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Isoflurane Reduces the Carbachol-evoked Ca^{2+} Influx in Neuronal Cells

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Background: **The authors previously reported that the isoflurane-caused reduction of the carbachol-evoked cytoplasmic Ca^{2+} transient increase ($[Ca^{2+}]_{cyt}$) was eliminated by K^+ or caffeine-pretreatment. In this study the authors investigated whether the isoflurane-sensitive component of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient involved Ca^{2+} influx through the plasma membrane.**

Methods: **Perfused attached human neuroblastoma SH-SY5Y cells were exposed to carbachol (1 mM, 2 min) in the absence and presence of isoflurane (1 mM) and in the absence and presence of extracellular Ca^{2+} (1.5 mM). The authors studied the effect of the nonspecific cationic channel blocker La^{3+} (100 μM), of the L-type Ca^{2+} channel blocker nitrendipine (10 μM), and of the N-type Ca^{2+} channel blocker ω-conotoxin GVIA (0.1 μM) on isoflurane modulation of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient. $[Ca^{2+}]_{cyt}$ was detected with fura-2 and experiments were carried out at 37°C.**

Results: **Isoflurane reduced the peak and area of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the presence but not in the absence of extracellular Ca^{2+}. La^{3+} had a similar effect as the removal of extracellular Ca^{2+}. ω-Conotoxin GVIA and nitrendipine did not affect the isoflurane sensitivity of the carbachol response although nitrendipine reduced the magnitude of the carbachol response.**

Conclusions: **The current data are consistent with previous observations in that the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient involves both Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry through the plasma membrane. It was found that isoflurane attenuates the carbachol-evoked Ca^{2+} entry. The isoflurane sensitive Ca^{2+} entry involves a cationic channel different from the L- or N- type voltage-dependent Ca^{2+} channels. These results indicate that isoflurane attenuates the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient at a site at the plasma membrane that is distal to the muscarinic receptor.**

THE role of muscarinic receptors in analgesia and anesthesia is controversial. In the brainstem muscarinic receptors modulate the level of consciousness,[1] and in cortical regions[2,3] and striatum[4] they affect memory and learning. At the spinal level muscarinic receptors inhibit glutamate release[5] and enhance γ-aminobutyric acid release.[6] Muscarinic agonists have been reported to enhance antinociceptive effects.[7–11] However, there are also reports showing that muscarinic block enhances the analgesic or anesthetic action of various drugs.[12,13] Halothane and isoflurane have been reported to depress muscarinic receptor function,[14–17] and inhibition of the muscarinic signaling has variable effects on the minimal alveolar anesthetic concentration of inhaled anesthetics.[18] Therefore, the role of muscarinic receptors in analgesia and anesthesia seems to be complex and unclear and hence it requires further study.

We have previously reported that isoflurane and halothane reduce the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient and that such an effect is eliminated in the presence of KCl.[19] In addition, we have shown that the isoflurane-sensitive component of the carbachol evoked $[Ca^{2+}]_{cyt}$ transient requires full caffeine-sensitive Ca^{2+} stores and that the elimination of the isoflurane-sensitivity of the carbachol response requires Ca^{2+} release through the ryanodine channels.[20] In SH-SY5Y cells activation of muscarinic receptors by carbachol stimulates the formation of inositol triphosphate (IP3).[21,22] In many cell types, including SH-SY5Y cells, stimulation of IP3 formation causes, *via* IP3 receptor activation, the release of Ca^{2+} from intracellular IP3-sensitive stores, as well as the entry of Ca^{2+} *via* plasma membrane cation channels, at times referred to as capacitative Ca^{2+} entry or store-operated Ca^{2+} channels.[21,23–27] In this study we used the human neuroblastoma SH-SY5Y cell line to investigate the modulation of the muscarinic response by isoflurane in a homogenous population of neuronal cells and to determine whether the isoflurane-sensitive component of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient involves Ca^{2+} release from intracellular stores or Ca^{2+} entry through the plasma membrane.

