

Mapping convulsants' binding to the GABA-A receptor chloride ionophore: A proposed model for channel binding sites

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Abstract

Gamma-aminobutyric acid (GABA) type A receptors play a key role in brain inhibitory neurotransmission, and are ligand-activated chloride channels blocked by numerous convulsant ligands. Here we summarize data on binding of picrotoxin, tetrazoles, β -lactams, bicyclophosphates, butyrolactones and neurotoxic pesticides to GABA-A ionophore, and discuss functional and structural overlapping of their binding sites. The paper reviews data on convulsants' binding sensitivity to different point mutations in ionophore-lining second trans-membrane domains of GABA-A subunits, and maps possible location of convulsants' sites within the chloride ionophore. We also discuss data on inhibition of glycine, glutamate, serotonin (5-HT₃) and *N*-acetylcholine receptors by GABA-A channel blockers, and examine the applicability of this model to other homologous ionotropic receptors. Positioning various convulsant-binding sites within ionophore of GABA-A receptors, this model enables a better understanding of complex architectonics of ionotropic receptors, and may be used for developing new channel-modulating drugs.

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Keywords: GABA-A receptors; Ionophore; Channel chemoconvulsants; Binding sites; Point mutagenesis

1. GABA and GABA-A receptor complex

Gamma-amino butyric acid (GABA) is the primary mediator of inhibitory transmission in the mammalian central nervous system (Akaike et al., 1987; Korpi et al., 2002; Leung and Xue, 2003). It has complex interactions with other neurotransmitter systems and acts through ionotropic A and metabotropic B type receptors (Martin and Dunn, 2002; Atack, 2003, 2005). Both receptors are a target for many endogenous and exogenous modulators that regulate normal and pathological brain mechanisms—sleep, memory, epilepsy and emotions (Kalueff and Nutt, 1997; Argyropoulos et al., 2000; Sandford et al., 2000; Nutt and Malizia, 2001; Vicini and Ortinski, 2004; Cryan and Kaupmann, 2004).

GABA-A receptors are crucial for controlling brain excitability, and represent ligand-gated ion channels composed of five subunits (belonging to eight families: α 1–6, β 1–3, γ 1–3, δ , ϵ , π , θ and ρ 1–3) around the ionophore (Baumann et al., 2001, 2002; Korpi et al., 2002; Rosahl, 2003; Vicini and

Ortinski, 2004). Each subunit of GABA-A receptors consists of four trans-membrane domains (TM1–4) (Perret et al., 1999; Engblom et al., 2002; Filippova et al., 2004) modulating the receptor activity. Binding of GABA opens up a Cl⁻ channel, leading to neuronal inhibition (Wooltorton et al., 1997; Olsen et al., 2004). GABA-A receptors contain binding sites for GABA agonists and antagonists, as well as numerous positive and negative modulators (Olsen et al., 1990; Mathews et al., 1996; El-Etr et al., 1998; Argyropoulos et al., 2000; Nutt and Malizia, 2001; Leung and Xue, 2003). Positive modulators of GABA-A receptors includes barbiturates, benzodiazepines, steroids, ethanol and γ -butyrolactones (Holland et al., 1995; Canney et al., 1998; Olsen et al., 2004; Atack, 2003, 2005; Rudolph and Mohler, 2006). Neurosteroid antagonists, benzodiazepine inverse agonists and chloride channel blockers negatively modulate the GABA-A receptor (Maksay, 1996; Akaike et al., 1987; Sousa and Ticku, 1997; Wooltorton et al., 1997; Leung and Xue, 2003) (Tables 1–3).

