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Functional pharmacology of cloned heterodimeric $GABA_B$ receptors expressed in mammalian cells

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1 In this study we report a new assay of heterodimeric γ-amino-butanoic acid subtype B ($GABA_B$) receptors where either $GABA_BR1a$ or $GABA_BR1b$ are co-expressed with $GABA_BR2$ and the chimeric G-protein Gαq-z5 in tsA cells. In this manner we obtained a robust response to $GABA_B$ agonists measured as increase in phosphoinositide hydrolysis.

2 We used this assay to characterize a number of commonly used $GABA_B$ receptor ligands. Both splice variants displayed the same rank order of agonist potency; 3-aminopropyl(methyl)phosphinic acid (SKF-97541) > GABA > (**R**)-4-amino-3-(4-chlorophenyl)butanoic acid ((**R**)-baclofen) > (**RS**)-4-amino-3-(5-chloro-2-thienyl)butanoic acid (BCTG) > 3-aminopropylphosphonic acid (3-APPA) and furthermore, the absolute agonist potency values were very close to each other.

3 3-APPA was a partial agonist displaying maximal responses of 41 and 61% compared to GABA at $GABA_BR1a$ and $GABA_BR1b$, respectively. The antagonist (**RS**)-3-amino-2-(4-chlorophenyl)-2-hydroxypropylsulphonic acid (2-OH-saclofen) displayed K_B values of 15 and 7.8 μM at $GABA_BR1a$ and $GABA_BR1b$, respectively.

4 The rank order of agonist potency as well as the absolute ligand potencies correspond very well with those previously reported in different tissues, and this study thus provides a functional assay of cloned $GABA_B$ receptors which should be a valuable tool for further characterization of $GABA_B$ ligands. Finally, we can conclude that the functional pharmacological profiles of the two $GABA_BR1$ splice variants are very similar.

Keywords: $GABA_B$ receptors; $GABA_BR1a$; $GABA_BR1b$; $GABA_BR2$; heterodimeric receptors; SKF-97541; (**R**)-baclofen; (**RS**)-4-amino-3-(5-chloro-2-thienyl)butanoic acid; 3-APPA; 2-OH-saclofen

Abbreviations: 2-OH-saclofen, (**RS**)-3-amino-2-(4-chlorophenyl)-2-hydroxypropylsulphonic acid; 3-APPA, 3-aminopropylphosphonic acid; ATD, amino terminal domain; (**R**)-baclofen, (**R**)-4-amino-3-(4-chlorophenyl)butanoic acid; BCTG, (**RS**)-4-amino-3-(5-chloro-2-thienyl)butanoic acid; DMEM, Dulbecco's modified Eagle medium; IP, inositol phosphate; GABA, γ-amino-butanoic acid; PBS, phosphate buffered saline solution; SKF-97541, 3-aminopropyl(methyl)phosphinic acid

Introduction

γ-Aminobutanoic acid (GABA) is the major inhibitory amino acid in the central and peripheral nervous system. The physiological effects of GABA are mediated by ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors, the latter being coupled to the Gαi class of G-proteins (Morishita *et al*., 1990). The $GABA_B$ receptor was first defined by its selective activation by the GABA analogue (**R**)-4-amino-3-(4-chlorophenyl)butanoic acid ((**R**)-baclofen) (Bowery *et al*., 1980; Hill & Bowery, 1981) and more recently a number of selective and very potent antagonists have been developed (Bittiger *et al*., 1993). Using one of these radiolabelled antagonists, two different splice variants of the $GABA_B$ receptor (named $GABA_BR1a$ and $GABA_BR1b$, respectively) were isolated by expression cloning (Kaupmann *et al*., 1997) which led several groups to identify a second subunit (Jones *et al*., 1998; White *et al*., 1998; Kaupmann *et al*., 1998a; Kuner *et al*., 1999; Ng *et al*., 1999a; Martin *et al*., 1999). Interestingly, although some of these groups have reported functional responses from cells transfected with either $GABA_BR1$ or $GABA_BR2$ alone (Kaupmann *et al*., 1997; 1998b; Kuner *et al*., 1999; Martin *et al*., 1999), several lines of evidence have shown that the two receptor subunits dimerize in order to form fully functional receptors with the expected pharmacological responses by agonists (Jones *et al*., 1998; White *et al*., 1998; Kaupmann *et al*., 1998a; Kuner *et al*., 1999; Ng *et al*., 1999b; Möhler & Fritschy, 1999). However, although transient expression functional assays are now available, the assays reported so far have either been limited by a low throughput or relatively weak responses. Thus, to this point only a couple of ligands have been characterized in functional assays based on heterodimeric cloned $GABA_B$ receptors compared to a much larger number of compounds characterized in binding experiments (Kaupmann *et al*., 1997; 1998a; Jones *et al*., 1998; White *et al*., 1998; Kuner *et al*., 1999; Ng *et al*., 1999b). In order to overcome these limitations on functional assays by designing a 'more sensitive and much-needed' functional assay system (Bettler *et al*., 1998), we used a strategy of co-transfection of the receptors with the chimeric G-protein named Gαq-z5 in which the five C-terminal aminoacids of Gαq have been replaced by the C-terminus of Gαz (a member of the Gαi subgroup). In this manner, receptors coupled to the Gαi class of G-proteins have previously been shown to mediate robust stimulative responses with the expected pharmacological profiles on e.g., monoamine receptors (Conklin *et al*., 1993; Bräuner-Osborne & Brann, 1996) and metabotropic glutamate receptors (Gomeza *et al*., 1996) and as shown in this paper a similar robust signal (generation of inositol phosphates) can be obtained from heterodimeric $GABA_B$ receptors when these are co-transfected with Gαq-z5.