Materials and Methods

Cell Culture and Solutions

SH-SY5Y human neuroblastoma cells were cultured in Roswell Park Memorial Institute 1640 medium with L-Glutamine, supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), and 12% fetal bovine serum at 37°C, in a humidified atmosphere containing 5% CO_2. All cell culture components were Gibco BRL products purchased from Life Technologies (Rockville, MD). Experiments were performed on monolayer of cells as previously reported.[20] Cells were plated on glass coverslips (25-mm diameter) at a density of 2–4 $\times$ 10^4 cells/ml (2 ml cell suspension/35 mm culture dish) and used when they formed a confluent monolayer ($\sim$10–16 days after plating). During experimentation cells were continuously perfused with a HEPES buffer containing (in mM)

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140 NaCl, 5 KCl, 5 $NaHCO_3$, 10 HEPES, 1 $MgCl_2$, 1.5 $CaCl_2$, 1 adenosine triphosphate, and 10 glucose (pH 7.4). Experiments were performed at 37°C and the temperature was controlled with a Dual Heater controller TC-344A and an inline heater SH-27B (Warner Instruments Inc, Hamden, CT). The exchange of the solution was carried out with a manifold. The solutions containing 1 mM carbachol, with and without 100 nM conotoxin GVIA, 10 μM nitrendipine, or 100 μM La^{3+}, were prepared using the HEPES buffer. Saturated isoflurane (Ohmeda Caribe Inc., Guayana, PR) solutions were prepared in HEPES buffer 24 h in advance in gas-tight containers and diluted to the final concentration (1 mM) immediately before use as previously described.[19]

Ca^{2+} Measurements

SH-SY5Y cells were loaded with the fluorescent Ca^{2+} indicator Fura-2[28] by incubating the cells attached on coverslips in the culture medium containing 5 μM of the acetoxymethyl ester of the dye (Fura-2 AM; Molecular Probes, Eugene, OR) for 30 min under culture conditions. After loading, cells were washed three times with the HEPES buffer, and the coverslips were placed into the perfusion chamber and perfused (250 μl/min) for 30 min with the HEPES buffer at 37°C before being exposed to the various drugs. The HEPES buffer without or containing the drugs was perfused at a speed of 250 μl/ml.

The perfusion chamber was set on an inverted microscope (DIAPHOT 300; Nikon, Melville, NY), equipped with a 40× oil-immersion objective (N.A.1.30; Nikon). The microscope was connected to a high-speed multiwavelength illuminator (DeltaRAM V; Photon Technology International Inc., Lawrenceville, NJ). The excitation wavelengths for Fura-2 (340 nm and 380 nm) were alternately generated by a monochromator (every 0.02 s). The emitted fluorescence (from the alternated excitation at 340 and 380 nm) from 15 to 20 cells was filtered with the fluorescence barrier filter BA 515 nm, collected with a photomultiplier (PMT01–710; Photon Technology International Inc.), and digitized at 50 Hz.

Data Analysis

Data collection and analysis was carried out using the software Felix (version 1.42a, Photon Technology International), Clampfit (Pclamp 8; Axon Instruments, Foster City, CA), and GraphPad Prism (GraphPad Software, Inc., San Diego, CA). For each treatment (corresponding to data in each figure), experiments were done under different conditions on sister cultures (same plating day) and on three to five culture sets (different plating days). The averaged traces shown in the figures were obtained by lining up the peak values for the evoked $[Ca^{2+}]_{cyt}$ transients. Unless otherwise indicated, the areas were obtained over a period of 300 s starting from the onset of the carbachol evoked $[Ca^{2+}]_{cyt}$ transient on each trace. In figures the data represent the delta ratio (Δ ratio) of the emission of Fura-2 at 515 nm generated by excitation at 340 and 380 nm (ratio 340/380).

Statistical Analysis

Comparison between different groups was performed using unpaired two-tailed Student *t* test when there was only one treatment (fig. 1) and one-way analysis of variance Newman-Keuls test when there was more than one treatment (figs. 2 and 3) using the GraphPad Prism (GraphPad Software, Inc.) software.

Results

Isoflurane Sensitivity of the Carbachol-evoked $[Ca^{2+}]_{cyt}$ Transient is Dependent on Extracellular Ca^{2+}

We have reported that isoflurane (1 mM) reduced the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 1A-C).[20] The isoflurane effect includes a reduction in the peak and area under the peak but not in the width at 50% peak height of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 1A, B, C, D). When the concentration of extracellular Ca^{2+} was reduced from 1.5 mM to 150 μM, carbachol still evoked a $[Ca^{2+}]_{cyt}$ transient but its magnitude was lower and its decay was speeded up, as indicated by the reduction in the width at 50% peak height (fig. 1E, F, G, H). These results are consistent with previous observations in SH-SY5Y cells and indicate that the carbachol-evoked $[Ca^{2+}]_{cyt}$ -transient results from Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry through the plasma membrane.[21,24,27] Interestingly, in the presence of low extracellular Ca^{2+}, the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient became insensitive to isoflurane (fig. 1E–H *versus* 1A–D).