Ionophore-lining TM2 are responsible for GABA-A channel activation and desensitization, ion selectivity and binding of various ionophore ligands (Buhr et al., 2001; Horenstein et al., 2001; Jensen et al., 2002; Scheller and Forman, 2002; Filippova et al., 2004). Similar structure is known for other ligand-gated

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Table 1
Comparative analysis of pharmacological properties of traditional GABA-A ionophore blockers (+ present, – absent, ? unclear or conflicting effects)

Properties	P	BPH	BL	PTZ	PC	LA
Character of channel blockage						
Competitive	–/+	–	–/+	+?	–?	+
Reversible	+	+	+	+	+	+
Voltage-dependent	+? ^a	+	+	+	–	+
Binding to closed channel	+	+	+		+?	+
Effects of channel state						
Open frequency		–		–	+	
Open duration	–	–		–	–	
Closed frequency	+	+			+	
Closed duration	+	+		+	+	
Molecular (structural) similarity						
Similarity to P	+	+	+	+	+	
Similarity to other ligands	BL, PTZ, BPH		PTZ	PC, BL	LA, PTZ	PC
Ability to displace BPH	+	+	+	+		
Hydrophobic molecule	+	+		–	–	
Ability to inhibit GABA agonists binding	–	–	–	–	–	+
References	I	II	III	IV	V	V, VI

P: picrotoxin; BPH: bicyclic phosphates; BL: butyrolactones; PTZ: pentylenetetrazole; PC: penicillin; LA: other lactam antibiotics.

^a Several studies have shown that picrotoxin inhibition of the channel may be voltage-dependent (Newland and Cull-Candy, 1992; Yoon et al., 1993; Lynch et al., 1995; Yakushiji et al., 1987). References: I (Newland and Cull-Candy, 1992), II (Hamann et al., 1990; Maksay et al., 1996; Sinkkonen et al., 2001), III (Holland et al., 1990, 1991, 1993; Maksay et al., 1994; Mathews et al., 1996), IV (Squires et al., 1984; Maksay et al., 1994; Huang et al., 2001), V (Akaike et al., 1987; Yakushiji et al., 1987; Twyman et al., 1992; Fujimoto et al., 1995; Behrends, 2000; Lindquist et al., 2004), VI (Fujimoto et al., 1995; Sugimoto et al., 2003).

ionotropic receptors—GABA receptors of invertebrates, *N*-acetylcholine, glycine, glutamate, and serotonin (5-HT₃) receptors (Ffrench-Constant et al., 1993; Baumann et al., 2001; Jentsch et al., 2001; Bloomquist, 2003; Das and Dillon, 2003, 2005; Newell et al., 2004; Olsen et al., 2004).

Picrotoxin and picrotoxinin, pentylenetetrazole (PTZ) and other tetrazoles, penicillin and other β -lactam antibiotics, thiobutyrolactones, bicyclic phosphates (such as *t*-butylbicyclic phosphorothionate TBPS), U-93631 and neurotoxic pesticides (NP) are traditional ionophore-blocking convulsant ligands (Squires et al., 1984; Hamann et al., 1990; Holland et al., 1991; Lindane, 1991; Twyman et al., 1992; Dillon et al., 1993, 1995; Wang et al., 1995; Le Corrionc et al., 2002; Omrani et al., 2003; Sugimoto et al., 2003; Vale et al., 2003; Hansen et al., 2004; Kaminski et al., 2004; Lindquist et al., 2004; Sinkkonen et al.,

2005). All these agents have cyclic structures, allowing to consider them as a common group of “cage convulsants” (Olsen et al., 1980; Hamon et al., 1998; Maksay et al., 1998; Rossi et al., 2001; Chen et al., 2006). Moreover, many of them share a substantial similarity in chemical and conformational structures (Table 1; Maksay, 1996), and also block other non-GABAergic ionotropic receptors (Table 3; Bloomquist, 2003; Vale et al., 2003).

Although mechanisms of action of non-ionophore modulators of GABA-A receptors have been extensively studied, the effector part of the receptor – its ionophore – is much less understood. It has long been thought that various convulsants inhibit ion influx by physically plugging ionophore (rev.: Perret et al., 1999; Behrends, 2000) when bound in different positions to a common “convulsant” binding pocket. Described in the

Table 2
Mutagenesis-based data on critical residues for binding of selected ionophore ligands to TM2 domains of GABA-A receptor subunits (legend as in Fig. 1)

TM2 residue	Ligands							References
	P	PTZ	BPH	BL	PC	LA	NP ^a	
2	Yes	No	Yes	No			Yes	I
3	Yes		Yes					I
6	Yes	Yes		Yes	Yes ^b	No	Yes	II
9	Yes	No	No	No	No			III
15	Yes							IV
19	Yes							IV

^a e.g., dieldrin.

