Br. J. Pharmacol. (1993), **108**, 711–716

The influence of the γ_{2L} subunit on the modulation of responses to $GABA_A$ receptor activation

A.L. Horne, P.C. Harkness, K.L. Hadingham, P. Whiting & [1]J.A. Kemp

Neuroscience Research Centre, Merck Sharp & Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex CM20 2QR

1 Whole-cell patch clamp recordings were made from L-cells transfected with 2 combinations of subunits of the $GABA_A$ receptor. Log concentration-response curves were constructed to γ-aminobutyric acid (GABA) on $\alpha_1,\beta_1,\gamma_{2L}$ containing cells and compared to those from α_1,β_1 containing cells. The effects of flunitrazepam, pentobarbitone and alphaxalone on the concentration-response relationships were also examined.

2 From the log concentration-response curves, GABA had a mean (± s.e.mean) $pEC_{50} = 5.2 \pm 0.09$ and slope factor = 1.7 ± 0.02 on $\alpha_1,\beta_1,\gamma_{2L}$ cells which were significantly different from the values obtained from α_1,β_1 cells where the $pEC_{50} = 5.6 \pm 0.02$ and the slope = 1.5 ± 0.02.

3 Flunitrazepam produced a parallel leftward shift of GABA concentration-response curves on $\alpha_1,\beta_1,\gamma_{2L}$ cells. The EC_{50} for flunitrazepam = 6.3 ± 2.7 nM. No increase in the maxima of the GABA concentration-response curves was found in the presence of flunitrazepam. Flunitrazepam did not potentiate responses from α_1,β_1 cells.

4 The log concentration-response curves from both populations of cells were shifted to the left by equal amounts by pentobarbitone. A significant increase in the maximal response to GABA was also produced by pentobarbitone. This occurred at lower concentrations of pentobarbitone on α_1,β_1 cells.

5 Alphaxalone produced leftward shifts of GABA log concentration-response curves of similar magnitudes in both populations of cells. Significant increases in the maxima were found at 100 nM in α_1,β_1 cells but not up to 1 μM in $\alpha_1,\beta_1,\gamma_{2L}$ cells.

6 These results provide further evidence of the modulatory role of the γ_{2L} subunit of the $GABA_A$ receptor containing α_1 and β_1 subunits. As well as influencing the apparent affinity of GABA and conferring benzodiazepine modulation, it also appeared to regulate the increase in maximal response produced in the presence of barbiturates and steroids. This latter effect may imply that barbiturates and steroids increase the channel open-state probability in the presence of GABA and that this effect is diminished by the presence of the γ_{2L} subunit.

Keywords: Concentration-response relationship; $GABA_A$ receptor; recombinant receptor; benzodiazepine; flunitrazepam; alphaxalone; pentobarbitone

Introduction

Potentiation of responses mediated by $GABA_A$ receptors has been observed with a number of compounds such as barbiturates (Nicoll, 1975), benzodiazepines (Choi *et al.*, 1977) and steroids (Harrison & Simmonds, 1984). These modulators selectively potentiate responses at this receptor, which has since been shown to be composed of a number of subunits that have been classified as members of α, β, γ etc. families (Schofield *et al.*, 1987; Pritchett *et al.*, 1989; see Luddens & Wisden, 1991 for review). Functional expression of γ-aminobutyric acid (GABA) receptors has provided a powerful tool to assess the pharmacological consequences and likely physiological occurrences of known combinations of these subunits (Schofield *et al.*, 1987; Pritchett *et al.*, 1989; Malherbe *et al.*, 1990; Puia *et al.*, 1990; 1991; Knoflach *et al.*, 1992).

Much of the preceding work on the modulation of responses of recombinant GABA receptors has involved measuring the %-potentiation of the response to a single concentration of agonist. Potentiation of individual responses produces a leftward shift of log concentration-response curves that has been well documented in intact preparations and isolated neurones (Barker & Ransom, 1978; Simmonds, 1981; Little, 1984). However, modulation of $GABA_A$ receptors by benzodiazepines, barbiturates and steroids is thought to involve separate sites on the receptor complex and is probably brought about by different mechanisms. Therefore, to investigate the underlying causes of $GABA_A$ receptor potentiation we have examined the effects of flunitrazepam, pentobarbitone and alphaxalone on the properties of the GABA log concentration-response curves produced in clonal cell lines stably transfected with known combinations of subunits. Furthermore, to investigate the effects of the γ_{2L} subunit we have compared the modulation of responses to GABA in cells transfected with $\alpha_1,\beta_1,\gamma_{2L}$ subunits (Hadingham *et al.*, 1992; Horne *et al.*, 1992) with those transfected with only α_1,β_1 subunits.

