ELSEVIER

Biochemical Pharmacology 62 (2001) 1229–1238

Biochemical Pharmacology

Inhibition of depolarization-induced [^{3}H]noradrenaline release from SH-SY5Y human neuroblastoma cells by some second-generation H_1 receptor antagonists through blockade of store-operated Ca^{2+} channels (SOCs)

Maurizio Taglialatela[a,*], Agnese Secondo[a], Angela Fresi[a], Barbara Rosati[b], Anna Pannaccione[a], Pasqualina Castaldo[a], Giovanna Giorgio[a], Enzo Wanke[b], Lucio Annunziato[a]

[a]*Unit of Pharmacology, Department of Neuroscience, University of Naples Federico II, School of Medicine, Via S. Pansini 5, I-80131 Naples, Italy*
[b]*Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy*

Received 27 December 2000; accepted 4 June 2001

Abstract

In the present study, the effect of the blockade of membrane calcium channels activated by intracellular Ca^{2+} store depletion on basal and depolarization-induced [^{3}H]norepinephrine ([^{3}H]NE) release from SH-SY5Y human neuroblastoma cells was examined. The second-generation H_1 receptor blockers astemizole, terfenadine, and loratadine, as well as the first-generation compound hydroxyzine, inhibited [^{3}H]NE release induced by high extracellular K^+ concentration ($[K^+]_e$) depolarization in a concentration-dependent manner (the IC_{50}s were 2.3, 1.7, 4.8, and 9.4 μM, respectively). In contrast, the more hydrophilic second-generation H_1 receptor blocker cetirizine was completely ineffective (0.1–30 μM). The inhibition of high $[K^+]_e$-induced [^{3}H]NE release by H_1 receptor blockers seems to be related to their ability to inhibit Ca^{2+} channels activated by Ca_i^{2+} store depletion (SOCs). In fact, astemizole, terfenadine, loratadine, and hydroxyzine, but not cetirizine, displayed a dose-dependent inhibitory action on the increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) obtained with extracellular Ca^{2+} reintroduction after Ca_i^{2+} store depletion with thapsigargin (1 μM), an inhibitor of the sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) pump. The rank order of potency for SOC inhibition by these compounds closely correlated with their inhibitory properties on depolarization-induced [^{3}H]NE release from SH-SY5Y human neuroblastoma cells. Nimodipine (1 μM) plus ω-conotoxin (100 nM) did not interfere with the present model for SOC activation. In addition, the inhibition of depolarization-induced [^{3}H]NE release does not seem to be attributable to the blockade of the K^+ currents carried by the K^+ channels encoded by the human Ether-a-Gogo Related Gene (I_{HERG}) by these antihistamines. In fact, whole-cell voltage-clamp experiments revealed that the IC_{50} for astemizole-induced hERG blockade is about 300-fold lower than that for the inhibition of high K^+-induced [^{3}H]NE release. Furthermore, current-clamp experiments in SH-SY5Y cells showed that concentrations of astemizole (3 μM) which were effective in preventing depolarization-induced [^{3}H]NE release were unable to interfere with the cell membrane potential under depolarizing conditions (100 mM $[K^+]_e$), suggesting that hERG K^+ channels do not contribute to membrane potential control during exposure to elevated $[K^+]_e$. Collectively, the results of the present study suggest that, in SH-SY5Y human neuroblastoma cells, the inhibition of SOCs by some second-generation antihistamines can prevent depolarization-induced neurotransmitter release.

Keywords: Second-generation antihistamines; Depolarization-induced norepinephrine release; SH-SY5Y human neuroblastoma cells; Ca^{2+} channels activated by Ca_i^{2+} store depletion; hERG K^+ channels; Long QT syndrome

1. Introduction

Elevation of intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) is crucial for exocytosis of neurotransmitters and hormones from neurons and endocrine cells [1]. In these excitable cells, the depolarization-induced opening of voltage-gated Ca^{2+} channels provides a major pathway for rapid Ca^{2+} influx [2]. Additional mechanisms controlling the changes

* Corresponding author. Tel.: +39-081-746-3318; fax: +39-081-746-3323.
E-mail address: mtaglial@unina.it (M. Taglialatela).

Abbreviations: hERG, human Ether-a-go-go Related Gene; SOC, Ca^{2+} currents activated by Ca_i^{2+} store depletion; NE, norepinephrine; $[K^+]_e$, extracellular K^+ concentration; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; HBS, HEPES-buffered saline; SERCA, sarcoplasmic-endoplasmic reticulum calcium ATPase; and TTX, tetrodotoxin.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00754-7

in $[Ca^{2+}]_i$ are also represented by Ca^{2+} entry though ionotropic receptors by the activation of membrane receptors linked to phospholipase C (PLC), leading to inositol(1,4,5)trisphosphate (IP_3)-dependent depletion of intracellular Ca^{2+} stores [3], and by Ca^{2+} release from ryanodine-sensitive stores [4]. In addition, it has been shown that, in SH-SY5Y human neuroblastoma cells, membrane depolarization in the absence of receptor stimulation may lead to PLC activation, IP_3 formation, and, consequently, to intracellular Ca^{2+} store depletion [5]. Emptying of intracellular Ca^{2+} stores activates a number of voltage-independent and Ca^{2+}-permeable currents denoted Ca^{2+} release-activated Ca^{2+} currents or Ca^{2+} store-operated currents (SOCs) [6]. Although the role of SOCs in secretion from non-excitable cells is firmly established, only recently has attention been given to the role played by the $[Ca^{2+}]_i$ increase that follows SOC activation in the process of neurotrasmitter release. In fact, Fomina and Nowicky [7] and Taylor and Peers [8] have shown that Ca^{2+} influx activated by Ca^{2+} store depletion may both directly trigger exocytosis and modulate excitation–secretion coupling in adrenal chromaffin and PC12 cells, respectively. Furthermore, in LAN-1 human neuroblastoma cells, $[Ca^{2+}]_i$ increase following muscarinic receptor stimulation appears to be largely due to voltage-insensitive, Gd^{3+}-blockable channels resembling SOCs [9].

Although the lack of specific pharmacological blockers has been considered a major obstacle in the clarification of the functional role played by Ca^{2+} release-activated Ca^{2+} channels, the recent availability of compounds possessing putative inhibitory action on SOCs [10,11] has allowed, in the present study, the investigation of the possible contribution of SOCs in the process of depolarization-induced noradrenaline (NE) release from SH-SY5Y human neuroblastoma cells. To this end, the inhibition exerted by several compounds belonging to the second-generation H_1 receptor blockers was evaluated on $[Ca^{2+}]_i$ by single-cell microfluorimetry in a model of SOC activation obtained with Ca_e^{2+} reintroduction after Ca^{2+} store depletion with the sarcoplasmic–endoplasmic reticulum calcium ATPase (SERCA) pump inhibitor thapsigargin. The rank order of potency for such pharmacological action by these compounds was compared to that exerted by the same molecules on high K^+-induced previously taken-up [^{3}H]NE release from the same cells.

The results obtained showed that a close relationship existed between the inhibition exerted by second-generation antihistamines on SOC activity and their inhibitory action on depolarization-induced [^{3}H]NE release. In addition, patch-clamp experiments in current- and voltage-clamp modes showed that the inhibition by second-generation antihistamines of the K^+ channels encoded by the human Ether-a-go-go Related Gene (hERG) expressed in SH-SY5Y cells did not modify the cell membrane potential in 100 mM K_e^+ and, therefore, that the inhibition of [^{3}H]NE release elicited by elevated $[K^+]_e$ could not be ascribed to their hERG-inhibitory properties.

