Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 478 (2003) 21–26
www.elsevier.com/locate/ejphar

Evidence for inhibitory effect of the agonist gaboxadol at human $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors

Morten Smith, Catarina E.L. Lindquist, Bryndis Birnir*

Molecular and Cellular Physiology, Department of Physiological Sciences, Lund University, Tornavagen 10 BMC F11, 22184 Lund, Sweden

Received 3 July 2003; received in revised form 13 August 2003; accepted 19 August 2003

Abstract

Gaboxadol (THIP; 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) is an agonist at $GABA_A$ receptors. THIP concentrations (0.01–50 mM) were applied rapidly to *Sf*9 cells expressing the human $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors. The EC_{50} values for the peak current in THIP alone or THIP plus 1 μM diazepam were 154 and 53 μM, respectively. In supersaturating THIP (10–50 mM) the rate of current decay increased and an off-current developed when THIP was rapidly removed. The mean currents measured over the first 4 s in 10 mM and higher THIP concentrations were 0.6 or less of the 1 mM THIP mean current. Diazepam (1 μM) increased the 4 s mean current when evoked by 10 to 20 mM THIP but not 50 mM THIP. No similar effects on the current time-course were recorded in supersaturating γ-aminobutyric acid (GABA) concentrations (50 and 80 mM). The results demonstrate an inhibitory as well as agonist effect of THIP at $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors.

Keywords: Ligand-gated; Anaesthetic; Benzodiazepine; γ-Aminobutyric acid; THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol); Inhibition

1. Introduction

γ-Aminobutyric acid (GABA) is the main inhibitory transmitter in the brain. $GABA_A$ receptors are chloride channels that are normally closed but when GABA binds to the receptors the channel is opened. Because of its wide distribution in the brain, the $GABA_A$ receptors are of major clinical significance and these receptors are the targets of many therapeutic drugs. Gaboxadol also called THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol), is a specific $GABA_A$ receptor agonist (Frolund et al., 2002) and is currently in clinical development as a hypnotic. THIP has been shown to promote deep sleep and sleep maintenance in addition to facilitate sleep initiation (Lancel and Faulhaber, 1996; Lancel, 1997; Faulhaber et al., 1997; Lancel and Langebartels, 2000; Mathias et al., 2001). THIP has also been shown to be neuroprotective when given together with diazepam in a rat cerebral ischemia model of delayed CA1 pyramidal cell death (Johansen and Diemer, 1991).

$GABA_A$ receptors are thought to be pentamers. Eighteen different mammalian subunits grouped into eight subunit families (α_{1-6}, β_{1-3}, γ_{1-3}, ρ_{1-2}, δ, ε, π, θ) have been identified to date (Barnard et al., 1998). Work on recombinant receptors indicates that the subunit composition of the receptor affects THIP efficacy. It is not clear what molecular properties of the receptors determine the agonist efficacy but the type of alpha subunit has been shown to be important (Ebert et al., 1994; Wafford et al., 1996). THIP efficacy is greater than GABA at $\alpha_6\beta x\gamma_{2S}$, it is a full agonist at $\alpha_5\beta_x\gamma_{2S}$ but it is only a partial agonist at $\alpha_{1-4}\beta_x\gamma_{2S}$ receptors. For receptors expressed in *Xenopus* oocytes and cell lines the apparent affinity for THIP ranges from 6 to 350 μM (Kusama et al., 1993; Ebert et al., 1994; Wafford et al., 1996; Thompson et al., 1999; Brown et al., 2002; Frolund et al., 2002, Storustovu and Ebert, 2003).

In this study, we examined the effect of supersaturating concentrations of THIP on the current-response in human $\alpha_1\beta_2\gamma_{2S}$ receptors expressed in *Sf*9 cells and how 1 μM diazepam modulated the current. Ten millimolar and higher THIP concentrations increased the initial rate of current decay and evoked an off-current when the drug application was terminated consistent with an inhibitory effect of THIP at supersaturating concentrations. Diazepam decreased the level of inhibition in a THIP concentration-dependent manner. The results demonstrate that THIP is both an agonist and an inhibitor at human $\alpha_1\beta_2\gamma_{2S}$ receptors.

