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PEPTIDES
Peptides 26 (2005) 1936–1943
www.elsevier.com/locate/peptides

The regulation of α-MSH release by GABA is mediated by a chloride-dependent $[Ca^{2+}]_c$ increase in frog melanotrope cells

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Received 1 October 2004; accepted 23 November 2004
Available online 28 June 2005

Abstract

In frog melanotrope cells, γ-aminobutyric acid (GABA) induces a biphasic effect, i.e. a transient stimulation followed by a more sustained inhibition of α-MSH release, and both phases of the GABA effect are mediated by $GABA_A$ receptors. We have previously shown that the stimulatory phase evoked by $GABA_A$ receptor agonists can be accounted for by calcium entry. In the present study, we have investigated the involvement of the chloride flux on GABA-induced $[Ca^{2+}]_c$ increase and α-MSH release. We show that GABA evokes a concentration-dependent $[Ca^{2+}]_c$ rise through specific activation of the $GABA_A$ receptor. The GABA-induced $[Ca^{2+}]_c$ increase results from opening of voltage-activated L- and N-type calcium channels, and sodium channels. Variations of the extracellular Cl^- concentration revealed that GABA-induced $[Ca^{2+}]_c$ rise and α-MSH release both depend on the Cl^- flux direction and driving force. These observations suggest for the first time that GABA-gated Cl^- efflux provokes an increase in $[Ca^{2+}]_c$ increase that is responsible for hormone secretion.

Keywords: α-MSH; $[Ca^{2+}]_c$; Chloride gradient; $GABA_A$ receptor; Melanotrope cell

1. Introduction

In amphibians, the intermediate lobe of the pituitary is composed of a single endocrine cell type, the melanotrope cell, which synthesizes the hormone α-MSH that plays a pivotal role in the process of skin color adaptation [24]. Extensive studies performed in frogs and toads have shown that the secretion of α-MSH is controlled by multiple factors, including classical neurotransmitters and neuropeptides [24,39,50,53]. For instance, in the frog *Rana esculenta*, the activity of melanotrope cells is stimulated by β-adrenergic [27] and muscarinic agonists [20], thyrotropin-releasing hormone [14,18,48] and neurotensin [17], and inhibited by dopamine [2,15,51], serotonin [26], adenosine [9,34,35], α-adrenergic agonists [27] and neuropeptide Y [8,12]. It has long been known that the neurotransmitter γ-aminobutyric acid (GABA) regulates the activity of several pituitary cell types [3,4,45,46]. In mammalian and amphibian melanotrope cells, GABA exerts a biphasic effect on α-MSH release, i.e. a transient stimulation followed by a more sustained inhibition [3,13,16,46]. In rat and porcine melanotrope cells, the dual effect of GABA can be ascribed to activation of both $GABA_A$ and $GABA_B$ receptors [13,46] while, in frog melanotrope cells, the stimulatory and inhibitory actions of GABA are exclusively mediated by the $GABA_A$ receptor [3,16].

The action of GABA at the $GABA_A$ receptor depends on the direction and the potency of the chloride driving force both controlled by the resting membrane potential (RMP) and the chloride equilibrium potential (ECl^-) [36]. In mature cells, the maintenance of low intracellular Cl^- concentrations ($[Cl^-]_i$) shifts the ECl^- towards values more negative than RMP. Thus, activation of $GABA_A$ receptors causes Cl^-

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doi:10.1016/j.peptides.2004.11.022

entry and hyperpolarization [23]. In contrast, in fetal and postnatal neurons exhibiting a relatively high $[Cl^-]_i$, ECl^- is more positive than RMP resulting in Cl^- efflux, depolarization and increase in intracellular calcium concentration ($[Ca^{2+}]_c$) through stimulation of voltage-gated Ca^{2+} channels [6,32]. Likewise, in frog melanotrope cells, activation of GABA-gated Cl^- channels evokes depolarization and $[Ca^{2+}]_c$ increase [16,29]. The purpose of the present study was to investigate the involvement of the chloride flux in the GABA-induced $[Ca^{2+}]_c$ increase and α-MSH release in frog melanotrope cells.

