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Two imidazoquinoxaline ligands for the benzodiazepine site sharing a second low affinity site on rat $GABA_A$ receptors but with the opposite functionality

Haesook K. Im, [2]Wha Bin Im, Jeffrey F. Pregenzer, Nancy C. Stratman, Philip F. VonVoigtlander & [1]E. Jon Jacobsen

CNS Diseases Research and [1]Research-Structural Analytical & Medicinal Chemistry, Pharmacia & Upjohn, Inc, 301 Henrietta Street, Kalamazoo, MI 49001, U.S.A.

1 Imidazoquinoxaline PNU-97775 and imidazoquinoline PNU-101017 are benzodiazepine site ligands with a second low affinity binding site on $GABA_A$ receptors, the occupancy of which at high drug concentrations reverses their positive allosteric activity via the benzodiazepine site, and may potentially minimize abuse liability and physical dependence.

2 In this study we discovered, with two imidazoquinoxaline analogues, that the functionality of the second site was altered by the nitrogen substituent on the piperazine ring moiety: PNU-100076 with a hydrogen substituent on the position produced a negative allosteric effect via the second low affinity site, like the parent compounds, while PNU-100079 with a trifluoroethyl substituent produced a positive allosteric response.

3 These functional characteristics were monitored with Cl^- currents measurements in cloned rat $\alpha x\beta 2\gamma 2$ subtypes of $GABA_A$ receptors expressed in human embryonic kidney 293 cells, and further confirmed in rat cerebrocortical membranes containing complex subtypes of $GABA_A$ receptors with binding of [^{35}S]-TBPS, which is a high affinity ligand specific for $GABA_A$ receptors with exquisite sensitivity to allosteric modulations.

4 This structure-functional relationship could be exploited to further our understanding of the second allosteric site of imidazoquinoxaline analogues, and to develop more effective benzodiazepine site ligands without typical side effects associated with those currently available on the market.

Keywords: $GABA_A$ $\alpha 1\beta 2\gamma 2$ receptor subtype; $GABA_A$ $\alpha 6\beta 2\gamma 2$ receptor subtype; benzodiazepine site; ligands of dual functionality; imidazoquinoxaline

Introduction

γ-Aminobutyric $acid_A$ ($GABA_A$) receptors are ligand-gated chloride channel complexes in the central nervous system, and their allosteric modulators have been therapeutically useful as hypnotics, anxiolytics and anticonvulsants (Hafely *et al*., 1985; Delorey & Olsen, 1992; Barnard *et al*., 1993; Sieghart, 1995). Recently we have found a group of imidazoquinoline and imidazoquinoxaline analogues which interact with two allosteric sites on recombinant $GABA_A$ receptors of rat $\alpha x\beta 2\gamma 2$ subtypes (Im *et al*., 1995; 1996). The drugs at low concentrations interact with the benzodiazepine site as positive allosteric modulators, but at high concentrations interact with a second low affinity site to reverse their positive allosteric action and also to inhibit GABA currents (Im *et al*., 1995; 1996). This dual functionality of the drugs, in principle, may minimize their liability to abuse, a side effect commonly associated with most benzodiazepines currently available on the market. Their second low affinity site thus seems to be of therapeutic interest, and has been shown to be distinct from those for benzodiazepines, barbiturates and neurosteroids (Im *et al*., 1995; 1996). In this study we describe the discovery of an imidazoquinoxaline analogue with a positive allosteric action for the second site, opposite to the other analogues. The key functional group of its reverse functionality appears to be the trifluoroethyl substituent on its piperazine ring moiety (Figure 1a). We describe here its functional properties as monitored with its actions on Cl^- currents in cloned $GABA_A$ receptors and [^{35}S]-*tert*-butylbicyclophosphorothionate (TBPS) binding in the receptors in rat brain membranes. [^{35}S]-TBPS is a high affinity ligand specific for $GABA_A$ receptors with exquisite sensitivity to allosteric modulations (Squires *et al*., 1983; Gee *et al*., 1986; Im & Blakeman, 1991; Squires & Saederup, 1993).

