Bioorganic & Medicinal Chemistry Letters 21 (2011) 6417–6419

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Halogen substituents on the aromatic moiety of the tetracaine scaffold improve potency of cyclic nucleotide-gated channel block

Sarah R. Kirk [a,†], Adriana L. Andrade [b,†], Kenneth Melich [b], Evan P. Jackson [a], Elysia Cuellar [a], Jeffrey W. Karpen [b,*]

[a] Department of Chemistry, Willamette University, 900 State Street, Salem, OR 97301, USA
[b] Program in Chemical Biology, Department of Physiology and Pharmacology, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Portland, OR 97239, USA

ARTICLE INFO

Article history:
Received 17 July 2011
Accepted 19 August 2011
Available online 27 August 2011

Keywords:
Ion channels
Local anesthetics
Pore blockers
cGMP
Retinal rods

ABSTRACT

A series of new tetracaine derivatives with substituents on the aromatic ring was prepared and evaluated for block of retinal rod cyclic nucleotide-gated (CNG) channels. Aromatic substitutions had little effect starting with the basic tetracaine scaffold, but electron-withdrawing substituents significantly improved the blocking potency of an octyl-tail derivative of tetracaine. In particular, halogen substitutions at either the 2- or 3-position on the ring resulted in compounds that were up to eight-fold more potent than the parent octyl-tail derivative and up to 50-fold more potent than tetracaine.

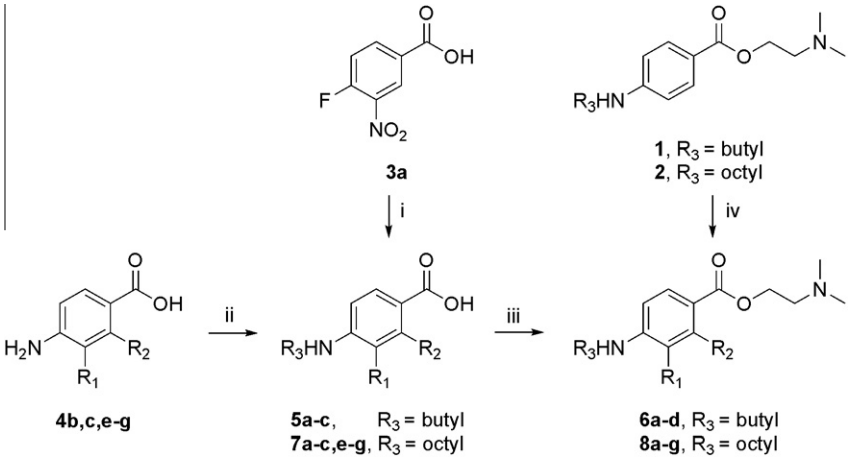
The cyclic nucleotide-gated (CNG) channels of retinal photoreceptors are non-selective cation conductances that regulate the membrane potential in response to light.[1,2] Unlike related voltage-gated potassium channels, these channels are directly activated by the binding of cGMP, and are minimally regulated by voltage. In photoreceptors, photons trigger a signaling cascade that leads to a decrease in cGMP levels and closure of channels. Lately, CNG channels have been receiving increasing attention for their role in some forms of retinitis pigmentosa (RP), a group of inherited retinal degeneration disorders.[3–9] In cGMP-phosphodiesterase (*pde6b*) deficient mice, a well-studied model for RP, excessive CNG channel activation is directly involved in rod photoreceptor degeneration.[10] Unfortunately, there is a dearth in pharmacological tools available to study the role of CNG channels in more depth and to develop therapeutics to treat certain forms of RP.

