Neuropharmacology 63 (2012) 368–373

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

Trifluoroacetate is an allosteric modulator with selective actions at the glycine receptor

Megan E. Tipps [1], Sangeetha V. Iyer, S. John Mihic*

Section of Neurobiology, Division of Pharmacology and Toxicology, Waggoner Center for Alcohol & Addiction Research, Institutes for Neuroscience and Cell & Molecular Biology, University of Texas at Austin, MC A4800, 2500 Speedway, MBB 1.148, Austin, TX 78712, USA

ARTICLE INFO

Article history:
Received 6 December 2011
Received in revised form
20 March 2012
Accepted 4 April 2012

Keywords:
Anesthetic metabolism
Allosteric modulation
Glycine receptor
$GABA_A$ receptor
5-HT3 receptor
Trifluoroacetate

ABSTRACT

Trifluoroacetic acid is a metabolite of the inhaled anesthetics halothane, desflurane and isoflurane as well as a major contaminant in HPLC-purified peptides. Ligand-gated ion channels, including cys-loop receptors such as the glycine receptor, have been the targets of peptide-based drug design and are considered to be likely candidates for mediating the effects of anesthetics *in vivo*, but the possible secondary contributions of contaminants and metabolites to these effects have not been studied. We used two-electrode voltage-clamp electrophysiology to test glycine, $GABA_A$ and 5-HT3 receptors expressed in *Xenopus* oocytes for their sensitivities to sodium trifluoroacetate.

Trifluoroacetate (100 μM–3 mM) enhanced the currents elicited by low concentrations of glycine applied to α1 homomeric and α1β heteromeric glycine receptors, but it had no effects when co-applied with a maximally-effective glycine concentration. Trifluoroacetate had no effects on α1β2γ2S $GABA_A$ or 5-HT3A receptors at any GABA or serotonin concentration tested.

The results demonstrate that trifluoroacetate acts as an allosteric modulator at the glycine receptor with greater specificity than other known modulators. These results have important implications for both the secondary effects of volatile anesthetics and the presence of contaminating trifluoroacetate in HPLC-purified peptides, which is potentially an important source of experimental variability or error that requires control.

1. Introduction

Trifluoroacetic acid is widely used in organic chemistry, particularly in peptide synthesis, where it functions as an ion-pairing agent during the HPLC purification step of peptide synthesis. The addition of trifluoroacetic acid increases the hydrophobicities of peptides by forming ionic pairs with their charged groups, favoring interactions between peptides and a hydrophobic stationary phase, thus enabling separation (García, 2005). It binds to the free amino termini of peptides as well as the side chains of positively charged lysine, histidine and arginine residues (Cornish et al., 1999), forming trifluoroacetate (TFA) salts. This ion pairing is extremely strong and requires an additional ion replacement step during purification to remove TFA from the purified peptides. Thus, HPLC-purified peptides are often prepared and used as TFA salts, resulting in purified peptides with varying levels of TFA contamination.

Trifluoroacetic acid is also a major metabolite of the volatile anesthetics halothane, isoflurane and desflurane (Cohen, 1971; Hitt et al., 1974). It is thought to be responsible for the development of halothane-induced hepatitis and neurotoxicity (Gut et al., 1995; Ma et al., 1990) and may play a role in the cardioprotective effects of isofluorane (Han et al., 2001).

A large number of protein targets of inhaled anesthetics have been identified, among them members of the cys-loop receptor family such as the glycine (GlyR) and γ-aminobutyric acid ($GABA_A$-R) receptors (Franks, 2006). Both the GlyR (Harrison et al., 1993; Mascia et al., 1996) and $GABA_A$-R (Wakamori et al., 1991; Nishikawa et al., 2002) are sensitive to clinically-relevant concentrations of a wide variety of volatile anesthetics that are hypothesized to interact with these receptors at defined molecular sites (Mascia et al., 2000). However, whether a metabolite of some of these anesthetics could also affect the functioning of these cys-loop receptors, and possibly contribute to anesthetic actions, has thus far not been investigated.

Abbreviations: GlyR, glycine receptor; MBS, Modified Barth's Saline; TFA, trifluoroacetate.

* Corresponding author. Tel.: +1 512 232 7174; fax: +1 512 232 2525.
E-mail address: mihic@mail.utexas.edu (S. John Mihic).
[1] Present address: Department of Behavioral Neuroscience, Oregon Health & Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97239-3098, USA.

