

Phosphinic, phosphonic and seleninic acid bioisosteres of isonipecotic acid as novel and selective GABA_C receptor antagonists

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Abstract

A number of amino acids bioisosterically derived from the specific GABA_A agonist, isonipecotic acid, were electrophysiologically characterized as antagonists at GABA_C ρ_1 receptors expressed in *Xenopus* oocytes. The phosphinic acid analogue of isonipecotic acid, piperidin-4-ylphosphinic acid (**2**), was comparable with the standard GABA_C antagonist, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA), in terms of potency and GABA_C versus GABA_A receptor selectivity. Whereas the phosphonic acid analogue, piperidin-4-ylphosphonic acid (**4**), was at least an order of magnitude weaker than piperidin-4-ylphosphinic acid as a GABA_C antagonist, the seleninic acid analogue, piperidin-4-ylseleninic acid (SEPI, **6**), was the most potent and selective GABA_C antagonist within the group of isonipecotic acid derived amino acids studied.

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1. Introduction

4-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system (CNS), inhibits neuronal activity through two classes of GABA receptors, ionotropic GABA_A and GABA_C receptors and metabotropic GABA_B receptors. The ionotropic GABA receptors are composed of five subunits. GABA_A receptors are heterooligomeric receptors assembling from a number of different subunits termed α_{1-6} , β_{1-4} , γ_{1-4} , δ , ϵ , π , whereas GABA_C receptors form homooligomeric receptors consisting of ρ subunits (Chebib and Johnston, 2000; Nayeem et al., 1994; Zhang et al., 2001).

The GABA system appears to be implicated in several neurological and psychiatric disorders, and in particular the GABA_C receptors are believed to be involved in certain inherited diseases of the eye (Bailey et al., 1999; Mehta and Ticku, 1999; Zhang et al., 2001). These aspects have focused interest on the GABA_C receptor as therapeutic target, making selective ligands important tools and potential thera-

peutic agents. To date, however, the physiological functions and pharmacological characteristics of the GABA_C receptors have not been studied as intensively as the GABA_A receptors (Chebib and Johnston, 2000).

cis-4-Aminocrotonic acid (CACA), a conformationally restricted analogue of GABA held in a folded conformation, is a moderately potent partial agonist on the GABA_C receptors, and has for a long time been used as a reference ligand for the GABA_C receptors. However, CACA has been shown to be a substrate for a GABA transporter (Chebib and Johnston, 2000). The most selective agonist and antagonist at GABA_C receptors, so far described, are (+)-*cis*-aminomethylcyclopropanecarboxylic acid ((+)-CAMP), and the methylphosphinic acid analogue of isoguvacine, (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA), respectively (Fig. 1A).

A number of amino phosphinic acids are moderately potent GABA_C antagonists (Chebib et al., 1997). So far very little is known about the pharmacological consequences of replacing the carboxyl groups of GABA analogues by other bioisosteric groups, such as the seleninic acid group. The potential of the seleninic acid group as a bioisostere of the carboxyl group of GABA has only been investigated

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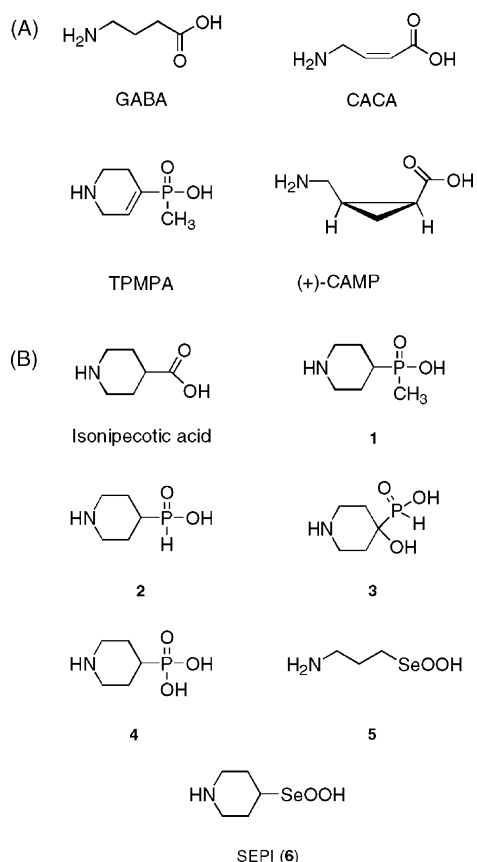


Fig. 1. (A) Structures of some GABA_C receptor ligands. (B) Structures of isonipepic acid and analogues of GABA and isonipepic acid.

at the GABA_A and GABA_B receptors (Ebert et al., 2001; Stuhr-Hansen et al., 2000).