2. Materials and methods

2.1. Cell culture

Human neuroblastoma SH-SY5Y cells (kindly provided from Dr. P.F.T. Vaughan, Dept. of Cardiovascular Studies, University of Leeds, Leeds, UK; passages 20–45) were cultured in Dulbecco's modified Eagle's medium (DMEM), containing d-glucose (4.5 g/L) and 5% fetal bovine serum (FBS), and incubated at 37° in a humidified atmosphere with 10% CO_2 in 100-mm plastic Petri dishes. In some experiments, the cells were differentiated by means of a 7–14 day treatment with retinoic acid (10 μM) [12]. For electrophysiological and microfluorimetry experiments, SH-SY5Y cells were seeded on glass coverslips (Fisher Scientific) coated with poly-L-lysine (30 μg/mL). All experiments were performed at room temperature (22–23°) 1–4 days after seeding.

2.2. [^{3}H]NE release from SH-SY5Y human neuroblastoma cells

The measurement of [^{3}H]NE release from SH-SY5Y human neuroblastoma cells follows a previously published protocol [13]. Briefly, the cells were grown in 24-well plastic plates until they reached confluence (≈250,000 cells/well). On the day of the experiment, the culture medium was removed and replaced with 0.5 mL of HEPES-buffered saline (HBS; 135 mM NaCl, 5 mM KCl, 0.6 mM $MgSO_4$, 2.5 mM $CaCl_2$, 10 mM HEPES, 6 mM d-glucose, 0.2 mM ascorbic acid, and 0.2 mM pargyline), containing 40 nM [^{3}H]NE (specific activity: 12Ci/mmol; Amersham). After 1 hr of incubation at 37°, the [^{3}H]NE-containing medium was removed and the cells were washed four–six times (5 min each wash) with 0.5-mL aliquots of HBS. After this washing period, the release of [^{3}H]NE was followed by exposing the cells for 5 min at 37° to 0.5 mL of HBS or to HBS plus the appropriate concentration of the drug under investigation, depending on the specific experimental protocol. After this period, the supernatant was collected in scintillation vials to assess the basal release and the cells were exposed to HBS containing elevated $[K^+]_e$ (30–140 mM), in the absence or presence of the appropriate concentration of the drug under investigation, depending on the specific experimental protocol. In order to keep the medium osmolarity constant, when the $[K^+]_e$ was increased, the extracellular Na^+ concentration was accordingly decreased. At the end of this period, the supernatant was collected to assess the depolarization-induced [^{3}H]NE release, and the cells were incubated overnight at −20° in 0.5 mL of H_2O. On the following day, the plates were thawed and the supernatant containing the intracellular content of

the lysed cells was centrifuged at 16,000 g for 7 min to pellet the membrane fraction; after centrifugation, an aliquot (typically 0.1 mL) was determined in a liquid scintillation counter (Beckman LS5000CE) to evaluate the intracellular content of [^{3}H]NE. The amount of [^{3}H]NE released into the medium was expressed as a percentage of the total amount of radioactivity in the cell monolayer at the beginning of the incubation period.

2.3. Single-cell videoimaging for $[Ca^{2+}]_i$ measurement

$[Ca^{2+}]_i$ was measured using a microfluorimetric technique, as previously reported [14]. Briefly, the cells grown on glass coverslips were loaded with 5 μM 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2^1-amino-5^1-methylphenoxy)-ethane-*N*,*N*,*N*^{1},*N*^{1}-tetraacetic acid pentaacetoxymethyl ester (FURA-2 AM) in Krebs–Ringer saline solution (5.5 mM KCl, 160 mM NaCl, 1.2 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM d-glucose, and 10 mM HEPES-NaOH, pH 7.4) for 1 hr at room temperature. At the end of the FURA-2AM loading period, the coverslip was introduced into a microscope chamber (Medical System Co.) on an inverted Nikon Diaphot fluorescence microscope. The cells were kept in Krebs–Ringer saline solution throughout the experiment. All the drugs tested were introduced into the microscope chamber by single-bolus fast injection. A 100-watt Xenon Lamp (Osram) with a computer-operated filter wheel bearing two different interference filters (340 and 380 nm) illuminated the microscopic field with UV light, alternating the wavelengths at an interval of 500 msec. The interval between each pair of illuminations was 3 sec, and the interval between filter movements was 1 sec. Emitted light was passed through a 400-nm dichroic mirror, filtered at 510 nm, and collected by a CCD camera (Photonic Science) connected to a light amplifier (Applied Imaging Ltd.). Images were digitized and analyzed with a Magiscan image processor (Applied Imaging Ltd.). At the end of each experimental session, calibration was performed according to the procedure described by Grynkievicz *et al.* [15]. In particular, the cells were lysed with ionomycin (2–10 μM) in the presence of 1.5 mM extracellular Ca^{2+}. Addition of ionomycin produced a rapid increase in fluorescence intensity that allowed us to calculate the R_{max} value. To determine the R_{min} value, the cells were subsequently exposed to a Ca^{2+}-free solution containing 1–20 mM EGTA. Given that K_d for Ca^{2+} of FURA-2 is 224 nM at 37°, the R_{min}, R_{max} values were introduced into the Grynkievicz formula to convert the values of fluorescence ratio between 340 and 380 nm into $[Ca^{2+}]_i$. The values were subtracted for background fluorescence obtained from images taken from a region of the coverslip devoid of cells. No interference was detected between any of the compounds utilized in the present study and the excitation or the emission spectra of FURA-2.

2.4. Patch-clamp electrophysiology

Membrane potential of SH-SY5Y cells was measured in current-clamp mode by means of a commercially available amplifier (Axopatch 200A, Axon Instruments) with the perforated-patch technique using amphotericin B (150 μg/mL) to gain access to the cell cytoplasm. The pipette solution contained (in mM): 10 NaCl, 140 K-aspartate, 2 $MgCl_2$, 10 HEPES, pH 7.4 with NaOH. The extracellular solution contained (in mM): 150 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, pH 7.4 with NaOH. In the experiments shown in Fig. 6, when the extracellular K^+ concentration ($[K^+]_e$) was increased to 100 mM, the extracellular Na^+ concentration was accordingly decreased. Membrane currents were recorded in voltage-clamp mode using the conventional whole-cell technique, using glass micropipettes of 3–7 MΩ resistance. No compensation was performed for pipette resistance and cell capacitance. The relatively small density of hERG K^+ channels in SH-SY5Y human neuroblastoma cells required the use of a high (100 mM) external K^+ concentration as a charge carrier. Therefore, the cells were perfused with an extracellular solution containing (in mM): 100 KCl, 10 EGTA, and 10 HEPES, pH 7.3 with KOH, and the pipettes were filled with (in mM): 110 CsCl, 10 tetraethylammonium-Cl, 2 $MgCl_2$, 10 EGTA, 8 glucose, 2 Mg-ATP, 0.25 cyclic adenosine monophosphate (cAMP) and 10 HEPES, pH 7.3. For Ca^{2+} current recordings, the cells were perfused with an extracellular solution containing (in mM): 10 $BaCl_2$, 125 NaCl, 1 $MgCl_2$, 10 HEPES, 300 nM Tetrodotoxin, pH 7.3. The pipettes were filled with (in mM): 110 CsCl, 10 tetraethylammonium-Cl, 2 $MgCl_2$, 10 EGTA, 8 glucose, 2 Mg-ATP, 0.25 cAMP, and 10 HEPES, pH 7.3. All the electrophysiological experiments were performed at room temperature (20–22°). No leak subtraction procedure was applied in analyzing the data.

2.5. Drugs and statistics

In order to obtain IC_{50} values for the effects of H_1 receptor antagonists on both depolarization-induced [^{3}H]NE release and SOC activity, the experimental data shown in Figs. 1 and 3, respectively, were fitted to the following binding isotherm: $Y = max/(1 + X/IC_{50})$, where X is the drug concentration.