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As pointed out previously, two different splice variants of $GABA_BR1$ have been identified differing in the distal amino terminal part of the amino-terminal of the receptor. The two splice variants show distinct expression patterns and different developmental regulation, indicating that they could have different physiological functions (Kaupmann *et al*., 1998b; Malitschek *et al*., 1998). Since it has recently been shown that GABA binds to the amino terminal domain (ATD) of the receptor (Galvez *et al*., 1999), it is tempting to speculate that the pharmacology of the splice variants could differ. So far, binding studies of the $GABA_BR1$ splice variants expressed alone (Kaupmann *et al*., 1997; 1998b) or in the presence of $GABA_BR2$ (Kaupmann *et al*., 1998a) have not revealed any such difference in binding affinity between the two splice variants. However, this does not rule out that the splice variants could differ in their pharmacological profiles in functional assays. Thus, in order to investigate this possibility we have also compared the pharmacology of a number of commonly used $GABA_B$ ligands (Figure 1) on the two splice variants of $GABA_BR1$ co-expressed with $GABA_BR2$ and Gαq-z5.

Methods

Cell culture and second messenger assay

tsA cells (a transformed HEK 293 cell line (Chahine *et al*., 1994)) were maintained at 37°C in a humidified 5% CO_2 incubator in Dulbecco's modified Eagle medium (DMEM) supplemented with penicillin (100 U ml^{-1}), streptomycin (100 mg ml^{-1}) and 10% foetal calf serum. Based on previously published protocols we decided to transfect the cells with a 5 fold excess of $GABA_BR2$ as compared to the $GABA_BR1$ splice variants (White *et al*., 1998; Kaupmann *et al*., 1998a). Two million cells were split into a 15 cm tissue culture plate and transfected with 1.4 μg $GABA_BR1a$-pcDNA3.1 or $GABA_BR1b$-pcDNA3.1, 7 μg $GABA_BR2$-pcDNA3.1 and 1.4 μg Gαq-z5-pcDNA the following day using SuperFect as a DNA carrier according to the protocol by the manufacturer (Qiagen, Hilden, Germany). In experiments where cells were only transfected with a subset of the plasmids, total DNA was maintained at the same level with empty pcDNA3 vector. The day after transfection, cells were split into two poly-D-lysine coated 24-well tissue culture plates in inositol-free growth medium containing 1 μCi ml^{-1} myo-[2-^{3}H]inositol (Amersham, Buckinghamshire, U.K.). Sixteen to twenty hours later, cells were washed with phosphate buffered saline solution (PBS) and incubated for 20 min in PBS containing 0.9 mM $CaCl_2$ and 1.05 mM $MgCl_2$ (PBS^{++}). Cells were then incubated for another 20 min in PBS^{++} containing 10 mM LiCl (PBS^{++}-LiCl). Finally, after the cells had been incubated in PBS^{++}-LiCl for 40 min with the indicated amounts of agonists, reactions were terminated by ice-cold 10 mM formic acid and total inositol phosphate (IP) generation was determined by ion-exchange chromatography as described previously (Nanevics *et al*., 1996). In the case of antagonist assays, cells were incubated in PBS^{++} for 20 min then in PBS^{++}-LiCl containing antagonist for 20 min and finally in PBS^{++}-LiCl containing both antagonist and 5 μM GABA for 40 min.