[2] Author for correspondence.

Methods

Cloned $GABA_A$ receptors

The stable cell lines expressing the indicated combinations of $\alpha 1$ or $\alpha 6$, $\beta 2$ and $\gamma 2$ subunits of $GABA_A$ receptors were derived by transfection of plasmids containing cDNA and a plasmid encoding G418 resistance into human embryonic kidney cells (HEK 293 cell) (Hamilton *et al*., 1993). After two weeks of selection in 1 mg ml^{-1} G418, resistant cells were assayed for the ability to express respective $GABA_A$ receptor mRNAs by Northern blotting. The cells expressing appropriate combinations of the mRNAs were used for electrophysiological measurements of GABA-induced Cl^- currents.

Electrophysiology

The whole cell patch clamp technique (Hamill *et al*., 1981) was used to record the GABA-mediated Cl^- currents in HEK 293 cells expressing various combinations of $GABA_A$ receptor subunits as described previously (Draguhn *et al*., 1990).

Briefly, patch pipettes were prepared from borosilicate glass tubes and were fire-polished to a tip resistance of 0.5–2 MΩ when filled with a solution containing (in mM): CsCl 140, EGTA 11, $MgCl_2$ 4, ATP 2 and HEPES 10, pH 7.3. Cells were bathed in an external solution containing (in mM): NaCl 135, KCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8 and HEPES 5, pH 7.2. GABA was dissolved in the external solution to a final concentration of 0.25 or 5 μM, and was applied with or without indicated drugs through a U-tube placed within 100 μm of the target cell. A submaximal concentration of GABA was chosen for individual receptor subtypes at which the actions of allosteric modulators are optimally measurable. The current was recorded with an Axopatch 1D amplifier and a CV-4 headstage (Axon Instrument Co.). A Bh-1 bath headstage was used to compensate for changes in bath potentials. GABA currents were measured at a holding potential of −60 mV at room temperature (21–24°C).

[^{3}H]-flunitrazepam and [^{35}S]-TBPS binding

Binding of [^{3}H]-flunitrazepam and its displacement by test ligands was measured in the cell membranes expressing a recombinant GABA$_A$ receptor, the $\alpha1\beta2\gamma2$ subtype, by use of filtration techniques as described elsewhere (Pregenzer *et al*., 1993). [^{3}H]-8-Azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate (Ro 15-4513) was used in the $\alpha6\beta2\gamma2$ subtype. Briefly, the incubation medium consisted of (mM): NaCl 118, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, HEPES/Tris (pH 7.3) 20, 30 μg membrane proteins, one of the radioactive ligands (6 nM) and test ligands at various concentrations in a final volume of 500 μl. The mixtures were incubated at 4°C for 60 min, filtered over a Whatman GF/B filter under vacuum, and washed. Non-specific binding was estimated in the presence of diazepam (or Ro 15-4513 for $\alpha6\beta2\gamma2$) at 200 μM, and was subtracted to compute specific binding. [^{35}S]-TBPS binding was measured, also by filtration techniques (Pregenzer *et al*., 1993), in the rat cerebrocortical synaptosomal membranes which were prepared from brain cortices of male Sprague-Dawley rats (150–180 g) as described elsewhere (Im & Blakeman, 1991). The synaptosomal membranes purified through a sucrose density gradient sedimentation were resuspended to a final concentration of 5 mg ml^{-1} in a solution containing 300 mM sucrose, 5 mM Tris/HCl, pH 7.5, and glycerol (a final concentration of 20%), and stored at −80°C. Briefly, the incubation medium for [^{35}S]-TBPS binding consisted of 1 M NaCl, 10 mM Tris/HCl (pH 7.4), [^{35}S]-TBPS (3 nM unless specified otherwise), 50 μg membrane proteins, and 4 μM GABA with or without 1 μM allosteric ligands in a total volume of 500 μl. The mixtures were incubated for 120 min at 24°C. Non-specific binding was estimated in the presence of 1 mM picrotoxin and was subtracted to compute specific binding.

