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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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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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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

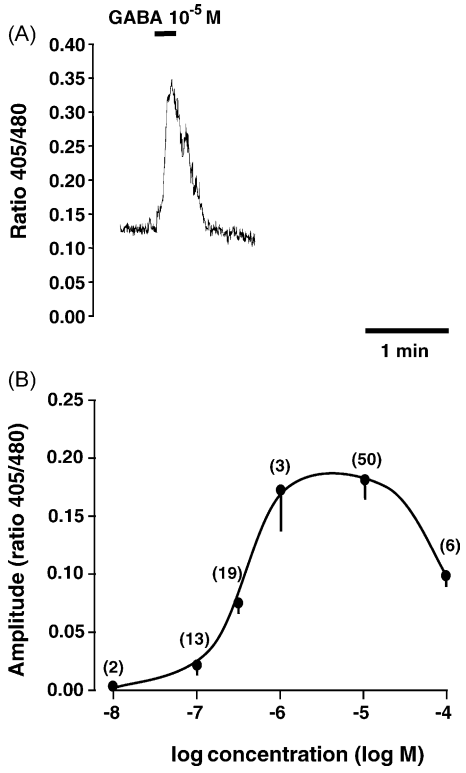

Fig. 1. Effect of GABA on cytosolic calcium concentration ($[Ca^{2+}]_c$) in cultured frog melanotrope cells. (A) Typical profile showing the effect of GABA (10^{-5} M, 15 s) on $[Ca^{2+}]_c$ in a melanotrope cell. (B) Effect of graded concentrations of GABA (10^{-8} M to 10^{-4} M, 15 s) on the amplitude of the $[Ca^{2+}]_c$ response. Each point represents the mean response (±S.E.M.) calculated from the number of recordings indicated in parentheses.

fluorescence ratio which peaked within 7 s and then decreased gradually during the next 30–60 s (Fig. 1A). Administration of graded concentrations of GABA (10^{-8} to 10^{-4} M) induced a dose-dependent increase in the $[Ca^{2+}]_c$ amplitude with a maximum effect at a concentration of 10^{-6} M and a half-maximum response at 3×10^{-7} M (Fig. 1B). When the cells were exposed to a high concentration of GABA (10^{-4} M), the $[Ca^{2+}]_c$ response declined (Fig. 1B).

3.2. Effect of GABA agonists and antagonist on $[Ca^{2+}]_c$

Application of the $GABA_A$ receptor antagonist SR95531 (10^{-5} M, 1 min) significantly reduced ($P < 0.001$) the GABA-induced $[Ca^{2+}]_c$ increase ($n = 16$; Fig. 2A and D), and the stimulatory effect of GABA was recovered after 70-s washout (Fig. 2A). The $GABA_A$ receptor agonist 3APS (10^{-5} M, 15 s; $n = 144$) mimicked the stimulatory effect of GABA on $[Ca^{2+}]_c$ (Fig. 2B and D). Incubation of melanotrope cells with the chloride channel blocker picrotoxin (15 min, 10^{-4} M; $n = 22$), did not affect basal $[Ca^{2+}]_c$ but totally abolished ($P < 0.001$) the 3APS-induced stimulation of $[Ca^{2+}]_c$ ($n = 22$; Fig. 2B and D). Exposure of melanotrope cells to the $GABA_B$ receptor agonist baclofen (10^{-5} M, 15 s; $n = 30$) had no effect on $[Ca^{2+}]_c$ (Fig. 2C and D).

Administration of repeated pulses of 3APS (10^{-5} M, 15 s; $n = 12$) on the same cell at 75-s intervals resulted in sequential rises in $[Ca^{2+}]_c$, with a slight attenuation of the amplitude of the Ca^{2+} transient (Fig. 3A). Similarly, prolonged exposure of frog melanotrope cells to 3APS (10^{-5} M, 4 min; $n = 6$) induced a sustained increase in $[Ca^{2+}]_c$ with gradual decline of the response (Fig. 3B).