All the reagents were purchased from Sigma Chemicals. Astemizole was provided by Janssen-Cilag; loratadine was obtained from Schering-Plough; cetirizine was generously donated by UCB Pharma. Nimodipine was from Calbiochem. When necessary, drugs were dissolved in DMSO at concentrations between 5 and 50 mM, and stock solutions were kept at −20°. Appropriate drug dilutions were prepared daily. Statistical significance between the data was obtained by means of the Student's *t*-test or by the ANOVA test followed by the Tukey test. When appropriate, the data are expressed as the means ± SEM. In microfluorimetry studies, the experiments were repeated at least 5 times on

separate coverslips. The $[Ca^{2+}]_i$ of 15–20 cells was measured in each coverslip. The mean of $[Ca^{2+}]_i$ given in the text refers to the average of the $[Ca^{2+}]_i$ measured in all the cells recorded in each experimental session.

3. Results

3.1. Effect of astemizole, terfenadine, loratadine, cetirizine, and hydroxyzine on 5 and 100 mM $[K^+]_e$-induced [^{3}H]NE release from SH-SY5Y human neuroblastoma cells

Exposure of undifferentiated SH-SY5Y human neuroblastoma cells to increasing $[K^+]_e$ caused a concentration-dependent increase of [^{3}H]NE release over the 5-min sampling period (Fig. 1A). This increase was statistically significant with 50 mM $[K^+]_e$, and reached a plateau at 75–140 mM $[K^+]_e$. Exposure of the cells to 1 mM of the Ca^{2+} chelator EGTA in the absence of extracellular Ca^{2+} did not affect basal (5 mM $[K^+]_e$) neurotrasmitter release, whereas it did prevent the 100-mM $[K^+]_e$-induced [^{3}H]NE release, thus suggesting that depolarization-induced [^{3}H]NE release in SH-SY5Y neuroblastoma cells was entirely dependent on extracellular Ca^{2+} entrance [13].

Fig. 1 (panels B–E) shows the effect of five H_1 receptor antagonists on [^{3}H]NE release from human neuroblastoma cells induced by exposure to 100 mM $[K^+]_e$. Astemizole (Fig. 1B) and terfenadine (Fig. 1C) caused a dose-dependent inhibition of [^{3}H]NE release, which was statistically significant at the concentration of 1 μM and was maximal at 10 μM (the IC_{50}s were 2.3 and 1.7 μM, respectively). On the other hand, although loratadine (Fig. 1D) also displayed inhibitory properties on [^{3}H]NE release induced by exposure to 100 mM $[K^+]_e$, its potency was slightly lower (IC_{50} of 4.8 μM) than that of astemizole or terfenadine. By contrast, the hydrophilic second-generation congener cetirizine (Fig. 1E) was completely devoid of any significant inhibitory action on [^{3}H]NE release induced by exposure to high $[K^+]_e$ at all the concentrations tested (0.1–30 μM). Interestingly, the more hydrophobic metabolic precursor of cetirizine, hydroxyzine (Fig. 1F), caused a dose-dependent inhibition of high $[K^+]_e$-evoked [^{3}H]NE release in concentrations ranging from 1 up to 30 μM. All of these drugs were unable to interfere with [^{3}H]NE release occurring in physiological (5 mM) $[K^+]_e$ (data not shown).

Since differentiation of neuroblastoma cells is known to affect the pattern of ion channel expression [16,17], we also investigated whether the treatment of SH-SY5Y cells with 10 μM retinoic acid (RA) for 7–14 days might modify the pharmacological modulation of [^{3}H]NE release by the H_1 receptor blockers. However, similar to the effects found in undifferentiated cells, 3 and 10 μM astemizole inhibited the depolarization-induced [^{3}H]NE release from differentiated cells with similar potency as in undifferentiated cells; the percent of [^{3}H]NE released during the 5-min high $[K^+]_e$

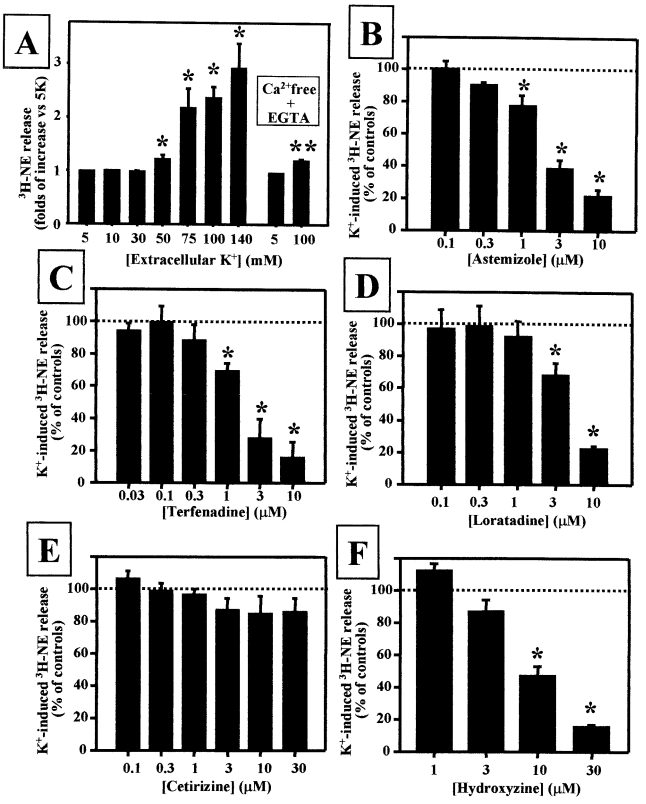

Fig. 1. Effect of increasing $[K_e^+]$ (panel A) and of the H_1 receptor antagonists astemizole (panel B), terfenadine (panel C), loratadine (panel D), cetirizine (panel E), and hydroxyzine (panel F) on [^{3}H]NE release from SH-SY5Y human neuroblastoma cells. Each value is the mean ± SEM of at least 3 determinations performed in triplicate for each experimental condition. In the experiments shown in the two columns on the right of panel A, the HBS was lacking Ca^{2+} ions, plus the addition of 1 mM of the Ca^{2+}-chelator EGTA. For panels B–F, the effect of different drug concentrations on [^{3}H]NE release, measured as described in the Methods section, was calculated as follows. The difference between the release occurring under stimulated (100 mM $[K_e^+]$) and in basal (5 mM $[K_e^+]$) conditions was calculated for control (no drug) cells and for each group exposed to a specific drug concentration. Then, this difference for the release occurring in each drug-exposed group was expressed as % of the control value. No statistically significant difference was found among the groups exposed to 5 mM $[K_e^+]$. Each value is the mean ± SEM of at least 3 determinations performed in triplicate. Asterisks denote values statistically different from the controls ($P < 0.05$). In panel A, the absolute value of [^{3}H]NE release occurring in 5 mM K_e^+ used as a reference value to normalize [^{3}H]NE release occurring in higher $[K_e^+]$, was 3.18 ± 0.26% of total cell radioactivity. In panels B–F, the [^{3}H]NE release absolute values were 5.21 ± 0.71% (5 mM K_e^+) and 10.07 ± 0.49% (100 mM K_e^+) of total cell radioactivity.

stimulation was 6.9 ± 0.57 in controls (N = 12), 5.35 ± 0.51 with 3 μM astemizole (N = 12; $P < 0.05$ vs controls), and 4.21 ± 0.20 in 10 μM astemizole N = 12; ($P < 0.05$ vs controls). Furthermore, also in RA-differentiated cells, cetirizine (1–10 μM) was unable to modify high $[K^+]_e$-induced [^{3}H]NE release; the percent of high $[K^+]_e$-induced [^{3}H]NE release was 8.20 ± 0.78, 8.17 ± 0.90, 6.76 ± 0.79 with 1, 3, and 10 μM cetirizine (N = 10; $P > 0.05$ vs controls). Similarly to the results obtained in undifferentiated cells, both astemizole and cetirizine failed to affect

[^{3}H]NE release occurring in 5 mM $[K^+]_e$ (data not shown). The inhibitory effect of terfenadine and loratadine on high $[K^+]_e$-induced [^{3}H]NE release was also similar in cells differentiated for 7–14 days with retinoic acid when compared to the effect observed in undifferentiated cells (data not shown).