Isoflurane Sensitivity of the Carbachol-evoked $[Ca^{2+}]_{cyt}$ Transient is Eliminated by Exposing the Cells to La^{3+}, a Nonselective Cationic Channel Blocker

Removal of extracellular Ca^{2+}, even for short periods, may induce partial depletion of intracellular Ca^{2+} stores. Hence, the elimination of the isoflurane-sensitive component of the carbachol-evoked cytoplasmic Ca^{2+} response may still involve reduction of Ca^{2+} release from intracellular store rather than elimination of Ca^{2+} entry through the plasma membrane. To distinguish between these possibilities we blocked Ca^{2+} entry through the plasma membrane by using the nonselective cationic channel blocker La^{3+}. La^{3+} has been shown to block various voltage-dependent Ca^{2+} channels,[29,30] as well as other cationic channels known as capacitative Ca^{2+} channels.[26] La^{3+} alone did not significantly affect the peak but decreased the area and reduced the width at 50% peak height of the carbachol-evoked $[Ca^{2+}]_{cyt}$ tran-

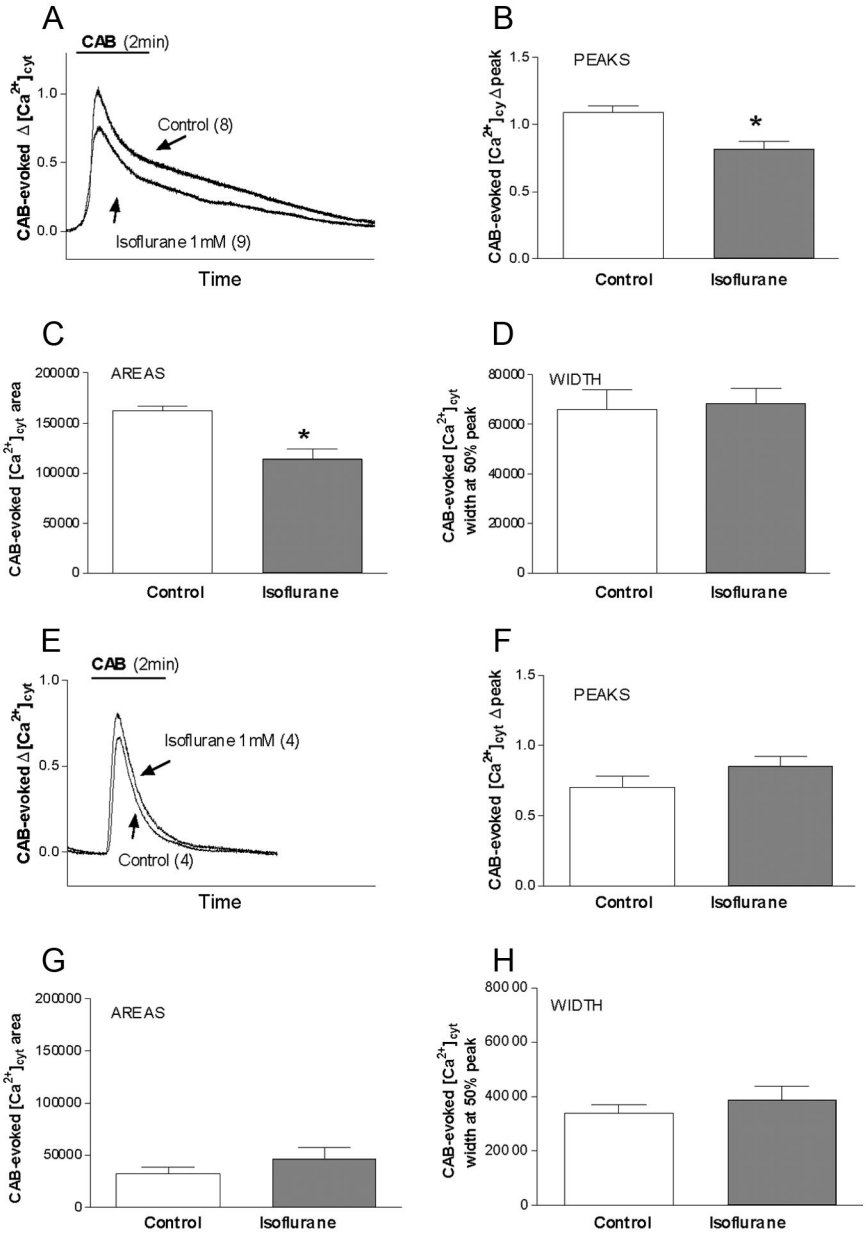

Fig. 1. Isoflurane reduces the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the presence, but not in the absence, of extracellular Ca^{2+}. The application of isoflurane was started 10 min before the 2 min carbachol stimulation. (*A–D*) Cells exposed to 1 mM carbachol (CAB) in the absence or presence of 1 mM isoflurane and in the presence of physiologic concentration of extracellular Ca^{2+} (1.5 mM). The averaged carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (*A*) and the corresponding measurements: peaks (*B*), areas (*C*), and widths at 50% peak height (*D*) of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the absence and presence of isoflurane. (*E–H*) Cells exposed to 1 mM carbachol in the absence or presence of 1 mM isoflurane after reducing the concentration of extracellular Ca^{2+} to 150 μM. The averaged carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (*E*), and the corresponding measurements: peaks (*F*), areas (*G*), and widths at 50% peak height (*H*) of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the absence and presence of isoflurane. Units: Peaks (Δ ratio 340/380), Areas (ratio 340/380* ms), and Width at 50% peak (ms). The horizontal bars in A and E indicate a 2-min period. The results were