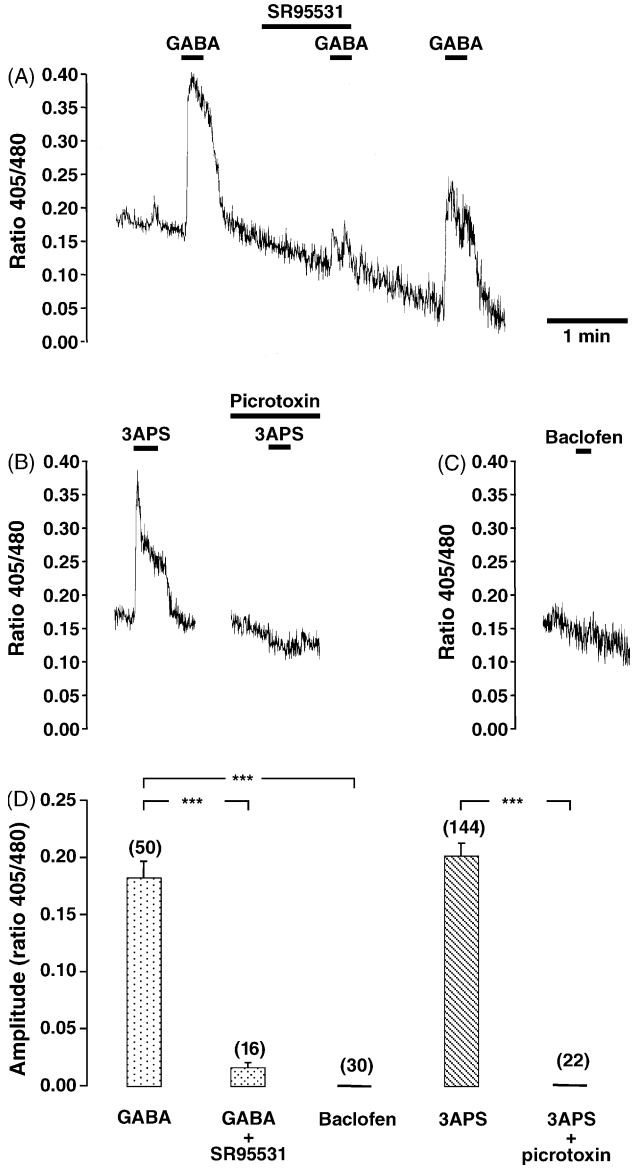

Fig. 2. Effects of GABA agonists and antagonist on $[Ca^{2+}]_c$ in cultured frog melanotrope cells. (A) Typical profile showing the effect of the $GABA_A$ receptor antagonist SR95531 (10^{-5} M, 65 s) on the GABA (10^{-5} M, 15 s)-induced $[Ca^{2+}]_c$ response. (B) Typical profile showing the effect of the $GABA_A$ receptor agonist 3APS (10^{-5} M, 15 s) on $[Ca^{2+}]_c$ in the absence or presence of the chloride channel blocker picrotoxin (10^{-4} M). (C) Typical profile showing the effect of the $GABA_B$ receptor agonist baclofen (10^{-5} M, 15 s) on $[Ca^{2+}]_c$. (D) Quantification of the effects of the GABA agonists and antagonist on $[Ca^{2+}]_c$. Each value represents the mean amplitude of the response (±S.E.M.) calculated from the number of recordings indicated in parentheses. $^{***}P < 0.001$ (one-way ANOVA followed by a Student–Newman–Keuls test).