We have conducted several studies on tetracaine derivatives to develop a potent CNG channel blocker for research and clinical use.[11–14] Tetracaine [2-(dimethylamino)ethyl 4-(butylamino)benzoate] **(1)** is a local anesthetic that blocks CNG channels with relatively high affinity.[15–17] Tetracaine binds to the channel from the intracellular side, and work on both voltage-gated sodium channels and CNG channels indicates that it interacts with the selectivity filter and pore region.[18–21] From our synthetic studies, we have found that increasing the hydrophobic character of the tail portion of tetracaine leads to improved CNG channel block.[13] Charge accumulation on the head group improves block as well, but in detriment to membrane permeability.[11] These findings replicate what has been determined in sodium channel mutagenesis studies, that tetracaine's interaction with the pore is bimodal, involving an electrostatic interaction with negatively charged residues in the selectivity filter and hydrophobic interactions with residues in the pore-lining helix.[18–20,22,23]

In this study we sought to examine the role of tetracaine's aromatic moiety in CNG channel block. Charged polyamines lacking aromatic groups block CNG channels with much lower affinity and permeate through the channel.[24] In sodium channels, the aromatic moiety is thought to interact with a hydrophobic binding site in the pore of the channel, and is responsible for high-affinity block.[25] This suggested aromatic–aromatic interaction with a phenylalanine residue remains to be evaluated in CNG channels. Alternatively, a cation-π interaction may be responsible for high-affinity block in CNG channels.[26–29] To assess the effect of electronic modifications to the aromatic ring, we synthesized a set of aromatic substituted derivatives of tetracaine (**1**) as well as a higher affinity octyl-tail derivative (**2**).[13,14] Substituents ranged from electron-donating (CH_3, CH_3O) to electron-withdrawing (F, Cl, Br, NO_2) located meta or ortho to the ester linkage. Scheme 1 outlines the synthesis of the eleven novel derivatives. Intermediates **5a** and **7a** were synthesized from commercially available 4-fluoro-3-nitrobenzoic acid (**3a**) by a nucleophilic aromatic substitution with *N*-butylamine or *N*-octylamine.[30] Intermediates **5b**,**c** and **7b**,**c**,**e**–**g** were obtained by reductive amination of commercially available

* Corresponding author. Tel.: +1 503 494 7463; fax: +1 503 494 4352.
E-mail address: karpenj@ohsu.edu (J.W. Karpen).
† These authors contributed equally to this work.

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.08.092

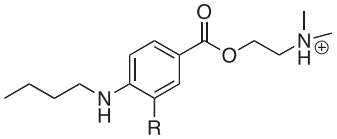


Scheme 1. R_1 and R_2 are defined in Tables 1 and 2; Reagents and conditions: (i) *N*-butylamine/*N*-octylamine, H_2O, $NaHCO_3$, 3 h at 150 °C; (ii) butanal/octanal, α-picoline-borane, MeOH, 1–24 h at rt; (iii) 1,1′-carbonyldiimidazole, 1,2-dimethoxyethane (glyme), 2 h at 60 °C followed by 2-(dimethylamino)ethanol, NaH, 16–24 h at rt; (iv) *N*-chlorosuccinimide, acetonitrile, 24 h, reflux.

derivatives of 4-aminobenzoic acid (**4b**,**c**,**e**–**g**) with butanal or octanal. The free carboxylic acid of the resulting alkylated intermediates (**5** and **7**) was then activated by 1,1′-carbonyldiimidazole and reacted with 2-(dimethylamino)ethanol to yield target compounds **6a**–**c** and **8a**–**c**,**e**–**g**.[14] Compounds **6d** and **8d** were made from **1** and **2** using *N*-chlorosuccinimide, via a synthesis adapted from Lazar et al.[31]

Heteromeric retinal CNG channels comprised of CNGA1 and CNGB1 subunits were expressed in *Xenopus laevis* oocytes, as described previously.[14,32] CNG channel currents were elicited with 2 mM cGMP at both positive (+50 mV) and negative (−50 mV) membrane potentials. Electrophysiological methods as well as data analysis have been previously described in more detail.[14] Potency of CNG channel block was assessed by fitting current amplitudes to the equation for block at a single binding site (see Tables 1 and 2). Apparent K_D values at +50 and −50 mV for tetracaine (**1**) and derivatives **6a–d** with various substituents at the 3-position are summarized in Table 1. Compound **6d** was the only compound in this series to have a slightly higher apparent affinity for CNG channels than compound **1** at both membrane potentials. This appeared to be unrelated to the electron-withdrawing character of Cl, as nitro at the same position (**6a**) did not improve block. The methyl group in **6c** had a slightly deleterious effect on block, again appearing to be unrelated to the electron-donating character, as compound **6b** with a methoxy group had no significant change in blocking potency. Overall, substitutions on the aromatic ring had only subtle effects on apparent affinity for CNG channels in this series.