expressed as mean ± SEM, except for panels A and E, where they were expressed only as means. Asterisks indicate a statistically significant difference (*$P < 0.001$, unpaired two-tailed Student *t* tests) between control and isoflurane. The baseline values (absolute ratio values before the addition of carbachol) were not statistically different ($P > 0.05$) between control and isoflurane in the presence of either high (1.5 mM) or low (150 μM) extracellular Ca^{2+} (data not shown). The data for parts A–C are taken from an earlier study and are shown here for comparison purposes. The (n) indicate the number of experiments for each condition.

sient (fig. 2). These results suggest that Ca^{2+} entry through the plasma membrane mostly contributes to the decay phase of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient, whereas the decrease in the carbachol-evoked $[Ca^{2+}]_{cyt}$ peak in low extracellular Ca^{2+} may reflect partial Ca^{2+} depletion from intracellular Ca^{2+} stores. In the presence of La^{3+}, isoflurane did not produce an additional change in the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 2). The effect of La^{3+} was stronger than the effect of isoflurane (fig. 2A *versus* fig. 1A), and the main difference was that La^{3+}, but not isoflurane, strongly decreased the width of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 1D *versus* fig. 2D). The latter suggests that the isoflurane effect appears to be mostly at the plasma membrane, probably by blocking a cationic channel.

Isoflurane Sensitivity of the Carbachol-evoked $[Ca^{2+}]_{cyt}$ Transient is not Eliminated by either ω-Conotoxin GVIA, an N-type Ca^{2+} Channel Blocker, or by Nitrendipine, an L-type Ca^{2+} Channel Blocker

Carbachol, through activation of muscarinic receptors, has been shown to affect voltage-dependent Ca^{2+} channels[31,32] and to allow Ca^{2+} entry through other nonselective cationic channels.[33–35] In SH-SY5Y cells, the predominant voltage-dependent Ca^{2+} channels are L-type and N-type.[36,37] We tested whether these voltage-dependent channels contributed to the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient and, if so, whether they were the isoflurane targets underlying the isoflurane reduction in the carbachol response. It was found that the N-type