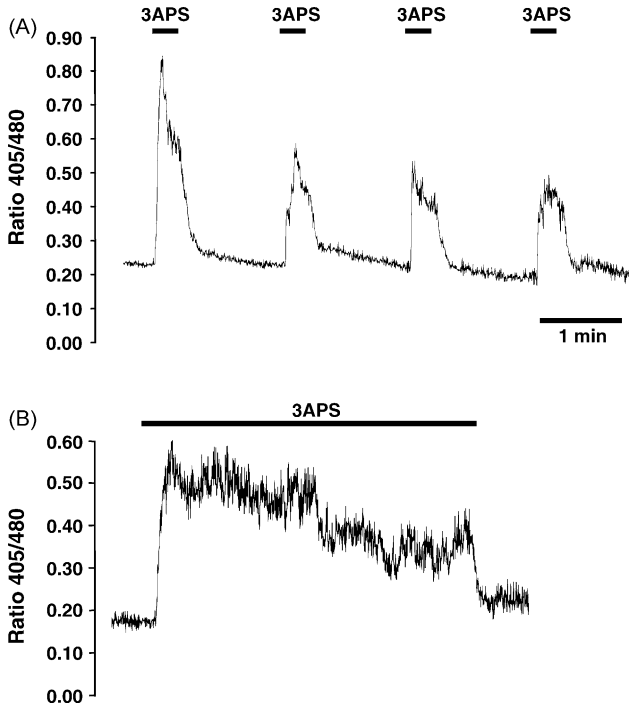

Fig. 3. Effect of repeated and prolonged administrations of 3APS on $[Ca^{2+}]_c$ in cultured frog melanotrope cells. (A) Typical profile showing the effect of four equimolar applications of 3APS (10^{-5} M each, 15 s) at 75-s intervals on $[Ca^{2+}]_c$. (B) Typical profile illustrating the effect of a prolonged infusion of 3APS (10^{-5} M, 4 min) on $[Ca^{2+}]_c$.

3.3. Source of Ca^{2+} involved in $GABA_A$ receptor-induced $[Ca^{2+}]_c$ increase

Incubation of melanotrope cells in Ca^{2+}-free Krebs Ringer's solution supplemented with 6 mM EGTA totally suppressed the stimulatory effect of 3APS (10^{-5} M, 15 s; $n = 16$) on $[Ca^{2+}]_c$ (Fig. 4A). After a 1.5-min washout, the response to 3APS was fully recovered (Fig. 4A).

Incubation of cultured melanotrope cells with the L-type Ca^{2+} channel blocker nifedipine (10^{-4} M, 30 min; $n = 37$) totally suppressed ($P < 0.001$) 3APS-induced $[Ca^{2+}]_c$ increase, while incubation with the N-type Ca^{2+} channel blocker ω-CgTx GVIA (10^{-6} M, 30 min; $n = 13$) significantly reduced ($P < 0.001$) the $[Ca^{2+}]_c$ response of melanotrope cells to 3APS (Fig. 4B). Concurrently, the Na^+ channel blocker TTX (10^{-6} M and 10^{-5} M, 10 min; $n = 27$ and 14, respectively) inhibited by 56% ($P < 0.001$) and 75% ($P < 0.001$) the $[Ca^{2+}]_c$ response to 3APS (Fig. 4B). None of these blockers had any effect on basal $[Ca^{2+}]_c$ (data not shown).

3.4. Chloride-dependence of 3APS-induced $[Ca^{2+}]_c$ increase and α-MSH secretion

Melanotrope cells perifused with Krebs Ringer's solution containing various chloride concentrations ($[Cl^-]_e$; from 42 to 671 mM) were exposed to 3APS (10^{-5} M, 15 s). Lowering $[Cl^-]_e$ from normal conditions (118 mM) did not significantly modify the amplitude of 3APS-induced $[Ca^{2+}]_c$ transient (Fig. 5A). However, elevating the $[Cl^-]_e$ to 214 and 671 mM provoked a concentration-dependent inhibition of the $[Ca^{2+}]_c$ response. The relationship between $[Cl^-]_e$ and theorical chloride reversal potentials can be determined from the Nernst equation $[ECl^- = RT/ZF \times \ln([Cl^-]_e/[Cl^-]_i)]$ assuming a resting $[Cl^-]_i$ equivalent to 26.5 mM (Fig. 5B). The dotted line represents the mean resting membrane potential (RMP) in melanotrope cells under physiological $[Cl^-]_i$ conditions [28]. An increase in $[Ca^{2+}]_c$ was obtained with 3APS when the ECl^- was more positive than the RMP. In contrast, 3APS failed to evoke calcium mobilization when ECl^- was equal to or more negative than the RMP. Perifusion of melanotrope cells with high (50 mM) KCl-containing medium provoked, as expected, a massive increase in $[Ca^{2+}]_c$. In these conditions, 3APS (10^{-5} M, 15 s) caused an inhibition of high KCl-induced $[Ca^{2+}]_c$ (Fig. 5C). Similarly, in cells exhibiting a high $[Ca^{2+}]_c$ in basal conditions, 3APS (10^{-5} M, 15 s) induced a decrease in $[Ca^{2+}]_c$ ($n = 3$; Fig. 5D).