Methods

Mouse fibroblasts were maintained and transfected as described previously (Whiting *et al.*, 1991; Hadingham *et al.*, 1992). Cell lines containing α_1,β_1 or $\alpha_1,\beta_1,\gamma_{2L}$ (Whiting *et al.*, 1990) were plated onto coverslips 5 days prior to recording and expression was induced with 100 nM dexamethasone. During electrophysiological recordings, cells were bathed in a solution comprising (mM): NaCl 137, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 5, D-glucose 11 and sucrose 13, the pH was adjusted to 7.2 with NaOH. Patch pipettes contained (mM): CsCl 130, $MgCl_2$ 1, HEPES 10 and EGTA 11. The pH was adjusted to 7.3 with HCl (~13 mM) and intracellular and extracellular solutions were approximately isotonic (~310

[1] Author for correspondence.

mOsm). Membrane currents were monitored with a List LM-EPC7 amplifier at a holding potential of −20 mV. Series resistance was not monitored or compensated for, as we previously found no evidence to suggest that significant clamp errors occurred during experiments similar to those described here (Horne et al., 1992).

Log concentration-response curves were constructed to incremental concentrations of GABA applied rapidly from a double barrelled pipette (Horne et al., 1992). GABA was applied for 2–3 s with a 1 min wash period allowed before the next application to avoid any possible problems caused by desensitization at the higher concentrations. Responses were measured at peak height. Concentration-response curves were redetermined in the presence of modulator and both curves were fitted to the logistic equation:

$$y = R_{max}/(1 + (EC_{50}/x)^H),$$

where, R_{max} = maximal response, EC_{50} = concentration producing half maximal response, H = slope factor.

Concentration-ratios were calculated from the quotient of the EC_{50}s obtained from the fitted curves in the absence and presence of modulator.

All bulk materials were obtained from BDH or FSA. GABA, flunitrazepam, EGTA, HEPES and pentobarbitone Na were obtained from Sigma. Flunitrazepam and alphaxalone were initially dissolved in ethanol at 10 mM, EGTA was initially dissolved in the minimum required CsOH. All other compounds dissolved into the bathing medium. All values are given as mean ± s.e.mean, and statistical analyses were by two-tailed Student's paired t test unless otherwise stated.

Results

GABA concentration-response curves

$\alpha_1,\beta_1,\gamma_{2L}$ GABA concentration-response curves were well fitted by the logistic equation (Figure 1) as reported previously for this cell line (Horne et al., 1992). From the values taken from individual curves a mean $pEC_{50} = 5.2 \pm 0.09$ and a mean slope factor = 1.66 ± 0.019 ($n = 96$) were obtained. A poorer fit to the data was obtained when the concentration-response curves were compared to a model of binding to 5 identical, independent sites (Figure 1) which, in turn, was a better fit than assuming any lower number of binding sites. When a second concentration-response curve was constructed on 5 cells, although a consistent reduction in maxima was obtained, there was no significant difference between the maxima ($P = 0.46$) or pEC_{50} ($P = 0.25$) of the initial and repeated curve (Table 1).