Furthermore, tetrodotoxin (TTX), at concentrations which completely eliminated voltage-dependent Na^+ currents in SH-SY5Y cells [16], was unable to modify either basal or high $[K^+]_e$-induced release of the catecholamine. In fact, basal [^{3}H]NE release from SH-SY5Y cells differentiated by a 7–14 day treatment with retinoic acid was 5.3 ± 0.25% (N = 10) in control cells and 5.4 ± 0.25% (N = 12) in 0.1 μM TTX-treated cells. The high $[K^+]_e$-induced release of the catecholamine was 11.2 ± 2.9% (N = 10) in control cells, and 14.4 ± 2.3% (N = 12) in 0.1 μM TTX-treated cells.

3.2. Effect of terfenadine, astemizole, loratadine, cetirizine, and hydroxyzine on $[Ca^{2+}]_i$ increase induced by depletion and subsequent refilling of Ca_i^{2+} stores in SH-SY5Y cells

The possible interference of terfenadine, astemizole, loratadine, and hydroxyzine with Ca^{2+} fluxes induced by the depletion and subsequent refilling of Ca_i^{2+} stores (SOC) was studied by depleting Ca_i^{2+} stores with the SERCA pump inhibitor thapsigargin (TG, 1 μM) in the absence of Ca_e^{2+}. This depletion is known to activate the plasma membrane channels involved in Ca^{2+} store refilling [18]. After Ca_i^{2+} store depletion, the subsequent reintroduction of 3 mM Ca_e^{2+} allows one to detect the possible inhibitory effects of compounds acting on SOC channels. Although in control cells the $[Ca^{2+}]_i$ response to Ca^{2+} depletion (Ca^{2+}-free + EGTA and TG) showed some variability among the cells, the reintroduction of 3 mM Ca_e^{2+} consistently induced an increase in $[Ca^{2+}]_i$ which peaked after 1–2 min and declined by 1.5 ± 0.5% of the peak in all the tested cells (mean ± SEM of 5 experimental sessions, each involving approximately 15–20 cells) over the subsequent 5-min period (Figs. 2A and 3). Astemizole (1–10 μM) (Fig. 2B), terfenadine (1–10 μM) (Fig. 2C), and loratadine (1–10 μM) (Fig. 2F), added after the $[Ca^{2+}]_i$ peak following Ca_e^{2+} reintroduction, caused a time- and concentration-dependent decline of $[Ca^{2+}]_i$, leading after 5 min to a marked suppression of $[Ca^{2+}]_i$. The IC_{50}s for such inhibition were 1.3, 1.7, and 5 μM for astemizole, terfenadine, and loratadine, respectively (Fig. 3). On the other hand, cetirizine (10 μM) (Fig. 2E) proved to be ineffective in this experimental model, whereas similar concentrations of its metabolic precursor hydroxyzine (1–10 μM) prevented the increase of $[Ca^{2+}]_i$ (Fig. 2D), with an IC_{50} of 3.8 μM. Fig. 3 represents a summary of the results and the IC_{50}s obtained with the different compound investigated on the described model of SOC activation.

The possible participation of voltage-activated Ca^{2+} channels (VGCC) in the increase of $[Ca^{2+}]_i$ elicited by Ca_e^{2+} reintroduction after Ca_i^{2+} store depletion was investigated by evaluating the effect of the simultaneous blockade of L- and N-type VGCC by nimodipine and ω-conotoxin GVIA, respectively, on $[Ca^{2+}]_i$ increase occurring in this experimental model (Fig. 4). Preincubation of SH-SY5Y cells for 5–7 min in the presence of nimodipine (1 μM) and ω-conotoxin GVIA (0.1 μM), failed to affect the $[Ca^{2+}]_i$ increase elicited by Ca_e^{2+} reintroduction after Ca_i^{2+} store depletion with the SERCA pump inhibitor thapsigargin (1 μM) in the absence of Ca_e^{2+}. In fact, the $[Ca^{2+}]_i$ increase elicited by 3 mM Ca_e^{2+} reintroduction was 57 ± 4 nM in controls and 46 ± 4 nM in nimodipine plus and ω-conotoxin-treated cells. This results suggested that L- and N- subtypes of VGCC are not involved in $[Ca^{2+}]_i$ increase elicited in the present model of SOC activation in SH-SY5Y cells. In addition, after exposure to nimodipine (1 μM) and ω-conotoxin GVIA (0.1 μM), astemizole (10 μM) and terfenadine (10 μM), added after Ca_e^{2+} reintroduction, produced a decline in $[Ca^{2+}]_i$ of 68 ± 4% and 67 ± 3% respectively, when compared to controls. These inhibitory values did not differ significantly when compared those obtained in cells which were not preincubated with the two voltage-gated Ca^{2+} channels blockers (see Fig. 3). This

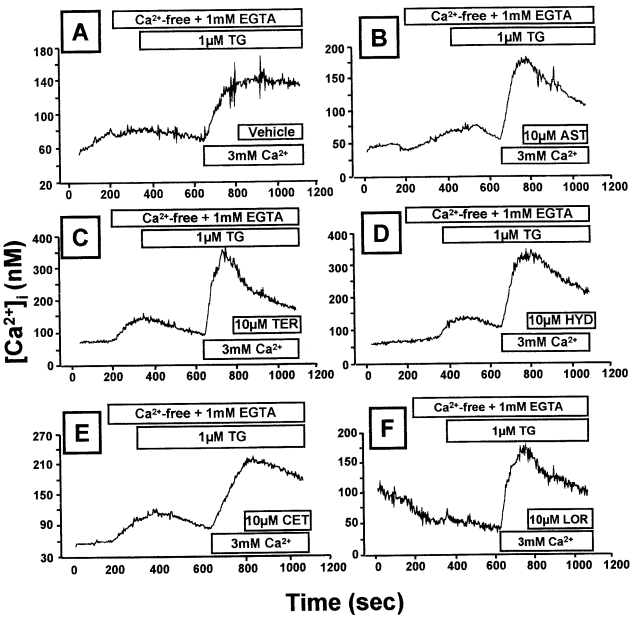

Fig. 2. Effect of astemizole (AST), terfenadine (TER), hydroxyzine (HYD), cetirizine (CET), and loratadine (LOR) on Ca^{2+} influx activated by Ca_i^{2+} store depletion (I_{SOC}) in SH-SY5Y cells. The following experimental model was used to investigate the effects of antihistamines on I_{SOC} activation: after removal of extracellular Ca^{2+} with 1 mM EGTA in a Ca_e^{2+}/free solution, the cells were treated with 1 μM thapsigargin; subsequently, 3 mM Ca_e^{2+} was reintroduced, producing an increase in $[Ca^{2+}]$. After 150 sec from Ca_e^{2+} reintroduction, the cells were exposed to vehicle (panel A), or to astemizole (10 μM, panel B), terfenadine (10 μM, panel C), hydroxyzine (10 μM, panel D), cetirizine (10 μM, panel E), or loratadine (10 μM, panel F), for the duration indicated by the respective bar. Each trace is representative of 15–30 cells for each experimental group studied in at least 3 different experimental sessions.