Chemicals

The following chemicals were prepared at the Pharmacia & Upjohn; *tert*-butyl 7-chloro-4,5-dihydro-5-[(1-(3,4,5-trimethyl)piperazino)carbonyl]-imidazo[1,5-a]quinoxaline-3-carboxylate (PNU-97775), *tert*-butyl 7-chloro-5-[(1-(3,5-dimethyl)piperazino)carbonyl]-imidazo[1,5a-]quinoline-3-carboxylate (PNU-101017), *tert*-butyl 7-chloro-4,5-dihydro-5-[(1-(3,5-dimethyl)piperazino)carbonyl]-imidazo[1,5-a]quinoxaline-3-carboxylate (PNU-100076), and *tert*-butyl 7-chloro-4,5-dihydro-5-[(1-(3,5-dimethyl-4-trifluoroethyl)piperazino)carbonyl]-imidazo[1,5-a]quinoxaline-3-carboxylate (PNU-100079).

Results

Both PNU-100076 and PNU-100079 interacted with the benzodiazepine site of the $\alpha1\beta2\gamma2$ subtype, as judged from their inhibition of [^{3}H]-flunitrazepam binding to the GABA$_A$ receptor, with K_i of 5.7±0.7 and 13.0±1.2 nM, respectively. We also examined their actions on GABA-induced Cl^- currents in the $\alpha1\beta2\gamma2$ subtype expressed in HEK 293 cells, using the whole-cell patch clamp technique. PNU-100076 at low concentrations (<1 μM) behaved like a partial positive allosteric modulator of the benzodiazepine site. The drug at 1 μM, for instance, increased the currents to 143±15% (mean±s.d., $n=7$) of that observed with GABA alone, and this action was disappeared in the presence of excess ethyl-8-fluoro-5,6 -dihydro-5-methyl- 6 -oxo-4H-imidazo[1,5a][1,4]benzodiazepine-3-carboxylate (Ro 15-1788), a benzodiazepine antagonist (Figure 1b). However, PNU-100076 at higher concentrations (>1 μM), progressively decreased the current level, e.g., to 115±4 and 55±9% of control at 5 and 20 μM, respectively (Figure 1c). Ro15-1788 did not reverse the inhibitory action of PNU-100076 at high concentrations (Figure 1b). In fact, the antagonist further reduced the current amplitude, probably due to elimination of the positive allosteric action of PNU-100076 via the benzodiazepine site. This biphasic action of PNU-100076 is analogous to that observed with the other analogues of this series such as PNU-97775 and PNU-101017 (Im *et al*., 1995; 1996). PNU-100079 also appeared to be a partial positive allosteric modulator of the benzodiazepine site, as the drug at 1 μM enhanced the currents to 135±14% (mean±s.d., $n=8$) above control, and in the presence of Ro 15-1788 at 10 μM, the currents reduced to 102±4% ($n=5$) ($P<0.01$ from Student's t test, unpaired) (Figure 1b and c). However, the drug at higher concentrations differed from the other analogues by enhancing the currents further, to 156±14, 175±19 and 199±14% of control at 5, 20 and 40 μM, respectively (Figure 1c). This current enhancement by PNU-100079 at the high concentrations was not blocked by Ro 15-1788 (Figure 1b).

