyl bicuculline ($n = 4$, data not shown). In contrast, the classical benzodiazepine, chlordiazepoxide, caused a small, concentration-dependent increase in the amplitude of the GABA responses, EC_{50} was 0.18 μM, $n = 6$ (Fig. 2b, filled squares), and did not show any additional actions. Propofol also positively modulated the amplitude of the GABA response in the optic nerve in a concentration-dependent manner, EC_{50} was 4.2 μM, $n = 7$ (Fig. 2b, open diamonds). To compare directly all three $GABA_A$ receptor modulators with their widely differing potentiating effects, we calculated the concentration of each compound that gave a 20% increase in the GABA response. The value of 20% was chosen since it was a level of potentiation that was achieved by all three compounds. For each compound, in separate experiments, the concentrations that gave a 20% potentiation were approximately 0.4 μM for both chlordiazepoxide and propofol ($n = 4$ for both compounds) while for pentobarbitone, the value was approximately 9 μM ($n = 4$). Also of note was the greater efficacy with which propofol potenti-

ated the GABA response compared with chlordiazepoxide (see Fig. 2b). Surprisingly, the maximum potentiating effect of propofol on the amplitude of the GABA response was greater than the 200% increase expected from the maximally observed GABA response (see Fig. 1b), and greater than we observed with high concentrations of pentobarbitone (see Fig. 2b). Like pentobarbitone, propofol has been shown to directly activate the $GABA_A$ receptor at high concentrations (Belelli et al., 1996). In contrast, we observed at concentrations as low as 3 μM that propofol caused a concentration-dependent, slow hyperpolarising response, shown in Fig. 3a. This was unlike the depolarising response characteristic of GABA or the direct depolarising effect of pentobarbitone, or the direct depolarising effect of propofol seen in other preparations (Hales and Lambert, 1991). Controls confirmed that these responses were not simply due to solution changes or the solvent. Unlike the direct depolarising influence of high concentrations of pentobarbitone ($n = 4$, data not shown), the direct propofol-induced hyperpolarisations were not blocked by the $GABA_A$ antagonist *N*-methyl bicuculline ($n = 3$). Rather, we observed a small increase in the hyperpolarisation after bicuculline application suggesting that a covert $GABA_A$-mediated propofol-induced depolarisation might underlie the more effective propofol-induced hyperpolarisation.

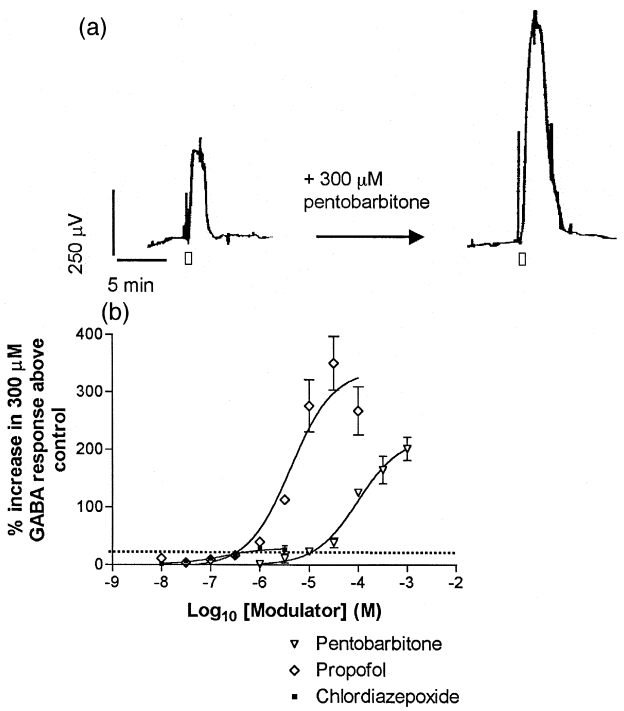

Fig. 2. (a) Pentobarbitone (300 μM) caused an increase in the amplitude of the GABA response, GABA, 300 μM. GABA was applied for 1 min at 25-min intervals. In the example shown, pentobarbitone was applied for 15 min. In (b), a summary of the concentration–response curves for the modulatory effects of the three chosen modulators is shown; error bars represent S.E.M. Pentobarbitone (open triangles) caused a concentration-dependent increase in the submaximal 300 μM GABA response, to the maximal possible level, approximately 200% as determined from the concentration–response curve to GABA (Fig. 1b). Predictably, the benzodiazepine, chlordiazepoxide (filled squares) potentiated GABA responses to a small extent and in a concentration-dependent manner. Propofol (open diamonds) lead to a concentration-dependent increase in the GABA response that was greater than the predicted maximum. The dotted line at 20% potentiation of the 300 μM GABA response indicates the potentiation achieved by all three modulators.