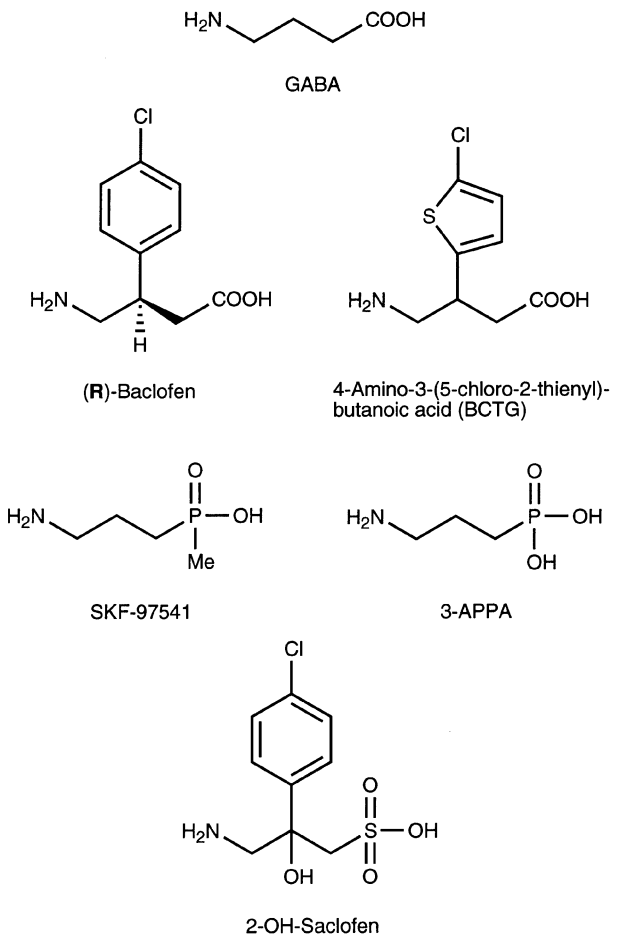

Figure 1 Structure of the $GABA_B$ ligands used in this study.

Materials

GABA and (**R**)-baclofen were obtained from Sigma (St. Louis, MO, U.S.A.). 3-Aminopropyl(methyl)phosphinic acid (SKF-97541), (**RS**)-4-amino-3-(5-chloro-2-thienyl)-butanoic acid (BCTG), 3-aminopropylphosphonic acid (3-APPA) and (**RS**)-3-amino-2-(4-chlorophenyl)-2-hydroxypropylsulphonic acid (2-OH-saclofen) were obtained from Tocris Cookson (Bristol, U.K). Culture media, serum and antibiotics for cell culture were obtained from Life Technologies (Paisley, U.K.). The rat $GABA_B$ receptor plasmids and the Gαq-z5 construct were generous gifts from Dr Janet Clark (National Institute of Health, Bethesda, MD, U.S.A.) and Dr Bruce Conklin (University of California, San Francisco, CA, U.S.A.), respectively. The pcDNA3 vector was obtained from Stratagene (La Jolla, CA, U.S.A.). The tsA cells were a generous gift from Dr Penelope S.V. Jones (University of California, San Diego, CA, U.S.A.).

Data analysis

All experiments were performed in triplicate and the results are given as mean ± s.e.mean of 3–5 experiments. Antagonist potencies were calculated from inhibition curves using the 'functional equivalent' of the Cheng-Prusoff equation $K_B = IC_{50}/(1 + ([A]/EC_{50}))$ (Craig, 1993), where [A] is the fixed agonist concentration.

Results

Initially, we tested whether it was necessary to express $GABA_BR1$, $GABA_BR2$ and Gαq-z5 simultaneously in order to stimulate IP formation. As expected, cells transfected with $GABA_BR1a$ and $GABA_BR2$ were unable to stimulate IP

formation when exposed to the agonist (**R**)-baclofen (Figure 2). In contrary, cells further co-transfected with Gαq-z5 gave a robust response to the agonist as a 4–6 fold increase in IP formation. As seen in Figure 2, the monomeric $GABA_BR1a$ or $GABA_BR2$ co-expressed with Gαq-z5 were also unable to respond to the agonist. Thus, in conclusion $GABA_BR1$, $GABA_BR2$ and Gαq-z5 all needed to be expressed in order to obtain a functional IP response. Cells transfected with Gαq-z5 alone did also not show any increase in IP formation to (**R**)-baclofen or any of the other ligands used in this study (data not shown).

