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The antiepileptic drug carbamazepine affects sodium transport in toad epithelium

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Abstract

The present work investigates the effects of the antiepileptic drug carbamazepine (CBZ) on sodium transport in the isolated skin of the toad *Pleurodema thaul*. A submaximal concentration of the drug (0.2 mM) applied to the outer surface of the epithelium increased the electrical parameters short-circuit current (I_{sc}) and potential difference (PD) by over 28%, whereas only a higher concentration (1 mM) induced over a 45% decrease in these parameters when applied to the inner surface. The amiloride test showed that the outer surface stimulatory effect was accompanied by an increase and the inner surface inhibitory effect by a decrease in the sodium electromotive force (E_{Na}). Exploration of these effects of CBZ on the outer surface showed that 0.2 mM increased net Na^+ (^{22}Na) influx by 20% and 0.6 mM CBZ decreased Na^+ mucosa–serosa flux by 19%, a result in agreement with the finding that higher concentrations of CBZ applied to the inner surface not only decreased E_{Na} but also sodium conductance (G_{Na}).

Keywords: Carbamazepine; Antiepileptic; Isolated toad skin; Sodium channel

1. Introduction

Epilepsy is a disorder of the brain due to a persistent predisposition to generate epileptic seizures. An epileptic seizure is a transient and recurrent occurrence of signs and/or symptoms due to abnormal or synchronous neuronal activity in the brain (Fisher et al., 2005). Abnormal potassium conductance across the neuronal membrane, defects in the voltage-sensitive calcium channels, or a deficiency in neuronal cell membrane ATPases may result in neuronal membrane instability and abnormal firing of these neurons (Gorrell, 2002). Antiepileptic drug therapy does not offer a permanent cure, but successful therapy can eliminate or reduce symptoms and arrest or slow the disease process. In view of the fact that restoration to health is not as yet possible, research approaches center on elucidating the cellular and molecular mechanisms of hyperexcitability, for a more accurate on-target quest for new active antiepileptic agents. Progress in techniques of electrophysiology has refined the analysis of seizure mechanisms from the electroencephalogram to individual ion channels on individual neurons (McNamara, 2001). Carbamazepine (CBZ) is a primary drug for the treatment of partial and tonic–clonic epileptic seizures (McNamara, 2001); it has also been used for the relief of pain associated with trigeminal neuralgia and for various psychiatric disorders. CBZ is an iminostilbene derivative, a dibenzazapine tricyclic compound (Yang and Kuo, 2002) which

Abbreviations: CBZ, Carbamazepine; I_{sc}, short-circuit current; PD, potential difference; E_{Na}, sodium electromotive force; G_{Na}, sodium conductance; G_{sh}, passive conductance; G_t, total conductance; EC_{50}, half-maximal excitatory concentration (IC_{50}); J^{Na}net, net Na^+ flux; $J^{Na}_{m\to s}$, active flux; $J^{Na}_{s\to m}$, passive flux.

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limits the repetitive firing of action potentials evoked by sustained depolarization of neurons due to slowing of the rate of recovery of voltage-activated Na^+ channels from inactivation. However, its aqueous solubility is limited and it is absorbed erratically after oral administration. Nevertheless, bioavailability of different forms of oral administration ranges from 75% to 85% (Gorrell, 2002), and its half-life ranges from 4 to 30 h (Cloyd and Remmel, 2000). Former use of the octanol/water partition coefficients to predict the ability of a molecule to cross membranes is insufficient, because this also depends on membrane charge and composition (Girault et al., 1997); CBZ is a lipophilic drug with the ability to increase its conversion to active metabolites by hepatic oxidative enzymes. The drug distributes rapidly to all tissues, binding to plasma proteins (75–85%), and concentrations in the cerebrospinal fluid appear to correspond to the concentration of free drugs in plasma (McNamara, 2001). The toxic effects of CBZ most commonly observed include diplopia, nystagmus, ataxia, dizziness and headache (Gorrell, 2002). Rash is the most frequent hypersensitivity reaction and may even progress to the Stevens–Johnson syndrome and toxic epidermolysis (Lin et al., 2005). Neurotoxicity was also induced by CBZ in cultured hippocampal neurons of the rat (Araujo et al., 2004).

