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Voltage-gated and background K^+ channel subunits expressed by the bushy cells of the rat cochlear nucleus

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Abstract

Bushy cells of the ventral cochlear nucleus produce a single, short latency action potential at the beginning of long depolarisations. In the present work an immunochemical survey was performed to detect the presence of K^+ channel subunits which may contribute to the specific membrane properties of the bushy cells.

The immunocytochemical experiments conducted on enzymatically isolated bushy cells indicated positive immunolabelling for several subunits known to be responsible for the genesis of rapidly inactivating K^+ currents. Bushy cells showed strong expression of Kv3.4, 4.2 and 4.3 subunits, with the lack of Kv1.4 specific immunoreaction. The Kv3.4-specific immunoreaction had a specific, patchy appearance. Bushy cells also expressed various members of the Kv1 subunit family, most notably Kv1.1, 1.2, 1.3 and 1.6. Weak positivity could be observed for Kv3.2 subunits. The positive immunolabelling for Kv3.4, Kv4.2 and Kv4.3 was confirmed in free-floating tissue slices. Voltage-clamp experiments performed on positively identified bushy cells in brain slices corroborated the presence and activity of Kv3.4 and Kv4.2/4.3 containing K^+ channels. Bushy cell showed strong immunopositivity for TASK-1 channels too.

The results presented in this work indicate that bushy cells possess several types of voltage-gated K^+ channel subunits whose activity may contribute to the membrane properties and firing characteristics of these neurones.

Keywords: Bushy cells; K^+ channel subunits; TASK channel; Immunochemistry; BDS-I; Phrixotoxin

1. Introduction

The cochlear nucleus (CN) is the first point in the auditory pathway concerned with the processing of auditory information carried by the acoustic nerve (Feketete et al., 1984; Moore, 1986; Rhode, 1991; Morest, 1993; Frisina, 2001). The timing and pattern of action potentials (AP) in the acoustic nerve are decoded to provide information on loudness, pitch, and location of the sound source. The latter function requires comparison of inputs from both cochleae and this binaural processing is conducted in nuclei of the superior olivary complex (SOC) and inferior colliculus (IC).

It has been described that bushy neurones fire a single, short latency AP at the beginning of long

Abbreviations: aCSF, artificial cerebrospinal fluid; AP, action potential; ATP, adenosine-5′-triphosphate; BDS-I, blood depressing substance-I; BSA, bovine serum albumin; CN, cochlear nucleus; DAPI, 4′,6-diamidino-2-phenylindole; DIC, differential interference contrast; EGTA, ethylene glycol-bis(β-aminoethylether)N,N,N′,N′-tetraacetic acid; FITC, fluorescein isothiocyanate; GIRK, G protein-regulated K^+ channel; GTP, guanosine-5′-triphosphate; Hepes, *N*-[2-hydroxyethyl]-piperazine-*N*′-[ethanesulfonic acid]; HVA, high-voltage-activated; IC, inferior colliculus; I_h, hyperpolarization-activated non-specific cationic current; Kv, voltage-gated K^+ channel; LVA, low-voltage-activated; NGS, normal goat serum; P, pore-forming loop; PaTx2, phrixotoxin-2; PB, phosphate buffer; PBS, phosphate-buffered saline; SOC, superior olivary complex; TASK, TWIK-related acid sensitive K^+ channel; T-BS, Tris-buffered saline; TMD, transmembrane domain; TTX, tetrodotoxin; TWIK, tandem of pore domains in weak inward rectifier K^+ channel

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depolarisations maintaining the temporal pattern of the incoming stimuli [primary-like or type II response (Oertel, 1983; Wu and Oertel, 1984; Oertel et al., 1990; Manis and Marx, 1991)], hence bushy neurones seem to be particularly important for sound source localisation. The characteristic firing pattern produced by bushy neurones is the consequence of several special features including their voltage-gated currents.

Mammalian K^+ channels are divided into three structural classes (e.g. see Coetzee et al., 1999; Rudy and McBain, 2001; Dodson and Forsythe, 2004). Voltage-gated K^+ channels consist of four subunits, each subunit containing six transmembrane domains (TMDs). Inward rectifier K^+ channels, G protein-regulated K^+ channels (GIRK) and the ATP-sensitive K^+ channels fall into the second class, having subunits with only two TMDs separated by a single pore-forming loop (2TM-P channels). In contrast to these channels, a new superfamily of K^+ channels, containing four TMDs and two pore-forming loops (4TM-PP channels) has been described more recently (Ketchum et al., 1995).