We then went on to test a series of commonly used $GABA_B$ receptor ligands (shown in Figure 1) in greater detail on tsA cells transiently transfected with either $GABA_BR1a$, $GABA_BR2$ and Gαq-z5 or $GABA_BR1b$, $GABA_BR2$ and Gαq-z5. As can be seen on the examples of concentration-response curves shown in Figure 3 and the derived pEC_{50} and pK_B values shown in Table 1, the pharmacological profiles of the two $GABA_BR1$ splice variants were almost identical. They both showed a similar rank order of agonist potency; SKF-97541 > GABA > (**R**)-baclofen > BCTG > 3-APPA. Interestingly, whereas SKF-97541, (**R**)-baclofen and BCTG were full agonists, 3-APPA was a partial agonist producing responses of 41 and 61% of a full GABA response on the $GABA_BR1a$ and

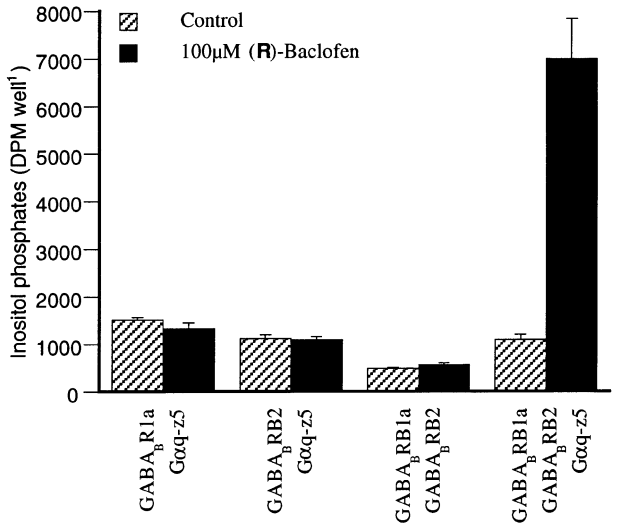

Figure 2 Generation of inositol phosphates by the $GABA_B$ agonist (**R**)-baclofen in tsA cells transfected with varying subsets of the $GABA_B$ receptor subunits and the chimeric G-protein Gαq-z5. After a 20 min preincubation in phosphate-buffered saline containing 0.9 mM $CaCl_2$ and 1.05 mM $MgCl_2$ (PBS^{++}) cells were incubated for further 20 min in the same buffer containing 10 mM LiCl (PBS^{++}-LiCl). Finally, after the cells had been incubated in PBS^{++}-LiCl for 40 min in the absence or presence of 100 μM (**R**)-baclofen, reactions were terminated by ice-cold 10 mM formic acid and total inositol phosphate generation was determined by ion-exchange chromatography. Data are the means±s.d.mean of representative experiments performed in triplicate.

Table 1 Agonist and antagonist potencies on heterodimeric $GABA_BR1$ / $GABA_BR2$ receptors in functional assay

	$GABA_BR1a$/ $R2$/Gαq-z5	*$GABA_BR1b$/ $R2$/Gαq-z5*
Agonist	pEC_{50}	
GABA	5.9±0.1	6.2±0.2
(**R**)-Baclofen	5.7±0.1	5.8±0.2
SKF-97541	6.4±0.1	6.5±0.2
3-APPA (% of maximal GABA response)	4.4±0.1 (41±5%)	4.4±0.2 (61±6%)
4-Amino-3-(5-chloro-2-thienyl)-butanoic acid (BCTG)	4.8±0.1	4.9±0.3
Antagonist	pK_B	
2-OH-Saclofen	4.9±0.1	5.1±0.1

tsA cells (a transformed HEK 293 cell line) were transiently transfected with $GABA_BR1a$ or $GABA_BR1b$, $GABA_BR2$ and the chimeric G-protein Gαq-z5. Functional responses were measured 2 days after transfection as total inositol phosphates generated during a 40 min exposure to ligand in the presence of 10 mM LiCl. In the case of antagonist assay, the cells were pre-incubated with antagonist for 20 min before the 40 min exposure to antagonist in the presence of 5 μM GABA and 10 mM LiCl. Values are mean±s.e.mean of 3–5 independent experiments.

