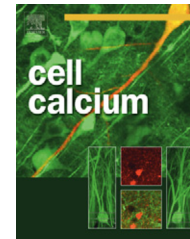
Cell Calcium (2008) 44, 521–532

journal homepage: www.elsevier.com/locate/ceca



GABA release by basket cells onto Purkinje cells, in rat cerebellar slices, is directly controlled by presynaptic purinergic receptors, modulating Ca^{2+} influx

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Received 15 June 2007; received in revised form 10 March 2008; accepted 12 March 2008
Available online 12 May 2008

KEYWORDS
Purkinje cell;
Cerebellar cortex;
Purinergic;
GABA;
GABA release;
Calcium influx;
Calcium stores;
Synaptic transmission;
P2X;
P2Y;
Presynaptic receptors;
IPSC

Summary In many brain regions, Ca^{2+} influx through presynaptic P2X receptors influences GABA release from interneurones. In patch-clamp recordings of Purkinje cells (PCs) in rat cerebellar slices, broad spectrum P2 receptor antagonists, PPADS (30 μM) or suramin (12 μM), result in a decreased amplitude and increased failure rate of minimal evoked GABAergic synaptic currents from basket cells. The effect is mimicked by desensitizing P2X1/3-containing receptors with α,β-methylene ATP. This suggests presynaptic facilitation of GABA release via P2XR-mediated Ca^{2+} influx activated by endogenously released ATP. In contrast, activation of P2Y4 receptors (using UTP, 30 μM, but not P2Y1 or P2Y6 receptor ligands) results in inhibition of GABA release. Immunological studies reveal the presence of most known P2Rs in ≥20% of GABAergic terminals in the cerebellum. P2X3 receptors and P2Y4 receptors occur in approximately 60% and 50% of GABAergic synaptosomes respectively and are localized presynaptically. Previous studies report that PC output is also influenced by postsynaptic purinergic receptors located on both PCs and interneurones.

The high Ca^{2+} permeability of the P2X receptor and the ability of ATP to influence intracellular Ca^{2+} levels via P2Y receptor-mediated intracellular pathways make ATP the ideal transmitter for the multisite bidirectional modulation of the cerebellar cortical neuronal network.
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0143-4160/$ — see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.ceca.2008.03.006

Introduction

The ATP-activated P2X receptor/channels (P2XRs) are highly Ca^{2+} permeable. Where purinergic synapses have been recorded in the brain, the amplitude of currents carried by ATP receptor/channels is small and thus it is likely that it is the calcium influx, rather than the depolarization caused by the influx of cations, which is important in their function. This is reflected in the fact that the location of P2XRs has often been found to be presynaptic [1—3] regulating the release of neurotransmitters including the inhibitory transmitter GABA [4—8]. Even where the P2X receptors are found postsynaptically they are thought primarily to regulate such functions as synaptic plasticity because of their calcium permeability [9]. In contrast to P2X receptors, P2Y receptors, including those in Purkinje neurones [10], are usually involved in release of Ca^{2+} from endoplasmic reticulum rather than Ca^{2+} influx. This P2Y-mediated Ca^{2+} release is activated by inositol trisphosphate, via phospholipase C (see [11], for review). Thus, purinergic transmission can in general be considered as a mechanism for regulation of internal Ca^{2+} concentration.

Over recent years purinergic and GABAergic transmission have been repeatedly shown to interact in different brain areas. In some cases ATP and GABA act as co-transmitters, both activating postsynaptic receptors [12]. More commonly however the purinergic receptors are presynaptic [13—15] and can regulate GABA release by altering the background level of calcium in the axon terminal [4—8].