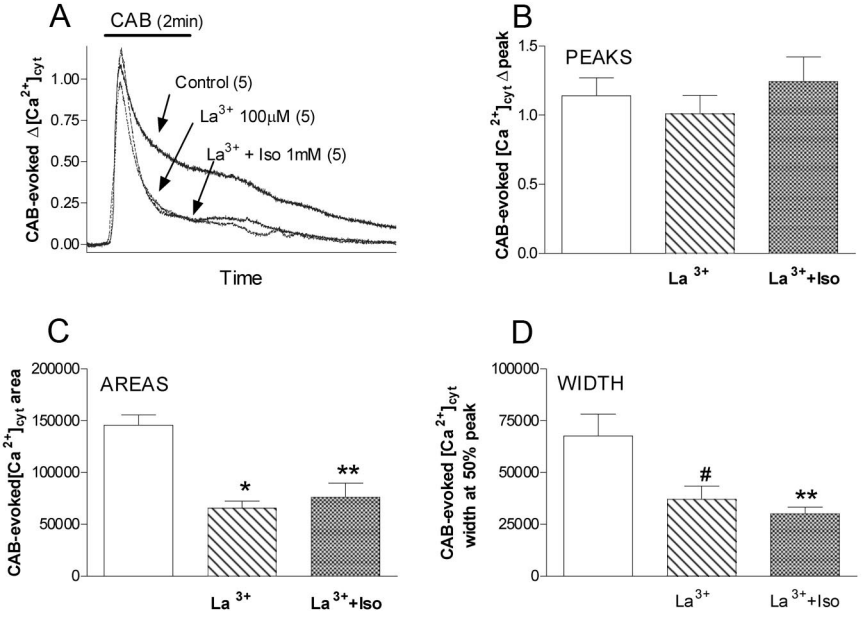

Fig. 2. La^{3+} reduces the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient and eliminates the isoflurane sensitivity of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient. The application of La^{3+} was started 10 min before the carbachol (CAB) stimulation and the application of 1 mM isoflurane (Iso) 5 min before the carbachol stimulation. The duration of the carbachol stimulation was 2 min. The averaged carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (*A*) and the corresponding measurements: peaks (*B*), areas (*C*), and widths at 50% peak height (*D*) of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the absence and presence of La^{3+}, or La^{3+} plus isoflurane. Units: Peaks (Δ ratio 340/380), Areas (ratio 340/380 * ms), and Width at 50% peak (ms). The horizontal bar in A indicates a 2-min period. The results were expressed as mean ± SEM, except for panel A where they were expressed only as means. Statistically significant differences with control are indicated as follows: *P < 0.001, **P < 0.01, #P < 0.05 (Newman-Keuls test). No statistical difference was found between the groups treated with La^{3+} and with La^{3+} plus isoflurane. The baseline values (absolute ratio values before the addition of carbachol) were not statistically different (P > 0.05) between control and the treated groups (data not shown).

Ca^{2+} channel blocker ω-conotoxin GVIA at a supramaximal concentration (100 nM) did not affect the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 3A), whereas the L-type Ca^{2+} channel blocker nitrendipine at a supramaximal concentration (10 μM) reduced the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient (fig. 3B). This indicated that under these conditions, exposure to carbachol increases Ca^{2+} entry through L-type, but not N-type, Ca^{2+} channels. Surprisingly, in the presence of nitrendipine, isoflurane further reduced the peak and area of the carbachol-