The $\alpha6\beta2\gamma2$ is useful for examining the second site, since its benzodiazepine site differs from that of the $\alpha1\beta2\gamma2$ subtype in that it does not interact with classical benzodiazepines, the imidazoquinoxaline and imidazoquinoline analogues (Luddens *et al*., 1990; Wieland *et al*., 1992; Im *et al*., 1995). PNU-100076 and PNU-100079 at 5 μM hardly inhibited [^{3}H]-Ro15-4513 (a benzodiazepine site ligand) binding to the $\alpha6\beta2\gamma2$ subtype. Consistent with the binding data, PNU-100076 showed no current enhancement in the $\alpha6\beta2\gamma2$ subtype, and monophasically inhibited GABA currents from their interaction with the second site (Figure 1d), like the parent compounds, PNU-97775 and PNU-101017 (Im *et al*., 1995; 1996). Analysis of the data showed its maximal inhibition of 50%, a half maximal concentration of 5.4±1.6 μM and a slope factor of 1 (Figure 1d). PNU-100079, on the other hand, monophasically enhanced GABA currents in the $\alpha6\beta2\gamma2$ subtype to a maximal level of 250%, with a half maximal concentration of 10.7±0.6 μM and a slope factor of 0.96 (Figure 1d). These results confirm the positive allosteric interaction of PNU-100079 with the second low affinity site, which is opposite to the actions of PNU-100076, PNU-97775 and PNU-101017.

[^{35}S]-TBPS binding in rat brain membranes, in the presence of exogenous GABA, has been shown to be highly sensitive to allosteric modulations of GABA$_A$ receptors; positive allosteric modulators potentiate GABA-induced inhibition of TBPS binding, and negative modulators enhance TBPS binding by reversing the inhibitory action of GABA (Gee *et al*., 1986; Im & Blakeman, 1991; Squires & Saederup, 1993). We compared