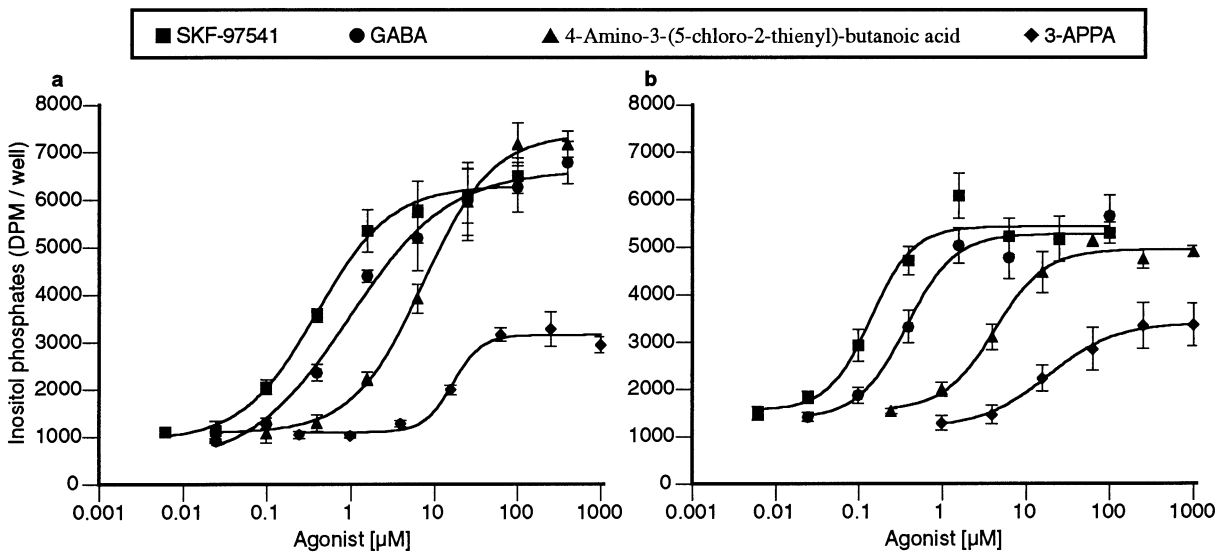

Figure 3 Concentration-response curves of some $GABA_B$ receptor agonists at tsA cells transfected with $GABA_BR1a$, $GABA_BR2$ and Gαq-z5 (a) or $GABA_BR1b$, $GABA_BR2$ and Gαq-z5 (b). Cell treatment and measurement of total inositol phosphates generated was performed as described in Figure 2.

$GABA_B$R1b splice variants, respectively (Figure 3 and Table 1).

Discussion

The first goal of these studies was to develop a functional assay of cloned $GABA_B$ receptors which could circumvent some of the limitations (e.g. low throughput and low signal) of previously reported assays (Kaupmann *et al*., 1997; 1998a; Jones *et al*., 1998; White *et al*., 1998; Kuner *et al*., 1999; Ng *et al*., 1999b; Martin *et al*., 1999) by using a strategy of co-transfection with the chimeric G-protein Gαq-z5. We and others have previously used this strategy to obtain robust stimulative responses from receptors coupled to the Gαi class of G-proteins on monoamine receptors (Conklin *et al*., 1993; Bräuner-Osborne & Brann, 1996) and metabotropic glutamate receptors (Gomeza *et al*., 1996), and in agreement with these results we obtained 4–6 fold increases in IP generation by agonist treatment of cells transfected with both subunits of the $GABA_B$ receptor and the chimeric G-protein (Figures 2 and 3). In agreement with several groups, we were only able to detect agonist responses in cells transfected with both subunits of the $GABA_B$ receptor (Jones *et al*., 1998; White *et al*., 1998; Kaupmann *et al*., 1998a; Ng *et al*., 1999b) whereas no response could be detected in cells transfected with either $GABA_B$R1/Gαq-z5 or $GABA_B$R2/Gαq-z5. Some groups have reported that weak functional responses can be obtained from cells transfected with either $GABA_B$R1 or $GABA_B$R2 alone (Kaupmann *et al*., 1997; 1998b; Martin *et al*., 1999; Kuner *et al*., 1999), but most results point to the fact that both $GABA_B$ receptor subunits have to be co-expressed in order to obtain the expected agonist potency and effector coupling (Jones *et al*., 1998; White *et al*., 1998; Kaupmann *et al*., 1998a; Kuner *et al*., 1999; Ng *et al*., 1999b; Möhler & Fritschy, 1999). Our results are in agreement with these observations as correct coupling to Gαq-z5 only occur when both $GABA_B$ subunits are present.