0028-3908/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2012.04.011

Based on multiple published reports of inhaled anesthetic modulation of ion channels and our previous work that identified novel peptides that act as allosteric modulators at the GlyR (Tipps et al., 2010), we tested TFA for its effects on the functioning of several cys-loop receptors. We found that TFA reversibly modulates GlyR responses and that these modulatory effects do not extend to other members of the cys-loop receptor family. These results are relevant to the development and testing of future peptide-based drugs, as well as highlighting possible secondary central nervous system effects following the administration and metabolism of some inhaled anesthetics.

2. Materials and methods

2.1. Oocyte isolation and DNA microinjection

Xenopus laevis were obtained from Nasco (Fort Atkinson, WI) and treated in accordance with an approved institutional animal care and use protocol at the University of Texas. Stage V and VI oocytes were surgically isolated, and receptor subunit cDNAs injected blindly into oocyte nuclei, as described previously (Welsh et al., 2010). The human glycine receptor α1 subunit cDNA was injected on its own to form homomeric receptors or with the β subunit cDNA in a 1:20 α1:β v/v ratio to form heteromeric receptors. Human $GABA_A$ α1, β2 and γ2S subunit cDNAs were combined in a 1:1:3 v/v ratio before being injected, while the mouse 5-HT3A subunit cDNA was injected alone. In each case, a total of 1.5 ng of cDNA (in 30 nL) was injected per oocyte. All chemicals were obtained from Sigma- Aldrich (St. Louis, MO). Mutation of serine-267 to glutamine (S267Q) in the GlyR α1 subunit was described previously (Findlay et al., 2002).

2.2. Electrophysiology

Oocytes were assayed for receptor expression one to four days after cDNA injection. Two high-resistance (0.5–10 MΩ) glass electrodes filled with 3 M KCl were used to impale the animal poles of isolated oocytes for electrophysiological recording. Cells were voltage-clamped at −70 mV using a Warner Instruments OC-725C oocyte clamp (Hamden, CT) and perfused with Modified Barth's Saline [MBS; 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM HEPES, 0.82 mM $MgSO_4 \cdot 7H_2O$, 0.33 mM $Ca(NO_3)_2$, 0.91 mM $CaCl_2$; pH 7.5] at a rate of 2 ml/min using a Masterflex USA peristaltic pump (Cole Parmer Instrument Co, Vernon Hills, IL) through 18-gauge polyethylene tubing. Perfusion tubing was washed with 100% ethanol for 60 s and rinsed with MBS for approximately five minutes before each oocyte was placed in the recording chamber. All drug solutions were prepared in MBS. Trifluoroacetate (100 μM–3 mM) was pre-applied to oocytes for 30 s before being co-applied with agonist for a further 10 or 45 s for the $GABA_A$ and glycine receptors or 10 or 90 s for 5-HT3A receptors. The 10 s applications were used when maximally-effective concentrations of agonists were applied. Clamping currents were acquired at 100 Hz using a PowerLab 4/30 digitizer and digitally filtered at 50 Hz using LabChart 7 software (ADInstruments, Bella Vista NSW, Australia). Peak current responses were visually determined from LabChart files, and TFA effects are expressed as percent changes in peak currents compared with the effects produced by agonists applied alone. Significant differences between experimental conditions were determined using one- or two-way ANOVAs or the Mann–Whitney Rank Sum test.

3. Results

3.1. Lack of non-specific membrane effects of TFA in oocytes

TFA is a chaotropic compound, and other compounds of this class are known to alter membrane integrity and function. Thus, we first tested TFA for its effects on holding currents in uninjected oocytes. When applied alone, 100 μM–3 mM TFA had no effects on the holding currents of uninjected oocytes across a range of command voltages (0 to −70 mV), nor did it elicit currents in oocytes expressing the tested receptors in the absence of the respective receptor agonists. These results suggest that TFA cannot directly gate channels and that it does not significantly alter the function of any of the other endogenous oocyte proteins responsible for the holding current (data not shown).

3.2. TFA acts as an allosteric modulator of the GlyR

TFA was tested for its effects on glycine, $GABA_A$ and serotonin (5-HT) 3A receptor function. To control for variability across oocytes, the concentration of agonist producing 5–10% of a maximal response (EC_{5-10}) was identified for each oocyte and used throughout the experiment. TFA (100 μM–3 mM) was pre-applied alone for 30 s before being co-applied with the EC_{5-10} agonist.