* Corresponding author. Tel.: +46-46-222-0675; fax: +46-46-222-7763.
E-mail address: bryndis.birnir@mphy.lu.se (B. Birnir).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.08.034

2. Materials and methods

2.1. Construction and expression of receptors

To allow expression of human α_1, β_2 and γ_{2S} $GABA_A$ receptor subunits from one virus, a triple promoter plasmid pBAC3 was created by ligating a 2.25 kB *Bst*X1/*Hin*d3 fragment, containing the triple promoter cassette from pAcAB3 (Pharmingen) into *Bst*X1/*Hin*d3 cut pBacPAK8 (Cromer, 1998). The result is a smaller triple promoter plasmid without a second unwanted *Eco*R1 site. To create the human β_2 amino acid sequence, the rat β_2 cDNA was mutated N323S, with the oligonucleotide GAGAAAGCTGCTAGCGCCAACAACGAG, using the USE mutagenesis kit (Pharmacia). This β_2 cDNA was subcloned into the *Bam*H1 site following a P10 promoter in pBAC3. The human α_1 cDNA was then subcloned into the *Bgl*II/*Eco*R1 sites following the other P10 promoter in β_2-pBAC3. Finally the γ_{2S} cDNA was subcloned into the *Xba*I/*Stu*1 sites following the polyhedrin promoter in $\alpha_1\beta_2$-pBAC3, creating $\alpha_1\beta_2\gamma_{2S}$-pBAC3. Recombinant baculoviruses were generated from each transfer plasmid by homologous recombination as described previously (Birnir et al., 1995). Techniques for general handling of *Sf*9 (*Spodoptera frugiperda*) cells, production of high titer viral stock and infection procedures have been described (Birnir et al., 1995).

2.2. Electrophysiology

Cells were infected for 1 h with the recombinant virus and then incubated at 25 ± 1 °C and used in electrophysiological experiments 24–40 h later. Currents were recorded using standard whole-cell, tight-seal recording techniques (Hamill et al., 1981). Whole-cell currents were recorded from voltage-clamped cells at a pipette potential of −40 mV. At this potential background-chloride current was minimized (Birnir et al., 1995). Patch electrodes were pulled from borosilicate glass capillaries (1.5 O.D. and 0.86 I.D., Harvard Apparatus, Edenbridge, UK). The electrodes were fire polished and had a resistance of 3 to 10 MΩ when filled with the pipette solution. All experiments were carried out at room temperature (20–22 °C) and series-resistance compensation used. The bath solution consisted of (in mM): 180 NaCl, 1 $CaCl_2$, 1 $MgCl_2$ and 10 MES (2-(*N*-morpholino)ethanesulfonicacid) adjusted to pH 6.2 with NaOH. The pipette solution consisted of (in mM): 178 NaCl, 1 $CaCl_2$, 1 $MgCl_2$, 5 EGTA (ethylene glycol-bis (*b*-aminoethylether)-tetraacetic acid) and 10 TES (*N*-tris(hydroxymethyl)) adjusted to pH 7.2 with NaOH. GABA (γ-aminobutyric acid, Sigma) and THIP (Lundbeck, AS) were dissolved in the bath solution whereas diazepam was first dissolved in dimethylsulfoxide (DMSO, Sigma).

Currents were monitored with a current-to-voltage converter (Axopatch 200B, Axon Instruments, Foster City, CA). Currents were examined for run-down of the current response by applying 1 mM THIP at the beginning and at the end of each experiment. Only experiments where the peak-current amplitudes evoked by the run-down test concentration differed by 20% or less were used. Data was digitized using analog-to-digital converter (DigiData 1322A, Axon Instruments) and analysed by pClamp analysis software (Axon Instruments). Results are presented as mean ± S.E.M. Differences between groups are considered significant for $P < 0.05$ using Student's *t*-test.