In order to determine whether the $GABA_B$ receptors were able to activate Gαq-z5 in the same manner as the endogenous G-proteins, we tested a number of commonly used $GABA_B$ ligands in greater detail. Furthermore, we also wanted to test whether the two $GABA_B$R1 splice variants differed in their functional pharmacological profile. First of all we found that both splice variants displayed very similar pharmacology when co-expressed with $GABA_B$R2 and Gαq-z5. This is in agreement with binding studies on cells expressing either $GABA_B$R1a or $GABA_B$R1b alone or in the presence of $GABA_B$R2 which were also unable to detect any pharmacological difference between the two splice variants (Kaupmann *et al*., 1997; 1998a,b; Malitschek *et al*., 1998). Thus, the function of the splice variation in the distal part of the ATD is still unknown. As pointed out recently, it is also possible that the splice variants interact with different membrane anchoring proteins causing distinct localization at the subcellular level (Möhler & Fritschy, 1999), but this remains to be proven by a different set of experiments than those presented in this study. The rank order of agonist potency was thus similar on both splice-variants; SKF-97541 > GABA > (**R**)-baclofen > BCTG > 3-APPA. Although, no previous study has looked at all these agonists simultaneously in functional experiments it can still be concluded that the rank order is in agreement with studies using tissue pharmacology or receptor binding. Thus, SKF-97541 has been reported to be more potent than baclofen (Knight & Bowery, 1996; Seabrook *et al*., 1990), GABA has been reported to be more potent than baclofen (Berthelot *et al*., 1991) which is more potent than BCTG (Berthelot *et al*., 1991; Lacey *et al*., 1993; Ong *et al*., 1997), and 3-APPA has been shown to be less potent than baclofen (Luzzi *et al*., 1986; Kerr *et al*., 1987). The actual pEC_{50} values of our study are also in agreement with those reported in tissues previously. Furthermore, 3-APPA has been shown to be a partial agonist in guinea-pig ileum where it both displayed agonist and antagonist actions (Luzzi *et al*., 1986; Kerr *et al*., 1987), which is in agreement with our findings of 3-APPA being a partial agonist. It should be noted that 3-APPA is a full agonist in the cat spinal cord (Kerr *et al*., 1987). We have previously shown that the intrinsic activity of partial agonists is highly dependent on the expression levels of receptors and G-proteins (Bräuner-Osborne *et al*., 1996) and this could explain why 3-APPA is a partial and full agonist in the ileum and spinal cord, respectively. We also tested the classical $GABA_B$ competitive antagonist 2-OH-saclofen. As shown in Table 1, this compound could also not discriminate between the $GABA_B$R1a ($pK_B = 4.9$) and $GABA_B$R1b ($p_{K_B} = 5.1$) splice variants. However, the potency of 2-OH-saclofen found in our assay is very similar to those found in tissue preparations such as the guinea-pig ileum (pA2 = 5.0) (Kerr *et al*., 1988).

In conclusion, we have reported a new method for functional testing of cloned heterodimeric $GABA_B$ receptors expressed in mammalian cells. The results using the method are in very nice agreement with previously published results from tissue preparations and since the assay has a fairly high throughput and a robust signal, the assay seems to be superior to previously reported functional assays. We can also conclude that at least in our functional assay, we were unable to detect any significant difference between the two $GABA_B$R1 splice variants. This is in agreement with previous reports using binding assays (Kaupmann *et al*., 1997; 1998a,b; Malitschek *et al*., 1998) and the physiological significance of the splice variation in the amino-terminal thus remains a puzzle which warrants further experiments to solve.

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References

BERTHELOT, P., VACCHER, C., FLOUQUET, N., DEBAERT, M., LUYCKX, M. & BRUNET, C. (1991). 3-Thienyl- and 3-furylaminobutyric acids. Synthesis and binding $GABA_B$ receptor studies. *J. Med. Chem.,* **34,** 2557–2560.

BETTLER, B., KAUPMANN, K. & BOWERY, N. (1998). $GABA_B$ receptors: drugs meet clones. *Curr. Opin. Neurobiol.,* **8,** 345–350.

BITTIGER, H., FROESTL, W., MICKEL, S.J. & OLPE, H.R. (1993). $GABA_B$ receptor antagonists: from synthesis to therapeutic applications. *Trends Pharmacol. Sci.,* **14,** 391–394.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature,* **283,** 92–94.