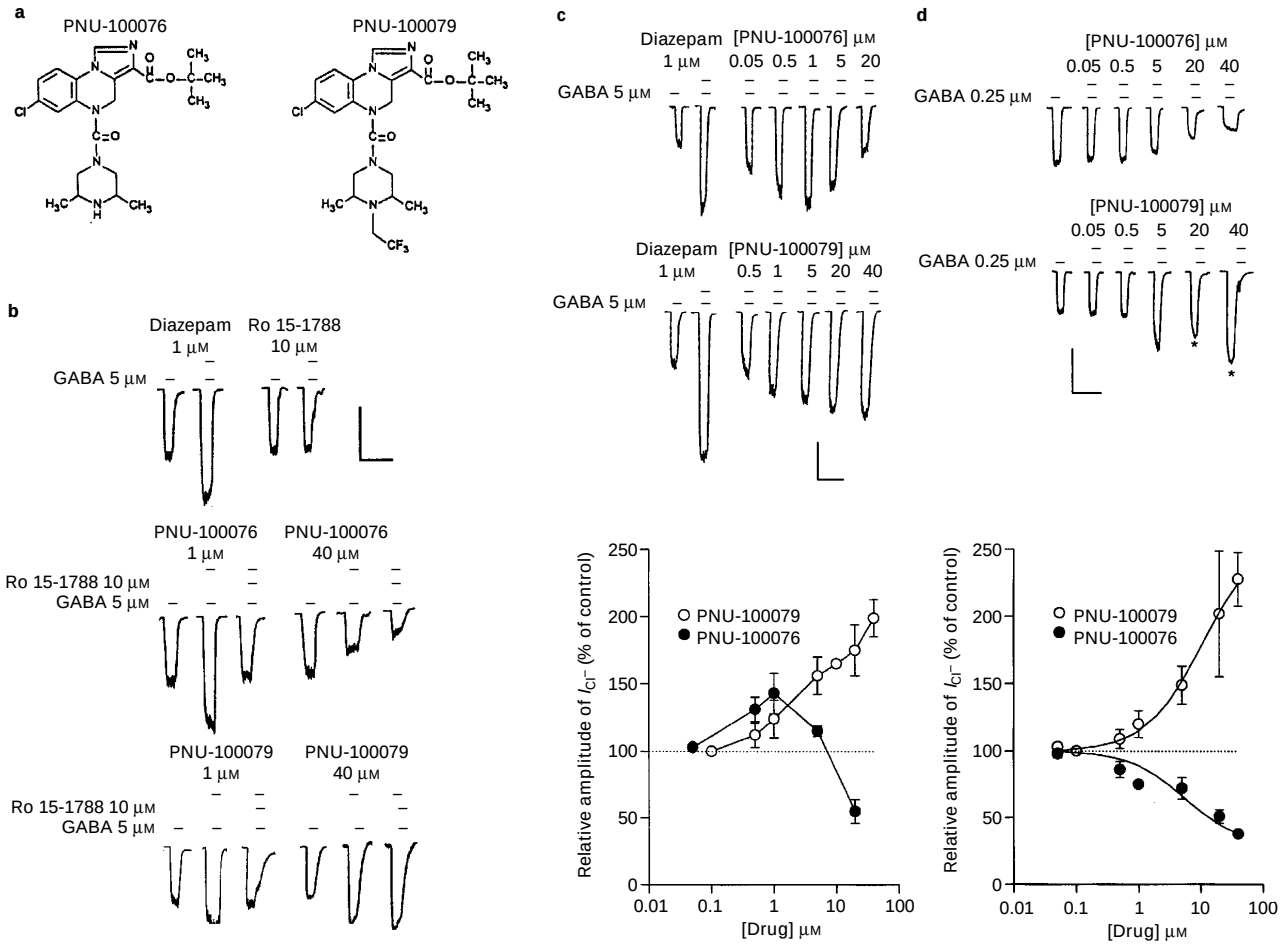

Figure 1 Drug actions on GABA-induced Cl^- currents. (a) Chemical structures of PNU-100076 and PNU-100079. (b) Reversal of the actions of PNU-100076 and PNU-100079 at low concentrations on Cl^- currents by Ro 15-1788, a benzodiazepine site antagonist, but no effects on their action at high concentrations. In the $\alpha1\beta2\gamma2$ subtype, Cl^- currents were induced with GABA at 5 μM in the presence of Ro 15-1788 (10 μM), PNU-100079, PNU-100076 or in combinations as indicated. Ro 15-1788 at 10 μM had no effect on the currents by itself. The vertical calibration bar represents 250 pA for all the traces except for the last three traces at the bottom (500 pA), and the horizontal bar 30 s. (c) Effects of PNU-100076 and PNU-100079 on GABA-induced Cl^- currents in the $\alpha1\beta2\gamma2$ subtype of GABA$_A$ receptors. Representative traces are shown in the upper panel, and the dose-response profiles in the lower panel. The relative amplitude was obtained upon normalization to the level observed with GABA alone. Each point represents the mean, and vertical lines show s.d., from 5 and 6 experiments for PNU-100076 and PNU-100079, respectively. The vertical calibration bar represents 250 pA, and the horizontal bar 30 s. (d) Effects of PNU-100076 and PNU-100079 on GABA-induced Cl^- currents in the $\alpha6\beta2\gamma2$ subtype of GABA$_A$ receptors. Cl^- currents were induced with GABA at 0.25 μM with or without indicated drugs. Representative traces are shown in the upper panel, and the dose-response profiles in the lower panel. Each point represents the mean and vertical lines show s.d. from 4 and 8 experiments for PNU-100076 and PNU-100079, respectively. The vertical calibration bar represents 250 pA but 500 pA for the traces with *, and the horizontal bar 30 s.

the effects of PNU-101017, PNU-100076 and PNU-100079 on [^{35}S]-TBPS binding in the rat cerebrocortical membranes which contain complex subtypes of GABA$_A$ receptors. PNU-101017 produced a biphasic dose-response profile (Figure 2). At low concentrations (<0.2 μM), the drug reduced TBPS binding, but at higher concentrations (>1 μM), it reversed its inhibition and subsequently increased TBPS binding well above the control (GABA alone). This is in agreement with its functional characteristics observed with GABA-induced Cl^- current measurements in cloned GABA$_A$ receptors; its positive allosteric modulation at low concentrations and its negative modulation at high concentrations (Im *et al*., 1996). PNU-100076 produced a biphasic dose-response profile similar to that for PNU-101017 (Figure 2). PNU-100079, on the other hand, differed from PNU-101017 and PNU-100076 in that its second phase showed more inhibition of TBPS binding, instead of stimulation (Figure 2). This is again in agreement with its positive allosteric interaction with the second site as observed with Cl^- current measurements in cloned GABA$_A$ receptors.