^b Partially sensitive (border of the binding pocket?). References: I (Gurley et al., 1995; Edwards and Lees, 1997; Jursky et al., 2000; Buhr et al., 2001), II (Sugimoto et al., 2002), III (Tierney et al., 1996; Lindquist et al., 2004), IV (Findlay et al., 2001; Dibas et al., 2002).

Table 3
Comparative analysis of the ability to inhibit different ionotropic receptors by GABA-active ionophore ligands (legend as in Fig. 1)

Receptors	Inhibition of ion channels						
	P	BPH	BL	PTZ	PC	LA	NP
GABA-A	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Invertebrate GABA	Yes	Yes			Yes		Yes
Acetylcholine N-type	Yes						
Glycine	Yes						Yes
5-HT ₃ serotonin	Yes	No		Yes			
Glutamate	Yes					No	
References	I	II		III	IV		I, V

References: I (Ffrench-Constant et al., 1993; Yoon et al., 1993; Lynch et al., 1995; Zhang et al., 1995; Etter et al., 1999; Shan et al., 2001, 2002; Le Corrionc et al., 2002; Bloomquist, 2003; Das et al., 2003; Das and Dillon, 2003, 2005; Erkkila et al., 2004), II (Maksay et al., 1996; Yagle et al., 2003), III (Das et al., 2003), IV (Fujimoto et al., 1995; Hosie et al., 2006), V (Vale et al., 2003).

literature as picrotoxin(in) site or receptor (Holland et al., 1991; Ito and Ho, 1994; Nobrega et al., 1995; Bell-Horner et al., 2000; Olsen et al., 2004; Das and Dillon, 2005), PTZ/TBPS site (Holland et al., 1990, 1993; Kalueff, 2002), convulsant, ionophore or channel site (Olsen et al., 1980, 1990; Peris et al., 1991; Maksay et al., 1996; Yagle et al., 2003), this convulsant-binding pocket of GABA-A receptors is currently poorly understood.

Mounting data indicates that different convulsants bind to overlapping but not identical sites, also showing multiple mechanisms of binding with different kinetics of association and dissociation (Holland et al., 1991; Twyman et al., 1992; Yoon et al., 1993; Hamon et al., 1998; Dibas and Dillon, 2000; Le Corronc et al., 2002; Mortensen et al., 2003; Sinkkonen et al., 2005). Some convulsant ligands (picrotoxin, bicyclophosphates, butyrolactones, β -lactams) seem to reach their binding sites in closed state of the channel, further confirming that simple plugging of ionophore is not the actual single mechanism of their action (Table 1; also see: Dibas et al., 2002; Hawthorne and Lynch, 2005 for discussion).

While several groups have modeled different aspects of ionophore functioning (Maksay, 1996, 2005; Baumann et al., 2002; Maksay et al., 2003; Chou, 2004; Chen et al., 2006; Muroi et al., 2006) and ligand binding (Twyman et al., 1992; Canney et al., 1998; Zhorov and Bregestovski, 2000; Shan et al., 2002; Vale et al., 2003; Horenstein et al., 2005), further studies modeling ionophore organization and its sites are needed. If successful, such attempts may increase our understanding of pathogenetic mechanisms of channelopathies (Felix, 2000) and facilitate the development of novel selective ionophore-targeting drugs (Eldefrawi and Eldefrawi, 1987; Bloomquist, 2003).

Since channel ligands are thought to bind to heterogeneous binding sites within ionophore, the important question is the positioning of these binding sites within the ionophore and relative to each other. Point mutagenesis data may give an important information on which TM2 residues may be critical for binding of different ligands. For example, a common critical residue for two convulsants implies overlapping of their binding sites, whereas different critical residues for these ligands suggests their distinct binding sites. As recent extensive data provides important insights into functional properties of ionophore receptors (Ffrench-Constant et al., 1993; Gurley et al., 1995; Perret et al., 1999), we will systematically evaluate the available literature on pharmacology and mutagenesis of GABA-A and other homologous ionotropic receptors, in order to develop a model of ionophore binding sites for different classes of GABA-active ligands.

