Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1573–1577

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Bioisosteric Determinants for Subtype Selectivity of Ligands for Heteromeric $GABA_A$ Receptors

Bjarke Ebert,[a,†] Martin Mortensen,[a] Sally A. Thompson,[b] Jan Kehler,[a,†] Keith A. Wafford[b] and Povl Krogsgaard-Larsen[a,*]

[a]*The Centre for Drug Design and Transport, Departments of Pharmacology and Medicinal Chemistry, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark*
[b]*Department of Pharmacology, Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow CM20 2QR, Essex, UK*

Received 29 January 2001; accepted 13 March 2001

Abstract—The potency and efficacy of a series of bioisosterically modified GABA analogues were determined electrophysiologically using heteromeric $GABA_A$ receptors expressed in *Xenopus* oocytes. These agonist parameters were shown to be strongly dependent on the receptor subunit combination. On the other hand, the antagonist potencies of the classical $GABA_A$ antagonists SR 95531 (**7**) and BMC (**8**) and also of **5g** and the phosphinic acid bioisosteres of **5a**, compounds **5f** and **6**, were essentially independent of the receptor subunit combinations.

4-Aminobutyric acid (GABA), which is the major inhibitory neurotransmitter in the central nervous system (CNS), operates through multiple receptors subdivided into the ionotropic $GABA_A$ and $GABA_C$ receptors and the metabotropic $GABA_B$ receptors.[1–4] The pentameric $GABA_A$ receptors are composed of subunits termed α, β, γ, δ, ε, π, and θ, most of which form groups of homologous proteins.[5,6] These heteromeric $GABA_A$ receptors are receptor complexes containing modulatory binding sites for a number of drugs including benzodiazepines, barbiturates, and neurosteroids, in addition to the GABA recognition sites.[1,5,6]

The $GABA_A$ receptors have been implicated in a variety of diseases and disease conditions, and in addition to modulatory $GABA_A$ receptor ligands, compounds interacting directly with the GABA recognition sites have considerable therapeutic interest as, for example, analgesic, hypnotic, or anxiolytic agents.[1,7,8] $GABA_A$ receptors are, however, ubiquitously and abundantly distributed in the CNS, and a prerequisite for the development of therapeutically useful direct acting GABAergic drugs is the design of ligands that selectively target restricted brain regions or subpopulations of neurones in the CNS. Accumulating evidence derived from in situ hybridization and immunocytochemistry studies actually seem to indicate distinct regional and neuronal distribution of $GABA_A$ receptors of different subunit composition.[1]

The recognition sites of $GABA_A$ receptors are formed by conserved protein regions, which may explain earlier observations of constant binding affinities of agonists and competitive antagonists irrespective of receptor subunit composition.[9] The GABA recognition sites are, however, located at interfaces between α and β subunits, and the functional selectivity of the few $GABA_A$ agonists so far studied may reflect different conformational changes of the interacting proteins forming the binding domains, induced by structurally different $GABA_A$ agonists.[9]

These studies have now been extended to include the new seleninic acid analogues of GABA and of the GABA agonist isonipecotic acid (**5a**), compounds **1** and **5d**, respectively, and the phosphinic (**5e**, **f** and **6**) and phosphonic (**5g**) acid analogues of **5a**. These studies also include 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol

*Corresponding author. Tel.: +45-3530-6511; fax: +45-3530-6040; e-mail: ano@dfh.dk
††Present address: Departments of Pharmacology and Medicinal Chemistry, H. Lundbeck A/S, 9 Ottiliavej, DK-2500 Valby-Copenhagen, Denmark.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00184-6

(THIP, **2**) and imidazole-4-acetic acid (IAA, **3**), both of which are systemically active $GABA_A$ agonists of clinical interest.[7,8,10]

The compounds 3-aminopropaneseleninic acid (**1**) and piperidin-4-ylseleninic acid (**5d**),[11] 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **2**),[12] isoguvacine (**4**),[13] piperidin-4-ylsulphonic acid (P4S, **5b**),[14] 5-(piperidin-4-yl)isothiazol-3-ol (Thio-4-PIOL, **5c**),[15] and piperidin-4-ylphosphinic acid (**5e**), methyl(piperidin-4-yl)phosphinic acid (**5f**), piperidin-4-ylphosphonic acid (**5g**) and 4-hydroxypiperidin-4-ylphosphinic acid (**6**)[16] were synthesized as described earlier. Imidazole-4-acetic acid (IAA, **3**) was obtained from Tocris Cookson, and all other compounds were purchased from Sigma Biochemicals.