Table 1
Dissociation constants for aromatic-substituted tetracaine (**1**) derivatives



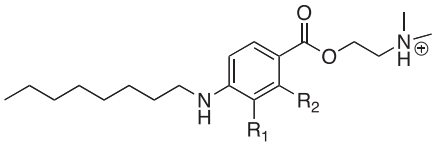
Compound[a]	R	$K_{D(+50)}$ (μM)[b]	$K_{D(-50)}$ (μM)[b]	*n*	σ_p[c]	r_W[d] (Å)
1	H	4.9 ± 1.8	21.8 ± 8.6	16	0.00	1.20
6a	NO_2	5.5 ± 3.2	19.6 ± 14.4	7	0.78	2.59
6b	OMe	4.3 ± 0.8	18.3 ± 4.4	7	−0.27	1.56
6c	Me	7.8 ± 3.1	28.1 ± 13.4	7	−0.17	1.72
6d	Cl	3.0 ± 1.0	12.8 ± 4.1	7	0.23	1.75

[a] Depicted as the predominant protonation state at pH 7.6.
[b] $K_{D(+50)}$ and $K_{D(-50)}$ are the apparent dissociation constants at +50 and −50 mV obtained from fits of the equation $I_{+B}/I_{-B} = K_D/(K_D + [B])$, where the left side is current in the presence of blocker divided by current in the absence of blocker, and [*B*] is blocker concentration.
[c] Hammett sigma constant at the *para*-position, which accounts for the net inductive and resonance effects. Positive values denote an electron-withdrawing substituent, and negative values an electron-donating substituent.
[d] Van der Waals radius; radius of a sphere that encloses the substituent.

In contrast to the butyl-tail derivatives, aromatic substituents in the octyl-tail series produced more dramatic results. Our previous study of ester linkage substitutions led us to conclude that compounds **1** and **2** bind at different locations in the CNG channel pore,[14] and the current results bear this out. Apparent K_D values at +50 and −50 mV for the octyl-tail series (compounds **2, 8a–g**) are presented in Table 2. Like compound **6d**, compound **8d** with a 3-Cl substituent had a higher affinity for CNG channels than compound **2**; however the effect was more pronounced with an approximately six-fold improvement compared to the 1.6-fold improvement in compound **6d**. To better understand the nature of the interaction of compound **8d**, we examined the effects of different halogen substituents 3-F and 3-Br (compounds **8e** and **f**), and position by introducing a 2-Cl substituent ortho to the ester (compound **8g**). All derivatives with halogen substituents, like compound **8d**, had superior blocking potency compared to compound **2**. The differences between them were small, and the patch-to-patch variability in block did not allow us to make fine structure–activity distinctions. Nonetheless, the derivatives in this group were up to eight-fold more potent than compound **2** and up to 50-fold more potent than tetracaine (**1**). A derivative with a strongly electron-donating 3-methoxy substituent (**8b**) blocked with roughly the same apparent affinity as **2**, while a derivative with a 3-methyl group (**8c**), weakly donating, gave a marginal improvement in apparent affinity. Interestingly, the strongly electron-withdrawing nitro derivative (**8a**) deviated from the general trend, blocking with a significantly lower apparent affinity than even **1**. The rationale for this result may be two-fold: (a) the nitro group is considerably larger than any of the other derivatives tested (Table 2, r_W), and (b) while the nitro group carries no net charge, both the nitrogen and oxygen carry formal charge which may negatively impact binding.

