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Research paper

Augmentation of glycine receptor alpha3 currents suggests a mechanism for glucose-mediated analgesia

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HIGHLIGHTS

- α3K, α3L, and α3L(P185L) glycine receptors were expressed in HEK 293 cells.
- Glucose potentiated α3L and α3K GlyR-mediated currents, shifting EC50 4- to 5- fold.
- The high-activity mutant α3L(P185L) was not further potentiated by glucose.
- Glucose was active below 10 mM, i.e. at physiological concentrations.
- Glycine receptor potentiation may account for glucose-mediated analgesia.

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ABSTRACT

The inhibitory glycine receptor (GlyR) mediates rapid synaptic inhibition in the mammalian central nervous system. Recently, glucose was identified as a positive modulator of α1 GlyRs. Here, recombinant human α3GlyRs with and without glucose treatment were studied using patch clamp methods. Similar to α1GlyRs, receptor variants α3L and α3K were potentiated by sugar. Glucose treatment reduced EC_{50} values of GlyR α3L and α3K by a factor of 4.5 and 3.3, respectively, without affecting maximum currents or desensitization. The high-activity mutant α3L(P185L) was not further potentiated by glucose. Potentiation of glycinergic signalling may underlie some of the analgetic effects of glucose.

1. Introduction

The inhibitory glycine receptor is a member of the cysteine loop superfamily of ligand gated ion channels [1], being one of the principal mediators of rapid synaptic inhibition in the mammalian central nervous system. GlyRs are involved in the control of muscle tone and movement as well as retinal signalling [2] and pain processing [3,4]. To date, five different subunits have been identified in mammals, four ligand-binding alpha subunits (α1–α4) and one β subunit [1]. Fine-tuning of neuronal receptor function is an essential mechanism for the central nervous system to adapt to physiological changes, learning, and repair of injury. In addition to modulation of receptor expression and subunit composition, numerous biochemical mechanisms, which are not yet fully understood, contribute to these adaptation processes. Recently, glucose and fructose were identified as modulators of human α1 and α1/β glycine receptors [5]. In presence of sugars, EC_{50} values of glycine were reduced up to 4.5- fold, while currents elicited by ivermectine, taurine or β-alanine were not affected [5].

The α3 subunit of the inhibitory glycine receptor is widely distributed within the central nervous system. Although expression of the GlyR α3 subunit increases with age, it is weakly expressed at all developmental stages compared to glycine receptor α1. Functional and immunohistochemical studies report GlyR α3 expression in several central nervous system regions including the hippocampus [6]. In the retina, GlyR α3 is found at synapses that are distinct from GlyR α1 locations [2]. Additionally, GlyR α3 has been identified in rat inhibitory synapses in the inner ear [7]. Most importantly, α3 GlyRs are found in spinal cord where they are key mediators of inflammatory pain signaling [4,8]. The receptors are located

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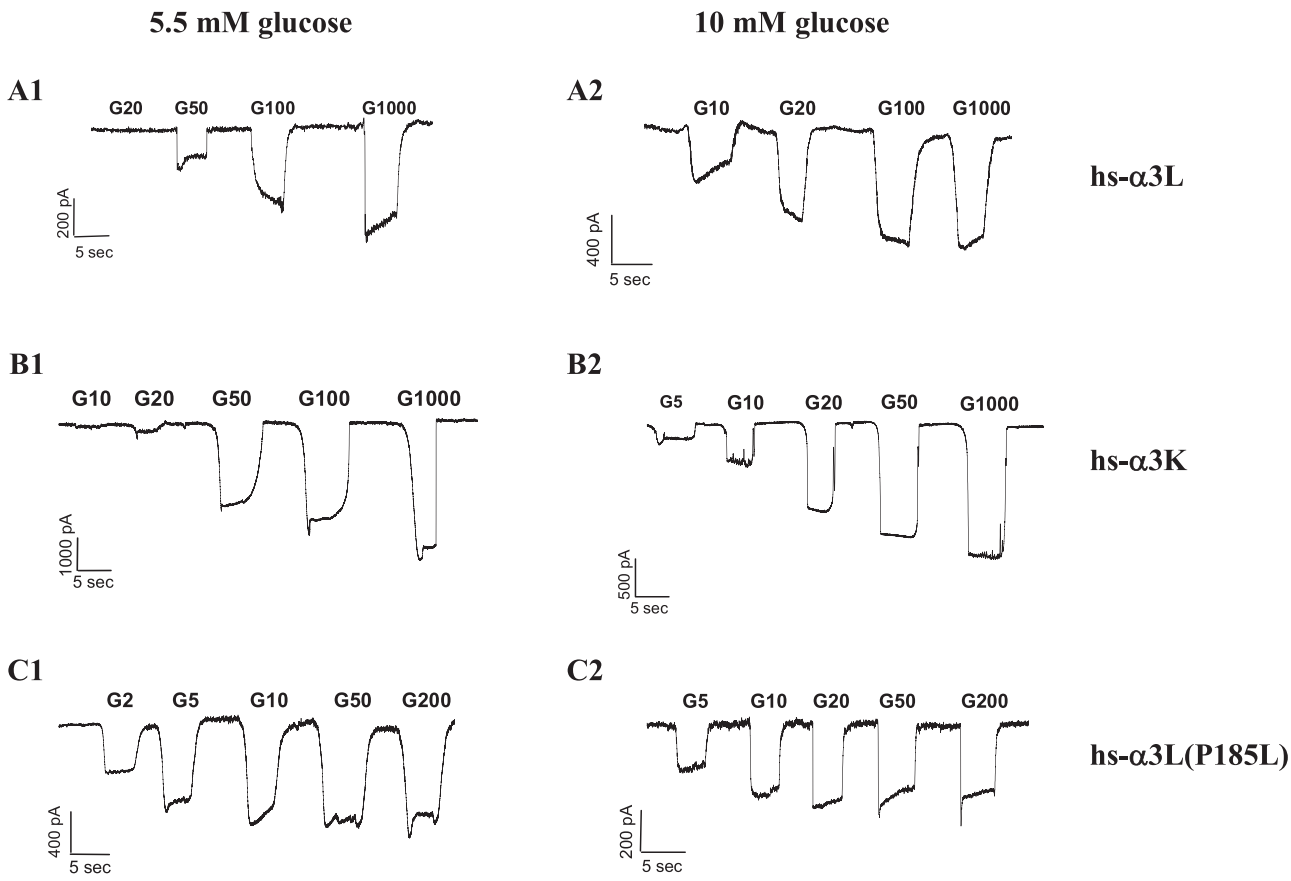