TFA acted as an allosteric modulator of the GlyR by enhancing the currents elicited by EC_{5-10} glycine in wild-type homomeric α1 GlyRs in a concentration-dependent and reversible manner [$F(3,31) = 4.73$, $p < 0.01$] (Fig. 1A,B). When co-applied with a saturating glycine concentration of 10 mM, TFA had no effect on wild-type GlyR (1.3 ± 6.7% potentiation, Fig. 1C), further supporting the conclusion that TFA is acting as an allosteric modulator of this channel, that acts by leftshifting the glycine concentration-

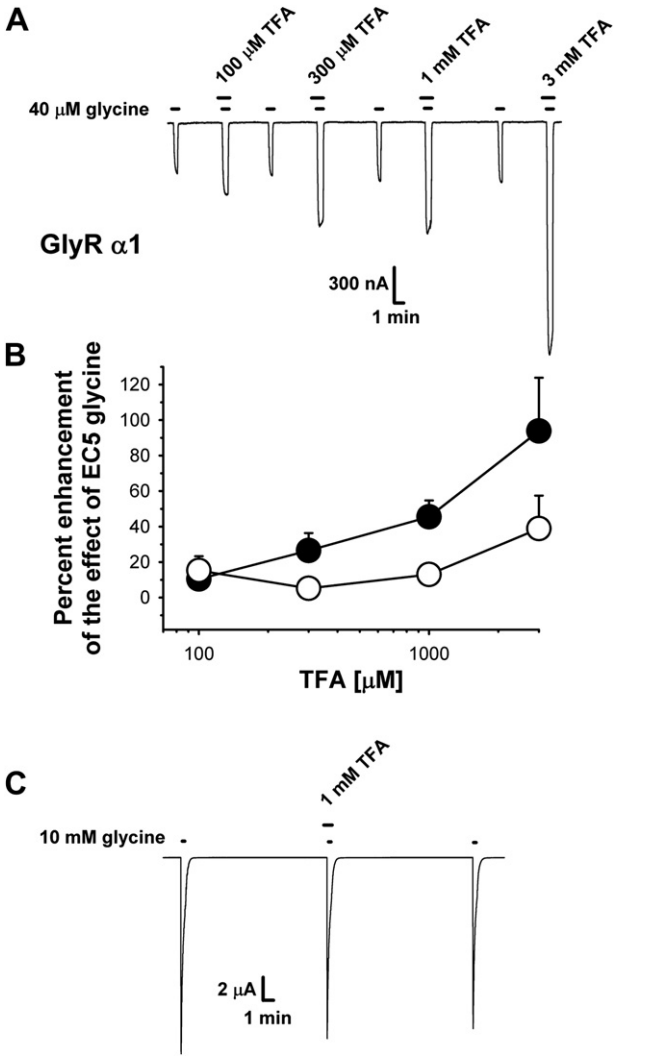

Fig. 1. Concentration-dependent enhancement of homomeric α1 GlyR function by TFA. (A) Sample tracings demonstrating the enhancing effects of TFA on glycine-mediated responses in oocytes voltage-clamped at −70 mV. For each oocyte the EC_{5-10} concentration of glycine (43 ± 5.1 μM glycine resulting in 5.9 ± 0.5% of maximal current) was first applied alone for 45 s. A 30 s pre-incubation of TFA preceded the co-application of TFA plus EC_{5-10} glycine for a further 45 s. TFA did not alter the holding current when applied alone. Reversibility of the effects of TFA is shown by intervening glycine-alone applications following 5–10 min washouts. (B) Summary of data showing the effects of 100 μM–3 mM TFA on wild-type α1 GlyR (solid symbols) as well as the S267Q α1 mutant (hollow symbols), which was almost completely resistant to TFA. Data are presented as the mean ± S.E.M. of 7–8 oocytes obtained from at least two batches of frogs. (C) Although TFA significantly enhanced responses to EC_{5-10} glycine in wild-type α1 GlyR, it had no enhancing effects on the responses elicited by a maximally-effective glycine concentration. A 30 s pre-incubation with 1 mM TFA preceded glycine application with TFA for a further 10 s.