2.3. Drug application

Drugs were applied by gravity feed via microperfusion tubes. All drugs were applied using this fast perfusion

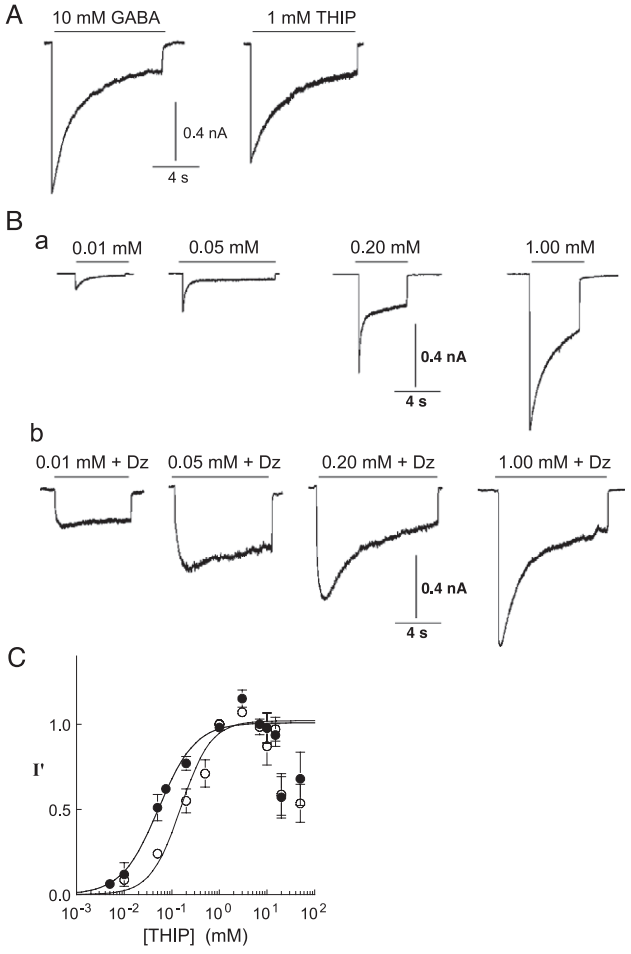

Fig. 1. THIP-activated currents are modulated by diazepam in $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors. (A) Currents evoked by 10 mM GABA or 1 mM THIP in the same cell. (Ba) Currents evoked in a cell by increasing concentrations of THIP. The currents were normalized to the peak-current amplitude recorded in response to 1 mM THIP. (Bb) Currents recorded in response to a test concentration of THIP in the presence of 1 μM diazepam. The currents were normalized to 1 mM THIP peak-current amplitude in the same cell. (C) The peak-current amplitude induced by THIP (open circles) or THIP plus 1 μM diazepam (filled circles) represented as a fraction of the 1 mM THIP-activated current is plotted as a function of the THIP concentration. The vertical bars show ± S.E.M. in three or more cells. Error bars are not visible if smaller than the symbol. A Hill-type equation (see Results) was fitted to the data and calculated constants are given in the text. Currents induced by 20 and 50 mM THIP were not included in the fit.

system. After establishing a whole-cell configuration, the cell was lifted off the bottom of the bath and placed in front of the drug delivery tubes and the bath flow (>10 ml/min) was turned on. The rate of solution exchange using this method is fast. For an open-tip electrode, the solution can be changed in less than a millisecond (Birnir et al., 1995).

3. Results

3.1. Characteristics of the THIP-activated current response

Whole-cell currents activated by saturating concentrations of GABA (10 mM) or THIP (1 mM) are shown in Fig. 1A. The current traces are from the same cell. The peak-current amplitudes to GABA and THIP were 1045 and 815 pA, respectively. The peak-current amplitude of the THIP-activated currents were on the average ($\pm$ S.E.M.) 0.68 ± 0.03 ($n = 10$) of the GABA-activated current in the same cell. This is in accordance with the observation that THIP is a partial agonist at $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors (Ebert et al., 1994).