In normal conditions, ($[Cl^-]_e = 118$ mM), 3APS induced a biphasic effect on α-MSH release from perifused melanotrope cells, i.e. a transient stimulation followed by a slight inhibition of hormone secretion (Fig. 6A). Reduction of

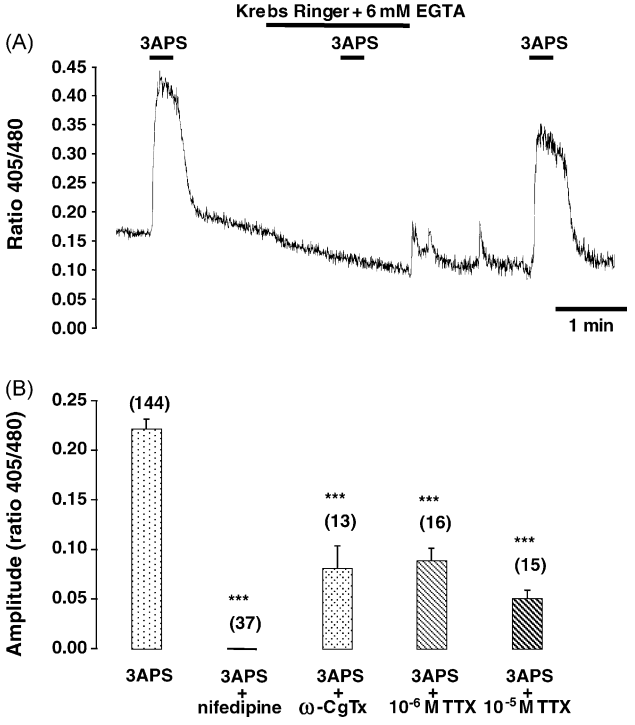

Fig. 4. Effect of EGTA, and Ca^{2+} and Na^+ channel blockers on 3APS-induced $[Ca^{2+}]_c$ increase in cultured frog melanotrope cells. (A) Typical profile illustrating the effect of 3APS (10^{-5} M, 15 s) on $[Ca^{2+}]_c$ in normal Krebs Ringer's solution and in calcium-free medium supplemented with 6 mM EGTA. (B) Quantification of the effects of the L-type Ca^{2+} channel blocker nifedipine (10^{-4} M), the N-type Ca^{2+} channel blocker ω-conotoxin GVIA (ω-GgTx, 10^{-6} M) and the Na^+ channel blocker tetrodotoxin (TTX, 10^{-6} M or 10^{-5} M) on 3APS (10^{-5} M, 15 s)-induced $[Ca^{2+}]_c$ increase. Each value represents the mean amplitude of the response (±S.E.M.) calculated from the number of recordings indicated in parentheses. $^{***}P < 0.001$ vs. 3APS alone (one-way ANOVA followed by a Student–Newman–Keuls test).