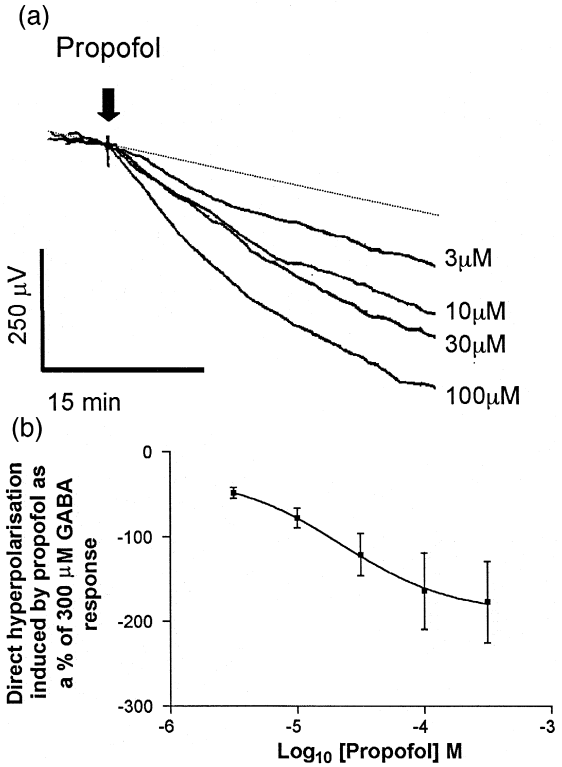

Fig. 3. (a) Shows the direct, concentration-dependent hyperpolarisation of the optic nerve by propofol. The effect is seen as a slow negative change in potential on top of the small negative drift in the baseline potential as shown by the dotted line. The concentration dependence of the hyperpolarisation is expressed in the individual experiments as a percentage of the response to 300 μM GABA in the same experiment, as shown in (b). It was difficult to reliably measure responses $< 50\%$ of the GABA response because of the slow onset of the hyperpolarisation in the presence of the slow baseline drift.

4. Discussion

We have shown that three known positive modulators of $GABA_A$ receptor function potentiated $GABA_A$ responses in the optic nerve preparation. The small extent, but high potency, with which the benzodiazepine, chlordiazepoxide potentiated GABA responses in the optic nerve contrasted with the low potency but maximal extent of potentiation by the anaesthetic/sedative compound pentobarbitone. Our results are broadly consistent with previous findings of both these classes of compound on the functional action of recombinant $GABA_A$ receptors expressed

in *Xenopus* oocytes (Sigel and Baur, 1988; Belelli et al., 1996; Fukami et al., 1999) and on $GABA_A$ergic synaptic inhibition in the hippocampus (Segal and Barker, 1984). However, our data contrast with previous findings with regard to the increased potency and efficacy of propofol compared with pentobarbitone (see Fig. 2a). Previous studies have shown propofol to potentiate $GABA_A$ responses with an EC_{50} of approximately 20 μM (Fukami et al., 1999).

Both the greater than expected maximal potentiating effect of propofol and the observation that it was equipotent with chlordiazepoxide in potentiation of GABA responses by 20%, were unexpected results. The data appeared to indicate an additional action for propofol in this preparation, which we believe to be best explained by direct, non-$GABA_A$ergic hyperpolarisation of the optic nerve by the drug. A hyperpolarisation would increase the driving force for movement of Cl^- through the $GABA_A$ receptor and, as we have shown, the result was a larger than expected maximal depolarisation in response to GABA and an increase in apparent potency. The precise mechanism that contributes to the direct hyperpolarisation by propofol remains unknown. However, given the smooth muscle relaxant (Cheng et al., 1996) and vasodilator properties of propofol (Kaye et al., 1999), together with the fact that other classes of anaesthetics open K^+ channels in neurones (Nicoll and Madison, 1982), an action of propofol to activate K^+ channels in the optic nerve is plausible. It is also possible that propofol activates a $GABA_B$ receptor linked K^+ channel, since functional $GABA_B$ receptors exist on optic nerve axons (Sun and Chiu, 1999).

In conclusion, our results show the optic nerve preparation to be a simple, robust model in which to study functional modulation of native $GABA_A$ receptor-mediated responses. The fact that three modulators, all of which are believed to act at different sites on the $GABA_A$ receptor (Belelli et al., 1996; Fukami et al., 1999), had significant effects indicates that the optic nerve $GABA_A$ receptor possesses the pentobarbitone, benzodiazepine and propofol sensitive sites. As such, the optic nerve presents a useful model of native $GABA_A$ receptors. In addition, the preparation also provides scope to detect non-$GABA_A$ergic-mediated responses of $GABA_A$ receptor modulators.

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