Effect of Halothane on cADP-Ribose-induced Ca\(^{2+}\) Release System in Tracheal Smooth Muscle

Eduardo N. Chini, M.D., Ph.D.,* Thomas F. Keller,† Yedatore S. Prakash, M.D., Ph.D.,† Christina M. Pabelick, M.D.,* Gary Sieck, Ph.D.§

The release of Ca\(^{2+}\) from intracellular stores is a widespread component in several signaling pathways.\(^1\)-\(^2\) It is well known that inositol-1,4,5-trisphosphate (IP\(_3\)) triggers Ca\(^{2+}\) release from intracellular stores;\(^3\); however, cells possess other intracellular Ca\(^{2+}\) releasing systems,\(^1\)-\(^3\) including the so-called Ca\(^{2+}\)-induced Ca\(^{2+}\) release system, mediated by the ryanodine receptor-channel (RyR).\(^1\)-\(^2\) Recently it was found that the endogenous nucleotide cADP-ribose (cADPR) is a potent activator of the RyR.\(^1\)-\(^2\) Recently it was found that halothane potentiates the cADPR-induced Ca\(^{2+}\) release,\(^4\)-\(^9\) and indeed, we have shown that the cADPR system is functional in porcine smooth muscle cells.\(^10\) In fact, in porcine airway smooth muscle cells cADPR has been shown to be a second messenger in several intracellular signaling pathways.\(^1\) Biosynthesis of cADPR from β-NAD\(^{2+}\) is catalyzed by adenosine diphosphate (ADP)-ribosyl cyclase, and cADPR is hydrolysis mediated by the cADPR hydrolase to ADP-ribose (ADPR).\(^1\)

Volatile anesthetics have multiple actions on intracellular Ca\(^{2+}\) homeostasis,\(^4\)-\(^9\) including activation of the RyR and sensitization of this channel to pharmacologic agonists such as caffeine and ryanodine.\(^4\)-\(^9\) Recently, we reported that halothane can sensitize the RyR to cADPR in sea urchin egg homogenates.\(^7\) It has been previously shown that the cADPR system is functional in porcine smooth muscle cells.\(^10\) In fact, in porcine airway smooth muscle cells cADPR has been shown to be a second messenger responsible for intracellular Ca\(^{2+}\) increase induced by acetylcholine.\(^10\) In the current study, we found that halothane potentiates the cADPR-induced Ca\(^{2+}\) release through the RyR in porcine airway smooth muscle cells. We propose that modulation of the cADPR signaling system by halothane may be an important component of the complex effect of this volatile anesthetic on intracellular Ca\(^{2+}\) homeostasis.

Materials and Methods

Microsomal Preparation Porcine Tracheal Smooth Muscle

Porcine tracheal smooth muscle was quickly dissected, chilled, and minced in ice-cold solution containing 0.25 M sucrose, 20 mM Tris-HCl (pH 7.2), and 20 μg/ml leupeptin. Microsomes were prepared by differential centrifugation as described before.\(^8\) Ca\(^{2+}\) uptake and release were measured in a medium containing 250 mM N-methyl glucamine, 250 mM potassium gluconate, 20 mM HEPES buffer (pH 7.2), 1 mM MgCl\(_2\), 2 U/ml creatine kinase, 1 mM adenosine triphosphate (ATP), 4 mM Pi, 25 μg/ml leupeptin, 20 μg/ml aprotinin, and 100 μg/ml soybean trypsin inhibitor and 3 μM fluo-3 was added. Fluo-3 fluorescence was monitored at 490 nm excitation and 535 nm emission in a 250-μl cuvette at 37°C with a circulation water bath and continuously mixed with a magnetic stirring bar, using a Hitachi spectrofluorometer (F-2000) (San Jose, CA). The addition of stock solutions of various substances did not exceed 2% of the homogenate volume in the cuvette. Changes in fluorescence were calibrated to known Ca\(^{2+}\) additions using separate samples of the same microsomal preparation.

Materials

Fluo-3 was purchased from Molecular Probes (Eugene, OR); IP\(_3\), oligomycin, and antimycin were from Calbiochem (San Diego, CA). All other reagents, of the highest purity grade available, were supplied from Sigma Chemical (St. Louis, MO).

The reported experiments were repeated at least three to six times. When appropriate, data are expressed as mean ± SD. The unpaired t test was used to evaluate statistical significance; P values < 0.05 were considered significant.

Results and Discussion

Activation of RyR by cADPR in Tracheal Smooth Muscle Microsomes

It has been previously shown that the RyR-cADPR system is present and functional in smooth muscle cells.\(^10\)-\(^12\) Furthermore, cADPR is able to activate the RyR in tracheal smooth muscle cells.\(^10\) Tracheal smooth muscle cell microsomes supplemented with an ATP-regenerative system sequester added Ca\(^{2+}\) into vesicular stores in an ATP-dependent manner and release Ca\(^{2+}\) in response to μM concentrations of cADPR (fig. 1). The cADPR-induced Ca\(^{2+}\) release was inhibited by several inhibitors of the RyR such as spermine, ruthenium red, and the specific cADPR inhibitor 8-Br-cADPR (fig. 1).\(^13\) However, Ca\(^{2+}\) release induced by cADPR was not in-

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Inhibited by 1 mg/ml heparin, a specific antagonist of the IP3 channel. These observations confirmed the evidence that cADPR activates Ca2+ release through the RyR in tracheal smooth muscle.

**Effect of Halothane on cADPR-Induced Ca2+ Release**

We investigated the effect of 350 μM halothane on the cADPR induced Ca2+ release. Figure 2 demonstrates the effect of halothane on cADPR-induced Ca2+ release, addition of 350 μM halothane did not produce any significant Ca2+ release by itself; however, it sensitized the Ca2+ release system to cADPR (fig. 2). The half-maximal concentration of cADPR was decreased more than four-fold by pretreatment of the microsomes with 350 μM halothane (fig. 2B), although the maximum Ca2+ release response to cADPR was not enhanced by halothane. Thus halothane increased the apparent affinity of the Ca2+-induced Ca2+ release to stimulation by cADPR. We also observed that 350 μM halothane had no effect steady-state Ca2+ levels in the microsomal preparations. The effect of halothane on the cADPR-induced Ca2+ release was abolished by the cADPR antagonist 8-Br-cADPR (fig. 3). Furthermore, the endoplasmic reticulum Ca2+-ATPase inhibitor thapsigargin was not able to potentiate Ca2+ induced by agonists of the RyR (data not shown). These observations further support the hypothesis that halothane at the concentration tested sensitizes the RyR.

In conclusion, we present evidence that halothane can interact with the new second messenger system modulated by cADPR in tracheal smooth muscle cells. It is possible that the effect of halothane on cADPR may play an important role in the complex effect of volatile anesthetics on intracellular Ca2+ homeostasis in these cells. Halothane can promote depletion of the intracellular Ca2+ stores by a mechanism that appears to involve leakage of Ca2+ through both the IP3 and RyR. Our current results indicate that halothane-induced Ca2+ leakage may involve sensitization of the RyR to endoge-
nous levels of intracellular cADPR. Increased sensitivity of the RyR to endogenous cADPR induced by halothane may lead to depletion of sarcoplasmic reticulum intravesicular Ca$^{2+}$ levels. This decrease in SR Ca$^{2+}$ will decrease the amount of Ca$^{2+}$ available for SR Ca$^{2+}$ release during agonist stimulation leading to decreased contraction.

References

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