BRÄUNER-OSBORNE, H. & BRANN, M.R. (1996). Pharmacology of muscarinic acetylcholine receptor subtypes (m1-m5): High throughput assays in mammalian cells. *Eur. J. Pharmacol.,* **295,** 93–102.

BRÄUNER-OSBORNE, H., EBERT, B., BRANN, M.R., FALCH, E. & KROGSGAARD-LARSEN, P. (1996). Functional partial agonism at cloned muscarinic acetylcholine receptors. *Eur. J. Pharmacol.,* **313,** 145 – 150.

CHAHINE, M., BENNETT, P.B., GEORGE JR, A.L. & HORN, R. (1994). Functional expression and properties of the human skeletal muscle sodium channel. *Pflügers. Arch.,* **427,** 136 – 142.

CONKLIN, B.R., FARFEL, Z., LUSTIG, K.D., JULIUS, D. & BOURNE, H.R. (1993). Substitution of three amino acids switches receptor specificity of Gq alpha to that of Gi alpha. *Nature,* **363,** 274 – 276.

CRAIG, D.A. (1993). The Cheng-Prusoff relationship: something lost in the translation. *Trends Pharmacol. Sci.,* **14,** 89 – 91.

GALVEZ, T., PARMENTIER, M.L., JOLY, C., MALITSCHEK, B., KAUPMANN, K., KUHN, R., BITTIGER, H., FROESTL, W., BETTLER, B. & PIN, J.P. (1999). Mutagenesis and modeling of the $GABA_B$ receptor extracellular domain support a Venus flytrap mechanism for ligand binding. *J. Biol. Chem.,* **274,** 13362 – 13369.

GOMEZA, J., MARY, S., BRABET, I., PARMENTIER, M.L., RESTITUITO, S., BOCKAERT, J. & PIN, J.-P. (1996). Coupling of metabotropic glutamate receptors 2 and 4 to Gα15, Gα16, and chimeric Gαq/i proteins: characterization of new antagonists. *Mol. Pharmacol.,* **50,** 923 – 930.

HILL, D.R. & BOWERY, N.G. (1981). ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline-insensitive $GABA_B$ sites in rat brain. *Nature,* **290,** 149 – 152.

JONES, K.A., BOROWSKY, B., TAMM, J.A., CRAIG, D.A., DURKIN, M.M., DAI, M., YAO, W.J., JOHNSON, M., GUNWALDSEN, C., HUANG, L.Y., TANG, C., SHEN, Q., SALON, J.A., MORSE, K., LAZ, T., SMITH, K.E., NAGARATHNAM, D., NOBLE, S.A., BRANCHEK, T.A. & GERALD, C. (1998). $GABA_B$ receptors function as a heteromeric assembly of the subunits $GABA_B$R1 and $GABA_B$R2. *Nature,* **396,** 674 – 679.

KAUPMANN, K., HUGGEL, K., HEID, J., FLOR, P.J., BISCHOFF, S., MICKEL, S.J., MCMASTER, G., ANGST, C., BITTIGER, H., FROESTL, W. & BETTLER, B. (1997). Expression cloning of $GABA_B$ receptors uncovers similarity to metabotropic glutamate receptors. *Nature,* **386,** 239 – 246.

KAUPMANN, K., MALITSCHEK, B., SCHULER, V., HEID, J., FRöSTL, W., BECK, P., MOSBACHER, J., BISCHOFF, S., KULIK, A., SHIGEMOTO, R., KARSCHIN, A. & BETTLER, B. (1998a). $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. *Nature,* **396,** 683 – 687.

KAUPMANN, K., SCHULER, V., MOSBACHER, J., BISCHOFF, S., BITTIGER, H., HEID, J., FROESTL, W., LEONHARD, S., PFAFF, T., KARSCHIN, A. & BETTLER, B. (1998b). Human gamma-aminobutyric acid type B receptors are differentially expressed and regulate inwardly rectifying K + channels. *Proc. Natl. Acad. Sci. U.S.A.,* **95,** 14991 – 14996.

KERR, D.I., ONG, J., JOHNSTON, G.A., ABBENANTE, J. & PRAGER, R.H. (1988). 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptors. *Neurosci. Lett.,* **92,** 92 – 96.

KERR, D.I.B., ONG, J., PRAGER, R.H., GYNTHER, B.D. & CURTIS, D.R. (1987). Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.,* **405,** 150 – 154.