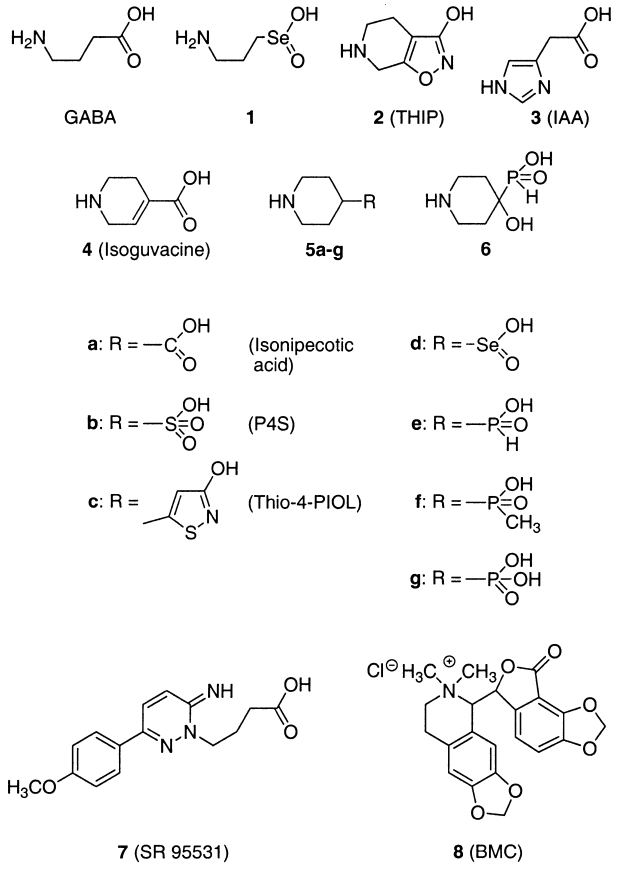

Figure 1. Structures of $GABA_A$ receptor ligands characterized in the present study.

The initial step in the electrophysiological[17] determination of comparative pharmacological profiles of GABA, the classical $GABA_A$ agonists THIP (**2**), isoguvacine (**4**), isonipecotic acid (**5a**), and P4S (**5b**),[18] and the new $GABA_A$ receptor ligands was construction of dose/response curves for all compounds under study at concentrations up to 1 mM (not illustrated). Compounds showing no significant response at this concentration were tested as antagonists. As described in the previous section, antagonist experiments were performed at a fixed concentration of antagonist (1 mM) and varying concentrations of GABA in order to determine K_i values, which are independent of the potency of the agonist.

Like GABA and isonipecotic acid (**5a**), the respective seleninic acid bioisosteres **1** and **5d** and also the phosphinic acid bioisostere of **5a**, compound **5e** (Fig. 1) were $GABA_A$ agonists, showing an agonist profile, which was highly dependent on the receptor subunit combination as illustrated in Table 1 and Figures 2 and 3. The agonist potency was frequently highest at receptors containing α5 or, in particular, α6 subunits and generally lowest at α4-containing receptors. Furthermore, the maximal response of $GABA_A$ agonists relative to that of GABA was shown to be dependent on the type of α subunit present. Compound **1** was a partial agonist at all subunit combinations tested, showing the lowest maximal response of α4-containing receptors. On the other hand, at this α4β3γ2 $GABA_A$ receptor, **5a** showed maximal agonist response but partial agonist responses at the other subunit combinations studied. The most efficacious partial agonist response of **5a** was observed at the α6β3γ2 receptor and was comparable with the relatively uniform effect elicited by isoguvacine (**4**) at all receptors tested (Table 1). Thus, high maximal response and exceptionally low potency at α4β3γ2 receptors, make isonipecotic acid (**5a**) a rather unique $GABA_A$ receptor ligand.