The voltage-dependent K^+ channels are further divided into eight subfamilies, but only members of the Kv1, Kv2, Kv3 and Kv4 subfamilies can produce functional homotetramers. Heterotetrameric channel formation is also possible between subunits belonging to the same subfamily, or belonging to the "electrically silent" families (Kv5, Kv6, Kv8 and Kv9). On the basis of their functional properties, depolarisation-activated K^+ channels fall into three major groups (for reviews see Rudy and McBain, 2001; Dodson and Forsythe, 2004), such as low-voltage-activated channels (i.e. Kv1.1, Kv1.2, Kv1.6); high-voltage-activated channels (i.e. Kv3.1, Kv3.2) and A-type (or rapidly inactivating) K^+ channels (i.e. Kv1.4, Kv4.2, Kv4.3). The current produced by Kv3.4 channels is a HVA one with distinct inactivation tendency (HVA A-current).

It has been noted earlier that bushy neurones have fast membrane time constants (Oertel, 1983; Manis and Marx, 1991), and it was suggested that this property might be the consequence of a low-threshold potassium conductance. Indeed, recent studies demonstrated the activity of low-threshold K^+ channels (Dodson et al., 2003; Rothman and Manis, 2003), and the experimental data indicated that these channels are most likely composed of Kv1.1, Kv1.2 and Kv1.6 subunits. Moreover, bushy cells possess a hyperpolarisation-activated non-specific cationic conductance (I_h), whose activity may also contribute to the relatively low input resistance of these cells (Cuttle et al., 2001). Bushy neurones express various types of depolarisation-activated Ca^{2+} currents too, and it was noted that the channels situated on the cell body and on the presynaptic terminals are not identical (Doughty et al., 1998). The presence of a transient outward current was also noted on bushy neurones, as it has been described that isolated bushy cells expressed high-threshold potassium conductances (Manis and Marx, 1991), showing some degree of inactivation. The presence of a somatic inactivating K^+ current component has also been noted in a thin slice preparation, and it appeared to be much less prominent in the presynaptic terminals of the bushy neurones (Dodson et al., 2003).

There are indications that neurones situated in the auditory system may express various members of the TASK channel family (Duprat et al., 1997; Leonoudakis et al., 1998; Reyes et al., 1998; Kim et al., 1999, 2000; Rajan et al., 2000; Ashmole et al., 2001; Kim and Gnatenco, 2001). More specifically, strong expression of TASK-1, TASK-3 and TASK-5-specific mRNA was noted in the ventral cochlear nucleus (Karschin et al., 2001), raising the possibility that certain neurones may possess these ionic channels, but hitherto no specific studies have been conducted to detect the presence of these channels on the individual cell types of the cochlear nucleus. As TASK-1 channels are known to be responsible for the high K^+ permeability of the cells at rest, playing very important roles in determining their resting membrane potential, input resistance and hence excitability (Duprat et al., 1997), it might be interesting to check for the presence of these channels in the cases of the bushy cells too.

In the present study a survey was conducted to detect the presence of various voltage-gated and background K^+ channel subunits expressed by the bushy cells of the rat ventral cochlear nucleus. Particular attention was given to those subunits that are known to contribute to the assembly of rapidly inactivating (transient) K^+ channels. The immunochemical experiments were mainly conducted on enzymatically isolated bushy cells of the rat cochlear nucleus, but the presence of those subunits which appeared to be strongly expressed by the isolated cells was confirmed by using immunohistochemistry on free-floating slices. The results of the present work indicate that bushy cells possess Kv3.4, Kv4.2 and Kv4.3 subunits known to be capable of producing transient K^+ current. The presence and functionality of these subunits were confirmed by using electrophysiology and subunit-specific channel blockers (Phrixotoxin-2 and BDS-I) on positively identified bushy neurones in slices. The present work also reports on the presence of TASK-1 specific immunoreactivity on enzymatically isolated bushy cells.

2. Materials and methods

2.1. Enzymatic isolation of the bushy cells

Bushy neurones were isolated by using a technique described earlier in details (Doughty et al., 1998; Rusznák et al., 2001). In short, 4–11-day-old rats were decap-