We examined whether PNU-100079 shares the common low affinity site (the second site) with PNU-101017 by analysis of their respective and combined actions on [^{35}S]-TBPS binding at equilibrium in the rat cerebrocortical membranes. First of all, to isolate their interaction with the low affinity site, we blocked their action via the high affinity benzodiazepine site with Ro 15-1788 at 10 μM. The benzodiazepine antagonist abolished the inhibition phase of TBPS binding by the drugs at low concentrations (<1 μM) (Figure 2). The dose-response profiles for TBPS binding in the presence of excess Ro 15-1788 thus became monophasic for both PNU-100079 and PNU-101017 (Figure 2). We observed only the stimulatory phase for PNU-101017 with a half-maximal concentration ($K_{0.5}^1$) of 8.9 ± 0.7 μM, and only the inhibitory phase for PNU-100079 with a half maximal concentration ($K_{0.5}^2$) of 2.1 ± 0.2 μM. To test their competitive interaction, we obtained the dose-

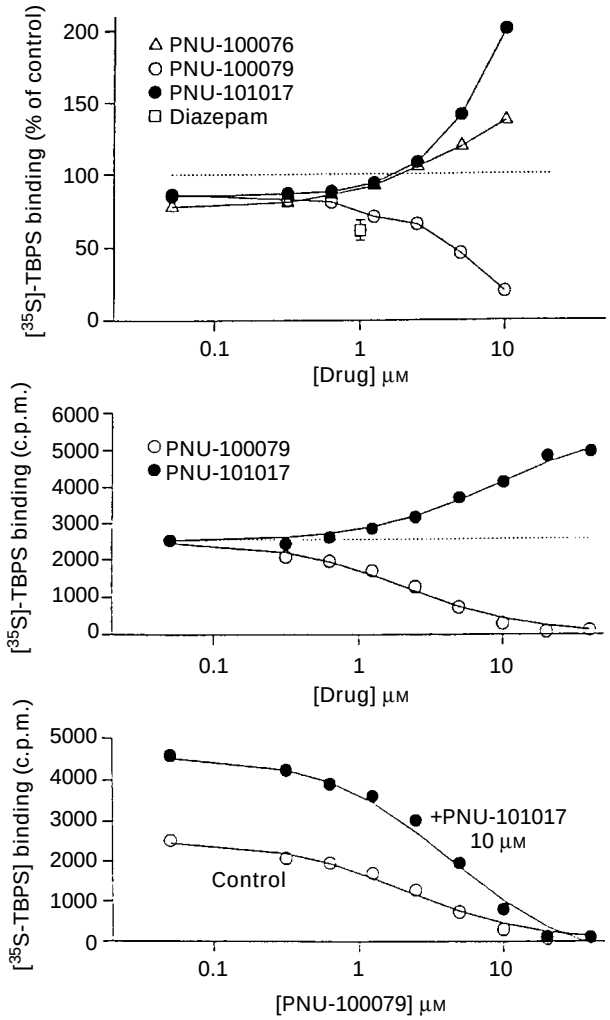

Figure 2 Effects of PNU-101017, PNU-100079 or in combination on GABA-sensitive TBPS binding in rat cerebrocortical membranes. TBPS binding in the presence of exogenous GABA at 4 μM was measured without or with the indicated drugs at various concentrations. The effect of diazepam at 1 μM was shown. The data were normalized to the level of TBPS binding observed with GABA alone, and represent the mean from three experiments and their standard errors were less than 10%.