Fig. 1B shows current traces recorded in response to 0.01, 0.05, 0.2 and 1 mM THIP alone (a) or together with 1 μM diazepam (b). In Fig. 1Ba, the currents were recorded from the same cell whereas in Fig. 1Bb the currents are from different cells. In response to THIP alone, the current increased rapidly to a peak value for all concentrations and then decayed to a steady-state plateau level. The peak-current value increased as the THIP concentration was increased from 0.005 to 1 mM. In 0.01 to 0.2 mM THIP plus 1 μM diazepam, the peak-current amplitude was

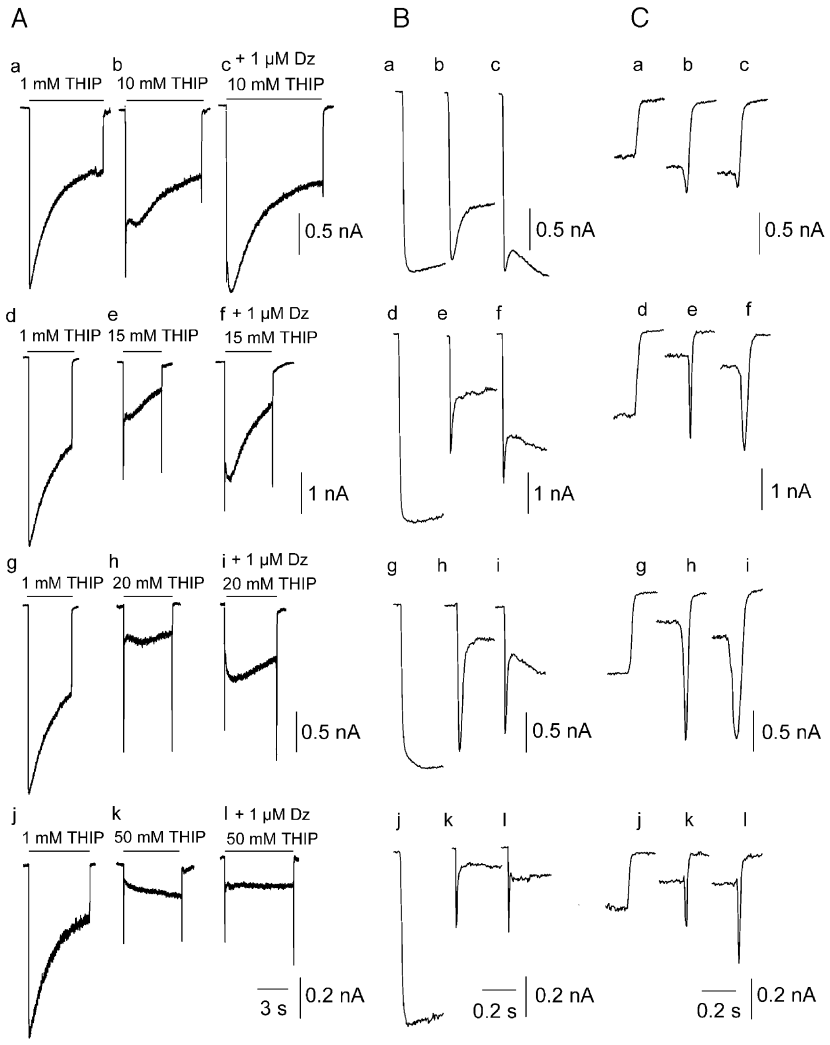

Fig. 2. The current time-course activated by supersaturating THIP concentration is multiphasic. (A) Current responses evoked in one cell to 1 mM THIP, test THIP concentration and test THIP concentration plus 1 μM diazepam. Test THIP concentrations were 10 (b, c), 15 (e, f), 20 (h, i) and 50 (k, l) mM THIP. The drugs were applied for the period indicated by the bars. (B) The initial phase of the current time-courses shown in (A). The increased rate of the current decay is apparent in the supersaturating THIP concentrations (10, 15, 20 or 50 mM) as compared to 1 mM THIP. (C) Off-currents from the time-courses shown in (A). A transient increase in the current amplitude (off-current) was recorded when supersaturating THIP concentrations were removed but not upon removal of 1 mM THIP.

enhanced and the rate of the current decay decreased as compared to currents evoked by THIP alone.