The results summarized in Table 1 emphasize that the effects of GABA and $GABA_A$ agonists are strongly dependent on the subunit combination of the $GABA_A$ receptor indicating that functional selectivity is an

Table 1. Agonist activity as function of $GABA_A$ receptor subunit composition[a]

	α1β3γ2		α2β3γ2		α3β3γ2		α4β3γ2		α5β3γ2		α6β3γ2	
	EC_{50}	MAX	EC_{50}	MAX	EC_{50}	MAX	EC_{50}	MAX	EC_{50}	MAX	EC_{50}	MAX
GABA	80	100	40	100	28	100	50	100	3.0	100	1.5	100
1	1400	30	1500	47	4900	33	2100	16	1900	58	3000	51
3	310	24	600	72	300	54	340	27	420	56	460	25
4	160	88	50	88	55	69	180	88	75	94	20	98
5a	620	46	420	53	370	46	300	104	690	57	160	83
5b	40	21	26	96	65	75	200	7.2	20	92	20	15
5d	200	3	170	2	180	28	160	0	420	3	240	13
5e	270	5	280	17	160	29	140	0	260	17	140	35

[a]Agonists were characterized at human $GABA_A$ receptors containing $\alpha_x\beta_3\gamma_{2s}$ ($x = 1$–6), expressed in *Xenopus* oocytes. EC_{50} values are in μM, and dose/response curves were fitted. The maximum responses (MAX), relative to that of GABA at the same oocyte, were calculated.[17] Values presented are mean values. SEM values for maximum responses were less than 10% and relative SEM of pEC_{50} values were less than 10%.

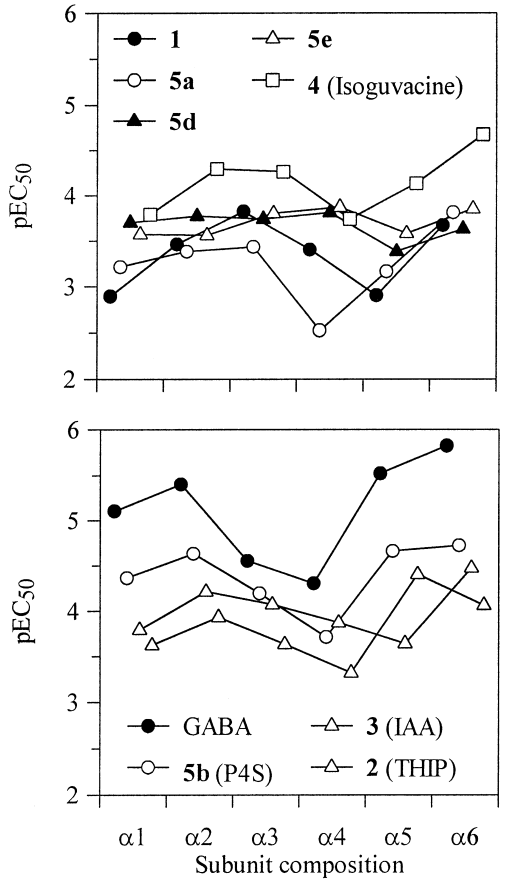

Figure 2. Agonist potencies as function of $GABA_A$ receptor subunit composition. Agonist potencies (EC_{50} values in μM) were determined at human $GABA_A$ receptors containing $\alpha_x\beta_3\gamma_{2s}$ ($x = 1$–6), expressed in *Xenopus* oocytes. Dose/response curves were constructed. Values are presented as pEC_{50} values.

important pharmacological parameter for $GABA_A$ receptor activating compounds. Thus P4S (**5b**) effectively activates α2β3γ2 and α5β3γ2 receptors but essentially blocks α4β3γ2 and α6β3γ2 receptors, whereas the structurally related bioisosteres, **5d** and **5e**, act as low-efficacy agonists at α3β3γ2 and α6β3γ2 receptors, but as essentially purely competitive antagonists at the other $GABA_A$ receptors tested (Table 1).

As illustrated in Figures 2 and 3, the pattern of $GABA_A$ receptor subunit dependence of the maximal responses of THIP (**2**) and IAA (**3**) is qualitatively similar to that of P4S (**5b**). THIP (**2**) as well as IAA (**3**) are active after systemic administration in man.[10,18] THIP (**2**) shows non-opioid analgesic effect and is currently the subject of clinical studies as an atypical hypnotic agent.[19] These observations have focused new interest on IAA (**3**) as a clinically useful agent and emphasize the therapeutic prospects of the progressive mapping of the subunit composition of the $GABA_A$ receptors in different regions of the CNS.[1]