response profile of TBPS binding for PNU-100079 in the presence of PNU-101017 at 10 μM, and fit the binding data to the following equation which is based on their competition for a single common site;

$$f = (a * [\text{PNU-101017}]/([\text{PNU-101017}] + K_{0.5}^{1}(1 + [PNU\text{-}100079]/K_{0.5}^{2})) + b) - (c * [\text{PNU-100079}]/([\text{PNU-100079}] + K_{0.5}^{2}(1 + [PNU\text{-}101017]/K_{0.5}^{1})),$$

where a is the net increase in TBPS binding by PNU-101017 at a given concentration (10 μM in this case), b is TBPS binding at the baseline (GABA alone), $K_{0.5}^{1}$ and $K_{0.5}^{2}$ are the half-maximal concentration for PNU-101017 and PNU-100079, respectively, and c is the net maximal inhibition of TBPS binding by PNU-100079. The first half of the equation represents the competitive block of PNU-101017-induced TBPS shift by PNU-100079, and the second half depicts the block of PNU-100079-induced TBPS shift by PNU-101017. The equation is simplified due to a fixed concentration of PNU-101017 at 10 μM, and variable concentrations of PNU-100079. The data representing their combined actions fitted well to the predicted curve by the equation (Figure 2). From this we propose that PNU-100079 interacts with the same low affinity site as PNU-101017. It appears that the N-substituent of the piperazine ring is the critical determinant for how the drug interacts with the second site, being a positive or negative allosteric modulator.

Discussion

Drugs acting on the benzodiazepine site of GABA$_A$ receptors are much safer than barbiturates, but are still liable to abuse and physical dependence. Recently, we discovered that several benzodiazepine site ligands with the imidazoquinoxaline and imidazoquinoline ring moiety interact with a second, low affinity binding site on GABA$_A$ receptors, the occupancy of which at high drug concentrations reverse their positive allosteric action on the benzodiazepine site, and may potentially minimize abuse liability and physical dependence (Im *et al*., 1995; 1996). Previous studies have shown that their second allosteric site seems to be distinct from those for barbiturates, neurosteroids and benzodiazepines (Im *et al*., 1995; 1996). Further studies are needed to understand their interactions with GABA$_A$ receptors at the molecular level.

We showed here that the functionality of the second site was altered by one nitrogen substitutent on the piperazine ring moiety of two imidazoquinoxaline analogues: PNU-100076 with a hydrogen substituent on the critical position induced a negative allosteric modulation via the second low affinity site, like the parent compounds, while PNU-100079 with a trifluoroethyl group produced a positive allosteric action. From these results it is proposed that the substituents of the piperazine ring moiety of these drugs are the key groups involved in their interaction with the second site. It would be of future interest to see how other modifications of the piperazine ring influence the affinity and functionality of ligands to the second site. Results from such studies would provide more insight into the molecular nature of the second site and would reveal structure-functional relationships which could be useful for developing more effective benzodiazepine site ligands without typical side effects associated with those currently available on the market.

References

BARNARD, E.C., SUTHERLAND, M., ZAMAN, M., MATSUTOMO, M., NAYNEEN, N., GREEN, T., DARLISSON, M.G. & BATESON, A.N. (1993). Multiplicity, structure and function in GABA$_A$ receptors. *Ann. New York Acad. Sci.*, **707,** 117–125.

DELOREY, T.M. & OLSEN, R.W. (1992). γ-Aminobutyric acidA receptor structure and function. *J. Biol. Chem.*, **267,** 16747–16750.

DRAGUHN, A., VERDOORN, T.A., EWERT, M., SEEBURG, P.H. & SAKMANN, B. (1990). Functional and molecular distinction between rat GABA$_A$ receptor subtypes by Zn^{2+}. *Neuron.*, **5,** 781–788.

