162. *Malignant Hyperpyrexia*

Effect of Halothane on Single Skinned Muscle Fibers

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Halothane is implicated to pathogenesis of malignant hyperpyrexia (MHP), but its exact mode of action is still controversial.^{1),2)} We reported that halothane released calcium (Ca) from the sarcoplasmic reticulum (SR).^{3),4)} We now describe the effects of halothane in 2 cases of MHP, using the skinned fiber preparation: Ca was released from SR at lower concentrations of halothane in MHP than in controls.

Methods. Biopsy was taken from rectus femoris in two patients, 38 and 29 year old males, who were recovered from episodes of MHP. Control muscle was obtained in the course of orthopedic operations from 5 adults without obvious neuromuscular complications.

Preparations of the skinned fiber and experimental procedures were essentially as previously described,⁵⁾ except that experiment was performed at 23°C. The skinned fiber was incubated for 2 minutes in solution containing 10^{-6} M free Ca ion (pCa 6). During the incubation, the fiber accumulated Ca in the SR space (Ca loading of the SR). In another experiment, we found that there was no accumulation of Ca ion by mitochondria at pCa 6. After Ca loading, a skinned fiber was exposed to certain concentration of halothane. After halothane was washed, the fiber was subsequently treated with 25 mM caffeine to deplete remaining Ca from the SR. The same procedure was repeated at each concentration of halothane (0.01, 0.03, 0.06, 0.1, 0.15, 0.2 or 0.3% v/v). The lowest concentration of halothane was determined as threshold where contraction was first induced in the fiber.

Response to halothane differed among different types of muscle fiber (unpublished observation). The type of muscle fiber under study was checked by its sensitivity to strontium ion.⁶⁾ Fibers prepared

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Fig. 1a and 1b. The effect of halothane on a skinned fiber from MHP (1a), and on a control fiber (1b).

All runs were made after Ca loading of the SR. Hx: x% (v/v) halothane in G-0.1, Caf: 25 mM caffeine in G-0.3, G: G-0.3. For further explanation, see in the text.

from MHP specimens mostly belonged to type 2 fiber, so presentation of results in this paper was limited to type 2 fibers.

Solutions. The solution for relaxation (G-2) contained 4 mM ATP- Na_2 , 4 mM MgSO_4 , 2 mM EGTA, 130 mM K-methansulfonate and 20 mM Tris-20 mM maleate (pH 6.8, $I=0.22$). In G-0.1 or G-0.3, EGTA was lowered to 0.1 mM or 0.3 mM, but the other components were as in G-2. In pCa 6 solution, only EGTA of G-2 was replaced with 10 mM EGTA and 3.3 mM CaSO_4 . Halothane or caffeine was dissolved in solution G-0.1 or G-0.3, respectively. Halothane was usually redistilled to remove thymol, a stabilizer. Halothane solutions were prepared immediately before use in stoppered tubes. 0.1% (v/v) halothane was approximately equivalent to 10 mM.

Results. 1) *Examples of typical records (Fig. 1a and 1b).* A skinned fiber from MHP developed tension when exposed to 0.01% halothane (Fig. 1a, middle). After halothane was washed, 25 mM caffeine produced another contraction. In the upper and lower record of Fig. 1a, the fiber was exposed to caffeine without prior halothane treatment. The magnitude of caffeine contracture (i.e. area under contraction curve) was assumed to indicate the amount of Ca in the SR. The size of caffeine contracture was smaller in the lower trace,

Fig. 2. Threshold of halothane concentration for tension generation. Threshold was determined as described in Methods in six fibers from 2 cases of malignant hyperpyrexia (MHP) and seven fibers from five controls.

suggesting Ca-accumulating capacity of MHP fiber was reduced in the course of experiment.

In a control fiber (Fig. 1b), halothane below 0.06% did not produce any tension. 0.1% halothane first induced a small tension. The magnitude of caffeine contracture was larger after exposure to halothane (2nd, 3rd or 4th record) than without previous halothane treatment (top and bottom records). This seemed due to the fact that halothane slightly enhanced sensitivity to Ca ion of the contractile system.⁴⁾ In the control fiber, Ca-accumulating activity of the SR was not appreciably altered during the course of experiment (Compare the magnitude of caffeine contracture between top and bottom record).

2) *Threshold of halothane for tension generation (Fig. 2).* In all 6 fibers examined from MHP patients, contracture appeared at concentrations of halothane below 0.06%. In fibers from controls, tension was never developed by halothane below 0.06%. The threshold was ranged between 0.1 and 0.3%.

Discussion and conclusion. In previous report, we demonstrated in guinea pig muscle that halothane induced contraction by releasing Ca from the SR.^{3),4)} In human skinned fiber, halothane also produced tension by a similar mechanism, since contraction was induced only after Ca loading of the SR.

In fibers from MHP, halothane induced contracture at lower concentrations than in controls. The Ca-accumulating capacity of SR seemed to deteriorate faster in MHP than in controls. Halothane is assumed to release Ca from the SR by enhancing "Ca-induced Ca release" mechanism.^{3),4)} In this respect, we speculate that the "Ca-induced Ca release" mechanism may be more easily activated in skeletal muscle of MHP. Caffeine, another accelerator of Ca-induced Ca release mechanism,⁷⁾ had been more effective in muscle of MHP.¹⁾ Further studies on this line are currently in progress.

In the present study, 0.01% halothane developed contracture in a fiber from MHP. This concentration may be approximate to that of body fluid during anesthesia. However, concentration of halothane itself discussed in the paper might be of little clinical relevance when the following factors are considered: In the present study, Ca in the SR after loading was much more than in a physiological resting state. Halothane was applied in solution containing 0.1 mM EGTA, whereas no similar Ca chelater is present in physiological environment. Free magnesium level was maintained at 0.9 mM, but there is little information on the intracellular level of this cation in human muscle. And analyses were performed at 23°C instead of 37°C.

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