This study describes very promising compounds for CNG channel research and possibly clinical usage. Compounds **8d**–**g** have a high apparent affinity for CNG channels in the 100–200 nM range, but without some of the drawbacks of APPA-tetracaine,[11] a derivative in which two propylamino groups in tandem were added to

Table 2
Dissociation constants for aromatic-substituted compound **2** derivatives



Compound[a]	R_1	R_2	$K_{D(+50)}$ (μM)[b]	$K_{D(-50)}$ (μM)[b]	*n*	σ_p[c]	r_W[d] (Å)
2	H	H	0.80 ± 0.50	1.72 ± 1.38	10	0.00	1.20
8a	NO_2	H	8.0 ± 5.8	12.6 ± 7.3	4	0.78	2.59
8b	OMe	H	1.25 ± 1.23	3.0 ± 3.4	5	−0.27	1.56
8c	Me	H	0.50 ± 0.42	1.41 ± 0.84	5	−0.17	1.72
8d	Cl	H	0.14 ± 0.04	0.38 ± 0.16	4	0.23	1.75
8e	F	H	0.20 ± 0.10	0.75 ± 0.86	5	0.06	1.47
8f	Br	H	0.14 ± 0.03	0.24 ± 0.10	4	0.23	1.85
8g	H	Cl	0.10 ± 0.09	0.22 ± 0.13	6	0.23	1.75

[a] Depicted as the predominant protonation state at pH 7.6.
[b] $K_{D(+50)}$ and $K_{D(-50)}$ are the apparent dissociation constants at +50 and −50 mV obtained from fits of the equation $I_{+B}/I_{-B} = K_D/(K_D + [B])$, where the left side is current in the presence of blocker divided by current in the absence of blocker, and [*B*] is blocker concentration.
[c] Hammett sigma constant at the *para*-position, which accounts for the net inductive and resonance effects. Positive values denote an electron-withdrawing substituent, and negative values an electron-donating substituent.
[d] Van der Waals radius; radius of a sphere that encloses the substituent.

the tertiary amine of tetracaine. APPA-tetracaine has an apparent affinity in the subnanomolar range; however its multiply charged head group renders it membrane-impermeant.[11] Other previously tested tetracaine derivatives with hydrophobic moieties have high apparent affinities for CNG channels, but not in the submicromolar range. The apparent K_D values we report here for compounds **8d–g** fall second only to APPA-tetracaine. Block by APPA-tetracaine shows specificity for CNG channels; the apparent K_D for sodium channels being approximately 100-fold higher.[11] The specificities of compounds **8d–g** for CNG channels and different CNG channel subtypes remain to be explored. We are particularly interested in synthesizing these high-affinity derivatives in the more hydrolytically stable thioamide forms.[14] We have previously demonstrated that substituting a thioamide for the ester linkage has a negligible effect on potency of CNG channel block for octyl-tail derivatives. Such compounds may prove to be highly effective pharmacological tools for CNG channel research.

Acknowledgments

This work was supported by NIH Grants EY009275 and MH071625 (J.W.K.). and NSF Grant MRI0821781 (S.R.K.). We thank the Bioanalytical Shared Resource at OHSU for mass spectrometry data, and Tapasree Banerji for technical assistance.

Supplementary data

Supplementary data (synthetic chemistry procedures and ^{1}H NMR, ^{13}C NMR, and ESI-MS data of compounds **6a–d** and **8a–g**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.08.092.