GEE, K.W., LAWRENCE, L.J. & YAMAMURA, H.I. (1986). Modulation of the chloride ionophore by benzodiazepine receptor ligands: Influence of γ-aminobutyric acid and ligand efficacy. *Mol. Pharmacol.,* **30,** 218 – 225.

HAFELY, W., KYBURZ, E., GERECKE, M. & MOHLER, H. (1985). Recent advances in the molecular biology of benzodiazepine receptors and in the structure activity relationship of their agonists and antagonists. *Adv. Drug. Res.,* **14,** 166 – 322.

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. *Pflügers. Arch.,* **391,** 85 – 100.

HAMILTON, B.J., LENNON, D.J., IM, H.K., IM, W.B., SEEBURG, P.H. & CARTER, D.B. (1993). Stable expression of cloned rat $GABA_A$ receptor subunits in a human kidney cell line. *Neurosci. Lett.,* **153,** 206 – 209.

IM, W.B. & BLAKEMAN, D.P. (1991). Correlation between $GABA_A$ receptor ligand-induced changes in t-butylbicyclophosphoro-[^{35}S]thionate binding and 36Cl- uptake in rat cerebrocortical membranes. *Mol. Pharmacol.,* **39,** 394 – 398.

IM, H.K., IM, W.B., PREGENZER, J.F., CARTER, D.B., JACOBSEN, E.J. & HAMILTON, B.J. (1995). Characterization of U-97775 as a $GABA_A$ receptor ligand of dual functionality in cloned rat $GABA_A$ receptor subtypes. *Br. J. Pharmacol.,* **115,** 19 – 24.

IM, H.K., IM, W.B., VONVOIGTLANDER, P.F., CARTER, D.B., MURRAY, B.H. & JACOBSEN, E.J. (1996). Characterization of U-101017 as a $GABA_A$ receptor ligand of dual functionality. *Brain Res.,* **714,** 165 – 168.

LUDDENS, H., PRITCHETT, D.B., KOHLER, M., KILLISCH, I., KEINANEN, K., MONYER, H., SPRENGEL, R. & SEEBURG, P.H. (1990). Cerebellar $GABA_A$ receptor selective for a behavioral alcohol antagonist. *Nature,* **346,** 648 – 651.

PREGENZER, J.F., IM, W.B., CARTER, D.B. & THOMSEN, D.R. (1993). Comparison of interactions of [^{3}H]muscimol, [^{35}S]t-butylbicyclophosphorothionate and [^{3}H]flunitrazepam with cloned γ-aminobutyric $acid_A$ receptors of the $\alpha 1\beta 2$ and $\alpha 1\beta 2\gamma 2$ subtype. *Mol. Pharmacol.,* **43,** 801 – 806.

SIEGHART, W. (1995). Structure and pharmacology of γ-aminobutyric $acid_A$ receptor subtypes. *Pharmacol. Rev.,* **47,** 181 – 234.

SQUIRES, R.F., CASIDA, J.E., RICHARDSON, M. & SAEDERUP, E. (1983). [^{35}S]t-butylbicyclophosphorothionate binds with high affinity to brain specific sites coupled to K-aminobutyric acid-A and ion recognition sites. *Mol. Pharmacol.,* **23,** 326 – 336.

SQUIRES, R.F. & SAEDERUP, E. (1993). Indomethacin/ibuprofen-like anti-inflammatory agents potentiate the γ-aminobutyric acid-antagonistic effects of several norfloxacin-like quinolone antibacterial agents on [^{35}S]t-butylbicyclophosphorothionate binding. *Mol. Pharmacol.,* **43,** 795 – 800.

WIELAND, H.A., LUDDENS, H. & SEEBURG, P.H. (1992). A single histidine in $GABA_A$ receptors is essential for benzodiazepine agonist binding. *J. Biol. Chem.,* **267,** 1426 – 1429.

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