2. Ionophore sites and their ligands

Table 1 summarizes known physiological and pharmacological properties of traditional GABA-A ionophore blockers. Table 2 shows point mutagenesis data for major GABA-A chemoconvulsants, outlining critical residues for each class of ionophore ligands. Table 3 describes the ability of ionophore blockers to inhibit other ionotropic (glycine, glutamate,

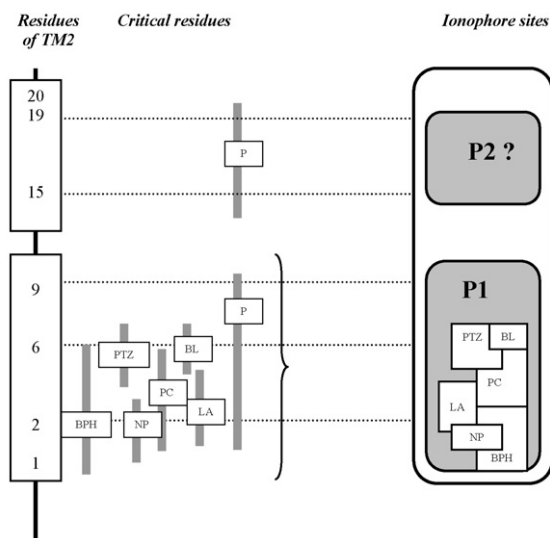


Fig. 1. Proposed model of convulsant-binding sites of GABA-A receptor ionophore. TM2 residues are conventionally numbered 1'–20' from the N-terminal bottom to the C-terminal extracellular end of the helix (see Olsen, 2006 for details). P: picrotoxin(in); BPH: bicyclophosphates; BL: butyrolactones; PTZ: pentylenetetrazole; PC: penicillin; LA: other lactam antibiotics; NP: neurotoxic pesticides; P1: “main” picrotoxin binding site; P2: hypothetical second “allosteric” picrotoxin site.

serotonin 5-HT₃ and *N*-acetylcholine) receptors. Fig. 1 shows a model of GABA-A ionophore, developed based on data in Tables 1–3.

2.1. Picrotoxin site

As picrotoxin effectively inhibits chloride influx in GABA-A (Newland and Cull-Candy, 1992) and other ionotropic receptors (Table 3), it represents a universal “reference” channel blocker (Das et al., 2003; Olsen, 2006) with whom other ligands may be compared (Table 1). While the exact location of picrotoxin binding to ionophore is still unknown (Huang et al., 2001), its sensitivity to mutations in residues 2, 3 and 6 of TM2 suggests that the site contains residues 2–6 (Table 2; Buhr et al., 2001). In line with this, Zhorov and Bregestovski (2000) suggested that picrotoxin penetrates deep inside the ionophore pore, binding with its hydrophobic moiety to residue 2 of TM2 (close to the pore) and forming hydrogen bonds with residue 6 in the middle of TM2. Importantly, while amino acid composition of residue 2 is variable in different ionotropic receptors, the composition of residue 6 is highly conservative, implying that it is crucial for picrotoxin binding to ionophore, and most likely representing the epicenter of its binding pocket (Fig. 1).

While residue 15 is critical for picrotoxin binding to glycine receptors, its mutation in GABA-A receptors inhibited (but not abolished) use-dependent ionophore block by picrotoxin, suggesting that this residue may be important for interplay between GABA and picrotoxin binding sites (Dibas et al., 2002). Other studies implicate residues 9 and 15 in the regulation of channel properties, such as desensitization, stabilization of open states and gating (Findlay et al., 2001;

Scheller and Forman, 2002). Taken together, this indirectly supports the possibility of a second “modulatory” (allosteric) binding site of picrotoxin that includes residues 15–19. In line with this, residue 17 is important for picrotoxin modulation of GABA-A receptors (Horenstein et al., 2001). The hypothesis of an additional allosteric picrotoxin site (Fig. 1) is also indirectly supported by recent data (Mortensen et al., 2003) showing that potency of picrotoxin binding is highly dependent on the level of spontaneous activity of GABA-A receptors.