Fig. 1. Whole-cell current responses of α3 glycine receptors.
HEK 293 cells were transfected with the indicated α3 GlyR subunits. 1 days post transfection, cells were cultured in standard MEM (minimum essential medium, 5.5 mM glucose), or in MEM with a total concentration of 10 mM glucose for 18–24 h. Whole-cell recording was performed in glucose-free recording buffers (see experimental section), at 21–23 °C, −50 mV. (A) α3L, (B) α3K, (C) α3L(P185L). G indicates glycine application, concentrations (in μM) are indicated.

at synaptic sites in laminae I and II of the dorsal horn where they inhibit the propagation of nociceptive signals to higher brain regions, serving as molecular substrate of pain sensitization by the inflammatory mediator prostaglandin E2 (PGE2)[9]. Two splice variants of human a3 GlyRs have been discovered, which differ by a 15-residue insert in the intracellular TM3-4 loop of the receptor protein. They show little difference in function, most prominent are moderate changes in EC_{50} and desensitization [10–12]. Human α3L(P185L) receptors, produced in the hippocampus through RNA editing, show high agonist affinity with a 5-to 10-fold reduction in glycine EC_{50} values [13].

Glucose is an essential nutrient and one of the main fuel molecules in the bloodstream. In recent years, glucose was found to exert analgetic effects in newborn babies [14–16], and in some forms of pain, including vascular pain during chemotherapy [17,18]. Here, we investigated the influence of glucose on recombinant human α3 glycine receptors. Physiological concentrations of glucose shifted EC_{50} of α3 GlyR -mediated current responses ~4-fold, while the gain-of function mutant hsGlyR α3L(P185L) was not further potentiated by glucose.

2. Materials and methods

Site-directed mutagenesis—single nucleotide exchanges corresponding to codon hsGlyR α3L(P185L) were introduced by PCR-mediated site-directed mutagenesis using an overlap extension PCR approach [19]. The mutated clone was sequenced across the PCR-generated sequence to verify successful mutagenesis (LGC, Berlin, Germany).

Cell Culture and transfection—HEK293 cells were grown in 10 cm tissue culture Petri dishes in MEM (Sigma, Deisenhofen, Germany) supplemented with 10% FBS (Invitrogen, Karlsruhe, Germany) and Penicillin/Streptomycin at 5% CO_2 and 37 °C in a water saturated atmosphere. For experiments, cells were plated on poly-L-lysine treated glass coverslips in 6 cm dishes. Transfection was performed 1 day after cell passage using 1.3 μg of receptor DNA, 1.3 μg of green fluorescence protein DNA and 2.6 μl GenCarrier (Epoch Life Sciences, Sugarland, TX, USA). The cell culture medium contained 1 g/l (5.5 mM) of glucose, which is the control value. Glucose was added to give the indicated total concentration in the growth/incubation medium. The total concentration of glucose is given in figures and tables.

Electrophysiological recordings and data analysis—HEK293 cells were transfected 2–4 days prior to electrophysiological recordings. For pre-treatment experiments, glucose (Sigma–Aldrich, Deisenhofen, Germany) in the indicated concentrations was added to the growth medium one day after transfection, and measurements performed 18–24 h later. Glucose-containing medium was removed from cells before recording and patch-clamp measurements were performed without glucose in the extracellular buffer. Current responses were measured at room temperature (21–23 °C) at a holding potential of −50 mV. Whole-cell recordings were performed using a HEKA EPC10 amplifier (HEKA Electronics, Lambrecht, Germany) controlled by Pulse software (HEKA Electronics). Recording pipettes were pulled from borosilicate glass (World Precision Instruments, Berlin, Germany) using a Sutter P-97 horizontal puller (Sutter, Novato, CA). Solutions were applied using an Octaflow system (NPI electronics, Tamm, Germany), where cells