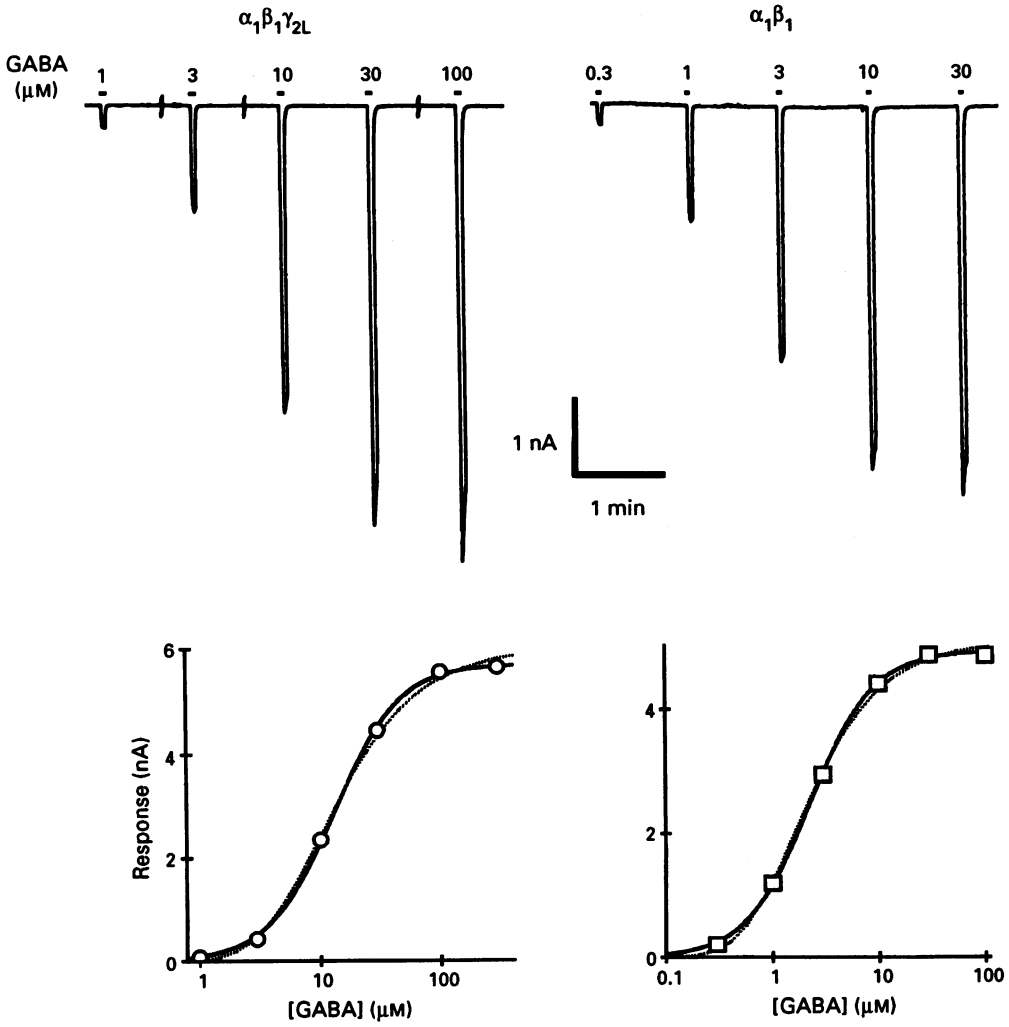

Figure 1 Log concentration-response curves to γ-aminobutyric acid (GABA). Top traces show continuous chart records of responses to incremental concentrations of GABA for receptors composed of $\alpha_1,\beta_1,\gamma_{2L}$ (left) and α_1,β_1 (right) subunits of the $GABA_A$ receptor. Responses were measured at peak height and plotted against log concentration of GABA as shown on the lower graphs which were taken from cells different from those illustrated above. These curves were well fitted by the logistic equation (see Methods, unbroken line). The broken line is an iterative fit to the data of the equation $y = R_{max}/(1 + EC_{50}/x)^5$ as a model of activation of 5 identical independent sites per receptor.