2.2. PTZ (tetrazole) site

Although early studies hypothesized PTZ binding to benzodiazepine site of GABA-A receptors, similarity to picrotoxin (in terms of molecular structure, use-dependent voltage-independent action and displacement of TBPS) prompted its activity at ionophore “convulsant” site (Table 1; Dibas and Dillon, 2000). Some butyrolactones and PTZ share stereo-structural similarity and synergetically affected TBPS binding (Maksay et al., 1994). This, and the ability of selected butyrolactones to inhibit binding of other cage convulsants—picrotoxin, PTZ or bicycphosphates (rev.: Dibas and Dillon, 2000; Huang et al., 2001), supports the idea that all these binding sites overlap. Finally, similar sensitivity of picrotoxin and PTZ binding to some mutations in TM2 further confirms this notion (Dibas and Dillon, 2000).

However, there are several distinctions between picrotoxin and PTZ actions, including different affinity and dynamics of association with ionophore and Cl⁻ current inhibition (Huang et al., 2001). Another dissimilarity is the lack of complex (competitive + non-competitive) effects in PTZ (Table 1), and insensitivity of PTZ binding to ablation of α -subunits (abolishing binding of picrotoxin and NP) (Huang et al., 2001). Finally, PTZ binding is insensitive to point mutations in residue 2, suggesting the location of this residue outside of the PTZ site (Huang et al., 2001), as indicated in Fig. 1.

2.3. TBPS (bicycphosphate) site

TBPS is a non-competitive ionophore blocker, traditionally thought to act via classical picrotoxin/convulsant site, although with different kinetics (Peris et al., 1991; Ito and Ho, 1994; Nobrega et al., 1995; Maksay et al., 1996; Luddens et al., 1998; Jursky et al., 2000; Sinkkonen et al., 2001, 2005). Since mutations in residue 2 are critical for TBPS binding to ionophore, it is possible to assume that its binding site includes this residue (Jursky et al., 2000). TM2 residues 1, 2 and 3 of β -subunits of GABA-A receptors were important to form TBPS binding site in chimeric receptors, implying that these residues form the TBPS binding pocket (Jursky et al., 2000) (Fig. 1).

2.4. Butyrolactone site

Earlier reports on competitive inhibition of TBPS binding by convulsant butyrolactones suggested that they bind to a common TBPS/picrotoxin “convulsant” ionophore site (Holland et al., 1991, 1995; Mathews et al., 1996; Canney et al.,

1998; Gonzales et al., 2004). This is also in line with similar chemical structures of these ligands (e.g., picrotoxin molecule contains a butyrolactone ring) (Williams et al., 1997). Likewise, butyrolactones share similar physiological and pharmacological mechanisms of action with PTZ (Maksay et al., 1994), and are able to allosterically modulate TBPS binding (Holland et al., 1990, 1991, 1993). Collectively, this implies that picrotoxin, PTZ, TBPS and butyrolactones may bind to overlapping ionophore binding sites (Fig. 1).

Although butyrolactone binding site is not yet identified (Gonzales et al., 2004), the sensitivity of butyrolactone binding to point mutations in residue 6 (Huang et al., 2001) indicates its location within a common binding area for these convulsants. Since mutations affecting picrotoxin binding also affect that of butyrolactones (Huang et al., 2001), it is indeed likely that binding sites for picrotoxin and butyrolactones significantly overlap (Fig. 1). In contrast, other studies have demonstrated anticonvulsant effects of some butyrolactones, suggesting either antagonism of the picrotoxin receptor, or a second positive (modulatory) “lactone” site (Holland et al., 1991, 1993, 1995; Williams et al., 1997; Gonzales et al., 2004).