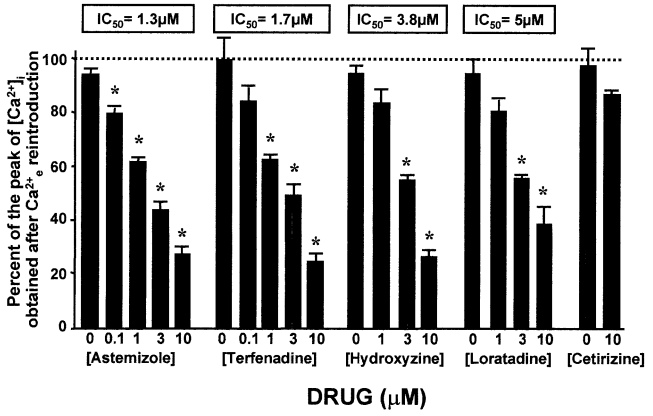

Fig. 3. Quantification of the effects of astemizole, terfenadine, loratadine, hydroxyzine, and cetirizine on Ca^{2+} influx activated by Ca_i^{2+} store depletion (I_{SOC}) in SH-SY5Y cells. The decline in $[Ca^{2+}]_i$ occurring upon extracellular Ca^{2+} reintroduction in SH-SY5Y cells, both under control conditions (no drug exposure) and upon perfusion with different drug concentrations, was calculated by averaging the last ten points (40 sec) of the trace during vehicle or drug exposure and dividing this value by the average of the last ten points (40 sec) of the peak of $[Ca^{2+}]_i$ measured immediately before vehicle or drug exposure. Both these values were preliminarily subtracted from the respective control $[Ca^{2+}]_i$ value recorded before extracellular Ca^{2+} reintroduction. The columns corresponding to the 0 drug value indicate the spontaneous decline of $[Ca^{2+}]_i$ for each experimental drug group. Each bar is the mean of the data obtained in at least 3 different experimental sessions in which 15–30 cells for each experimental group were studied. Asterisks denote values that are statistically different from controls ($P < 0.05$).

result suggested that the inhibition exerted by astemizole and terfenadine of $[Ca^{2+}]_i$ increase elicited by Ca_e^{2+} reintroduction after Ca_i^{2+} store depletion in SH-SY5Y cells did not involve their possible interaction with VGCC. On the other hand, whole-cell patch-clamp experiments revealed that VGCC in non-differentiated SH-SY5Y cells were too small to be amenable to pharmacological analysis (data not shown) [12,19].

3.3. Effect of second-generation antihistamines on hERG K^+ channels constitutively expressed in SH-SY5Y human neuroblastoma cells (I_{HERG})

Since it has been reported that some second-generation antihistamines can block hERG K^+ channels, we evaluated the pharmacological profile for the blockade of these channels, which are constitutively present in SH-SY5Y human neuroblastoma cells, by the four second-generation antihistamines utilized in the present study. For this purpose, a voltage-clamp protocol which allowed us to detect a K^+-selective inward tail current displaying the biophysical properties of I_{HERG} was used [20,21]. Particularly, in order to minimize the contribution of other classes of K^+ channels also expressed in SH-SY5Y human neuroblastoma cells, I_{HERG} was activated by depolarizing, to 0 mV, the cell for 10 sec from a holding potential of −60 mV; this value of membrane potential fully activated I_{HERG} and completely

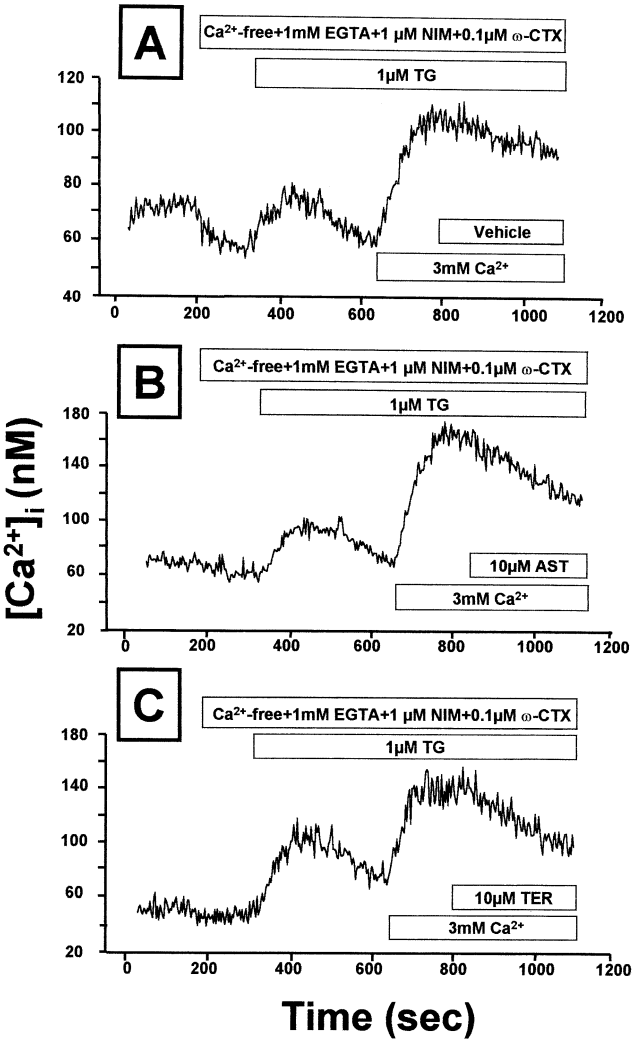

Fig. 4. Effect of nimodipine (NIM, 1 μM) plus ω-conotoxin GVIA (ω-CTX, 0.1 μM) on astemizole- and terfenadine-induced inhibition of Ca^{2+} influx activated by Ca_i^{2+} store depletion (I_{SOC}) in SH-SY5Y cells. The model is identical to that shown in Fig. 2, except that nimodipine (1 μM) plus ω-conotoxin GVIA (0.1 μM) were added in the 1 mM EGTA plus Ca_e^{2+}-free solution, and perfused throughout the experiments. After 150 sec from Ca_e^{2+} reintroduction, the cells were exposed to vehicle (panel A) or to astemizole (10 μM, panel B), or terfenadine (10 μM, panel C) as indicated by the respective bars. Each trace is representative of 15–30 cells for each experimental group studied in at least 3 different experimental sessions.

inactivated the delayed rectifier K^+ current. Subsequently, the cell was repolarized to increasingly negative voltages (from 0 to −140/−180 mV in −20 mV steps), each repolarizing pulse lasting 100 msec. Fig. 5A shows representative current traces from single undifferentiated SHSY-5Y human neuroblastoma cells exposed to the same concentration (3 μM) of the four different H_1 receptor antagonists astemizole, terfenadine, loratadine, and cetirizine. After superfusion with astemizole or terfenadine for 5 min, the I_{HERG} inward current was almost completely suppressed (≥80% blockade). On the other hand, the same concentration of loratadine caused a 40% blockade of I_{HERG}, whereas cetirizine (3 μM) was completely devoid of any inhibitory action.

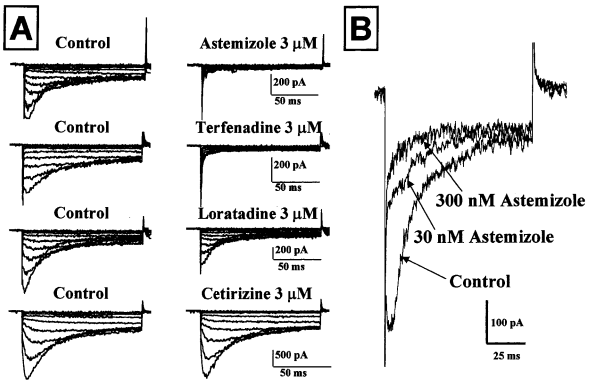

Fig. 5. Effect of the four H_1 receptor antagonists astemizole, terfenadine, loratadine, and cetirizine on I_{HERG} constitutively expressed in SH-SY5Y human neuroblastoma cells. Panel A. Representative current traces recorded in the whole-cell configuration of the patch-clamp technique from four different SHSY-5Y human neuroblastoma cells under control conditions, and after 5-min perfusion with each of the four different H_1 receptor antagonists at 3 μM. Holding potential: −60 mV; test potential 0 mV for 10 sec; return potentials: from 0 to −140/−180 mV in −20 mV steps for 100 msec. Only the last 150 msec of each pulse are shown. *Panel B.* Effect of 30 and 300 nM astemizole in a single SH-SY5Y human neuroblastoma cell. Holding potential: −60 mV; test potential 0 mV for 10 sec; return potential: −120 mV. Only the last 150 msec of each pulse are shown. After recording the control traces, 30 and 300 nM astemizole were perfused on the cell, each for five minutes. The experiment shown is representative of four, each giving comparable results.