The relationship between the THIP concentration and the peak whole-cell current in the absence (open circles) and presence (filled circles) of 1 μM diazepam is shown in Fig. 1C. The solid line through the data points is a fit of a Hill-type equation:

$$I = \mathrm{Imax}[\mathrm{THIP}]^h/([\mathrm{EC}_{50}]^h + [\mathrm{THIP}]^h)$$

to the data. The values for 20 and 50 mM THIP were not included in the fit because of the large reduction in the peak-current amplitude. The curves were constructed from data obtained from 29 (open circles) and 36 (filled circles) cells. Each data point represents the mean normalised current (I') ± S.E.M. for three or more cells. The EC_{50} is the concentration that gave half-maximal current response and h is the Hill coefficient. The EC_{50} value and h for THIP alone was 154 ± 32 μM and 1.4 ± 0.3 whereas in the presence of 1 μM diazepam it was 53 ± 8 μM and 1.1 ± 0.2, respectively. Diazepam did not increase the maximal peak-current value above what was obtained in saturating THIP concentration.

3.2. Inhibition of the current response by 10 to 50 mM THIP

We examined in greater detail the inhibition of the THIP-activated current that became apparent in supersaturating THIP concentrations. The results are shown in Figs. 2 and 3. Currents activated by 1 mM or 10 to 50 mM THIP in the absence or presence of 1 μM diazepam in the $\alpha_1\beta_2\gamma_{2S}$ receptors are shown in Fig. 2. The supersaturating THIP concentrations clearly affected the current time-course. The currents initially decayed faster (Fig. 2A, B) than currents evoked by the saturating 1 mM THIP concentration and gave an off-current response when the drug application was rapidly terminated (Fig. 2A, C). In 10, 15 and 20 mM THIP, a second peak or rebound peak-current appeared after the initial current decay (Fig. 2A, B) and was particularly prominent in the presence of 1 μM diazepam. The amplitude of the rebound current in 10, 15 or 20 mM THIP plus 1 μM diazepam as a fraction of the peak-current amplitude in 1 mM THIP was 0.81 ± 0.16 (n = 6), 0.41 ± 0.10 (n = 3) and 0.30 ± 0.04 (n = 4), respectively. In 50 mM THIP, a rebound peak-current did not develop.

In order to examine further the effect of the supersaturating THIP concentrations on the current time-course, we measured the mean current over the first 4 s. The mean current reflects both the peak-current value and the rate of current decay. The results are shown in Fig. 3A and the histograms show the averages as a fraction of the 1 mM THIP current response from three or more cells. The mean current decreased with increasing THIP concentrations (Fig. 3A). The mean current over the first 4 s of the drug application for 3, 7, 10, 15, 20 and 50 mM THIP as a fraction of the current evoked by 1 mM THIP in the same cell were 0.9, 0.8, 0.6, 0.5, 0.3 and 0.2 (Fig. 3A). Diazepam enhances subsaturating THIP-activated currents and increased the apparent affinity for the agonist (Fig. 1). The effect of diazepam on the apparent inhibition by THIP of the THIP-activated current is shown in Fig. 3B. The currents were measured over the first 4 s of the drug application and are shown as a fraction of the current evoked by the same concentration of THIP alone in the same cell. The histograms are the averages from three or more cells. In 1 to 7 mM THIP, currents evoked in the presence or absence of diazepam were similar (Fig. 3B) whereas in 10, 15 and 20 mM THIP 1 μM diazepam significantly decreased the apparent inhibition (Fig. 3B). The fractional increase in the 10, 15 and 20 mM THIP plus diazepam were 1.6, 1.8 and 1.7 of the corresponding current response in THIP alone. In 50 mM THIP, diazepam did not significantly affect the mean current as compared to currents evoked by 50 mM THIP alone. The effect of diazepam on the fractional increase in the mean current evoked by supersaturating THIP, e.g. 10 mM THIP by 1.6 ± 0.2 (n = 4) was comparable to the fractional increase obtained in low, subsaturating THIP, e.g. 0.01 mM THIP (1.6 ± 0.2, n = 5).

