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INFLAMMATORY MEDIATOR-INDUCED MODULATION OF $GABA_A$ CURRENTS IN HUMAN SENSORY NEURONS

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Abstract—The purpose of the present study was to characterize the properties of A-type GABA receptor ($GABA_A$ receptor) currents in human sensory neurons. Neurons were obtained from adult organ donors. $GABA_A$ currents were recorded in isolated neurons. Both large inactivating low-affinity currents and smaller persistent high-affinity currents were present in all of the 129 neurons studied from 15 donors. The kinetics of human $GABA_A$ currents were slower than those in rat sensory neurons. GABA currents were completely blocked by bicuculline (10 μM), and persistent currents were activated by the δ-subunit-preferring agonist, 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol (THIP). The GABA current equilibrium potential was ~20 mV more hyperpolarized than in rat neurons. Both low- and high-affinity currents were increased by inflammatory mediators but via different second messenger pathways. These results highlight potentially important species differences in the properties of ion channels present in their native environment and suggest the use of human sensory neurons may be a valuable tool to test compounds prior to use in humans.

Key words: dorsal root ganglion, intracellular Cl^- concentration, patch clamp, protein kinase A, protein kinase C, tyrosine kinase.

INTRODUCTION

Behavioral pharmacological data from rodents indicate that A-type GABA receptor ($GABA_A$ receptor) signaling is critically involved in the modulation of nociception at sites throughout the central nervous system (CNS) (Price et al., 2009). Particularly important is the gating of afferent input to the spinal cord. While data from rodent models also suggest that post-synaptic inhibitory circuitry in the spinal cord dorsal horn is likely to be important for regulation of nociceptive threshold, particularly in the presence of injury, available electrophysiological, pharmacological and morphological data suggest that pre-synaptic inhibition of afferent input is the dominant mechanism for inhibition of somatosensory input into the CNS (Eccles et al., 1962, 1963; Nishi et al., 1974; Mokha et al., 1983; Hiura et al., 1998; Reeve et al., 1998; Rudomin and Schmidt, 1999; Bae et al., 2000; Olave et al., 2002; Sutherland et al., 2002; Sokal and Chapman, 2003; Vesselkin et al., 2003; Weng and Dougherty, 2005). Virtually all dorsal root ganglion (DRG) neurons from rat respond to GABA with a rapidly activating, bicuculline-sensitive anion current (Oyelese et al., 1995; Zhu et al., 2012a). However, in contrast to neurons in the CNS, activation of $GABA_A$ receptors on primary afferents results in membrane depolarization. This paradoxical response is thought to be due to a relatively high concentration of intracellular Cl^- as a result of the persistent expression of the Na^+–K^+–Cl^--co-transporter, NKCC1 into adulthood.

Despite the wealth of knowledge of $GABA_A$ signaling in rodents and the growing interest in the use of $GABA_A$ receptor ligands for the treatment of pain, there is only one report of $GABA_A$ currents in human sensory neurons (Maddox et al., 2004). Interestingly, the currents from adult human DRG neurons described in this study were resistant to the classical $GABA_A$ receptor antagonists bicuculline and picrotoxin (Maddox et al., 2004). And while there appear to be limited differences between human and rodent $GABA_A$ receptor homologs in heterologous expression systems, evidence of unique pharmacological properties of the $GABA_A$ currents in human sensory neurons raises the possibility that the differences are due to processes unique to the native environment. Thus, given the therapeutic potential of $GABA_A$ receptor ligands suggested by preclinical data (Witschi et al., 2011), and in light of growing concerns over the extent to which preclinical data translate to human clinical conditions (Seok et al., 2013), we sought to further characterize $GABA_A$ currents in human sensory neurons.

Our results suggest that while there are many similarities between the $GABA_A$ currents present in rodent and human sensory neurons, there are marked

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Abbreviations: CNS, central nervous system; DRG, dorsal root ganglion; GABA, γ-aminobuteric acid; $GABA_A$ receptor, A-type GABA receptor; PKA, protein kinase A; PKC, protein kinase C; THIP, agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-3-ol; TK, tyrosine kinase.

