

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

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RELEASE of  $\text{Ca}^{2+}$  from intracellular stores is a widespread component in several signaling pathways.<sup>1-2</sup> It is well known that inositol-1,4,5-tris-phosphate ( $\text{IP}_3$ ) triggers  $\text{Ca}^{2+}$  release from intracellular stores<sup>1</sup>; however, cells possess other intracellular  $\text{Ca}^{2+}$  releasing systems,<sup>1-3</sup> including the so-called  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$  release system, mediated by the ryanodine receptor-channel (RyR).<sup>1-2</sup> Recently it was found that the endogenous nucleotide cADP-ribose (cADPR) is a potent activator of the RyR,<sup>1-2</sup> and this nucleotide has been proposed to be a second messenger in several intracellular signaling pathways.<sup>1</sup> Biosynthesis of cADPR from  $\beta$ -NAD is catalyzed by adenosine diphosphate (ADP)-ribosyl cyclase, and cADPR is hydrolysis is mediated by the cADPR hydrolase to ADP-ribose (ADPR).<sup>1</sup>

Volatile anesthetics have multiple actions on intracellular  $\text{Ca}^{2+}$  homeostasis,<sup>4-9</sup> including activation of the RyR and sensitization of this channel to pharmacologic agonists such as caffeine and ryanodine.<sup>4-9</sup> Recently, we reported that halothane can sensitize the RyR to cADPR in sea urchin egg homogenates.<sup>7</sup> It has been previously shown that the cADPR system is functional in porcine smooth muscle cells.<sup>10</sup> In fact, in porcine airway smooth muscle cells cADPR has been shown to be a second messenger responsible for intracellular  $\text{Ca}^{2+}$  increase induced by acetylcholine.<sup>10</sup> In the current study, we found that halothane potentiates the cADPR-induced  $\text{Ca}^{2+}$  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  $\text{Ca}^{2+}$  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  $\mu\text{g}/\text{ml}$  leupeptin. Microsomes were prepared by differential centrifugation as described before.<sup>8</sup>  $\text{Ca}^{2+}$  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  $\text{MgCl}_2$ , 2 U/ml creatine kinase, 4 mM phosphocreatine, 1 mM adenosine triphosphate (ATP), 4 mM Pi, 25  $\mu\text{g}/\text{ml}$  leupeptin, 20  $\mu\text{g}/\text{ml}$  aprotinin, and 100  $\mu\text{g}/\text{ml}$  soybean trypsin inhibitor and 3  $\mu\text{M}$  fluo-3 was added. Fluo-3 fluorescence was monitored at 490 nm excitation and 535 nm emission in a 250- $\mu\text{l}$  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  $\text{Ca}^{2+}$  additions using separate samples of the same microsomal preparation.

### Materials

Fluo-3 was purchased from Molecular Probes (Eugene, OR);  $\text{IP}_3$ , 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  $\pm$  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.<sup>10-12</sup> Furthermore, cADPR is able to activate the RyR in tracheal smooth muscle cells.<sup>10</sup> Tracheal smooth muscle cell microsomes supplemented with an ATP-regenerative system sequester added  $\text{Ca}^{2+}$  into vesicular stores in an ATP-dependent manner and release  $\text{Ca}^{2+}$  in response to  $\mu\text{M}$  concentrations of cADPR (fig. 1). The cADPR-induced  $\text{Ca}^{2+}$  release was inhibited by several inhibitors of the RyR such as spermine, ruthenium red, and the specific cADPR inhibitor 8-Br-cADPR (fig. 1).<sup>13</sup> However,  $\text{Ca}^{2+}$  release induced by cADPR was not in-

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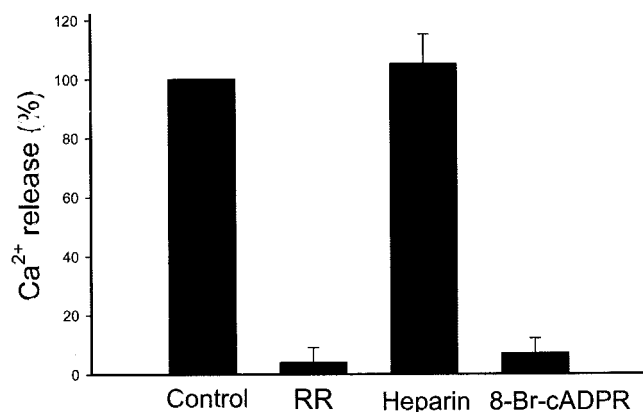


Fig. 1. Effect of inhibitors on Ca<sup>2+</sup> release induced by cADPR. Experiments were carried as described in Materials and Methods. Values are mean  $\pm$  SD. The Ca<sup>2+</sup> release induced by 10  $\mu$ M cADPR was tested in microsomes pretreated with either no addition (control), 30  $\mu$ M ruthenium red (RR), 1 mg/ml heparin (Heparin), or 10  $\mu$ M 8-Br-cADPR (a specific cADPR antagonist).

hibited by 1 mg/ml heparin, a specific antagonist of the IP<sub>3</sub> channel.<sup>13</sup> These observations confirmed the evidence that cADPR activates Ca<sup>2+</sup> release through the RyR in tracheal smooth muscle.