We examined if supersaturating concentration of an agonist generally caused inhibition of the receptors by applying 50 and 80 mM GABA to the cells and compared it to the current response to a saturating (10 mM) GABA concentration (Fig. 4). In the $\alpha_1\beta_2\gamma_{2s}$ receptors in this study

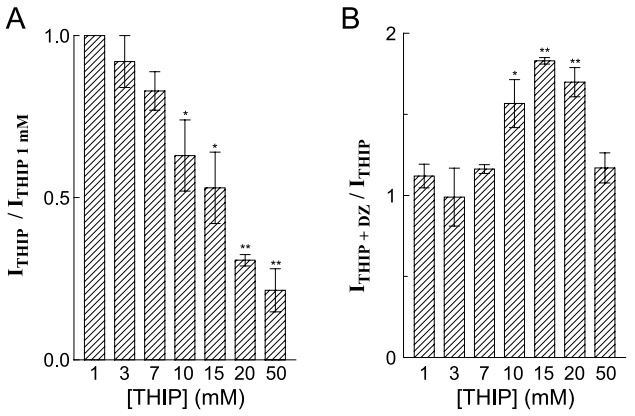

Fig. 3. (A) Supersaturating THIP concentrations inhibit the THIP-activated current. The THIP-activated current was measured over the first 4 s of the exposure to the drug and normalized to the 4 s current response to 1mM THIP alone in the same cell. Each bar shows the average fractional currents in three or more cells and the vertical lines show the S.E.M. The currents were compared to values obtained when the test concentration was 1 mM THIP. Significant decrease in the mean current was recorded in 10, 15, 20 and 50 mM THIP (*P<0.05, **P<0.01). (B) Diazepam reduces the current inhibition by 10, 15 and 20 but not 50 mM THIP. The THIP-activated current was recorded and then after a wash-out period the cell was exposed to the same concentration of THIP plus 1 μM diazepam. The THIP plus diazepam activated current was measured over the first 4 s of the exposure to the drugs and normalized to the 4 s current response to the THIP alone in the same cell. Each bar shows the average fractional currents in three or more cells and the vertical lines show the S.E.M. Significant increase in the mean current was recorded in 10, 15 and 20 but not 50 mM THIP plus 1 μM diazepam (*P<0.05, **P<0.01).

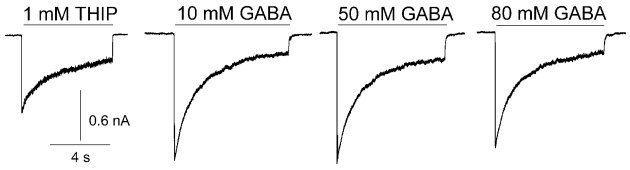

Fig. 4. Supersaturating GABA concentrations do not change the GABA-activated current time-course. Currents evoked by 1 mM THIP, 10 mM, 50 mM and 80 mM GABA in the same cell. The peak-activated currents were 0.92, 1.53, 1.56 and 1.40 nA, respectively. The drugs were applied for the period indicated by the bar.

supersaturating GABA concentrations only caused a slight reduction in the peak GABA-activated current and no off-current was recorded. The 50 and 80 mM activated peak-currents were 0.92 ± 0.2 ($n=4$) and 0.87 ± 0.02 ($n=3$), respectively, of the 10 mM GABA-activated current in the same cell. The 1 mM THIP-activated peak-current was 0.80 ± 0.10 ($n=4$) and 0.75 ± 0.05 ($n=7$) of the 50 and 80 mM GABA-activated currents and a similar fraction (0.68 ± 0.03, $n=10$) of the saturating (10 mM) GABA-activated current.

4. Discussion

In this study, maximal THIP activation of $\alpha_1\beta_2\gamma_{2S}$ receptors was about 0.7 of the saturating (10 mM) and supersaturating (50 and 80 mM) peak-current response to GABA and is similar to what has been reported for the receptors expressed in oocytes where it was 0.76 of the GABA response (Ebert et al., 1994). At all concentrations, THIP-activated currents rose to a peak-current value and then decayed. Diazepam decreased the rate of the current decay by subsaturating THIP concentrations and increased the apparent affinity of the receptors for THIP about threefold. Diazepam appeared to decrease the apparent inhibition by THIP of the THIP-activated current response that became evident in 10 mM and higher THIP concentrations. The inhibition was manifested in increased rate of the initial current decay, an off-current that developed when 10 to 50 mM THIP were removed rapidly and decreased mean current. However, as the THIP concentration was increased from 20 to 50 mM the effectiveness of diazepam in enhancing the current response or relieving the inhibition decreased.