As the entire output of the cerebellar cortex is carried by the firing of Purkinje cells, any mechanism able to modulate the firing pattern of Purkinje cells will influence cerebellar function. Purkinje cells fire spontaneously, even in the absence of glutamate input, and the pattern of firing is very strongly influenced by high conductance, high frequency GABAergic inputs [16]. At least under experimental conditions, this inhibitory input is largely conveyed by two types of inhibitory interneurones, the basket cells and the stellate cells. We have recently demonstrated that, under specific conditions which may prevail during motor activity, the GABA release from the Lugaro cell may also be very important [17].

Considering the importance of GABA transmission in influencing Purkinje cell firing and the many reports that the cerebellum is rich in ATP (P2) receptors [18—21], the interaction between P2 receptors and GABAergic transmission in the cerebellum is clearly of interest. Recently two different groups have approached this question. Saitow et al. [22] reported a role for metabotropic P2Y receptors, demonstrating both a short-term increase in the firing rate of the afferent and a postsynaptic long-term potentiation of GABAergic synaptic activity. In particular, they implicated P2Y receptors in increasing action potential frequency in a population of Lugaro cells but suggested that other GABAergic interneurones may also be recruited. Another group not only showed similar effects of P2Y1 receptors but also implicated P2Y2 and P2Y4 receptors and P2X5 receptors on the soma of basket and stellate cells [23]. The same group later showed that the effects of ATP develop over the first postnatal weeks with the full expression of receptors and effects on release being functional by postnatal day 14 (P14) in rats [24], in agreement with the reported expression of P2X receptor in the cerebellum [25].

Thus, it is clear that, by the end of the second postnatal week, purinergic receptors become important in the control of Purkinje cell function by influencing the firing patterns of interneurones. However, in other brain areas P2 receptors have mostly been found on presynaptic terminals, directly influencing release via Ca^{2+} influx, rather than being located somato-dendritically, controlling the firing of the presynaptic cell (reviewed in [8,13,14]. Thus, in the present study, we specifically investigate the role of P2 receptors on the axon terminals of GABAergic neurones. We use patch-clamp techniques to record synaptic currents in acute brain slices and show that purinergic receptors can modulate GABA release bidirectionally at the level of the GABAergic terminals. By combining pharmacological studies with an immunological characterization of P2 receptors in sub-synaptic fractions and in GABAergic cerebellar terminals, we find that P2X3-containing and P2Y4 receptors are the most likely subtypes involved in the facilitation and inhibition of GABA release, respectively. We go on to investigate how such a complex distribution of P2 receptor-mediated Ca^{2+} modulation, reported here on axons and previously in the soma/dendritic compartments, will affect the firing rates of individual Purkinje cells.

Methods

Electrophysiology

Animals

Male Wistar rats (13—15 days old) were obtained from the University College London animal facility and were killed by decapitation under Home Office License.

Preparation of cerebellar slices

Sagittal cerebellar slices (400 μm thick) were prepared using standard methods [26]. Briefly, the cerebellum was cut along the midline through the vermis and glued on this surface for slicing. Slices were maintained in bubbled Krebs solution at 34 °C for 20—30 min. They were then transferred, within 30 min of slicing, to a second chamber containing fresh bubbled Krebs solution at 34 °C before being allowed to cool to room temperature. All recordings were made at room temperature (21—24 °C) unless otherwise stated.

Solutions. The bath solution (Krebs) contained (in mM): 125 NaCl, 2.4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 1.1 NaH_2PO_4, 25 glucose, and was bubbled with 95% O_2—5% CO_2. In all experiments, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydro[f]quinoxaline-7-suphonamide (NBQX; 20 μM) and 7-chlorokynurenate (10 μM) were also included in the bath solution to inhibit ionotropic glutamate receptors. The intracellular solution contained (in mM): 140 CsCl; 5 HEPES; 10 EGTA; 2 MgATP; and 2 $CaCl_2$; pH 7.4 with CsOH.

NBQX was dissolved in DMSO at 500× its final concentration. Aliquots of the concentrated NBQX were frozen at −20 °C and diluted into Krebs solution at the time of the experiment. 7-chlorokynurenate was dissolved in NaOH at

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