#### Effect of Halothane on cADPR-Induced Ca<sup>2+</sup> Release

We investigated the effect of 350  $\mu$ M halothane on the cADPR induced Ca<sup>2+</sup> release. Figure 2 demonstrates the effect of halothane on cADPR-induced Ca<sup>2+</sup> release, addition of 350  $\mu$ M halothane did not produce any significant Ca<sup>2+</sup> release by itself; however, it sensitized the Ca<sup>2+</sup> 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  $\mu$ M halothane (fig. 2B), although the maximum Ca<sup>2+</sup> release response to cADPR was not enhanced by halothane. Thus halothane increased the apparent affinity of the Ca<sup>2+</sup>-induced Ca<sup>2+</sup> release to stimulation by cADPR. We also observed that 350  $\mu$ M halothane had no effect steady-state Ca<sup>2+</sup> levels in the microsomal preparations. The effect of halothane on the cADPR-induced Ca<sup>2+</sup> release was abolished by the cADPR antagonist 8-Br-cADPR (fig. 3). Furthermore, the endoplasmic reticulum Ca<sup>2+</sup>-ATPase inhibitor thapsigargin was not able to potentiate Ca<sup>2+</sup> 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 Ca<sup>2+</sup> homeostasis in these cells. Halothane can promote depletion of the intracellular

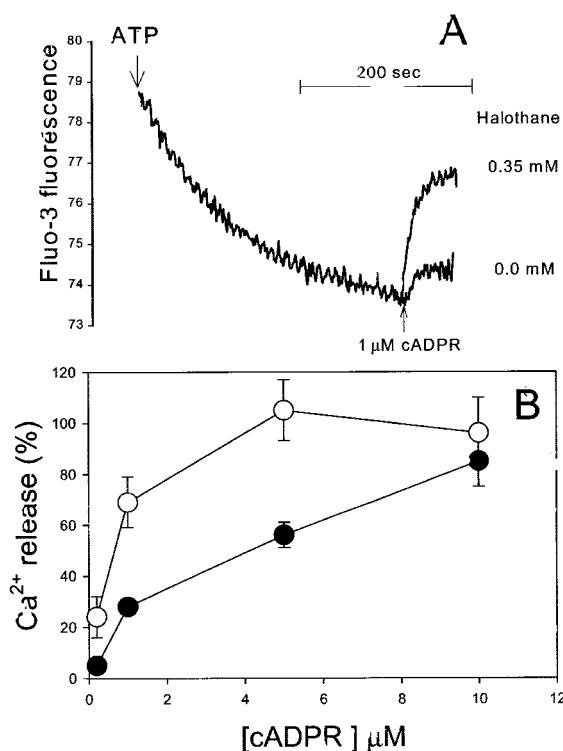


Fig. 2. Effect of halothane on cADPR-induced Ca<sup>2+</sup> release in tracheal smooth muscle microsomes. In (A) Ca<sup>2+</sup> uptake was initiated by the addition of 1 mM ATP in the presence or not of 0.35 mM halothane after the uptake reached steady state cADPR was added and Ca<sup>2+</sup> release was monitored with fluo-3 (as described in Materials and Methods). In (B) dose dependence for cADPR is shown. Ca<sup>2+</sup> release was induced by different concentrations of cADPR in the absence, or in the presence of 0.35 mM halothane.

Ca<sup>2+</sup> stores by a mechanism that appears to involve leakage of Ca<sup>2+</sup> through both the IP<sub>3</sub> and RyR.<sup>6</sup> Our current results indicate that halothane-induced Ca<sup>2+</sup> leakage may involve sensitization of the RyR to endoge-