In this paper, we report the pharmacological characterization of the GABA_A agonist isonipepic acid and a series of isosterically derived seleninic, phosphinic and phosphonic acid analogues of GABA and isonipepic acid (Fig. 1B) at functional human GABA_C ρ₁ receptors expressed in *Xenopus* oocytes.

2. Experimental procedures

2.1. Materials

Sources of chemicals were as follows: piperidin-4-ylphosphinic acid (2), 4-hydroxypiperidin-4-ylphosphinic acid (3), and piperidin-4-ylphosphonic acid (4) (Kehler et al., 1998), 3-aminopropaneseleninic acid (5) and piperidin-4-ylseleninic acid SEPI, 6 (Stuhr-Hansen et al., 2000), were synthesized as described previously. All other chemicals were obtained through regular commercial sources.

2.2. Electrophysiological recording

Xenopus laevis was anaesthetized with 0.17% ethyl 3-aminobenzoate and a lobe of the ovaries was carefully

removed. The lobe was placed in oocyte releasing buffer 2 (OR2) (82.5 mM NaCl, 2 mM KCl, 1 mM MgCl₂·6H₂O, 5 mM HEPES, pH 7.5) with 2 mg/ml collagenase A (Boehringer Mannheim) for 2 h. Defolliculated oocytes were then rinsed with frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂·6H₂O, 1.8 mM CaCl₂, 5 mM HEPES, pH 7.5) supplemented with 2.5 mM pyruvate, 0.5 mM theophylline and 50 μg/ml gentamycin. Stage V–VI oocytes were collected.

Human ρ₁ cDNA in pcDNA (Invitrogen, San Diego, CA, USA) was provided by Dr. George Uhl (National Institute for Drug Abuse, Baltimore, MD, USA). After linearization of the plasmid containing ρ₁ cDNA with restriction enzyme Xba 1, cRNA was synthesized using the 'Mmessage Mmachine' kit from Ambion (Austin, TX, USA).

ρ₁ cRNA (10 ng/50 nl) was injected into defolliculated stage V–VI *Xenopus* oocytes and the oocytes were then incubated at 16 °C on an orbital shaker for 3–8 days prior to recording.

Receptor activity was measured by two electrode voltage clamp recording using a Geneclamp 500 amplifier (Axon Instruments, Foster City, CA, USA), a MacLab 2e recorder (AD Instruments, Sydney, NSW, Australia) and Chart version 3.5 program. Oocytes were voltage clamped at –60 mV and continuously superfused with frog Ringer solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂·2H₂O, 1.8 mM CaCl₂·2H₂O, 5 mM HEPES). For receptor activation measurements, the indicated concentrations of drugs were added to the buffer solution.

Antagonist activity was measured as a p*K*_i value. The p*K*_i value was determined by doing a GABA dose–response curve as a control followed by a GABA concentration–response curve in the presence of a fixed antagonist concentration on the same oocyte (*n* ≥ 3). Only one concentration of each antagonist was used. 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 (DR), calculated as the ratio between the EC₅₀ value of GABA in the presence and absence of antagonist (DR = EC₅₀(GABA + antagonist)/EC₅₀(GABA)), was transformed to a *K*_i value by the equation: p*K*_i = log(DR – 1) – log[antagonist].

3. Results

A series of seleninic, phosphinic and phosphonic acids isosterically derived from GABA and isonipepic acid, were characterized on human GABA_C ρ₁ receptors expressed in *Xenopus* oocytes using two electrode voltage clamp technique. The activities (*K*_i values) of these compounds on human GABA_C ρ₁ receptors are summarized in Table 1, and the results are compared against the effects (*K*_i or EC₅₀ values) they have on human GABA_A α₁β₃γ₂ receptors (Ebert et al., 2001).