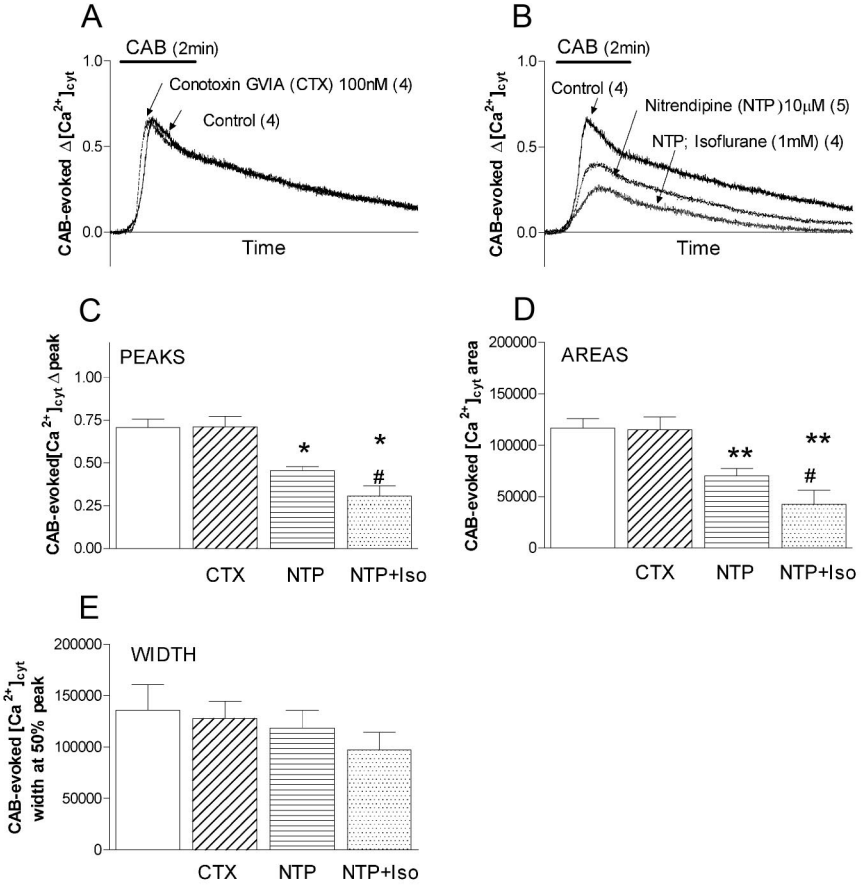

Fig. 3. Nitrendipine a L-type Ca^{2+} channel blocker reduces the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient but does not eliminate the isoflurane sensitivity of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient. The application of the calcium channel blocker was started 10 min before the carbachol (CAB) stimulation; and the application of isoflurane (1 mM) 5 min before the carbachol stimulation. The duration of the carbachol stimulation was 2 min. (*A*) The averaged carbachol-evoked $[Ca^{2+}]_{cyt}$ transients in the absence and presence of ω-conotoxin GVIA (CTX, 100 nM). (*B*) The averaged carbachol-evoked $[Ca^{2+}]_{cyt}$ transients in the absence and presence of nitrendipine (NTP, 10 μM); and of NTP plus 1 mM isoflurane (Iso). The corresponding measurements: peaks (*C*), areas (*D*), and widths at 50% peak height (*E*) of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient in the absence and presence of either ω-conotoxin GVIA, NTP, and NTP plus isoflurane (NTP+Iso). Units: Peaks (Δ ratio 340/380); Areas (ratio 340/380 * ms) and Width at 50% peak (ms). The horizontal bars in A and B indicate a 2-min period. The results were expressed as mean in panels A and B and as mean ± SEM in panels C, D, and E. Statistically significant differences from control are indicated as follows: *P < 0.001, **P < 0.01; and statistically significant differences from the NTP group are indicated as follows: #P < 0.05 (Newman-Keuls test). The baseline values (absolute ratio values before the addition of carbachol) were not statistically different (P > 0.05) between control and the treated groups (data not shown).