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differences. These included biophysical properties of the evoked currents, which were more slowly activating and inactivating in human DRG neurons, as well as the response to inflammatory mediators. That is, in contrast to the selective potentiation of high-affinity $GABA_A$ currents previously observed in rat DRG neurons (Lee and Gold, 2012), both low- and high-affinity currents were potentiated in human sensory neurons via what appeared to be distinct second messenger pathways. These results highlight potentially important species differences in the properties of ion channels present in their native environment and suggest the use of human sensory neurons may be a valuable tool with which to test compounds developed in heterologous expression systems and validated in rodent models prior to the use in humans.

EXPERIMENTAL PROCEDURES

Human subjects

L4 and L5 DRG were collected from organ donors with the consent of family members for the use of their loved one's tissue for research purposes.

DRG collection

Following the collection of tissue needed for transplantation purposes, L4 and L5 DRG were accessed via a ventral approach. Briefly, the lumbosacral trunk was found running medially to the psoas major. Blunt dissection was used to follow the spinal nerves of the L4 and L5 ganglia to their respective foramen in the vertebral column. An oscillating autopsy saw (Mopec, Oak Park, MI, USA) was used to cut through the vertebral bodies above and below the L4 and L5 ganglia, respectively. Cuts were then made through the vertebral pedicles, with care to keep the saw blade angled above the ganglia. A Virchow skull breaker was then used to lift the ventral surface of the spinal column off en bloc, exposing the ganglia and cauda equina. Ganglia were carefully freed of connective tissue, the central and peripheral processes were cut and the ganglia were placed in ice-cold collection media (Table 1) of the following composition: 124.5 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1 mM $CaCl_2$, and 30 mM HEPES. The pH of the collection media was adjusted to 7.35 with NaOH and filter sterilized for storage at 4 °C for no more than two weeks.

With this approach, it was possible to recover all four ganglia in well under 15 min. The time between cross clamp and the start of the DRG isolation was usually between 45 min and an hour, and as a result, we were generally able to have the DRG in collection media in a little over an hour.

In an initial set of pilot experiments, we sought to determine how long the DRG could sit in collection media prior to dissociation before the overall health of the isolated DRG neurons began to deteriorate, as manifest by a poor yield of neurons, high resting intracellular Ca^{2+} concentrations and/or depolarized (> -40 mV) resting membrane potential. This was to determine the maximal travel time, and therefore the farthest hospital we could use for the recovery of tissue. Results from this analysis suggested that ~3 h was the outside limit.

Isolation of DRG neurons

The protocol employed was adapted from that developed by Baumann et al. (2004). Ganglia were transferred to fresh collection media and carefully freed of connective tissue and fat under a dissecting microscope. "Cleaned" tissue was then cut into small (~2–5 mm^3) pieces and these pieces were transferred to dissociation media (Table 1) containing 1 mg/ml of collagenase P (Roche Bioscience,

Table 1. Solutions for collection dissociation and culturing of human DRG neurons

	Collection media	Dissociation media	Broth	Basal media	Complete media
Purpose	Collection and transfer to DRG	Enzymatic treatment and quenching enzymatic treatment	Added to dissociation media	Percoll spins	Plating and culture
Composition	129.5 mM NaCl, 5 mM KCl, 1.2 mM $MgS04_2$, 1 mM $CaCl_2$, 30 mM HEPES	Collection media + Broth at 25 ml/l	200 mM glucose, 400 mM NaCl, 0.9% phosphoric acid (w/w)	L-15 media containing: 60-mg imidizole, 15-mg aspartic acid, 15-mg glutamic acid, 15-mg cystine, 5-mg β-alanine, 10-mg myo-inositol, 10-mg cholineCl, 5-mg p-aminobenzoic acid, 25-mg fumaric acid, 2-mg vitamin B12 and 5-mg of lipoic acid (which was first dissolved in methanol at a concentration of 1 g/2.5 ml)	Basal Media containing: heat inactivated fetal bovine serum (1:10 with basal media), to which is added (to a final concentration): 50 ng/ml nerve growth factor (NGF 2.5S), 0.3 mg/ml glutamine, 0.225 mg/ml glucose, 2.55 mg/ml ascorbic acid, and 0.12 mg/ml glutathione, and 0.2% (w/v) $NaHCO_3$
Notes	Adjust pH to 7.35 with NaOH, sterile filter, store at 4 °C for < 2 wks	Made fresh every time	Diluted in double distilled H_2O. Sterile filtered. Stored at −20 °C	Components added to 500-ml bottle of L-15. Stored at −4 °C for < 1 wk	Made fresh every time