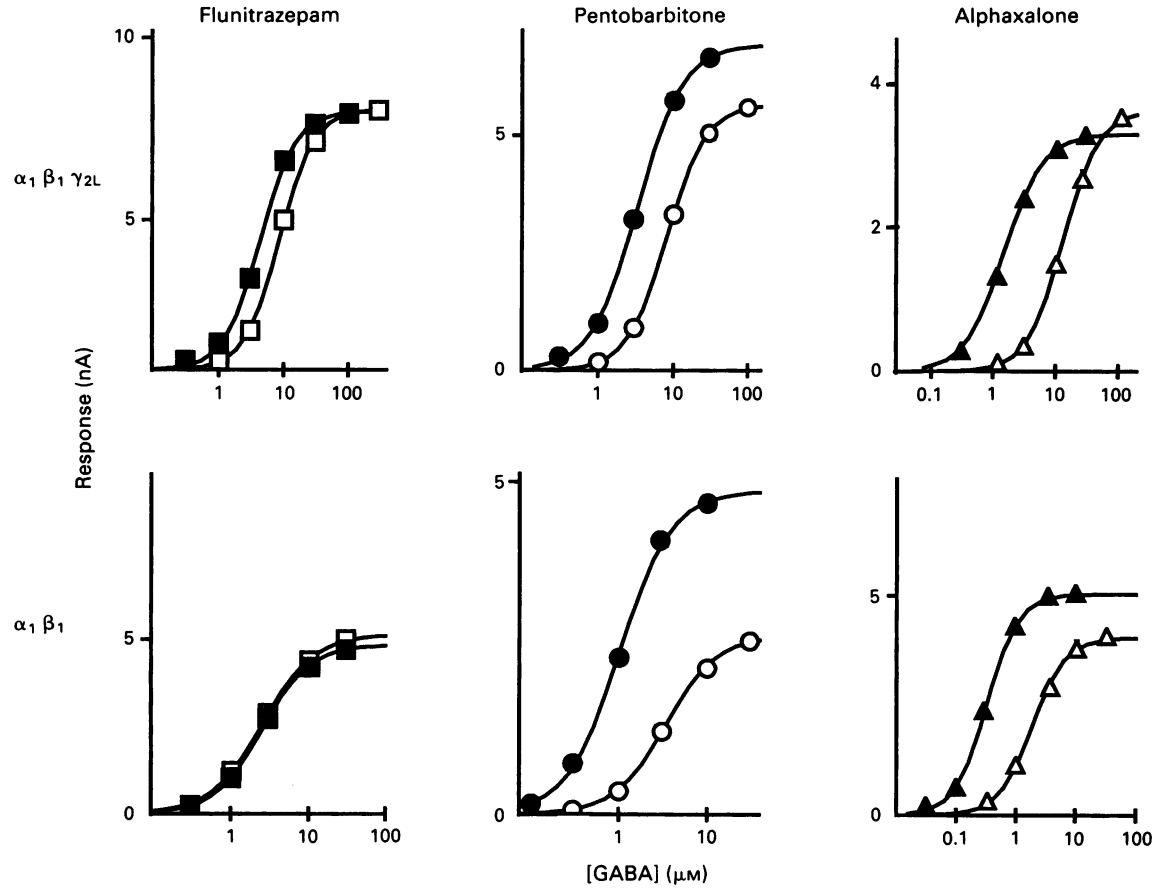

Figure 2 Modulation of γ-aminobutyric acid (GABA) concentration-response relationships. Each plot shows a control curve (open symbols) and one repeated in the presence of either 300 nM flunitrazepam, 30 μM pentobarbitone or 1 μM alphaxalone (filled symbols) for both combinations of subunits. All are fitted to the logistic equation. Each pair shows data taken from the same cell. Potentiation by flunitrazepam was only seen when the γ_{2L} subunit was present. With pentobarbitone and alphaxalone, potentiation was associated with an increase in maxima of the concentration-response curve on α_1,β_1 receptors. This increase in maxima was only seen with $\alpha_1,\beta_1,\gamma_{2L}$ in the presence of 30 μM pentobarbitone and not at all in alphaxalone up to 1 μM.

Table 1 Changes in the maxima of γ-aminobutyric acid (GABA) log concentration-response curves produced by flunitrazepam, pentobarbitone and alphaxalone

	$\alpha_1,\beta_1,\gamma_{2L}$		α_1,β_1	
Treatment	*Δmax*	n	*Δmax*	n
Control	−4.3 ± 4.2	5	−13.7 ± 2.9	7
Flunitrazepam				
300 nM	−5.2 ± 2.6	4	−13.5 ± 4.6	4
Pentobarbitone				
3 μM	−8.3 ± 2.7	3	+10.3 ± 4.3	4
10 μM	+0.9 ± 2.0	3	+35.1 ± 10.0*	3
30 μM	+12.6 ± 4.0*	4	+77.9 ± 18.8*	4
Alphaxalone				
1 nM	–	–	−7.7 ± 2.6	3
10 nM	+1.3 ± 5.0	4	−10.4 ± 4.7	3
30 nM	+0.2 ± 1.9	3	–	–
100 nM	−7.6 ± 5.1	3	+32.1 ± 15.3*	5
300 nM	−8.3 ± 1.1	3	–	–
1000 nM	−11.8 ± 1.8	3	+18.2 ± 7.9*	4

Log concentration-response curves were produced in cells transfected with the indicated combinations of subunits of the GABA$_A$ receptor. A second curve was constructed either in the absence (control) or presence of the stated concentration of modulator. Changes in the maxima (Δmax) are indicated as a mean percentage increase (+) or decrease (−) compared to the initial curve. Statistical analyses were performed on the untransformed maximal values.
*Statistically significant increase.

α_1,β_1 Log concentration-response curves for α_1,β_1 were also better fitted by the logistic equation than one describing up to 5 independent sites (Figure 1). A mean pEC_{50} of 5.6 ± 0.02 and a slope factor = 1.45 ± 0.019 (n = 58) were obtained. The probability of these mean values being identical to those from $\alpha_1,\beta_1,\gamma_{2L}$ cells was very low ($P<0.001$ for both EC_{50} and slope).

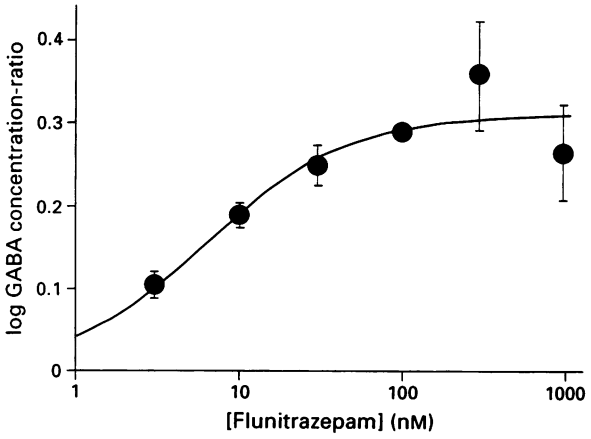

Figure 3 Log concentration-response curve to flunitrazepam. γ-Aminobutyric acid (GABA) concentration-ratios were calculated and plotted against the corresponding concentration of flunitrazepam. Each value is the mean ± s.e.mean (vertical bars) of 4 individual shifts.