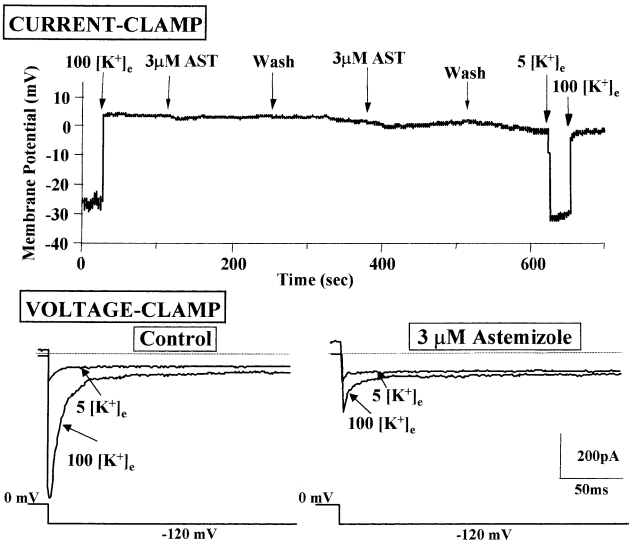

Fig. 6. Effect of astemizole on the membrane potential in a single SH-SY5Y human neuroblastoma cell. The top panel shows the result of a current-clamp recording in a single SH-SY5Y cell exposed to the experimental conditions indicated by the arrows. Note the lack of effect of astemizole (3 μM) in interfering with the depolarization induced by 100 mM $[K^+]_e$. The bottom panel shows the K^+ currents recorded in the voltage-clamp mode from the same cell shown in the top panel, using two different extracellular solutions (5 mM and 100 mM $[K^+]_e$) before and after cell exposure to astemizole (3 μM). Note the complete blockade of I_{HERG}. Holding potential: 0 mV; test potential: −120 mV. Only the last 200 msec of each pulse are shown. The experiment shown is representative of four, each giving comparable results.

In order to verify whether I_{HERG} inhibition by astemizole might correlate with the ability of the second-generation H_1 receptor blocker to prevent high $[K^+]_e$-induced [^{3}H]NE release from SH-SY5Y human neuroblastoma cells, we studied the concentration-dependence of I_{HERG} blockade by astemizole in these cells. As shown in Fig. 5B, I_{HERG} was 66 ± 8% blocked by 30 nM astemizole (N = 4) and 79 ± 5% (N = 4) by 300 nM astemizole. Therefore, the IC_{50} for astemizole blockade of I_{HERG} was 6.4 nM. The fact that the IC_{50} for astemizole-induced blockade of I_{HERG} was about 300-fold lower than that calculated for its inhibition of depolarization-induced [^{3}H]NE release from SH-SY5Y cells suggested that I_{HERG} inhibition by astemizole was not responsible for such pharmacological effects. This hypothesis was further supported by the observation that, when the cell membrane potential of SH-SY5Y cells was directly measured by means of the current-clamp technique in the whole-cell configuration of the patch-clamp, 3 μM astemizole, a concentration which caused approximately a 50% inhibition of [^{3}H]NE release and completely suppressed I_{HERG} in the same cells (Fig. 6B), failed to interfere with the changes in SH-SY5Y membrane potential elicited by high $[K^+]_e$-induced depolarization (Fig. 6A). After exposure to 3 μM astemizole, the cell membrane potential in the presence of 100 mM K_e^+ changed by −2.4 ± 0.4 mV (N = 4, $P > 0.05$). As expected, cetirizine, which did not interfere with either I_{HERG} or depolarization-induced ^{3}H]NE release, did not affect SH-SY5Y cell membrane potential under resting (5 mM K_e^+) or stimulated (100 mM K_e^+) conditions (data not shown).

4. Discussion

The results of the present study suggest that in SH-SY5Y human neuroblastoma cells, a useful *in vitro* model of human sympathetic neurons, the high $[K^+]_e$-induced release of previously taken-up [^{3}H]NE is prevented by the H_1 receptor antagonists terfenadine, astemizole, loratadine, and hydroxyzine. On the other hand, cetirizine, a potent piperazine H_1 receptor antagonist, failed to prevent high $[K^+]_e$-induced [^{3}H]NE release. The possibility that the inhibitory property on [^{3}H]NE release displayed by the above-mentioned congeners derives from their ability to interact with H_1 receptors seems to be ruled out by the observation that some (i.e. astemizole, terfenadine, loratadine, hydroxyzine) were able to prevent [^{3}H]NE release, whereas others (i.e. cetirizine), although provided of potent antagonistic activity at H_1 sites, were ineffective.

The molecular mechanism responsible for the observed inhibition of $[K^+]_e$-stimulated [^{3}H]NE release by some H_1 receptor antagonists seems to be related to their ability to inhibit Ca^{2+} channels activated by Ca_i^{2+} store depletion (SOCs). In fact, in a widely used model for SOC activation such as the $[Ca^{2+}]_i$ increase obtained with Ca_e^{2+} reintroduction after Ca_i^{2+} store depletion with the SERCA pump inhibitor thapsigargin [22], astemizole, terfenadine, loratadine, and hydroxyzine, but not cetirizine, displayed a dose-dependent inhibitory action. The rank order of potency for

SOC inhibition by these compounds closely correlated with their inhibitory properties on depolarization-induced [^{3}H]NE release in SH-SY5Y human neuroblastoma cells. Although such parallelism does not demonstrate that SOCs are the only mechanism responsible for depolarization-induced [^{3}H]NE release, the data obtained are suggestive of the existence of a tight link between these two phenomena.

The inhibitory effect exerted by micromolar concentrations of the second-generation antihistamines astemizole, terfenadine, and loratadine on Ca^{2+}-permeable SOC channels in SH-SY5Y cells is in line with the results of Fisher *et al*. [10,11], obtained in rat basophylic leukemia RBL-2H3 cells, which showed that astemizole inhibited both Ca^{2+} fluxes mediated by SOCs and β-hexoseaminidase release. Subsequently, the same group of investigators [23], by studying the inhibitory effects of a long series of astemizole derivatives, concluded that SOC inhibition was strictly correlated to the degree of lipophilicity of their chemical structure. In accordance with these findings obtained with astemizole, an inhibition of SOCs was also observed in the present study with micromolar concentrations of terfenadine, loratadine, and of the first-generation H_1 receptor antagonist hydroxyzine, three molecules all displaying elevated lipophilicity [24]. Interestingly, cetirizine, which is the main *in vivo* metabolite of hydroxyzine and is much more hydrophilic than its metabolic precursor due to the presence of a ionizable carboxyl group, failed to affect SOCs in SH-SY5Y human neuroblastoma cells.

The novel role for Ca^{2+}-permeable SOCs in depolarization-induced [^{3}H]NE release from SH-SY5Y cells seems also to be supported by the results showing that, in the same cell line, exposure to elevated $[K^+]_e$, by enhancing $[Ca^{2+}]_i$ via the opening of VGCC, is able to trigger an activation of phospholipase C, leading to a rapid and transient increase in polyphoshoinositide hydrolysis and to IP_3 accumulation [5]. This intracellular mediator is known to cause the release of Ca^{2+} from internal stores, leading to Ca^{2+} store depletion and subsequent Ca^{2+} refilling from the extracellular space via Ca^{2+}-permeable channels [6]. Furthermore, it has recently been shown that, in neurons, SOCs also participate in the refilling of ryanodine-sensitive Ca^{2+} stores which follows the Ca^{2+}-induced Ca^{2+} release process [4]. Thus, the present results suggest that, during exposure to elevated $[K^+]_e$, the activation of SOCs plays an important role in allowing Ca^{2+} to enter the cytoplasm and trigger NE release; blockade of this entry pathway for Ca^{2+} by lipophylic H_1 receptor antagonists may prevent [^{3}H]NE release evoked by elevated $[K^+]_e$. This hypothesis seems to be supported by the recent evidence that SOCs regulate exocytosis in adrenal chromaffin cells [7] and in clonal PC12 cells [8].