2.5. Penicillin and lactam site(s)

Despite early studies (implying action via benzodiazepine site; Shirashi et al., 1993), later data demonstrated binding of penicillin and other β -lactams to an ionophore site, with a physical blockage of GABA-A channel (Twyman et al., 1992; Fujimoto et al., 1995; Lindquist et al., 2004) and complex multiphasic kinetics (Katayama et al., 1992). Structural similarity and interference with psychopharmacological effects of picrotoxin and PTZ (Kalueff, 2002) confirms that penicillin, like other ionophore ligands, acts via a common convulsant-binding site. Sensitivity of penicillin binding to mutations in TM2 residue 6 (Table 2) indicates that it may be a common site for picrotoxin, PTZ and penicillin (Fig. 1).

However, the fact that such mutation completely abolished picrotoxin binding, but only reduced penicillin binding (Sugimoto et al., 2002), suggests that residue 6 may be on a border of penicillin binding pocket, as suggested in Fig. 1. Insensitivity of penicillin (but not picrotoxin) binding to a point mutation in residue 9 indicates that this residue is not a critical element of penicillin binding site, and is most likely located outside the penicillin site (Tierney et al., 1996; Lindquist et al., 2004). Given partial effects of residue 6 mutations on penicillin binding, this indirectly suggests that penicillin binding site may be located below residue 6 of TM2 (Fig. 1).

Notably, other β -lactams, such as cephalosporines and penems, are known as competitive voltage-independent inhibitors of GABA-A ionophore, strikingly dissimilar to non-competitive voltage-dependent action of penicillin (Fujimoto et al., 1995; Sugimoto et al., 2002, 2003). Collectively, this implies different mechanisms of action (and binding sites) of penicillin and other lactams (Sugimoto et al., 2003). Since mounting data shows heterogeneity of penicillin and other β -lactam binding sites, it is possible to assume distinct binding sites for penicillin and other β -lactams. Insensitivity of

β -lactam binding to mutated residue 6 (Sugimoto et al., 2002) suggests that this additional “lactam” binding site is not within penicillin binding pocket (see model in Fig. 1).

2.6. Neurotoxic pesticides

NP, such as lindane, α -endosulphan and dieldrin, share structural similarity (and compete for the binding site) with picrotoxin, inhibit TBPS binding, induce seizures and block Cl^- currents through ionophore (Lindane, 1991; Ffrench-Constant et al., 1993; Edwards and Lees, 1997; Le Corrionc et al., 2002; Kaminski et al., 2004). Together, this implies similar mechanisms of their action, also see (Chen et al., 2006) for discussion. However, some differences of insect GABA receptors in sensitivity to picrotoxin and NP blockage (Le Corrionc et al., 2002), and of rat and fish GABA-A receptors to TBPS and lindane (Thompson et al., 1990), suggest that binding sites of these ligands are overlapping but not identical. Sensitivity of some NP-mediated effects to point mutation in residue 2 (Edwards and Lees, 1997) (Table 2) suggests the location of NP site(s) close to this residue (Fig. 1), rather than to residue 6. Positioning bicyclophosphate site close to NP binding site in this model is also in line with numerous above-mentioned data on overlapping pharmacological mechanisms of their action.

3. Non-GABA-A receptors

A substantial homology in molecular structures of different ionophore receptors (Eldefrawi and Eldefrawi, 1987; Yoon et al., 1993; Vassilatis et al., 1997; Bloomquist, 2003; Erkkila et al., 2004) implies similar actions of their ionophore ligands (Table 3); also see (Thompson et al., 1999; Horenstein et al., 2001; Jensen et al., 2002; Chen et al., 2006) for discussion. Can the model suggested for GABA-A receptor ionophore (Fig. 1) be generally applied to other ionophore channels? While mounting neurogenetic data generally supports this notion, it also reveals some interesting receptor-specific differences.