KNIGHT, A.R. & BOWERY, N.G. (1996). The pharmacology of adenylyl cyclase modulation by $GABA_B$ receptors in rat brain slices. *Neuropharmacology,* **35,** 703 – 712.

KUNER, R., KÖHR, G., GRÜNEWALD, S., EISENHARDT, G., BACH, A. & KORNAU, H.-C. (1999). Role of heteromer formation in $GABA_B$ receptor function. *Science,* **283,** 74 – 77.

LACEY, G., BERTHELOT, P., VACCHER, C., FLOUQUET, N., VACCHER, M.P., DEBAERT, M. & CURTIS, D.R. (1993). Thienyl-GABA derivatives as specific baclofen agonists in the rat and cat spinal cord in vivo. *Neurosci. Lett.,* **159,** 64 – 66.

LUZZI, S., FRANCHI-MICHELI, S., CIUFFI, M., PAJANI, A. & ZILLETTI, L. (1986). GABA-related activities of amino phosphonic acids on guinea-pig ileum longitudinal muscle. *J. Auton. Pharmacol.,* **6,** 163 – 169.

MALITSCHEK, B., RÜEGG, D., HEID, J., KAUPMANN, K., BITTIGER, H., FRÖSTL, W., BETTLER, B. & KUHN, R. (1998). Developmental changes of agonist affinity at $GABA_B$R1 receptor variants in rat brain. *Mol. Cell. Neurosci.,* **12,** 56 – 64.

MARTIN, S.C., RUSSEK, S.J. & FARB, D.H. (1999). Molecular identification of the human $GABA_B$R2: cell surface expression and coupling to adenylyl cyclase in the absence of $GABA_B$R1. *Mol. Cell Neurosci.,* **13,** 180 – 191.

MORISHITA, R., KATO, K. & ASANO, T. (1990). $GABA_B$ receptors couple to G proteins Go, Go* and Gi1 but not to Gi2. *FEBS Lett.,* **271,** 231 – 235.

MÖHLER, H. & FRITSCHY, J.-M. (1999). $GABA_B$ receptors make it to the top – as dimers. *Trends Pharmacol. Sci.,* **20,** 87 – 89.

NANEVICS, T., WANG, L., CHEN, M., ISHII, M. & COUGHLIN, S.R. (1996). Thrombin receptor activating mutations. *J. Biol. Chem.,* **271,** 702 – 706.

NG, G.Y., MCDONALD, T., BONNERT, T., RIGBY, M., HEAVENS, R., WHITING, P., CHATEAUNEUF, A., COULOMBE, N., KARGMAN, S., CASKEY, T., EVANS, J., O'NEILL, G.P. & LIU, Q. (1999a). Cloning of a novel G-protein-coupled receptor GPR 51 resembling $GABA_B$ receptors expressed predominantly in nervous tissues and mapped proximal to the hereditary sensory neuropathy type 1 locus on chromosome 9. *Genomics,* **56,** 288 – 295.

NG, G.Y.K., CLARK, J., COULOMBE, N., ETHIER, N., HEBERT, T.E., SULLIVAN, R., KARGMAN, S., CHATEAUNEUF, A., TSUKAMOTO, N., MCDONALD, T., WHITING, P., MEZEY, E., JOHNSON, M.P., LIU, Q., KOLAKOWSKI, JR, L.F., EVANS, J.F., BONNER, T.I. & O'NEILL, G.P. (1999b). Identification of a $GABA_B$ receptor subunit, gb2, required for functional $GABA_B$ receptor activity. *J. Biol. Chem.,* **274,** 7607 – 7610.

ONG, J., KERR, D.I.B., VACCHER, C. & BERTHELOT, P. (1997). Actions of thienyl analogs of baclofen at $GABA_B$ receptors in rat neocortical slices. *Eur. J. Pharmacol.,* **329,** 133 – 136.

SEABROOK, G.R., HOWSON, W. & LACEY, M.G. (1990). Electrophysiological characterization of potent agonists and antagonists at pre- and postsynaptic $GABA_B$ receptors on neurones in rat brain slices. *Br. J. Pharmacol.,* **101,** 949 – 957.

WHITE, J.H., WISE, A., MAIN, M.J., GREEN, A., FRASER, N.J., DISNEY, G.H., BARNES, A.A., EMSON, P., FOORD, S.M. & MARSHALL, F.H. (1998). Heterodimerization is required for the formation of a functional $GABA_B$ receptor. *Nature,* **396,** 679 – 682.

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