Conversion of the low-efficacy partial $GABA_A$ agonist **5e** into the structurally related phosphinic acids **5f** and **6** and substitution of a phosphonic acid group for the phosphinic acid group of **5e** to give **5g** result in $GABA_A$ antagonists. In agreement with earlier observations for $GABA_A$ antagonists,[9] the potencies of these antagonists were essentially independent of the subunit composition of the receptors studied (Table 2). The potencies of these $GABA_A$ antagonists were lower than that earlier reported for the bioisostere of **5a** containing a 3-isothiazolol group (**5c**),[9] and markedly lower than those of the classical $GABA_A$ antagonists SR 95531 (**7**) and BMC (**8**) (Fig. 1). The antagonists are envisaged to bind to and stabilize a distinct inactive receptor conformation,[9,20,21]

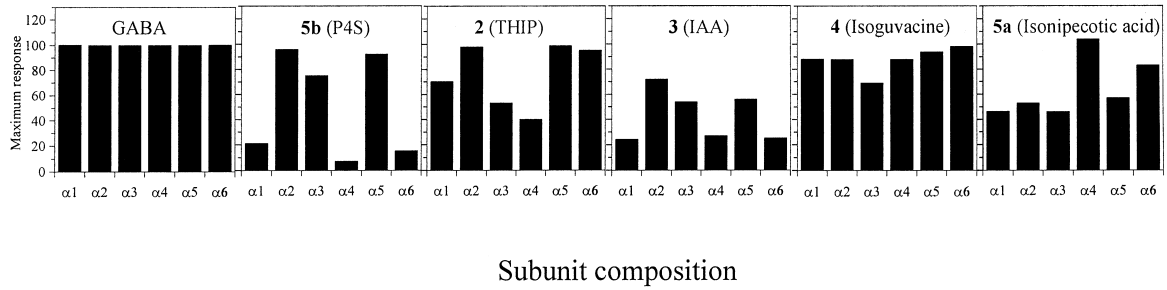

Figure 3. Maximum response of different agonists as function of $GABA_A$ receptor subunit composition. Agonists were characterized at human $GABA_A$ receptors containing $\alpha_x\beta_3\gamma_{2s}$ ($x = 1$–6), expressed in *Xenopus* oocytes. Dose/response curves were fitted and the maximum response, relative to that of GABA at the same oocyte, was calculated.[17]

Table 2. Antagonist potencies as function of $GABA_A$ receptor subunit composition[a]

	K_i values in μM					
	α1β3γ2	α2β3γ2	α3β3γ2	α4β3γ2	α5β3γ2	α6β3γ2
5f	310	600	300	340	420	460
5g	3300	2100	3800	3100	2800	4800
6	1400	1500	4900	2100	1900	3000

[a]Antagonist potencies (K_i values in μM) were determined at human $GABA_A$ receptors containing $\alpha_x\beta_3\gamma_{2s}$ ($x = 1$–6), expressed in *Xenopus* oocytes by parallel shift of GABA dose/response curves in the presence of a fixed concentration of antagonist. Values presented are mean values. Relative SEM of pK_i values were less than 10%.

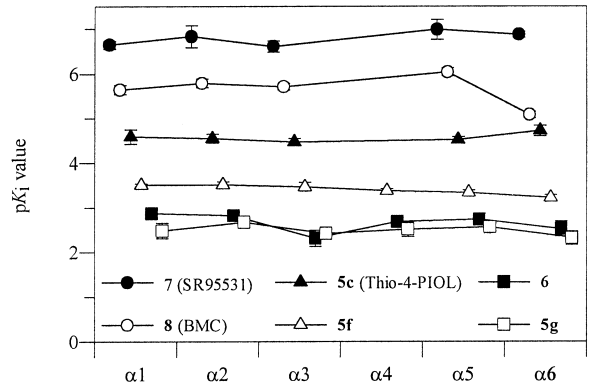

Figure 4. Antagonist potencies as function of $GABA_A$ receptor subunit composition. Antagonist potencies (K_i values in μM) were determined at human $GABA_A$ receptors containing $\alpha_x\beta_3\gamma_{2s}$ ($x = 1$–6), expressed in *Xenopus* oocytes by parallel shift of GABA dose/response curves in the presence of a fixed concentration of antagonist. Values are presented as pK_i values ± SEM.

implying that the molecular mechanisms underlying receptor blockade are less complex than those involved in the transformation of agonist receptor binding to receptor activation, as reflected by the data summarized in Table 2 and Figure 4.