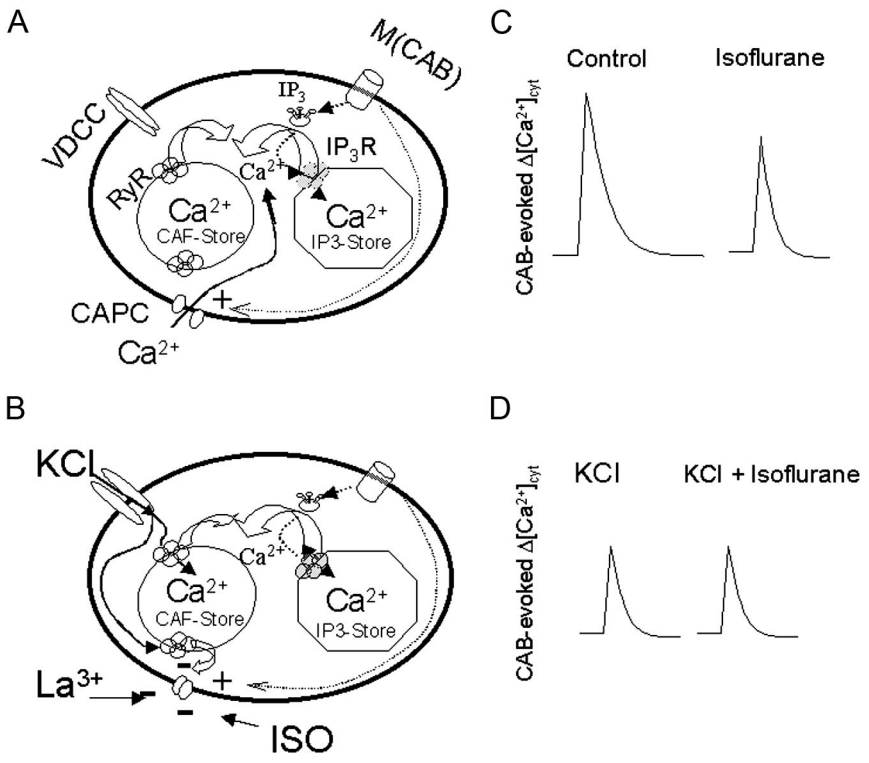

Fig. 4. Postulated mechanism for the isoflurane-sensitivity of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient. (*A*) We postulate that in SH-SY5Y cells there is an isoflurane-sensitive cationic channel at the plasma membrane (CAPC) that is activated (+) through activation of the muscarinic receptors (M). On its activation, CAPC allows Ca^{2+} entry through the plasma membrane. (*B*) Ca^{2+} influx through the CAPC is inhibited (-) by isoflurane (ISO), La^{3+} and possibly by either an interaction with the ryanodine-sensitive Ca^{2+} release channel (RYR) or by a Ca^{2+} release through RYR channels located at the caffeine-sensitive Ca^{2+} stores (CAF-stores). Under control conditions, the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient results from Ca^{2+} release from the IP3-sensitive Ca^{2+} stores (IP3-stores) and from at least some of the caffeine-sensitive Ca^{2+} stores,[19] and finally from the Ca^{2+} influx through a putative isoflurane-sensitive plasma membrane cationic channel (CAPC). The carbachol-evoked $[Ca^{2+}]_{cyt}$ transient is reduced by isoflurane before (*C*) but not after treatment with either KCl or La^{3+} (*D*). Exposure to KCl depolarizes the plasma membrane leading to opening of voltage-dependent Ca^{2+} channels (VDCC), which in turn leads to opening of RYR channels by a process called Ca^{2+}-induced Ca^{2+} release. *IP3* = inositol triphosphate; *IP3R* = IP3-sensitive Ca^{2+} channels.

evoked $[Ca^{2+}]_{cyt}$ transient (fig. 3C, D) without affecting the width at 50% peak (fig. 3E). Therefore, the isoflurane effect on the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient is not attributable to the isoflurane effects on the L-type or N-type Ca^{2+} channels but to an isoflurane effect on a La^{3+}-sensitive plasma membrane cationic channel.

Discussion

As previously reported,[21,24,27] it was found that in the human neuroblastoma cell line SH-SY5Y cells, the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient involves both Ca^{2+} release from intracellular Ca^{2+} stores and Ca^{2+} entry through the plasma membrane. Moreover, we found that the blocking effect of isoflurane on the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient appears to be mediated by blocking the carbachol-evoked Ca^{2+} entry through the plasma membrane. This isoflurane sensitive Ca^{2+} entry involves a cationic channel that is different from the L-type or N-type voltage-dependent Ca^{2+} channels. These results together with our previous observations[19,20] indicate that at the concentrations used, isoflurane blocks only part of the carbachol-evoked $[Ca^{2+}]_{cyt}$ response, apparently at a site at the plasma membrane that is distal to the muscarinic receptor.

In SH-SY5Y cells the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient is blocked by atropine[20,38] and is resistant to the N-type channel blocker (ω-conotoxin).[38] Previously, it was found that the carbachol-evoked $[Ca^{2+}]_{cyt}$ increase was also resistant to a maximal effective concentration (1 μM) of the L-type channel blocker dihydropyridine +PN 200–110.[38] However, in this study we found that the L-type channel blocker, nitrendipine (10 μM) reduced the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient without eliminating its sensitivity to isoflurane. This was surprising because volatile anesthetics are known to reduce the magnitude of L-type Ca^{2+} channel currents.[39,40] If L-type channels are contributing to the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient, blocking them should reduce the isoflurane sensitivity of the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient, which did not occur. One possible explanation is that at the high concentration of nitrendipine used in this study, nitrendipine may be reducing the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient by interfering with the G-protein-linked muscarinic receptors rather than by blocking L-type Ca^{2+} currents.[41–43]

Because La^{3+}, but not N-type or L-type Ca^{2+} channel blockers, eliminated the isoflurane action on the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient, isoflurane may be reducing the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient by blocking a cationic channel at the plasma membrane. As La^{3+}, but not isoflurane, reduced the width at 50% peak, it indicates that SH-SY5Y cells may express several nonvoltage dependent cationic channels that mediate Ca^{2+} influx upon muscarinic activation and that isoflurane acts only in a subgroup of these channels.