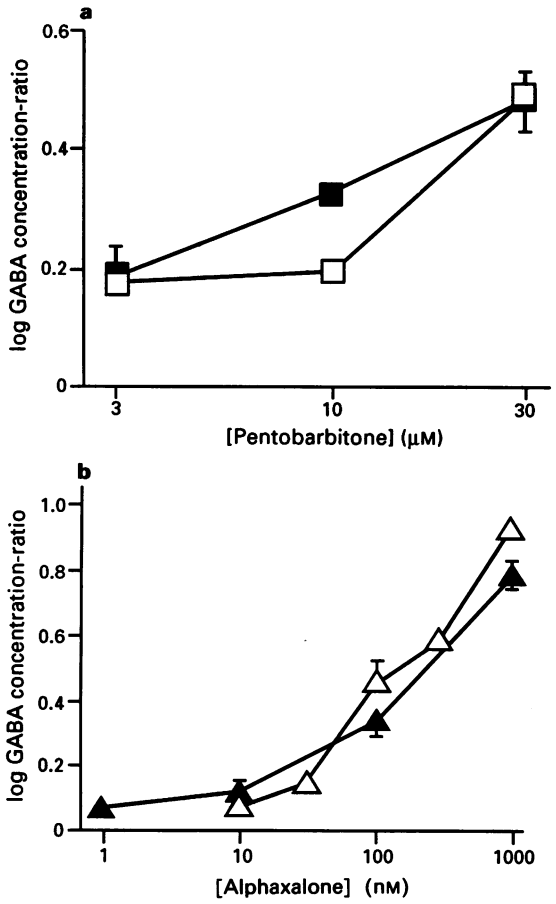

Figure 4 Log concentration-response curves to pentobarbitone and alphaxalone. γ-Aminobutyric acid (GABA) concentration-ratios were calculated in the presence of pentobarbitone (a) or alphaxalone (b) for both $\alpha_1,\beta_1,\gamma_{2L}$ (open symbols) and α_1,β_1 (filled symbols) combinations of subunits.

When a second concentration-response curve was produced on 7 of these cells a consistent and significantly different maximum ($P = 0.023$) was obtained (Table 1) but no significant difference in pEC_{50} was found between the initial and repeated curve.

Flunitrazepam

$\alpha_1,\beta_1,\gamma_{2L}$ Flunitrazepam (3–1000 nM) produced parallel shifts to the left of the log concentration-response curve to GABA (Figure 2) with no significant increase in maximum (Table 1). Individual log concentration-ratios were plotted against log flunitrazepam concentration and the logistic equation was fitted (Figure 3). From this plot, an $EC_{50} = 6.4 \pm 2.7$ nM, slope factor $= 0.99 \pm 0.49$ and maximum log concentration-ratio $= 0.31 \pm 0.03$ were obtained.

α_1,β_1 Flunitrazepam (300 nM) produced no significant change in either the EC_{50} or the maximum of the concentration-response curve to GABA (Figure 2, Table 1).

Pentobarbitone

$\alpha_1,\beta_1,\gamma_{2L}$ Pentobarbitone (3–30 μM) produced parallel shifts of the GABA concentration-response curves (Figure 2). GABA concentration-ratios were calculated at the level of the EC_{50} and plotted against log pentobarbitone concentration (Figure 4). Pentobarbitone (30 μM) produced a significant increase in the maximum ($P = 0.041$; Table 1) and a significant decrease in the slope factor (1.41 ± 0.08 compared to 1.64 ± 0.11 in controls: $P = 0.04$) of the GABA concentration-response curve. No significant difference in the maxima of the curves was produced by lower concentrations of pentobarbitone.

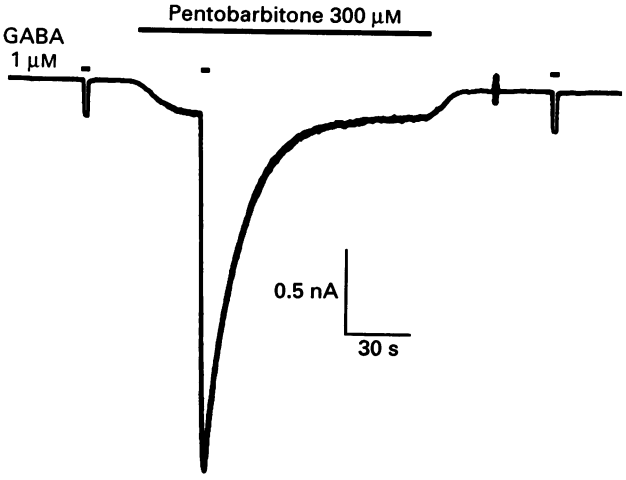

Figure 5 Direct activation of the γ-aminobutyric acid (GABA) receptor by pentobarbitone. The trace shows a response to GABA before, during and after the addition of 300 μM pentobarbitone from a cell transfected with α_1,β_1 subunits of the $GABA_A$ receptor.

At higher concentrations pentobarbitone has been shown to evoke an inward current in these cells (Horne *et al.*, 1992). Pentobarbitone (300 μM) evoked an inward current 0.2 ± 0.06 times that of 3 μM GABA and produced a potentiation $= 282 \pm 66.8\%$ ($n = 4$) of this response.

α_1,β_1 Leftward shifts of the log concentration-response curves were produced by pentobarbitone (Figure 2). Concentration-ratios were calculated from the GABA EC_{50}s and these are compared to those from $\alpha_1,\beta_1,\gamma_{2L}$ cells in Figure 4. These shifts were non-parallel, producing increases in the maxima of these curves (Table 1) that were significantly different at 10 μM ($P = 0.0001$) and 30 μM ($P = 0.033$). The slope factor was also significantly different in pentobarbitone (e.g. control $= 1.49 \pm 0.04$ compared to 1.32 ± 0.05 in 30 μM pentobarbitone: $P = 0.04$).