Table 1
Pharmacology of the compounds listed in Fig. 1B on GABA_C ρ_1 receptors and GABA_A $\alpha_1\beta_3\gamma_2$ receptors

Compound	GABA _C human ρ_1 receptors,	GABA _A human $\alpha_1\beta_3\gamma_2$ receptors	
	K_i (μM) ($pK_i \pm \text{S.E.M.}$)	K_i (μM) ^a	EC ₅₀ (μM) ^a
TPMPA	3.2 (5.49 \pm 0.11)	320 ^b	–
1	4.2 ^c	310	–
Isonipectic acid	>300 ^d	–	620
2	2 (5.67 \pm 0.02)	–	270
3	15 (4.82 \pm 0.04)	1400	–
4	51 (4.29 \pm 0.03)	3300	–
5	5 (5.30 \pm 0.00)	–	1400
SEPI (6)	0.95 (6.02 \pm 0.01)	–	200

^a From Ebert et al. (2001).

^b From Murata et al. (1996). Inhibition as a K_b value of rat brain GABA_A receptors expressed in oocytes.

^c From Johnston et al. (1998).

^d No agonist nor antagonist activity at 300 μM .

The compounds were first screened to determine agonist activity, by activation of the receptor or antagonist activity, by blocking the activation of the receptor by 1 or 3 μM of GABA (Fig. 2). The GABA_A agonist isonipectic acid was shown to be inactive on GABA_C ρ_1 receptors, whereas the remaining compounds were shown to be competitive antagonists at this receptor. No agonist activity was seen at a 300 μM concentration of the compounds.

The phosphinic acid analogue of isonipectic acid, compound **2**, was shown to be a potent antagonist on ρ_1 receptors, ($K_i = 2 \mu\text{M}$). When a hydroxy group was introduced at the 4-position of the piperidine ring of **2**, resulting in **3**, there was a significant reduction in activity. Replacing the carboxylic acid group of isonipectic acid with a phosphonic acid group as in **4**, resulted in a marked decrease in the activity at GABA_C ρ_1 receptors. Phosphonic acid, **4**, was shown to be 25-fold lower in activity than the corresponding phosphinic acid analogue, **2**.

The seleninic acid analogue of GABA, **5**, was shown to be a potent antagonist on ρ_1 receptors ($K_i = 5 \mu\text{M}$), which

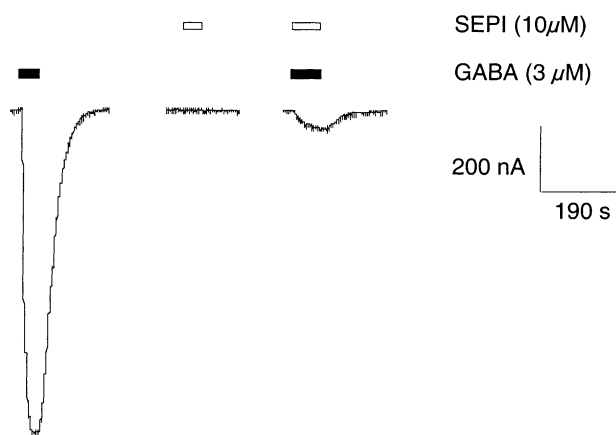


Fig. 2. At homooligomeric ρ_1 receptors expressed in *Xenopus* oocytes, GABA (3 μM) (duration indicated by filled bar) activates the receptor while SEPI (**6**) (10 μM) does not activate the receptor (duration indicated by unfilled bar). However, when SEPI (10 μM) is co-applied with GABA (3 μM), the GABA response is reduced.

is comparable to the potency of TPMPA. Furthermore, the corresponding seleninic acid analogue of isonipectic acid, SEPI (**6**), was found to be the most potent of the compounds studied to date ($K_i = 0.95 \mu\text{M}$). The inhibition of a 3 μM GABA response by 10 μM SEPI is shown in Fig. 2.

4. Discussion

GABA_C receptors were discovered only a couple of decades ago (Drew et al., 1984), and, so far, very few selective ligands have been identified for these receptors. CACA is a selective GABA_C agonist and a substrate for the GABA transporter (Biedermann et al., 1994; Chebib et al., 1997). Recently, (+)-CAMP was identified as the most selective agonist at the GABA_C receptors (Chebib and Johnston, 2000).