There are at least two possible candidates for the isoflurane-sensitive plasma-membrane cationic channel, an inositol IP3-activated IP3-channel and a capacitative Ca^{2+} channel. There is evidence suggesting the presence of plasma membrane-IP3 receptors in mammalian

neurons. IP3-activated inward Ba^{2+} currents have been recorded in excised inside-out patches of primary cultured Purkinje cells[44] and in olfactory neurons of rat.[45] However, halothane has been shown to increase, rather than decrease, Ca^{2+} currents through IP3 receptors.[46] Moreover, it has been argued that there are no IP3 receptors on the plasma membrane but a group of IP3 receptors located very close to the plasma membrane that on activation in turn activate cationic channels on the plasma membrane.[47,48] Capacitative Ca^{2+} influx is mediated by channels that are opened in response to depletion of intracellular Ca^{2+} stores.[49] There appear to be various types of capacitative Ca^{2+} channels.[49,50] Opening of capacitative channels after activation of G-protein-linked receptors, such as muscarinic receptors, involves receptor-mediated activation of phospholipase C and Ca^{2+} release by IP3.[49] Isoflurane has been shown to inhibit the histamine-induced Ca^{2+} influx in primary cultures of human endothelial cells.[51] In rat glioma C6 cells, volatile anesthetics appear to have different inhibitory effects on capacitative Ca^{2+} influx such that strong inhibition is observed with halothane but not with enflurane.[52] It is then possible that isoflurane is inhibiting muscarinic-activated capacitative Ca^{2+} influx in the SH-SY5Y cells.

We would like to postulate that this isoflurane-sensitive cationic channel contributes either to the anesthetic potency or to the side effects of isoflurane. The previously reported variable effects of muscarinic blockers (as with other G-protein linked receptors) on the minimal alveolar anesthetic concentration of inhaled anesthetics might in part reflect differences in magnitude of the muscarinic-mediated modulation of various cationic channels. Although the muscarinic-mediated activation of the isoflurane-sensitive cationic channel might reduce the isoflurane potency, the muscarinic-mediated inhibition of voltage-dependent channels[31,32] might increase the isoflurane potency. The net effect of a muscarinic agent on the isoflurane potency for reducing the muscarinic-evoked increases in $[Ca^{2+}]_{cyt}$ would then depend on the contribution of each of the cationic channels in the different brain and spinal regions where the muscarinic agents are applied.

We previously reported that the isoflurane-action on the carbachol-evoked $[Ca^{2+}]_{cyt}$ transient required that the caffeine-sensitive Ca^{2+} stores were not depleted (by either KCl or caffeine pretreatment) and that ryanodine-sensitive Ca^{2+} release channels were open.[19,20] One possible explanation is that there is an open conformation of ryanodine-sensitive Ca^{2+} release channels that interacts with the muscarinic-activated cationic channels and prevents their opening. Another explanation is that because of distinct spatial distribution, Ca^{2+} release through the ryanodine-sensitive channels blocks the isoflurane-sensitive cationic channels, whereas Ca^{2+} release through IP3-sensitive channels opens the isoflurane-sensitive cationic channels.

In summary, we postulate that in SH-SY5Y cells there is an isoflurane-sensitive cationic channel at the plasma membrane that is activated by carbachol and inhibited by La^{3+}, isoflurane, and, possibly, through an interaction with the ryanodine-sensitive Ca^{2+} release channel or by a Ca^{2+} release through these channels (fig. 4). As discussed above, a possible candidate is a isoflurane-sensitive capacitative Ca^{2+} channel. This potential target of isoflurane may serve as a site at which isoflurane may affect at least some of the actions of most of the G-protein linked receptors. The magnitude of the isoflurane effect on a given G-protein linked receptor would then be determined in part by the ability of the receptor to activate these isoflurane-sensitive capacitative Ca^{2+} channels.

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