The molecule of GABA is highly flexible, and it has been hypothesized that different conformations of GABA are recognized and bound by different GABA recognition sites. There is strong evidence supporting the view that GABA interacts with its transporters in a conformation different from the conformation(s) recognized by the receptors.[7,10,18] During the interaction with the former class of recognition sites, the conformational flexibility of GABA may be a factor of importance,[22] but the present studies seem to indicate that this structural parameter does not play a significant role during receptor activation. Thus, although the molecular flexibility of compound **1** is comparable with that of GABA, **1** is markedly less potent and efficacious than GABA at all subtypes of GABA receptors studied (Table 1). The ring of isoguvacine (**4**) is less flexible than that of isonipecotic acid (**5a**). Still **4** is more potent and generally more efficacious as a $GABA_A$ agonist than **5a**. THIP (**2**), which is essentially conformationally rigid, is only slightly less potent than **4** and shows an efficacy profile similar to those of **4** and **5a** (Table 1 and Fig. 2).

The structure of the carboxyl bioisosteric groups of the compounds under study is, on the other hand, a factor of key importance for the pharmacology of the ligands. There is strong evidence that an arginine residue at the $GABA_A$ receptor recognition sites is directly involved in the binding of the anionic part of receptor ligands.[23] The results of recent structure/activity studies strongly suggest that the anionic 3-isoxazolol groups of $GABA_A$ agonists like THIP (**2**) and anionic groups of analogues of Thio-4-PIOL (**5c**) showing predominant or full $GABA_A$ antagonist effects exhibit different modes of interaction with the guanidinium group of arginine.[23] The interaction of seleninic, sulphonic, phosphinic, and phosphonic acid groups with the guanidinium group of arginine remains to be studied in detail, but computational chemistry studies along the lines described[23] may shed light on the molecular mechanisms underlying the structure/activity relationships described here.

Acknowledgements

This work was supported by the Lundbeck Foundation and the Danish Medicinal Research Council. The secretarial assistance of Anne Nordly is gratefully acknowledged.

References and Notes

1. Stephenson, F. A. In *Amino Acid Neurotransmission*; Stephenson, F. A., Turner, A. J., Eds.; Portland: London, 1998; pp 65–92.
2. Chebib, M.; Johnston, G. A. R. *J. Med. Chem.* **2000**, *43*, 1427.
3. Marshall, F. H.; Jones, K. A.; Kaupmann, K.; Bettler, B. *Trends Pharmacol. Sci.* **1999**, *20*, 396.
4. *GABA: Receptors, Transporters and Metabolism*; Tanaka, C., Bowery, N. G., Eds.; Birkhäuser: Basel, 1996.
5. Barnard, E. A.; Skolnick, P.; Olsen, R. W.; Mohler, H.; Sieghart, W.; Biggio, G.; Braestrup, C.; Bateson, A. N.; Langer, S. Z. *Pharmacol. Rev.* **1998**, *50*, 291.
6. Bonnert, T. P.; McKernan, R. M.; Farrar, S.; le Bourdellès, B.; Heavens, R. P.; Smith, D. W.; Hewson, L.; Rigby, M. R.; Sirinathsinghji, D. J.; Brown, N.; Wafford, K. A.; Whiting, P. J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9891.
7. Krogsgaard-Larsen, P.; Frølund, B.; Kristiansen, U.; Frydenvang, K.; Ebert, B. *Eur. J. Pharm. Sci.* **1997**, *5*, 355.
8. *The GABA Receptors*; Enna, S. J., Bowery, N. G., Eds.; Humana: Totowa, 1997.
9. Ebert, B.; Thompson, S. A.; Suonatsou, K.; McKernan, R.; Krogsgaard-Larsen, P.; Wafford, K. A. *Mol. Pharmacol.* **1997**, *52*, 1150.
10. Krogsgaard-Larsen, P.; Hjeds, H.; Falch, E.; Jørgensen, F. S.; Nielsen, L. *Adv. Drug Res.* **1988**, *17*, 381.
11. Stuhr-Hansen, N.; Ebert, B.; Krogsgaard-Larsen, P.; Kehler, J. *Org. Lett.* **2000**, *2*, 7.
12. Krogsgaard-Larsen, P. *Acta Chem. Scand.* **1977**, *B31*, 584.
13. Krogsgaard-Larsen, P.; Roldskov-Christiansen, T. *Eur. J. Med. Chem.* **1979**, *14*, 157.
14. Krogsgaard-Larsen, P.; Falch, E.; Schousboe, A.; Curtis, D. R.; Lodge, D. *J. Neurochem.* **1980**, *34*, 756.
15. Frølund, B.; Kristiansen, U.; Brehm, L.; Hansen, A. B.; Krogsgaard-Larsen, P.; Falch, E. *J. Med. Chem.* **1995**, *38*, 3287.
16. Kehler, J.; Ebert, B.; Dahl, O.; Krogsgaard-Larsen, P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3241.
17. The cDNAs encoding α1–α6, β3, and γ2 subunits have been described previously.[24–26] *Xenopus* oocytes were removed from anaesthetized frogs and manually defolliculated with fine forceps. After mild collagenase treatment to remove follicle cells type IA (0.5 mg/mL) for 6 min, the oocyte nuclei were directly injected with 10–20 nL of injection buffer (88 mM NaCl, 1 mM KCl, 15 mM HEPES, at pH 7.0, nitrocellulose filtered) containing different combinations of human $GABA_A$ subunit cDNAs (6 ng/mL) engineered into the expression vector pCDM8 or pcDNAAmp. Following incubation for 24 h, oocytes were placed in a 50 μL bath and perfused with modified Barth's medium (MBS) consisting of 88 mM NaCl, 1 mM KCl, 10 mM HEPES, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$, 2.4 mM $NaHCO_3$, pH 7.5. Cells were impaled with two 1–3 MΩ electrodes containing 2 M KCl and voltage

