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Non-immobilizers put to the test: F6 and the $GABA_A$ receptor

Misha Perouansky *, Robert A. Pearce

Department of Anesthesiology, University of Wisconsin Medical School, Madison WI, USA

Abstract. In the ongoing search for the mechanisms of anesthetic action, a promising paradigm introduced approximately a decade ago was the use of non-immobilizers [D.D.,Koblin, et al., Polyhalogenated and perfluorinated compounds that disobey the Meyer–Overton hypothesis, Anesth. Analg. 79 (6) (1994) 1043–1048] [1]. These drugs have physicochemical properties similar to those of anesthetics but, except for amnesia, do not induce an anesthetic state. Since the $GABA_A$ receptor figures prominently in the list of general anesthetic targets, we investigated the effects of F6 (1,2-dichlorohexafluorocyclobutane, a.k.a. 2N) in vitro on expressed and native $GABA_A$ receptors and in vivo on field potentials in the hippocampus. The experiments in vitro were aimed at analyzing the interaction of F6 with the $GABA_A$ receptor, and in vivo on testing the hypothesis that nonconvulsive seizures cause F6-induced amnesia. We used three different types of preparations: transfected HEK-293 cells combined with a rapid drug application system for studies of expressed receptors; acute hippocampal slices from juvenile rats for investigations of synaptic and extrasynaptic receptors; and multisite electrodes chronically implanted into adult rats for experiments on hippocampal field potentials. All animal experiments were conducted in accordance with APS guidelines and with the approval of the IACUC. We found that with expressed receptors, F6 blocked currents mediated by $\alpha_1\beta_2$ receptors with an EC50 of 8 μM (approx. 0.5 times predicted MAC) but did not alter current kinetics. By contrast, $\alpha_1\beta_2\gamma_2$s-containing receptors were insensitive to F6. Native $GABA_A$ receptors at subsynaptic and extrasynaptic locations on hippocampal pyramidal cells were also insensitive to F6. In vivo recordings showed no evidence of F6-induced nonconvulsive seizure activity at amnestic concentrations. We conclude that F6 causes amnesia neither by altering inhibition of hippocampal pyramidal neurons nor by inducing nonconvulsive hippocampal seizures. Instead, amnesia may result from effects on other types of hippocampal neurons or on receptors other than the $GABA_A$ receptor. © 2005 Published by Elsevier B.V.

Keywords: Volatile anesthetic; Whole-cell recording; Expressed receptors; Extrasynaptic receptors; Mechanism of anesthesia; Isoflurane; Inhibition; Seizure; Pro-convulsant; Amnesia; Memory

* Corresponding author.
E-mail address: mperouansky@wisc.edu (M. Perouansky).

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1. Introduction

More than two decades ago, proteins were recognized as potential targets of general anesthetics. In the following years, more and more proteins were found to be affected to some degree by anesthetic drugs, and the problem of separating relevant from irrelevant effects arose. This problem was compounded by the fact that anesthesia is essentially an 'in vivo' state without an adequate 'in vitro' model. In an attempt to experimentally address this problem, anesthetic-like compounds termed non-anesthetics were introduced more than a decade ago [1]. These peculiar drugs cause either none or only some of the behavioral effects of anesthetics at concentrations predicted to be anesthetic by their lipophilicity. Members of a subgroup of non-anesthetics that cause amnesia without immobility have been termed 'non-immobilizers' [2].

Their experimental application follows the following reasoning: if a process on the molecular or cellular level is similarly affected by a non-anesthetic/non-immobilizer and an anesthetic, this process is unlikely to contribute to the 'anesthetic state' (a notable exception being amnesia for non-immobilizers). The expectation has been that application of this modification of 'Occam's razor' would dramatically reduce the number of candidate targets for pivotal roles in the mechanisms of anesthesia.

The best-studied of these drugs is the non-immobilizer 1,2-dichlorohexafluorocyclobutane (abbreviated F6 or 2N). We investigated its interaction with the $GABA_A$ receptor, a recognized target for many injectable and inhalational anesthetic drugs, because the potentiation of $GABA_A$ receptors causes sedation, hypnosis and amnesia, whereas its suppression can cause seizures. Specifically, we wished to determine whether the behavioral effects of F6 could be related to modulation of hippocampal $GABA_A$ receptors, as this structure is important for some forms of memory formation and is susceptible to seizure involvement.

2. Materials and methods

We studied synaptic $GABA_A$ receptors of hippocampal pyramidal cells in acute brain slices obtained from young Sprague–Dawley rats. Extrasynaptic receptors were obtained in the same preparation by harvesting nucleated patches and exposing them to exogenous GABA pulses [3].

Receptors of known subunit composition were transiently expressed in HEK 293 cells using complementary DNA (cDNA) encoding the rat $GABA_A$ receptor subunits α_1 (FLAG tagged) [4], β_2, or γ_2s, inserted into the multiple cloning site of the mammalian expression vector pCEP4 (Invitrogen, Carlsbad, CA). Cells were cotransfected with $\alpha_1\beta_2$ or $\alpha_1\beta_2\gamma_2$s subunits using Lipofectamine 2000.

2.1. Electrophysiology

Recordings were performed at room temperature (22–24 °C) on the stage of an Olympus BX50WI upright microscope. Patch clamp recordings were made using an Axopatch 200A patch clamp amplifier (Axon Instruments, Foster City, CA, USA) and pClamp 8.0 software (Axon Instruments). Data were low-pass filtered at 5 kHz, sampled at 10 kHz (Digidata 1200, Axon Instruments) and stored online on a computer hard disk.