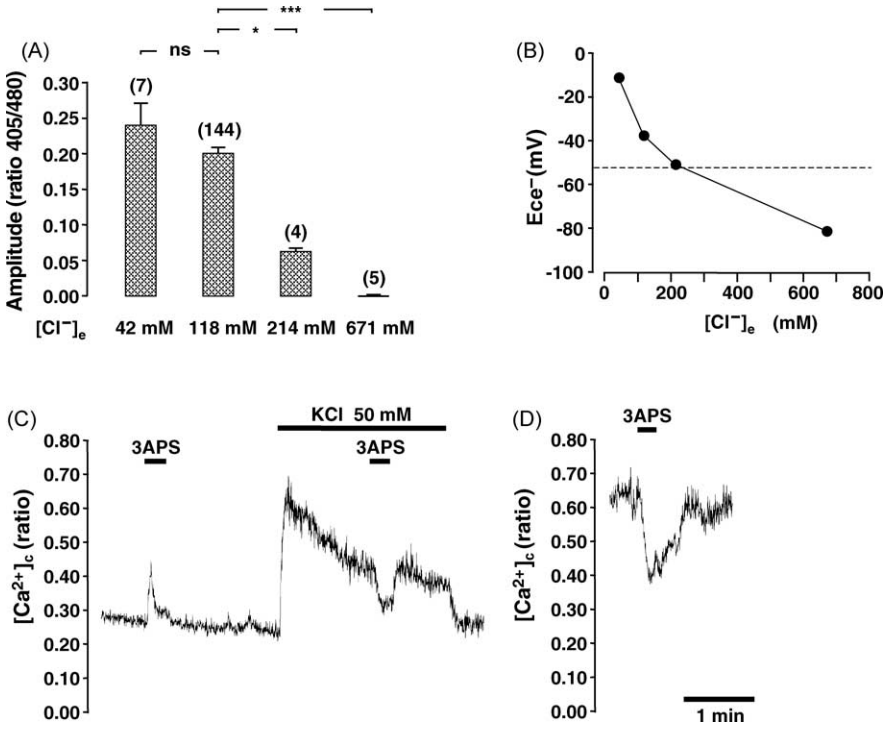

Fig. 5. Effect of extracellular chloride concentrations ($[Cl^-]_e$) and the depolarized state of the melanotrope cell on 3APS-induced $[Ca^{2+}]_c$ increase. (A) Effect of graded concentration of $[Cl^-]_e$ (42 to 671 mM) on 3APS (10^{-5} M, 15 s)-induced $[Ca^{2+}]_c$ increase. Each point represents the mean amplitude of the response (±S.E.M.) calculated from the number of recordings indicated between parentheses. (B) Relationship between $[Cl^-]_e$ in Krebs Ringer's solution and theorical Cl^- reversal potentials calculated from the Nernst equation. The dotted line represents the mean resting membrane potential (RMP) under physiological $[Cl^-]_i$ in melanotrope cells. (C) Typical profile showing the effect of 3APS (10^{-5} M, 15 s) on $[Ca^{2+}]_c$ in normal Krebs Ringer's solution and in a high (50 mM) KCl-containing medium. (D) Typical profile showing the effect of 3APS (10^{-5} M, 15 s) in a cell exhibiting a high basal $[Ca^{2+}]_c$. ns, not statistically significant, $^{*}P < 0.05$, $^{***}P < 0.001$ (one-way ANOVA followed by a Student–Newman–Keuls test).