Direct effects were also observed with 300 μM pentobarbitone on these cells (Figure 5). A current $= 0.98 \pm 0.16$ times that to 1 μM GABA, a concentration equi-effective to 3 μM on $\alpha_1,\beta_1,\gamma_{2L}$ cells, was evoked and at this concentration the response to 1 μM GABA was potentiated by $737 \pm 261\%$ ($n = 4$).

Alphaxalone

$\alpha_1,\beta_1,\gamma_{2L}$ Alphaxalone (10–1000 nM) produced parallel shifts of the log concentration-response curve with no change in the maximum or slope factor (Figure 2, Table 1). As for the other modulators, log concentration-ratio was plotted against log alphaxalone concentration (Figure 4).

α_1,β_1 Alphaxalone produced non-parallel leftward shifts of the concentration-response curves producing significant increases in the maxima at 0.1 and 1 μM (Figure 2, Table 1). Concentration-ratios are plotted in Figure 4. No significant difference in the slope factor was produced.

Discussion and conclusions

The sigmoidal form of log concentration-response curves has long been successfully modelled assuming a dynamic reversible association with a finite number of receptor sites (Hill, 1909; see Mackay, 1977 for overview). This approach continues to provide strong evidence that the response is essentially

a function of the proportion of the available receptors that are occupied by the agonist, obtaining a maximum when, at least in voltage-clamped single cells, all receptors are activated. From this consideration the EC_{50} of an agonist should represent the concentration at which half the receptor population is activated and thus provide an estimate of the affinity of the agonist for the receptor. Whilst this estimate may, for a number of reasons, prove to be inaccurate (e.g. Colquhoun, 1987), we have assumed that the EC_{50} provided our best measure for comparison when changes in affinity occur.

Modelling the log concentration-response curves obtained from both combinations of receptors using the logistic equation provided good fits to the experimental data. As reported earlier for $\alpha_1,\beta_1,\gamma_{2L}$ receptors (Horne *et al.*, 1992) the concentration-response curves to α_1,β_1 receptors were also no better fitted by assuming up to 5 identical, independent binding sites (Colquhoun & Ogden, 1988; Bean, 1990). Thus, for both combinations the possibility remains that cooperativity of binding occurs within the receptor complex under our experimental conditions and, therefore, this property appears not to be dependent upon the γ_{2L} subunit. As with the remainder of this discussion our data provide no evidence as to whether or not any effect of the γ_{2L} subunit is dependent upon the 8 amino acid insert that produces the long variant (Whiting *et al.*, 1990).

The γ_{2L} subunit produced a significantly steeper slope factor when it was incorporated into the receptor complex. As suggested by Verdoorn *et al.* (1990) this may have resulted from the inclusion of an extra binding site for GABA within the complex. As those workers also recorded responses to GABA from receptors comprising each of the subunits alone, it seems improbable that the γ_{2L} subunit replaced another that does not have a binding site for GABA. An alternative explanation could be that the number of subunits per receptor was increased when the γ_{2L} was included, with this subunit acting as an insert to the α, β complex, although this would seem unlikely. Perhaps a more plausible explanation is that the γ_{2L} may increase the effectiveness of the proposed cooperative process. Whilst these possibilities are not easy to investigate by this approach, it does seem clear that the γ_{2L} decreased the potency of GABA at the receptor. Whether this represented a real decrease in affinity for the recognition site or merely resulted from the inclusion of an additional site is unclear.

Concentration-response curves proved to be reasonably reproducible when repeated on the same cell. A significantly different (lower) maximal response was found on the second curve in α_1,β_1 cells. When the γ_{2L} subunit was present this decrease was also a common but not significantly different observation. The pEC_{50}s were not significantly different for the control and repeated curve for both populations of cells. Thus, these findings probably reflect a slight 'run-down' of responses over the timecourse of the experiments but this did not appear to compromise the results obtained.

The concentration-response curves were shifted to the left when the GABA response was potentiated. In the case of the α_1,β_1 combination the shift was often non-parallel with an increase in the maximum response. Therefore, the EC_{50} was always used as a measure for comparison as outlined above. When the two receptor combinations were compared, there was little difference in the magnitude of the shift of the concentration-response curve produced by alphaxalone over the concentration-ranges tested (Puia *et al.*, 1990). Similarly both receptors were sensitive to pentobarbitone both for potentiation of GABA and direct receptor activation. There was, however, a difference in the magnitude of the potentiation produced by the 10 μM concentration of pentobarbitone. It is difficult to explain this finding. It may represent differences in the apparent affinity for pentobarbitone at the two receptors, although this would seem unlikely as concentrations immediately above and below 10 μM did not produce different effects. This was difficult to investigate further as higher concentrations of pentobarbitone directly activated the receptor. In contrast to alphaxalone, the slope factor of the GABA concentration-response curve was significantly different (lower) in the presence of pentobarbitone. This occurred whether the γ_{2L} subunit was present or not. Whilst it is not easy to interpret this change in slope factor it presumably emphasized that whilst the modulation of GABA by barbiturates and steroids has been found to be similar mechanistically, their loci of action may be different.