For example, residues 2, 6, 15 and 19 of TM2-domain of glycine receptor β -subunits are critical determinants for picrotoxin binding, whereas residue 6 is important for picrotoxin binding to glutamate and serotonin 5-HT₃ receptors, although reducing but not abolishing picrotoxin sensitivity in the latter (Lynch et al., 1995; Shan et al., 2001; Dibas et al., 2002; Das and Dillon, 2005). Residue 6 of TM2 is critical for picrotoxin and NP binding by insect GABA receptors (Ffrench-Constant et al., 1993; Jursky et al., 2000). Unlike GABA-A and glycine receptors, residue 2 is not required for picrotoxin binding to 5-HT₃ receptors, reflecting different functions of this residue in different receptors (see, however, an additional modulatory role of residue 7 in Das and Dillon, 2005). Interestingly, while picrotoxin binding to glycine receptors is use- and voltage-independent (Lynch et al., 1995), it was use-dependent for *N*-acetylcholine (Erkkila et al., 2004) and glutamate (Etter et al., 1999) receptors, suggesting that amino acids in position 15 (different in these receptors) may modulate use-dependent character of picrotoxin binding (Dibas et al., 2002).

There are other examples of receptor-specific differences in ionophore binding of convulsant ligands. For instance, in addition to GABA-A receptors, NP also inhibit glycine receptors (Table 3), supporting common mechanism of their action at ion channels (Vale et al., 2003). While NP lindane was equally effective in blocking both receptors, endosulphan and dieldrin were more active at GABA-A channels (Vale et al., 2003); also see GABA-A-selectivity for another related compound BIDN (Hamon et al., 1998). Likewise, TBPS binds to GABA-A receptors but shows much weaker binding to invertebrate GABA receptors (Yagle et al., 2003). Collectively, these findings further confirm the notion that different ionophore ligands may have complex interactions with ionophores at different receptors (Das and Dillon, 2003; Hosie et al., 2006).

4. Concluding remarks

Mounting data evidences that ionophore binding sites of GABA-A and some other ionotropic receptors demonstrate sufficient homology and show heterogeneous overlapping binding sites for different convulsant ligands (Fig. 1). Ligand-binding area of ionophore can be considered as a “big picrotoxin binding pocket”, representing a conservative basis for clustering sites of other channel ligands. The ability of some mutant channels to be picrotoxin-resistant and yet sensitive to other similar ligands (e.g., data for penicillin in Tierney et al., 1996) suggests a relative autonomy of ionophore blockage by different ligands. Moreover, many classes of convulsant drugs discussed here have been suggested to have dual mechanisms of ionophore action, including both inhibitory and stimulatory effects (e.g., Williams et al., 1997 (butyrolactones); Dibas and Dillon, 2000 (PTZ); Kalueff, 2002 (penicillin)). In line with this, Lynch et al. (1995) have shown that a single mutation in glycine receptor may convert picrotoxin from antagonist into allosteric potentiator. Given similarity of various ionophore receptors, it is possible to expect that similar phenomenon may exist for other receptors, including GABA-A.

Finally, it is possible to assume that GABA-A ionophore binding sites may have complex 3D architectonics, determining the accessibility for, and interactions with, various channel convulsants. Clearly, an in-depth analysis of 3D structures of GABA-A receptor channels may improve the present ionophore model (Fig. 1) and help clarify the impact of individual TM2 point mutations on binding of different channel ligands. Moreover, in addition to ligand binding, the effects on channel functions may be related to transduction mechanisms (Miyazawa et al., 2003; Unwin, 2005). Recent homology 3D models of receptor channels (Miyazawa et al., 2003; Maksay, 2005; Reeves et al., 2005), based on the electron microscopic structures of the nicotinic receptor channel (Unwin, 1995, 2003, 2005), may be a useful approach to further modeling of GABA-A receptor ionophore.

In conclusion, although the model of ionophore suggested here (Fig. 1) needs further sophistication and elaboration, it paves the way to reconstructing ionophore binding pockets in GABA-A and other receptors, based on data from neurogenetics and

neurochemistry. Understanding how different binding sites may be located relative to each other would help design new selective ligands that will target several overlapping sites or bind simultaneously to several distinct neighboring sites. Based on modulation of “ionophore” binding sites, this may lead to creation of novel classes of selective GABA-ergic channel-active neurotropic drugs (also see Lynch et al., 1995; Dawson et al., 2000).

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