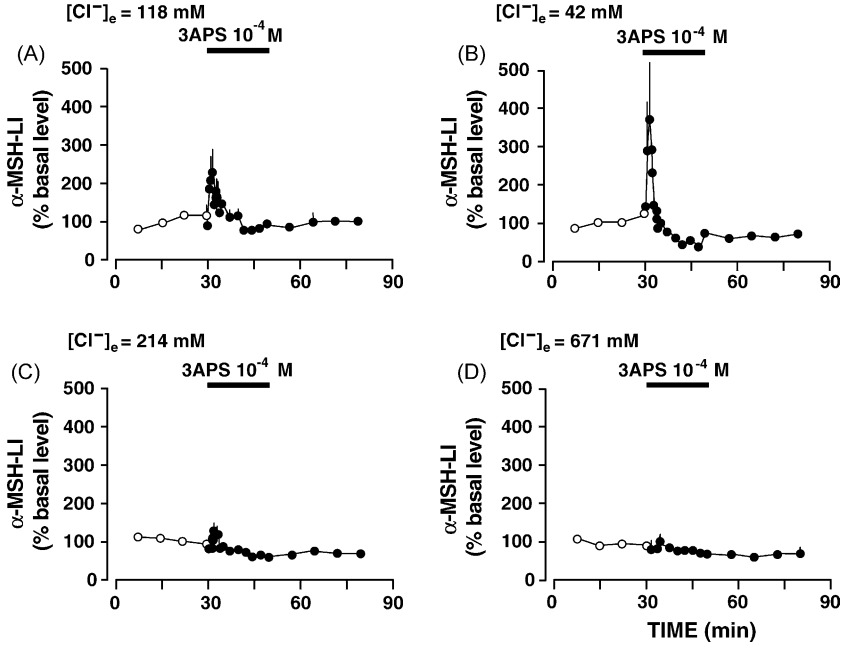

Fig. 6. Effect of graded concentration of $[Cl^-]_e$ (42 to 671 mM) on 3APS-induced release of α-MSH secretion from perifused neurointermediate lobes. The profiles represent the mean secretion pattern (±S.E.M.) of four independent experiments. For each experiment, the spontaneous level of α-MSH release (100% basal level) was calculated as the mean hormone secretion in the four consecutive fractions collected before the onset of 3APS (◯—◯). The mean secretion rate of α-MSH, in basal condition, was 30.02 ± 3.72 pg/min per NIL.

$[Cl^-]_e$ to 42 mM markedly enhanced the stimulatory effect of 3APS on α-MSH secretion (Fig. 6B) whereas augmentation of $[Cl^-]_e$ to 214 and 671 mM decrease in a concentration-dependent manner the secretory response induced by 3APS (Fig. 6C and D).

4. Discussion

In pituitary melanotrope and lactotrope cells, GABA induces a dual response consisting of a rapid and transient stimulation followed by a more sustained inhibition of the secretory activity [3,4,7,16,49]. In frog melanotrope cells, both phases are essentially mediated through activation of $GABA_A$ receptors [3,16]. Using an electrophysiological gramicidin-perforated patch-clamp approach, to maintain the physiological $[Cl^-]_i$, we have previously found that, in this cell model, GABA provokes a depolarization underlined by a chloride efflux [29,30]. Here, we show that the increase in $[Ca^{2+}]_c$ and the biphasic secretory response induced by $GABA_A$ receptor activation are entirely dependent on the Cl^- flux direction and driving force.

In frog melanotrope cells, GABA caused a robust and sustained increase in $[Ca^{2+}]_c$. This effect was mimicked by the specific $GABA_A$ receptor agonist 3APS but not by the $GABA_B$ receptor agonist baclofen. Moreover, the GABA-induced $[Ca^{2+}]_c$ rise was markedly inhibited by the $GABA_A$ receptor antagonist SR95531 and totally suppressed by the chloride channel blocker picrotoxin. These findings demonstrate that the $GABA_A$-gated Cl^- channel receptor mediates the stimulatory effect of GABA on $[Ca^{2+}]_c$ in frog melanotrope cells. A similar $[Ca^{2+}]_c$ increase through activation of $GABA_A$ receptors has been reported in newborn rat pituitary cells [1,21] and lactotrope cells [31], in toad melanotrope cells [44], as well as in immature rat neurons and *Xenopus* spinal neurons on which GABA acts as an excitatory neurotransmitter rather than an inhibitory factor [10,37,38,42].

Exposure of frog melanotrope cells to increasing concentrations of GABA provoked a gradual rise in $[Ca^{2+}]_c$ although, for high doses of GABA, the calcium response decayed suggesting the existence of a desensitization/inactivation process. The dose-response relationship revealed that the concentration of GABA inducing half-maximum increase in $[Ca^{2+}]_c$ was in the same range as that required to obtain half-maximum stimulation of GABA-evoked chloride current [28] and α-MSH release [3], confirming that the $[Ca^{2+}]_c$ elevation is involved in the stimulatory effect of GABA in frog melanotrope cells.