Other drugs that do not share a binding site with GABA but act at the $GABA_A$ receptors have been shown to have multiple effects on the receptors, e.g. pentobarbital and propofol are modulators, agonists and inhibitors at the receptors depending on the concentration (Barnard et al., 1998). Whether these multiple effects involve one or more binding sites is not clear. The competitive inhibitors bicuculline and SR95531 have overlapping binding sites with GABA. The drug 4PS (poperidine-4-sulfonic acid) is normally a partial agonist at $GABA_A$ receptors but has been shown to be a competitive inhibitor at $\alpha_4\beta_1\gamma_{2S}$ receptors with similar affinity for the binding site as it has at receptors where it is a partial agonist (Wafford et al., 1996). Current inhibition by supersaturating concentrations of GABA (30 to 100 mM) has been recorded in $GABA_A$ receptors in outside-out patches from cultured hippocampal neurons (Mercik et al., 2002). Similar to the results in supersaturating THIP in this study, the inhibitory effect of GABA was manifested in reduced current amplitudes and sometimes rebound-currents but differed in that no off-currents were recorded in the hippocampal patches. However, it appears that the inhibition by GABA of the GABA-activated current may depend on the receptor subtype as the current time-course evoked by the supersaturating GABA in this study was similar to the saturating current evoked by 10 mM GABA. An off-current is associated with inhibition by acetylcholine of nicotinic acetylcholine receptors (Maconochie and Steinbach, 1998) that belong to the same superfamily of ligand-gated receptors as the $GABA_A$ receptors. The block by acetylcholine of the receptors is thought to be due to an open-channel block mechanism where a single acetylcholine molecule occludes the open channel (Maconochie and Steinbach, 1995). THIP has an overlapping binding site with GABA and presumably also with the competitive inhibitors. We cannot determine from our data if the inhibitory effect of THIP originates from the same or different binding site as the agonist effect or if THIP is an open channel blocker at supersaturating concentrations. The ability of diazepam to partially relieve the inhibition at 10 to 20 mM THIP concentrations suggests that the diazepam affects the receptor complex at saturating agonist concentrations despite that no current enhancement is normally detected.

THIP is a partial agonist at $\alpha_1\beta_2\gamma_{2S}$ receptors. At full receptor occupancy partial agonists produce a lower response than full agonists do. The molecular mechanism that accounts for the reduced maximal response to partial agonists is not known. As we used fast drug application the reduced peak-current in response to saturating THIP concentration (1 mM) as compared to the response evoked by saturating GABA concentration (10 mM) cannot be attributed to pharmacology of desensitised receptors. It is possible that the inhibitory effect of THIP clearly manifested at 10 mM and higher concentrations is present at lower concentrations but to a different degree. If inhibition is present at 1 mM THIP it may contribute to the reduced peak-current amplitude of the saturating current response as compared to the saturating GABA evoked peak-current. It is plausible that the degree of self-block by an agonist is dependent on the receptor subtype. It remains for further studies to determine if the inhibitory effect of THIP, as well as GABA, can be detected at other $GABA_A$ receptors subtypes where it is a super, full or partial agonist.

Acknowledgements

We thank the Swedish Medical Research Council (Y0924), Vetenskapsrådet (K2002-33X-13408-03A), Åke

Wibergs Stiftelse, Segerfalks Stiftelsen, Crafoordska Stiftelsen, Swärds Stiftelse and the Medical Faculty Lund University for financial support.

References

Barnard, E.A., Skolnick, P., Olsen, R.W., Mohler, H., Sieghart, W., Biggio, G., Braestrup, C., Bateson, A.N., Langer, S.Z., 1998. International Union of Pharmacology. XV. Subtypes of γ-aminobutyric acid A receptors: classification on the basis of subunit structure and receptor function. Pharmacol. Rev. 50, 291–313.

Birnir, B., Tierney, M.L., Pillai, N.P., Cox, G.B., Gage, P.W., 1995. Rapid desensitization of $\alpha_1\beta_1$ $GABA_A$ receptors expressed in *Sf*9 cells under optimised conditions. J. Membr. Biol. 148, 193–202.