Another possibility that should be taken into account to explain the inhibitory properties of astemizole, terfenadine, loratadine, and hydroxyzine on high $[K^+]_e$-induced [^{3}H]NE release from SH-SY5Y cells could be their potential direct inhibitory effect on voltage-gated Ca^{2+} channels [25]. In fact, the inhibition of these channels [12,13,26,27] was able to prevent catecholamine release from SH-SY5Y cells [13]. However, this possibility seems unlikely since the simultaneous addition of nimodipine and ω-conotoxin GVIA, in concentrations which effectively blocked L- and N-type Ca^{2+} channels as well as [^{3}H]NE release from SH-SY5Y cells [26,28], did not modify $[Ca^{2+}]_i$ increase in the model of SOC activation utilized in the present study, and did not interfere with the inhibition of $[Ca^{2+}]_i$ increase by those H_1-receptor blockers inhibiting [^{3}H]NE release. Furthermore, astemizole, when used in concentrations which effectively suppressed NE release in the present study, was unable to inhibit high voltage-activated Ca^{2+} currents (mainly of the L-subtype) in pituitary GH_3 cells [29].

Several studies have recently shown that second-generation antihistamines such as terfenadine [30,31], astemizole [32], and loratadine [33] all share the ability to inhibit the K^+ channels encoded by the human Ether-a-go-go Related Gene (hERG) [34]. These channels appear to be crucial for membrane potential control in cardiac [35], neuronal [20, 21], microglial cells [36], and astrocytes [37]; thus, the possibility existed that, under conditions of elevated $[K^+]_e$, the pharmacological blockade of hERG K^+ channels might have impeded membrane depolarization from occurring, leading to a reduced entry of Ca^{2+} though voltage-gated Ca^{2+} channels and, consequently, to the inhibition of neurotransmitter release. In order to rule out the possibility that the inhibitory action on high $[K^+]_e$-induced [^{3}H]NE release could be the consequence of the effect of hERG K^+ channel inhibition on membrane potential in SH-SY5Y cells, the effect of hERG K^+ channel blockade by astemizole was evaluated by current-clamp electrophysiology. The data obtained demonstrated that, under depolarizing conditions, the inhibition of hERG K^+ channels by astemizole was unable to modify SH-SY5Y membrane potential, and therefore that the pharmacological inhibition of [^{3}H]NE release observed with these H_1 receptor blockers could not be ascribed to their interference with membrane depolarization. In addition, these experiments seem to suggest that, under these depolarizing conditions, hERG K^+ channels do not play a relevant role in membrane potential control. Another crucial point that should be underlined and which favors the hypothesis of SOC inhibition as a primary mechanism for [^{3}H]NE release inhibition by H_1 receptor antagonists is the existence of a 2- to 300-fold difference in the potency of astemizole-induced hERG inhibition (IC_{50}: 6 nM) versus [^{3}H]NE release (IC_{50}: 2.3 μM) or SOC (IC_{50}: 1.3 μM) inhibition.

In addition, since it has been shown that terfenadine can interfere with voltage-dependent Na^+ channels in guinea pig ventricular myocytes [38], the possibility existed that the inhibition of [^{3}H]NE release by this H_1 receptor antagonist could be ascribed to this pharmacological action. However, this possibility seems to be ruled out since tetrodotoxin, at concentrations which completely eliminated voltage-dependent Na^+ currents in SH-SY5Y cells [16,

present study], was unable to modify either basal or high-$[K^+]_e$-induced release of the catecholamine.

The present results showing that, in a cellular model of human postganglionic sympathetic neurons, Ca^{2+} channels activated by Ca_i^{2+} store depletion may participate in high $[K^+]_e$-induced NE release, besides their physiological relevance, might also have important pharmacological implications [39]. In fact, the inhibition of peripheral catecholamine release by these H_1 receptor blockers may represent an additional risk factor in patients undergoing polymorphic ventricular tachycardias because of the direct inhibition of cardiac K^+ channels by these drugs [40,41]. Indeed, in this clinical setting, the activation of catecholaminergic β receptors represents a protective mechanism to counteract the concomitant bradycardia, as suggested by the observation that isoproterenol administration represents a first-choice treatment for such life-threatening arrhythmias [42,43]. Therefore, it seems possible to speculate that the impairment of catecholamine outflow through SOC inhibition may contribute to the occurrence of the arrhythmic episode.

In conclusion, the present results suggest that Ca^{2+} currents activated by Ca_i^{2+} store depletion might have a prominent role in depolarization-induced NE release from SH-SY5Y human neuroblastoma cells, and that pharmacological agents able to block this Ca^{2+}-influx pathway might prevent high $[K^+]_e$-induced release of the sympathetic neurotransmitter. Although the present results have been obtained in cells of neoplastic origin, it seems reasonable to speculate that SOCs might be involved in depolarization-induced NE release in non-cancer cells as well.

Acknowledgments

The authors are indebted to Dr. P. Cilli (Janssen-Cilag, Rome, Italy) for the generous supply of astemizole; and to Drs. M. Olivotto and A. Arcangeli (Univ. of Florence, Italy) and to Dr. P.F.T. Vaughan (University of Leeds, Leeds, UK) for providing the SH-SY5Y human neuroblastoma cells. The study was supported by the following grants: Telethon 1058 to M.T.; National Research Council (CNR) 99.02614.CT04, 98.03149.CT04 and 97.01238.PF49 to M.T., and 95.02857.CT04 to L.A.; MURST COFIN 1999 to M.T. and MURST COFIN 2000 to L.A.; MURST-CNR Biotechnology Program L.95/95 N. 98.00062.PF31 to L.A.; Regione Campania to L.A.