2. Materials and methods

2.1. Animals

Adult male frogs (*Rana esculenta*; body weight, 40–50 g) were obtained from a commercial source (Couétard, Saint-Hilaire de Riez, France). The animals were housed in a temperature-controlled room (8 ± 0.5 °C) under running water on a 12-h dark, 12-h light regimen (lights on from 06:00 a.m. to 08:00 p.m.). Animal manipulations were carried out according to the recommendations of the French Ethical Committee and under the supervision of authorized investigators.

2.2. Chemicals and reagents

GABA, 3-aminopropane sulfonic acid (3APS), tetrodotoxin (TTX), picrotoxin, SR95531, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), sodium acetate, tris[hydroxymethyl]aminomethane (Trizma base), nifedipine, bovine serum albumin (BSA; fraction V), collagenase type IA, baclofen, ω-conotoxin GVIA (ω-CgTx GVIA), Leibovitz culture medium (L15), the antibiotic-antimycotic solution and kanamycin were purchased from Sigma (St-Quentin Fallavier, France). Indo-1-pentaacetoxymethylester was purchased from Molecular probes (Leiden, The Netherlands). Fetal bovine serum (FBS) was from Cambrex Bio Science (Verviers, Belgium).

2.3. Cell culture

Neurointermediate lobes (NIL) were collected in a Krebs Ringer's solution consisting of 112 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 15 mM HEPES, 2 mg/ml glucose and 0.3 mg/ml BSA (pH 7.4). The NIL were enzymatically dissociated by collagenase type IA (1 μg/ml) in a Ca^{2+}-free Ringer's solution. The cell suspension was rinsed and transferred into the perifusion chambers or plated on poly-L-lysine-coated glass coverslips, in 35-mm culture dishes. Cultured cells were maintained in L15 culture medium adjusted to *Rana esculenta* osmolarity (*f*L15; L15/water, 1:0.4, v/v) and supplemented with 0.2 mg/ml glucose, 82 μg/ml $CaCl_2$, 15 mM HEPES, 1% each of the kanamycin and antibiotic-antimycotic solutions, and 10% FBS. Cultured cells were kept at 21 °C in a humidified atmosphere for 4–7 days. The culture medium was renewed every 72 h.

2.4. Cytosolic calcium measurement

Cytosolic calcium concentration ($[Ca^{2+}]_c$) was monitored by a dual emission microfluorimeter system as previously described [18]. Briefly, melanotrope cells were incubated in a Krebs Ringer's solution containing 5 μM Indo-1-pentaacetoxymethylester in the dark at room temperature for 1 h. The fluorescence emission of Indo-1, induced by excitation at 355 nm, was measured at two wavelengths (405 and 480 nm) by separate photometers (PI; Nikon, Champigny-sur Marne, France). The three signals were continuously recorded using an AS1-type acquisition card with the JAD-FLUO program (Notocord System, Croissy-sur-Seine, France). In Krebs Ringer's solution containing 42 mM $[Cl^-]$, NaCl was replaced by sodium acetate. In Krebs Ringer's solution containing 214 and 671 mM $[Cl^-]$, chloride concentrations were elevated by HCl (10N) and pH adjusted with Trizma base buffer. Test substances were delivered in the vicinity of recorded cells by means of a superfusion system. Results were expressed as the mean amplitude of Ca^{2+} increase ± S.E.M.

2.5. Measurement of α-MSH release

The perifusion system used to determine the effect of test substances on α-MSH secretion has been previously described [47]. Briefly, NIL were suspended in a Bio-Gel P2 matrix and perifused with the Krebs Ringer's solution at a constant flow rate (0.3 ml/min) and temperature (24 °C). After a 1.5-h stabilization period, the perifusion effluent from each column was collected as 7.5-min fractions during the stabilization periods and as 1- or 2.5-min fractions during administration of the secretagogues. The concentration of α-MSH was measured in each fraction by using a double-antibody radioimmunoassay procedure [52]. The perifusion profiles were expressed as percentages of the basal secretion rate calculated as the mean profiles of α-MSH release (±S.E.M.) from at least three independent experiments.

2.6. Statistical analysis

The statistical significance of differences was determined by analysis of variance (ANOVA) followed by a Student–Newman–Keuls comparison test.

3. Results

3.1. Effect of GABA on $[Ca^{2+}]_c$

Exposure of cultured melanotrope cells to a 15-s pulse of 10^{-6} M GABA induced a substantial increase in the 405/480