Brown, N., Kerby, J., Bonnert, T.P., Whiting, P.J., Wafford, K.A., 2002. Pharmacological characterization of a novel cell line expressing human $\alpha_4\beta_3\delta$ $GABA_A$ receptors. Br. J. Pharmacol. 136, 965–974.

Cromer, B.A., 1998. PhD thesis. John Curtin School of Medical Research. Australian National University, Canberra, Australia.

Ebert, B., Wafford, K.A., Whiting, P.J., Krogsgaard-Larsen, P., Kemp, J.A., 1994. Molecular pharmacology of γ-aminobutyric acid type A receptor agonists and partial agonists in oocytes injected with different α, β, and γ receptor subunit combinations. Mol. Pharmacol. 46, 957–963.

Faulhaber, J., Steiger, A., Lancel, M., 1997. The $GABA_A$ agonist THIP produces slow wave sleep and reduces spindling activity in NREM sleep in humans. Psychopharmacology 130, 285–291.

Frolund, B., Ebert, B., Kristiansen, U., Liljefors, T., Krogsgaard-Larsen, P., 2002. $GABA_A$ receptor ligands and their therapeutic potentials. Curr. Top. Med. Chem. 2, 817–832.

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J., 1981. Improved patch-clamp techniques for high resolution current recording from cells and cell-free membrane patches. Pflugers Arch. 391, 85–100.

Johansen, F.F., Diemer, N.H., 1991. Enhancement of GABA neurotransmission after cerebral ischemia in the rat reduces loss of hippocampal CA1 pyramidal cells. Acta Neurol. Scand. 84, 1–6.

Kusama, T., Spivak, C.E., Whiting, P., Dawson, V.L., Schaeffer, J.C., Uhl, G.R., 1993. Pharmacology of GABA rho 1 and GABA α/β receptors expressed in *Xenopus* oocytes and COS cells. Br. J. Pharmacol. 109, 200–206.

Lancel, M., 1997. The $GABA_A$ agonist THIP increases non-REM sleep and enhances non-REM sleep-specific delta activity in the rat during the dark period. Sleep 20, 1099–1104.

Lancel, M., Faulhaber, J., 1996. The $GABA_A$ agonist THIP (gaboxadol) increases non-REM sleep and enhances delta activity in the rat. NeuroReport 7, 2241–2245.

Lancel, M., Langebartels, A., 2000. γ-Aminobutyric acid A ($GABA_A$) agonist 4,5,6, 7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol persistently increases sleep maintenance and intensity during chronic administration to rats. J. Pharmacol. Exp. Ther. 293, 1084–1090.

Maconochie, D.J., Steinbach, J.H., 1995. Block by acetylcholine of mouse muscle nicotinic receptors, stably expressed in fibroblasts. J. Gen. Physiol. 106, 113–147.

Maconochie, D.J., Steinbach, J.H., 1998. The channel opening rate of adult- and fetal-type mouse muscle nicotinic receptors activated by acetylcholine. J. Physiol. 506, 53–72.

Mathias, S., Steiger, A., Lancel, M., 2001. The $GABA_A$ agonist gaboxadol improves the quality of post-nap sleep. Psychopharmacology 157, 299–304.

Mercik, K., Zarnowska, E.D., Mandat, M., Mozrzymas, J.W., 2002. Saturation and self-inhibition of rat hippocampal $GABA_A$ receptors at high GABA concentrations. Eur. J. Neurosci. 16, 2253–2259.

Storustovu, S., Ebert, B., 2003. Gaboxadol: in vitro interaction studies with benzodiazepines and ethanol suggest functional selectivity. Eur. J. Pharmacol. 467, 49–56.

Thompson, S.A., Smith, M.Z., Wingrove, P.B., Whiting, P.J., Wafford, K.A., 1999. Mutation at the putative $GABA_A$ ion-channel gate reveals changes in allosteric modulation. Br. J. Pharmacol. 127, 1349–1358.

Wafford, K.A., Thompson, S.A., Thomas, D., Sikela, J., Wilcox, A.S., Whiting, P.J., 1996. Functional characterization of human γ-aminobutyric acid A receptors containing the α_4 subunit. Mol. Pharmacol. 50, 670–678.