As reported by Pritchett *et al.* (1989), the inclusion of the γ_{2L} subunit was required before flunitrazepam modulated the GABA response. No significant effect was found on α_1,β_1 receptors whilst concentration-dependent shifts in $\alpha_1,\beta_1,\gamma_{2L}$ concentration-response curves were produced. From these shifts an EC_{50} was calculated for flunitrazepam of about 6 nM. Whilst this is slightly smaller than the value we obtained earlier (Horne *et al.*, 1992) for flunitrazepam (~18 nM) the values fall within each others 95% confidence limits and so are unlikely to represent a significant difference.

An increase in the maximum of the GABA concentration-response curve was produced by pentobarbitone on both receptors and by alphaxalone on α_1,β_1 receptors. Flunitrazepam, by contrast, produced shifts in $\alpha_1,\beta_1,\gamma_{2L}$ curves with no increase in maximum. It may be that the different mechanisms by which these compounds modulate GABA underlie the differences on the maxima. Thus, whilst flunitrazepam has been shown to increase the frequency of openings, both pentobarbitone and alphaxalone-like steroids have been shown to produce an increase in the mean open time of the activated channel (Study & Barker, 1981; Callachan *et al.*, 1987). That the increase in maxima produced by these compounds may be due to a reduction of desensitization seems unlikely as pentobarbitone increased rather than decreased the fade of the response seen with high concentrations of GABA. The increase in frequency produced by flunitrazepam would therefore presumably be limited to submaximal concentrations, and then only when the γ_{2L} subunit was present. These data are consistent with benzodiazepines exerting their effects through an increase in the affinity of GABA for its recognition sites.

If the increase in maxima produced by pentobarbitone and alphaxalone was caused by an increase in channel open time, then the γ_{2L} subunit appears to influence this effect as well. Of the concentrations of pentobarbitone tested, only the highest (30 μM) produced a significant increase in the maxima when the γ_{2L} subunit was present whereas 10 μM was effective, in this respect, on α_1,β_1 receptors. Similarly, whilst alphaxalone up to 1 μM produced no increase in the maxima of concentration-response curves in $\alpha_1,\beta_1,\gamma_{2L}$ cells, 100 nM was sufficient to produce this effect when the γ_{2L} subunit was omitted from the combination of subunits. These results may imply that either the γ_{2L} subunit limits the maximal increase in channel open time produced by the modulator or that, in the absence of a modulator, the probability of channel opening during agonist occupation is limited when this subunit is not present. Therefore, in the presence of a modulator there would be a greater opportunity for an increase in channel open time and, thus an increase in the maximal response. This would also indicate that the probability of channel activation following the binding of GABA is increased by the inclusion of the γ_{2L} subunit.

In summary, our data provide further evidence that the γ_{2L} subunit regulates the pharmacology of the GABA$_A$ receptor. As previously reported by Pritchett *et al.* (1989), this subunit was required before α_1,β_1 containing receptors were modulated by flunitrazepam. Although we obtained no evidence for a γ_{2L} regulation of the change in GABA affinity produced by pentobarbitone and alphaxalone, this subunit did alter the increase in the maximum potentiation of the GABA response produced by these modulators.

We would like to thank Alison Macaulay for maintenance of the cultured cell lines and Roy Hammans, Andy Butler and Susan Shadbolt for photographic assistance.

References

BARKER, J.L. & RANSOM, B. (1978). Pentobarbitone pharmacology of mammalian central neurones grown in tissue culture. *J. Physiol.*, **280**, 355–372.

BEAN, B.P. (1990). ATP-activated channels in rat and bullfrog sensory neurones: concentration-dependence and kinetics. *J. Neurosci.*, **10**, 1–10.

CALLACHAN, H., COTTRELL, N.Y., HATHER, N.Y., LAMBERT, J.J., NOONEY, J.M. & PETERS, J.A. (1987). Modulation of the $GABA_A$ receptor by progesterone metabolites. *Proc. R. Soc. B*, **231**, 359–369.

CHOI, D.W., FARB, D.H. & FISHBACH, G.D. (1977). Chlordiazepoxide selectivity augments GABA action in spinal cord cell cultures. *Nature*, **269**, 342–344.

COLQUHOUN, D. (1987). Affinity, efficacy, and receptor classification: is the classical theory still useful? In *Perspectives on Receptor Classification*. ed. Black, J.W., Jenkinson, D.H. & Gerskowitch, V.P. pp. 103–114. New York: Alan R. Liss.

COLQUHOUN, D. & OGDEN, D.C. (1988). Activation of ion channels in the frog endplate by high concentrations of acetylcholine. *J. Physiol.*, **395**, 131–159.

HADINGHAM, K.L., HARNESS, P.C., MCKERNAN, R.M., QUIRK, K., LE BOURDELLES, B., HORNE, A.L., KEMP, J.A., BARNARD, E.A., RAGAN, C.I. & WHITING, P.J. (1992). Stable expression of mammalian type A γ-aminobutyric acid receptors in mouse cells: demonstration of functional assembly of benzodiazepine-responsive sites. *Proc. Natl. Acad. Sci. U.S.A.*, **89**, 6378–6382.