References

[1] Tsien RW, Tsien RY. Calcium channels, stores and oscillations. Annu Rev Cell Biol 1990;6:715–60.
[2] Hofmann F, Biel M, Flockerzi V. Molecular basis for Ca^{2+} channel diversity. Annu Rev Neurosci 1994;17:399–418.
[3] Berridge MJ. Neuronal calcium signalling. Neuron 1998;21:13–26.
[4] Peng Y. Ryanodine-sensitive component of calcium transients evoked by nerve firing at presynaptic nerve terminals. J Neurosci 1996;16:6703–12.
[5] Smart D, Wandless A, Lambert DG. Activation of phospholipase C in SH-SY5Y neuroblastoma cells by potassium-induced calcium entry. Br J Pharmacol 1995;116:1797–1800.
[6] Parekh AB, Penner R. Store depletion and calcium influx. Physiol Rev 1997;77:901–30.
[7] Fomina AF, Nowycky MC. A current activated on depletion of intracellular Ca^{2+} stores can regulate exocytosis in adrenal chromaffin cells. J Neurosci 1999;19:3711–22.
[8] Taylor SC, Peers C. Store-operated Ca^{2+} influx and voltage-gated Ca^{2+} channels coupled to exocytosis in Pheochromocytoma (PC12) cells. J Neurochem 1999;73:874–80.
[9] Fatatis A, Bassi A, Monsurrò MR, Sorrentino G, Mita GD, Di Renzo GF, Annunziato L. LAN-1: a human neuroblastoma cell line with M1 and M3 muscarinic receptor subtypes coupled to intracellular Ca^{2+} elevation and lacking Ca^{2+} channels activated by membrane depolarization. J Neurochem 1992;59:1–9.
[10] Fischer MJ, Paulussen JJ, Kok-Van Esterick EE, Van der Heijden VS, De Mol NJ, Janssen LH. Effect of the antiallergics astemizole and norastemizole on Fc ε RI receptor-mediated signal transduction processes. Eur J Pharmacol 1997;322:97–105.
[11] Fischer MJ, Paulussen JJ, de Mol NJ, Janssen LH. Dual effect of the anti-allergic astemizole on Ca^{2+} fluxes in rat basophilic leukemia (RBL-2H3) cells: release of Ca^{2+} from intracellular stores and inhibition of Ca^{2+} release-activated Ca^{2+} influx. Biochem Pharmacol 1998;55:1255–62.
[12] Seward EP, Henderson G. Characterization of two components of the N-like, high threshold-activated calcium channel current in differentiated SH-SY5Y cells. Pfluger Arch 1990;417:223–30.
[13] Murphy NP, Ball SG, Vaughan PF. Potassium- and carbachol-evoked release of [^{3}H]Noradrenaline from human neuroblastoma cells, SH-SY5Y. J Neurochem 1991;56:1810–5.
[14] Cataldi M, Taglialatela M, Guerriero S, Amoroso S, Lombardi G, Di Renzo GF, Annunziato L. Protein tyrosine kinases activate while protein tyrosine phosphatases inhibit L-type calcium channel activity in pituitary GH_3 cells. J Biol Chem 1996;271:9441–6.
[15] Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440–50.
[16] Toselli M, Tosetti P, Taglietti V. Functional changes in sodium conductances in the human neuroblastoma cell line SH-SY5Y during in vitro differentiation. J Neurophysiol 1996;76:3920–7.
[17] Arcangeli A, Rosati B, Cherubini A, Crociani O, Fontana L, Passani B, Wanke E, Olivotto M. Long-term exposure to retinoic acid induces the expression of IRK1 channels in HERG channel-endowed neuroblastoma cells. Biochem Biophys Res Commun 1998;244:706–11.
[18] Fatatis A, Caporaso R, Iannotti E, Bassi A, Di Renzo GF, Annunziato L. Relationship between time of activation of phospholipase C-linked plasma membrane receptors and reloading of intracellular Ca^{2+} stores in LAN-1 human neuroblastoma cells. J Biol Chem 1994;269:18021–7.
[19] Forsythe ID, Lambert DG, Nahorski SR, Lindsdell P. Elevation of cytosolic calcium by cholinoceptor agonists in SH-SY5Y human neuroblastoma cells: estimation of the contribution of voltage-dependent currents. Br J Pharmacol 1992;107:207–14.
[20] Arcangeli A, Bianchi L, Becchetti A, Faravelli L, Coronnello M, Mini E, Olivotto M, Wanke E. A novel inward-rectifying K^+ current with a cell-cycle dependence governs the resting potential of mammalian neuroblastoma cells. J Physiol (Lond) 1995;489:455–71.
[21] Bianchi L, Wible B, Arcangeli A, Taglialatela M, Morra F, Castaldo P, Crociani O, Rosati B, Faravelli L, Olivotto M, Wanke E. *herg* encodes a K^+ current highly conserved in tumors of different histogenesis: a selective advantage for cancer cells? Cancer Res 1998;58:815–22.
[22] Treiman M, Caspersen C, Christensen SR. A tool coming of age: thapsigargin as an inhibitor of sarco-endoplasmic reticulum Ca^{2+}-ATPases. Trends Pharmacol Sci 1998;19:131–5.

[23] Fischer MJ, Paulussen JJ, Tollenaere JP, De Mol NJ, Janssen LH. Structure–activity relationships of store-operated Ca^{2+} channels and exocytosis. Eur J Pharmacol 1998;350:353–61.

[24] Timmerman H. Why are non-sedating antihistamines non-sedating? Clin Exp Allergy 1999;29(Suppl 3):13–8.

[25] Liu S, Melchert RB, Kennedy RH. Inhibition of L-type Ca^{2+} channel current in rat ventricular myocytes by terfenadine. Circ Res 1997;81: 202–10.

[26] McDonald RL, Vaughan PF, Peers C. Muscarinic (M_1) receptor mediated inhibition of K^+-evoked [^{3}H]noradrenaline release from human neuroblastoma (SH-SY5Y) cells via inhibition of L- and N-type Ca^{2+} channels. Br J Pharmacol 1994;113:621–7.

[27] Reeve HL, Vaughan PF, Peers C. Calcium channel currents in undifferentiated human neuroblastoma (SH-SY5Y) cells: action and possible interactions of dihydropyridines and ω-conotoxin. Eur J Neurosci 1994;6:943–52.

[28] Morton AJ, Hammond C, Mason WT, Henderson G. Characterization of the L- and N-type calcium channels in differentiated SH-SY5Y neuroblastoma cells: calcium imaging and single channel recordings. Mol Brain Res 1992;13:53–61.

[29] Secondo A, Taglialatela M, Cataldi M, Giorgio G, Valore M, Di Renzo GF, Annunziato L. Pharmacological blockade of ERG K^+ channels and Ca^{2+} influx through store-operated channels exerts opposite effects on intracellular ea^{2+} oscillations in pituitary GH_3 cells. Mol Pharmacol 2000;58:1115–28.

[30] Roy ML, Dumaine R, Brown AM. HERG, a primary human ventricular target of the non-sedating antihistamine terfenadine. Circulation 1996;94:817–23.

[31] Taglialatela M, Pannaccione A, Castaldo P, Giorgio G, Zhou Z, January CT, Genovese A, Marone G, Annunziato L. The molecular basis for the lack of HERG K^+ channels block-related cardiotoxicity by the H_1 receptor blocker cetirizine as compared to other second-generation antihistamines. Mol Pharmacol 1998;54:113–21.

[32] Suessbrich H, Waldegger S, Lang F, Busch AE. Blockade of HERG channels expressed in Xenopus oocytes by the histamine receptor antagonists terfenadine and astemizole. FEBS Lett 1996; 385:77–80.

[33] Crumb WJ. Loratadine blockade of K^+ channels in human heart: comparison with terfenadine under physiological conditions. J Pharmacol Exp Ther 2000;292:261–4.

[34] Warmke JW, Ganetzky B. A family of potassium channel genes related to eag in *Drosophila* and mammals. Proc Natl Acad Sci USA 1994;91:3438–42.

[35] Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. Cell 1995;81:299–307.

[36] Zhou W, Cayabyab FS, Pennefather PS, Sclichter LC, DeCoursey TE. HERG-like K^+ channels in microglia. J Gen Physiol 1998;111: 781–94.

[37] Emmi A, Wenzel J, Schwartzkroin PA, Taglialatela M, Castaldo P, Bianchi L, Nerbonne J, Robertson GA, Janigro D. Do glia have heart? Expression and functional role for Erg-like currents in hippocampal astrocytes. J Neurosci 2000;20:3915–25.

[38] Ming Z, Nordin C. Terfenadine blocks time-dependent Ca^{2+}, Na^+, and K^+ channels in guinea pig ventricular myocytes. J Cardiovasc Pharmacol 1995;26:761–9.

[39] Zhang MQ. Chemistry underlying the cardiotoxicity of antihistamines. Curr Med Chem 1997;4:187–200.

[40] Woosley RL. Cardiac actions of antihistamines. Annu Rev Pharmacol Toxicol 1996;36:233–52.

[41] Taglialatela M, Timmerman H, Annunziato L. Cardiotoxic potential and CNS effects of first generation antihistamines. Trends Pharmacol Sci 2000;21:52–6.

[42] Schwartz PJ, Locati EH, Napolitano C, Priori SG. The long QT syndrome. In: Zipes DP and Jalife J, editors. Cardiac electrophysiology. From cell to bedside. Philadelphia: W. B. Saunders Co., 1995. p. 788–811.

[43] Honig P, Baranjuk JN. Adverse effects of H_1-receptor antagonists in the cardiovascular system. In: Simons EF and Simons R, editors. Histamine and H_1-receptor antagonists in allergic diseases. New York: Marcel Dekker Inc., 1996. p. 383–412.