References and notes

1. Fesenko, E. E.; Kolesnikov, S. S.; Lyubarsky, A. L. *Nature* **1985**, *313*, 310.
2. Nakamura, T.; Gold, G. H. *Nature* **1987**, *325*, 442.
3. Farber, D. B.; Lolley, R. N. *J. Neurochem.* **1977**, *28*, 1089.
4. Bowes, C.; Li, T.; Danciger, M.; Baxter, L. C.; Applebury, M. L.; Farber, D. B. *Nature* **1990**, *347*, 677.
5. Pierce, E. A. *Bioessays* **2001**, *23*, 605.
6. Pacione, L. R.; Szego, M. J.; Ikeda, S.; Nishina, P. M.; McInnes, R. R. *Annu. Rev. Neurosci.* **2003**, *26*, 657.
7. Olshevskaya, E. V.; Calvert, P. D.; Woodruff, M. L.; Peshenko, I. V.; Savchenko, A. B.; Makino, C. L.; Ho, Y. S.; Fain, G. L.; Dizhoor, A. M. *J. Neurosci.* **2004**, *24*, 6078.
8. Nishiguchi, K. M.; Sokal, I.; Yang, L.; Roychowdhury, N.; Palczewski, K.; Berson, E. L.; Dryja, T. P.; Baehr, W. *Invest. Ophthalmol. Vis. Sci.* **2004**, *45*, 3863.
9. Trifunovic, D.; Dengler, K.; Michalakis, S.; Zrenner, E.; Wissinger, B.; Paquet-Durand, F. *J. Comp. Neurol.* **2010**, *518*, 3604.
10. Paquet-Durand, F.; Beck, S.; Michalakis, S.; Goldmann, T.; Huber, G.; Muhlfriedel, R.; Trifunovic, D.; Fischer, M. D.; Fahl, E.; Duetsch, G.; Becirovic, E.; Wolfrum, U.; van Veen, T.; Biel, M.; Tanimoto, N.; Seeliger, M. W. *Hum. Mol. Genet.* **2011**, *20*, 941.
11. Ghatpande, A. S.; Uma, R.; Karpen, J. W. *Biochemistry* **2003**, *42*, 265.
12. Strassmaier, T.; Uma, R.; Ghatpande, A. S.; Bandyopadhyay, T.; Schaffer, M.; Witte, J.; McDougal, P. G.; Brown, R. L.; Karpen, J. W. *J. Med. Chem.* **2005**, *48*, 5805.
13. Strassmaier, T.; Kirk, S. R.; Banerji, T.; Karpen, J. W. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 645.
14. Andrade, A. L.; Melich, K.; Whatley, G. G.; Kirk, S. R.; Karpen, J. W. *J. Med. Chem.* **2011**, *54*, 4904.
15. Quandt, F. N.; Nicol, G. D.; Schnetkamp, P. P. *Neuroscience* **1991**, *42*, 629.
16. Schnetkamp, P. P. *Biochemistry* **1987**, *26*, 3249.
17. Schnetkamp, P. P. *J. Gen. Physiol.* **1990**, *96*, 517.
18. Sunami, A.; Dudley, S. C., Jr.; Fozzard, H. A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14126.
19. Ragsdale, D. S.; McPhee, J. C.; Scheuer, T.; Catterall, W. A. *Science* **1994**, *265*, 1724.
20. Ragsdale, D. S.; McPhee, J. C.; Scheuer, T.; Catterall, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9270.
21. Fodor, A. A.; Black, K. D.; Zagotta, W. N. *J. Gen. Physiol.* **1997**, *110*, 591.
22. Catterall, W. A. *Novartis Found. Symp.* **2002**, *241*, 206.
23. Nau, C.; Wang, G. K. *J. Membr. Biol.* **2004**, *201*, 1.
24. Guo, D.; Lu, Z. *J. Gen. Physiol.* **2000**, *115*, 783.
25. Li, H. L.; Galue, A.; Meadows, L.; Ragsdale, D. S. *Mol. Pharmacol.* **1999**, *55*, 134.
26. Heginbotham, L.; MacKinnon, R. *Neuron* **1992**, *8*, 483.
27. Karlin, A.; Akabas, M. H. *Neuron* **1995**, *15*, 1231.
28. Sussman, J. L.; Harel, M.; Frolow, F.; Oefner, C.; Goldman, A.; Toker, L.; Silman, I. *Science* **1991**, *253*, 872.
29. Dougherty, D. A. *Science* **1996**, *271*, 163.
30. Skinner, P. J.; Cherrier, M. C.; Webb, P. J.; Sage, C. R.; Dang, H. T.; Pride, C. C.; Chen, R.; Tamura, S. Y.; Richman, J. G.; Connolly, D. T.; Semple, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6619.
31. Lazar, C.; Kluczyk, A.; Kiyota, T.; Konishi, Y. *J. Med. Chem.* **2004**, *47*, 6973.
32. Strassmaier, T.; Karpen, J. W. *J. Med. Chem.* **2007**, *50*, 4186.