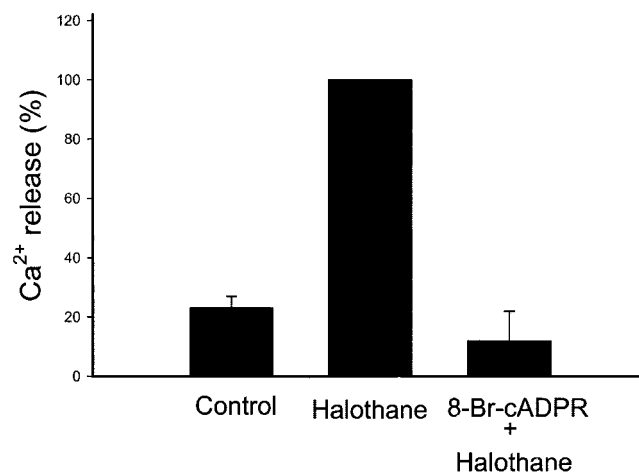


Fig. 3. Effect of 8-Br-cADPR on halothane induced sensitization of the cADPR-induced Ca<sup>2+</sup> release. Experiments were carried out as described in Materials and Methods. Values are mean  $\pm$  SD. The Ca<sup>2+</sup> release induced by 1  $\mu$ M cADPR was tested in microsomes pretreated with no addition (control), 350  $\mu$ M halothane (halothane), or 350  $\mu$ M halothane and 10  $\mu$ M 8-Br-cADPR (halothane + 8-Br-cADPR).

nous levels of intracellular cADPR. Increased sensitivity of the RyR to endogenous cADPR induced by halothane may lead to depletion of sarcoplasmic reticulum intravesicular  $\text{Ca}^{2+}$  levels. This decrease in SR  $\text{Ca}^{2+}$  will decrease the amount of  $\text{Ca}^{2+}$  available for SR  $\text{Ca}^{2+}$  release during agonist stimulation leading to decreased contraction.

## References

1. Dousa, TP, Chini EN, Beers KW: Adenine nucleotide dinucleotide diphosphate: Emerging second messengers acting via intracellular  $\text{Ca}^{2+}$  release. *Am J Physiol Cell Physiol* 1996; 271:C1007-24
2. Coronado, R, Morrisette J, Sukhareva M, Vaughan DM: Structure and function of ryanodine receptor. *Am J Physiol Cell Physiol* 1994; 266:C1485-504
3. Chini, EN, Beers KW, Dousa TP: Nicotinic acid adenine dinucleotide phosphate (NAADP) triggers a specific calcium release system in sea urchin eggs. *J Biol Chem* 1995; 270:3216-23
4. Beltran, M, Bull R, Donoso P, Hidalgo C:  $\text{Ca}^{2+}$ - and pH-dependent halothane stimulation of  $\text{Ca}^{2+}$  release in sarcoplasmic reticulum from frog muscle. *Am J Physiol Cell Physiol* 1996; 271:C540-6
5. Blanck TJ, Peterson CV, Baroody B, Tegazzin V, Lou J: Halothane, enflurane, and isoflurane stimulate calcium leakage from rabbit sarcoplasmic reticulum. *ANESTHESIOLOGY* 1992; 76:813-21
6. Pabelick, CM, Prakash YS, Kannan MS, Warner DO, Sieck GC: Effects of halothane on sarcoplasmic reticulum calcium release channels in porcine airway smooth muscle cells. *ANESTHESIOLOGY* 2001; 95:207-15
7. Chini EN: Effect of volatile anesthetics on cADP-ribose-induced  $\text{Ca}^{2+}$  release system. *J Appl Physiol* 2001; 91:516-21
8. Chini EN, Walker H: FK506 (tacrolimus) increases halothane-induced  $\text{Ca}^{2+}$  release from skeletal muscle sarcoplasmic reticulum. *ANESTHESIOLOGY* 2000; 92:1361-5
9. Connelly TJ, Coronado R: Activation of the  $\text{Ca}^{2+}$  release channel of cardiac sarcoplasmic reticulum by volatile anesthetics. *ANESTHESIOLOGY* 1994; 81:459-69
10. Prakash YS, Kannan MS, Walseth TF, Sieck GC: Role of cyclic ADP-ribose in the regulation of  $[\text{Ca}^{2+}]_i$  in porcine tracheal smooth muscle. *Am J Physiol* 1998; 274:C1653-60
11. White TA, Johnson S, Walseth TF, Lee HC, Graeff RM, Munshi CB, Prakash YS, Sieck GC, Kannan MS: Subcellular localization of cyclic ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase activities in porcine airway smooth muscle. *Biochim Biophys Acta* 2000; 1498(1):64-71
12. De Toledo FGS, Cheng J, Liang M, Chini EN, Dousa TP: ADP-ribosyl cyclase in rat vascular smooth muscle cells properties and regulation. *Circ Res* 2000; 86:1153-9
13. Chini EN, Beers KW, Chini CS, Dousa TP: Specific modulation of cyclic ADP-ribose induced  $\text{Ca}^{2+}$  release by polyamines. *Am J Physiol* 1995; 269:C1042-7