HARRISON, N.L. & SIMMONDS, M.A. (1984). Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res.*, **323**, 287–292.

HILL, A.V. (1909). The mode of action of nicotine and curari, determined from the form of the contraction curve and the method of temperature coefficients. *J. Physiol.*, **39**, 361–373.

HORNE, A.L., HADINGHAM, K.L., MACAULAY, A.J., WHITING, P. & KEMP, J.A. (1992). The pharmacology of recombinant $GABA_A$ receptors containing bovine α_1, β_1, γ_{2L} subunits stably transfected into mouse fibroblast L-cells. *Br. J. Pharmacol.*, **107**, 732–737.

KNOFLACH, F., BACKUS, K.H., GILLER, T., MALHERBE, P., FLIMLIN, P., MOHLER, H. & TRUBE, G. (1992). Pharmacological and electrophysiological properties of recombinant $GABA_A$ receptors comprising the α_3,β_1 and γ_2 subunits. *Eur. J. Neurosci.*, **4**, 1–9.

LITTLE, H.J. (1984). The effects of benzodiazepine agonists inverse agonists and Ro15-1788 on the responses of the superior cervical ganglion to GABA *in vitro*. *Br. J. Pharmacol.*, **83**, 57–68.

LUDDENS, H. & WISDEN, W. (1991). Function and pharmacology of multiple $GABA_A$ receptors. *Trends Pharmacol. Sci.*, **12**, 49–51.

MACKAY, D. (1977). A critical survey of receptor theories of drug action. In *Kinetics of Drug Action*. ed. van Rossum, J.M. Berlin: Springer-Verlag.

MALHERBE, P., SIGEL, E., BAUR, R., PERSOHN, E., RICHARDS, J.G. & MOHLER, H. (1990). Functional characteristics and sites of gene expression of the $\alpha_1,\beta_1,\gamma_2$-isoform of the rat $GABA_A$ receptor. *J. Neurosci.*, **10**, 2330–2337.

NICOLL, R.A. (1975). Pentobarbital: action on frog motoneurons. *Brain Res.*, **96**, 119–123.

PRITCHETT, D.B., SONTHEIMER, H., SHIVERS, B.D., YMER, S., KETTENMANN, H., SCHOFIELD, P.R. & SEEBURG, P.H. (1989). Importance of a novel $GABA_A$ receptor subunit for benzodiazepine pharmacology. *Nature*, **338**, 582–585.

PUIA, G., SANTI, M., VICINI, S., PRITCHETT, D.B., PURDY, R.H., PAUL, S.M., SEEBURG, P.H. & COSTA, E. (1990). Neurosteroids act on recombinant human $GABA_A$ receptors. *Neuron.*, **4**, 759–765.

PUIA, G., VICINI, S., SEEBURG, P.H. & COSTA, E. (1991). Influence of recombinant γ-aminobutyric acid-$_A$ receptor subunit composition on the action of allosteric modulators of γ-aminobutyric acid gated Cl^- currents. *Mol. Pharmacol.*, **39**, 691–696.

SCHOFIELD, P.R., DARLISON, M.G., FUJITA, N., BURT, D.R., STEPHENSON, F.A., RODRIGUEZ, H., RHEE, L.M., RAMACHANDRAN, J., REALE, V., GLENCORSE, T.A., SEEBURG, P.H. & BARNARD, E.A. (1987). Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature*, **328**, 221–227.

SIMMONDS, M.A. (1981). Distinction between the effects of barbiturates, benzodiazepines and phenytoin on responses to γ-aminobutyric acid receptor activation and antagonism by bicuculline and picrotoxin. *Br. J. Pharmacol.*, **73**, 739–747.

STUDY, R.E. & BARKER, J.L. (1981). Diazepam and (−)pentobarbital: fluctuation analysis reveals different mechanisms for potentiation of γ-aminobutyric acid responses in cultured central neurons. *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 7180–7184.

VERDOORN, T.A., DRAGUHN, A., YMER, S., SEEBURG, P.H. & SAKMANN, B. (1990). Functional properties of recombinant rat $GABA_A$ receptors depend upon subunit composition. *Neuron*, **4**, 911–928.

WHITING, P., MCKERNAN, R.M. & IVERSEN, L.L. (1990). Another mechanism for creating diversity in γ-aminobutyrate type A receptors: RNA splicing directs expression of two forms of the γ2 subunit, one of which contains a protein kinase C phosphorylation site. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 9966–9970.

WHITING, P., SCHOEPFER, R., LINDSTROM, J. & PRIESTLEY, T. (1991). Structural and pharmacological characterization of the major brain nicotinic acetylcholine receptor subtype stably expressed in mouse fibroblasts. *Mol. Pharmacol.*, **40**, 463–472.

(Received August 26, 1992
Revised October 20, 1992
Accepted October 28, 1992)