In the absence of extracellular calcium, 3APS failed to increase $[Ca^{2+}]_c$ indicating that activation of $GABA_A$ receptors provokes the opening of voltage-activated calcium channels (VACCs). Mammalian and amphibian melanotrope cells express several combinations of low and/or high threshold VACCs, depending on the cell preparations and species [5,11,33,40,41]. In particular, frog melanotrope cells express at least two types of high threshold VACCs, i.e. a rapidly inactivating current that is blocked by ω-CgTx GVIA (N-current) and a sustained current that is sensitive to nifedipine (L-current) [5,33]. We found that the calcium influx generated by 3APS was totally abolished by nifedipine and partially inhibited by ω-CgTx GVIA and the sodium channel blocker TTX. These data indicate that the $GABA_A$ receptor-evoked $[Ca^{2+}]_c$ increase can be primarily ascribed to activation of L-type VACCs and, to a lesser extent, to N-type VACCs and Na^+ channels.

Studies conducted on *Xenopus* larvae and fetal rat spinal neurons [25,38], newborn mouse hypothalamic neurons [19] and frog melanotrope cells [29] have shown that GABA can induce either hyperpolarization or depolarization depending on both the $[Cl^-]_i$ and the resting membrane potential (RMP). If the RMP is less negative than the ECl^-, the resulting Cl^- efflux causes depolarization. In frog melanotrope cells, the experimental value of ECl^- has been shown to be approximately −37.5 mV, indicating a resting $[Cl^-]_i$ of 26.5 mM which leads to a depolarizing effect of GABA [29]. The Ca^{2+} response evoked by a prolonged application of the $GABA_A$ receptor agonist or a high KCl concentration slightly decayed, suggesting the participation of an inactivation of VACCs in the desensitization process of the 3APS-induced $[Ca^{2+}]_c$ rise.

In melanotrope cells, action potential-driven calcium entry is thought to be directly responsible for basal and stimulated hormone secretion [7,16,33,43]. By varying the $[Cl^-]_e$, we showed that, in frog melanotrope cells, the 3APS-induced $[Ca^{2+}]_c$ rise depends on the Cl^- gradient. When the ECl^- (calculated from the Nernst equation) is less negative than the RMP, activation of the $GABA_A$ receptor induces a Cl^- influx and the resulting hyperpolarization is responsible for the absence of calcium response. Conversely, when the ECl^- is more positive than the RMP, the amplitude of 3APS-induced $[Ca^{2+}]_c$ increase is directly related to the intensity of the Cl^- driving force. Similarly, the stimulatory and inhibitory effects of the $GABA_A$ receptor agonist 3APS on α-MSH release were also dependent on the Cl^- driving force. This Cl^- efflux should only occur via $GABA_A$ receptor channels since the amplitude of the depolarization and the drop of the membrane resistance induced by GABA were not affected by administration of Na^+, K^+ and Ca^{2+}-voltage-dependent channel blockers, thus excluding, for instance, the possible contribution of a Ca^{2+}-activated Cl^- conductance in the GABA-induced Cl^- efflux [29]. Altogether, these observations indicate that the early transient stimulation of α-MSH release can be accounted for by GABA-induced outward chloride current, membrane depolarization and activation of sodium and calcium channels, leading to exocytosis. Subsequently, prolonged activation of $GABA_A$ receptors provokes the opening of a large number of chloride channels, the shunt of voltage-dependent conductances and the arrest of the firing activity, causing inhibition of α-MSH release.

The pituitary hormone α-MSH, that provokes pigment dispersion in dermal melanophores, plays a crucial role in the physiological process of skin color adaptation (also called homochromy) in various vertebrate species, notably in

amphibians [24]. Previous studies have shown that, in frog, dopamine and neuropeptide Y exert a sustained inhibitory effect on α-MSH release [12,15] and thus are probably involved in long-lasting white background adaptation [22]. In contrast, GABA, that has a dual effect on α-MSH release, may serve during short-term adaptation. Indeed, low GABA levels would mainly stimulate α-MSH secretion in dark background-adapted animals, while high GABA levels may be required to inhibit α-MSH secretion during the initial steps of adaptation to white background.

In conclusion, the present study has demonstrated that, in frog melanotrope cells, activation of $GABA_A$ receptors causes a chloride efflux that is responsible for membrane depolarization and calcium channel activation. Our data also indicate that the $[Ca^{2+}]_c$ increase and the biphasic effects on α-MSH release induced by GABA both depend on the chloride driving force.

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