In earlier work (Suwalsky et al., 2001; Suwalsky et al., 2002; Suwalsky et al., 2003; Suwalsky et al., 2004) we have shown that several agents affect the activation not only of voltage-sensitive Na^+ channels but also the activation of epithelial ligand-gated sodium channels such as those present in the isolated amphibian skin. Visceral antinociceptive affects of CBZ in noxious colorectal distension (Su et al., 2002) suggest a peripheral sodium channel blocking action. In addition, although several ion channels are known to be implicated in the generation of epileptic seizures, it has recently been shown that Na^+ channel inhibition is the primary mechanism for CBZ and probably also for newer antiepileptic agents (Armijo et al., 2002). This prompted us to explore the effect of this antiepileptic on Na^+ transport in the isolated toad skin. As previously mentioned, CBZ inhibits high-frequency neuronal firing because it reduces the rate of recovery of Na^+ channels from inactivation (McNamara, 2001; Farber et al., 2002; Lingamanemi and Hemmings, 2003). It is not known whether CBZ depresses epithelial Na^+ transport since the Na^+ channels in the outer (mucosal or apical) surface of the membrane of epithelial cells (ENaCs) (Kellenberger and Schild, 2002) belong to another family with structure different from voltage-gated channels: they are amiloride-sensitive and belong to the ENaC-Degenerin family (Alvarez de la Rosa et al., 2000) and they serve diverse functions varying from Na^+ absorption across epithelia to being the receptors for neurotransmitters in the nervous system. The large electrochemical gradient for Na^+ existing across the outer surface of the membrane provides the driving force for the entry of Na^+ into the cell, and together with the in series basolateral (serosal or inner) membrane extrusion step supplied by the Na^+, K^+-ATPase, is responsible for the transepithelial transport of Na^+ from the outer to the inner side of the skin (Horisberger, 2003). This process was examined by: (a) measurement of the potential difference (PD) and of the short-circuit current (I_{sc}), the amount of current which brings the PD across the skin down to zero, and is identified with the Na^+ transport (Hoffmann, 2001), before and after exposure to several concentrations of CBZ; (b) analysis of the results using Isaacson's amiloride test (Isaacson, 1977), which quantifies the changes produced by drugs on each of the components of the equivalent electrical circuit; and (c) determination of net ^{22}Na flux across the epithelium.

2. Materials and methods

Experiments were performed on male and female toads of the species *Pleurodema thaul* (7–16 g) collected from fresh water ponds. They were kept in bins containing tap water at room temperature (18–22 °C) at least 24 h prior to use and fed on sow bugs (*Oniscus asellus*). The abdominal skin was dissected from pithed toads and samples were carefully washed in toad Ringer's solution, stretched and mounted between two halves of a modified perspex Ussing chamber. A circular area of 1 cm^2 was exposed to 3 ml Ringer's bathing solution on each side. The composition of the solution was (mM): Na^+ 114, K^+ 2.5, Cl^- 117.5, Ca^{2+} 2.0, $HC0_3^-$ 2.3, glucose 11, and phosphate-buffered to pH 7.4. The bathing medium was oxygenated by means of an Elite 800 aerator (R.C. Hagen, W. Yorkshire, UK). The I_{sc} was monitored with non-polarizable Ag/AgCl electrodes placed at 15 mm distance from the skin and connected to a voltage-clamp circuit (G. Métraux Electronique, Crissier, Switzerland) set to keep the PD across the skin at zero mV. The PD was measured with calomel-agar electrodes at intervals of 2 min for 4 s. Both parameters were displayed on a two-channel Cole Parmer recorder. Thirty to sixty minutes after steady readings had been obtained, CBZ was applied in the solution bathing either the outer or the inner surface of the skin in the final concentrations specified in the text. Sodium electromotive force (E_{Na}), sodium conductance (G_{Na}) and passive conductance (G_{sh}) were determined by Isaacson's amiloride test equation: $I_{sc}/PD = I_{sc}/E_{Na} + G_{sh}$. Amiloride was obtained from Merck, Sharp and Dohme. Measurements were made by solving the equation graphically for skin samples with and without CBZ. In each case two points (with and without amiloride) were obtained (Suwalsky et al., 2004). To measure net sodium flux [$(J^{Na}net)$ = active flux$(J^{Na}_{m\to s})$ − passive flux$(J^{Na}_{s\to m})$], 1 μCi ^{22}Na (New England Nuclear, Boston, Mass) was added to the mucosal solution, and 30 min were allowed to ensure a steady state of transfer of ^{22}Na. Samples were then removed from either the mucosal or the serosal bathing solution at 15 min intervals, before and after the addition of 0.2 mM CBZ to the mucosal side of the short-circuited skin, and analyzed in a model RM 73301 alpha nuclear automatic spectrometer.