clamped between −40 and −70 mV. The cell was continuously perfused with saline at 4–6 mL/min, and drugs were applied in the perfusate. GABA or $GABA_A$ agonists were applied until the peak of the response was observed, usually 30 s or less. At least 3 min wash time was allowed between each agonist application to prevent desensitization. Data from each oocyte were analyzed with respect to the maximum response, relative to either the plateau level for a full concentration/response curve for GABA or the response to 3 mM GABA (no difference). Concentration/response curves were calculated using a non-linear squares fitting program to the equation $f(x) = B_{max}/(1 + (EC_{50}/x^n)$ where x is the drug concentration, EC_{50} is the concentration of drug eliciting a half-maximal response, and n is the Hill coefficient. Antagonist experiments were carried out with 3–5 concentrations of GABA followed by 3–5 concentrations of GABA in the presence of the antagonist. The shift of the dose/response curve to GABA was determined in the response range, where the two obtained curves were parallel. The dose ratio, calculated as the ratio between the concentration of GABA in the presence and absence of antagonist, respectively, was transformed to a K_i value by the equation: log (dose ratio −1) = −log (K_i)−log ([antagonist]).

18. Krogsgaard-Larsen, P.; Frølund, B.; Jørgensen, F. S.; Schousboe, A. *J. Med. Chem.* **1994**, *37*, 2489.
19. Lancel, M.; Langebartels, A. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 1084.
20. Macdonald, R. L.; Olsen, R. W. *Annu. Rev. Neurosci.* **1994**, *17*, 569.
21. Thompson, S. A.; Bonnert, T. P.; Whiting, P. J.; Wafford, K. A. *Toxicol. Lett.* **1998**, *100*, 233.
22. Krogsgaard-Larsen, P.; Frølund, B.; Frydenvang, K. *Curr. Pharm. Des.* **2000**, *6*, 1193.
23. Frølund, B.; Tagmose, L.; Liljefors, T.; Stensbøl, T. B.; Engblom, C.; Kristiansen, U.; Krogsgaard-Larsen, P. *J. Med. Chem.* **2000**, *43*, 4930.
24. Hadingham, K. L.; Garrett, E. M.; Wafford, K. A.; Bain, C.; Heavens, R. P.; Sirinathsinghji, D. J.; Whiting, P. J. *Mol. Pharmacol.* **1996**, *49*, 253.
25. Hadingham, K. L.; Wafford, K. A.; Thompson, S. A.; Palmer, K. J.; Whiting, P. J. *Eur. J. Pharmacol.* **1995**, *291*, 301.
26. Hadingham, K. L.; Wingrove, P. B.; Wafford, K. A.; Bain, C.; Kemp, J. A.; Palmer, K. J.; Wilson, A. W.; Wilcox, A. S.; Sikela, J. M.; Ragan, C. I.; Whiting, P. J. *Mol. Pharmacol.* **1993**, *44*, 1211.