Murata et al. (1996) synthesized the first selective GABA_C antagonist, TPMPA, derived from isoguvacine, which is a GABA_A agonist with no effect on GABA_B receptors but with moderately potent agonist effects at GABA_C receptors. In addition, it was observed that (3-aminopropyl)methylphosphinic acid, a GABA_B agonist, had little effect on GABA_A receptors (Froestl et al., 1995a; Froestl et al., 1992; Froestl et al., 1995b; Olpe et al., 1990; Olpe et al., 1993), but potent antagonist effects on GABA_C receptors (Woodward et al., 1993). Thus, TPMPA was synthesized as a structural hybrid of isoguvacine and (3-aminopropyl)methylphosphinic acid, being 100-fold selective for GABA_C as compared to GABA_A receptors, and 500-fold more effective at GABA_C than at GABA_B receptors (Murata et al., 1996).

Isosteric replacement of the carboxyl group of the GABA_A agonist, isonipectic acid, with a phosphinic or a phosphonic acid group had substantial effect on the pharmacology of the compounds on GABA_C ρ_1 receptors. Thus, the phosphinic acid analogue, **2**, as well as the saturated analogue of TPMPA, compound **1**, were shown to be equipotent with TPMPA (Johnston et al., 1998). Based

on these results, the flexibility of the piperidine ring appears to be a structural parameter of limited importance for antagonist potency at GABA_C receptors.

Introduction of a hydroxy group in the 4-position of the piperidine ring in **2** to give **3** results in a seven-fold decrease in the antagonist potency, suggesting that the hydroxy group may prevent optimal positioning of the acid group into the binding site. The phosphonic acid analogue of isonipecotic acid, compound **4**, showed a further decrease in antagonist potency as compared to the phosphinic acid analogue **2**. This observation is in agreement with the relative GABA_C antagonist potencies reported for the phosphinic and phosphonic acid analogues of GABA (Chebib and Johnston, 2000; Chebib et al., 1997). The observed reduction in potency following replacement of a phosphinic by a phosphonic acid group may reflect that the diacidic phosphonic acid group in contrast to the monoacidic phosphinic acid group does not fit optimally into the binding site of the GABA_C receptor. Although compounds **2** and **3** have been shown to have little effect on GABA_B receptors (B. Ebert, unpublished), these compounds showed weak effects on GABA_A receptors (Table 1). The phosphinic acid analogue, **2**, acts as a low-efficacy partial agonist at human GABA_A $\alpha_1\beta_3\gamma_2$ receptors, whereas the phosphonic acid analogue as well as the hydroxy substituted analogue, compounds **4** and **3**, respectively, are weak competitive GABA_A antagonists (Ebert et al., 2001).

Like the phosphinic and phosphonic acid analogues **2–4**, the amino seleninic acids **5** and **6** were shown to be GABA_C receptor antagonists. The seleninic acid analogue of GABA, compound **5**, was shown to be equipotent with TPMPA. The seleninic acid analogue of isonipecotic acid, SEPI (**6**), was, however, shown to be the most potent compound tested in this study, showing a potency some three-fold higher than that of TPMPA.

Recently, the seleninic acid analogue of GABA, **5**, was described as a potent GABA_B agonist and a low-efficacy partial agonist at cloned human GABA_A receptors (Ebert et al., 2001; Stuhr-Hansen et al., 2000). In contrast, the seleninic acid analogue of isonipecotic acid, SEPI (**6**), was shown to be a very low-efficacy partial GABA_A agonist with no effect at the GABA_B receptors (Stuhr-Hansen et al., 2000). Furthermore, compound **5** and SEPI, respectively, show 280-fold and 200-fold selectivity for GABA_C receptors as compared with GABA_A receptors (Table 1).

Using a series of compounds structurally related to GABA and the GABA_A agonist, isonipecotic acid, we have shown that replacement of the carboxylic acid group with isosteric groups results in a profound change in pharmacological profile. Conversion of the selective GABA_A agonist, isonipecotic acid, into the structurally related phosphinic and phosphonic acids **2–4** and the seleninic acid SEPI (**6**) resulted in GABA_C antagonists. SEPI was shown to be more potent than TPMPA on GABA_C ρ_1 receptors, displaying a higher selectivity for GABA_C receptors relative to GABA_A receptors than TPMPA. Furthermore, SEPI showed no effect

at the GABA_B receptors (Stuhr-Hansen et al., 2000), and is therefore considered to be a useful pharmacological tool as a selective GABA_C receptor antagonist.

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