Halothane Changes Free Fatty Acid Contents of Heart and Liver Tissues in Guinea Pigs

HALOTAN KOBAY KALP VE KARACİĞER DOKULARININ SERBEST YAĞ ASİDİ İÇERİĞİNİ DEĞİŞTİRİR

Mustafa KAVUTCU*, Murat KAÇMAZ*, Bayazit DİKMEN**, Ömer KURTİPEK**, Servet ÇETE***, H. Serdar ÖZTÜRK*, Orhan CANBOLAT*

* Dept. of Biochemistry, Medical School of Ankara University,
** Dept. of Anesthesiology, Numune Hospital and Medical School of Ankara University,
*** Dept. of Biochemistry, Gazi University, Science and Education Faculty, Ankara, TURKEY

Summary

The effects of halothane on free fatty acid levels of heart and liver tissues were determined. Fourteen guinea pigs were used in the experiments. Seven of the subjects were seperated as control group and other subjects were exposed to halothane (1.5 % v/v) and oxygen (100%) mixture for the period of 30 min for 3 days. The animals were then killed by cervical dislocation and their livers and hearts were removed. Tissues were first washed with deionized water to discard blood and then homogenized in a homogenize/: Free fatty acid analyses of the tissues were made by using gas chromatographic method. In the halothane-treated animals, saturated free fatty acid (12:0, 14:0, 16:0, 18:0) levels were found decreased and mono unsaturated free fatty acid (18:1) level increased in the heart and liver tissues compared with those of control animals.

Results suggest that exposure to halothane may cause alterations in free fatty acid composition of the heart and liver tissues.

Key Words: Halothane, Fatty acids. Heart, Liver

T Klin J Med Res 1998, 16:84-87

Özet

Halotanın kobay kalp ve karaciğer dokularındaki serbest yağ asidi düzeyine olan etkileri araştırıldı. Çalışmada 14 kobay kullanıldı. Deneklerin 7 tanesi kontrol grubu olarak kullanıldı. Geri kalan deneklere, halotan (%ıl.5v/v) ve oksijen (%100) karışımı 3 gün boyunca 30'ar dakika süre ile verildi. Servikal dislokasyon ile öldürülen hayvanların karaciğer ve kalp dokuları çıkarılıp deiyonize su ile yıkanarak kandan temizlendi ve homojen ize edildi. Dokuların serbest yağ analizleri gaz kromatograjisi ile yapıldı. Halotanla muamele edilen hayvanlarda kontrol grubuna nisbetle doymuş serbest yağ asidi (12:0, 14:0, 16:0, 18:0) düzeyleri düşük bulunurken, tekli doymamış serbest yağ asidi (18:1) düzeyi yüksek olarak bulundu.

Sonuçlar halotana maruz kalcın kalp ve karaciğer dokularındaki serbest yağ asidi kompozisyonunun değişebildiğini göstermektedir.

Anahtar Kelimeler: Halotan, yağ asitleri, kalp, karaciğer

T Klin Araştırma 1998, 16:84-87

Membrane function is known to be dependent on the interaction between each of its components (1). The changes occurring in lipid fraction due to several factors are likely to influence membrane stability and function (2). It has been suggested that altered phospholipid and fatty acid (FA) compositions in membrane structure might make them more susceptible to exogenous membrane perturbations as occured in halothane toxicity (3). Multilayer sticking of anesthetic agents within the membrane has been proposed as the mechanism by which the membrane stability is altered (4). Evidence is accumulating that generalized membrane alterations may exist within all cell membranes including those of non-muscle cells (5-9).

Anesthesia-induced malignant hypcrthennia (MH) is a potentially fatal disease that is believed to result from an uncontrollable increase in myoplasmic Ca2+ upon challenge with halogenated anesthetics including halothane (10-12). Since MH syndrome in human is only manifest on exposure to

Received: July 13, 1998

Correspondence: Orhan CANBOLAT
Dept. of Biochemistry,
Medical School of Ankara University,
06100 Sıhhiye, Ankara, TURKEY

anesthetic agents (11), it seems important to examine possible reasons of halothane-induced structural changes occurring at tissue and organ levels. Some researchers have suggested possible link between altered FA metabolism, and MH (13,14). Therefore, we think that investigation of the effects of halothane on tissue free fatty acid (FFA) levels might be helpful in elucidating the mechanisms leading to MH triggered by some volatile anaesthetics.

Materials and Methods

Animals: Fourteen guinea pigs (2 months age and approx. 450 g weight) were used throughout the experiments.This study was performed according to the National and Institutional Animal Care Committees and approved by Ethical Committee of our institution. The animals were fed on a laboratory diet during that time. Seven of the subjects were seperated as control group and other subjects were exposed to halothane (1.5 % v/v) and oxygen (100%) mixture at the rate of 2L/min for the period of 30 min for 3 days. Gas mixture inspiration was made by using face masks. At the end of the experiments, the animals were killed by cervical dislocation and their livers and hearts were taken and kept in an ice bath until homogenisation for about one hour.

Preparation and analysis: Livers and hearts were first washed with dcionized water to discard blood and then homogenized (B.Braun Melsungen model) at 1000 U for about 3 min. FFA analyses were made by gas chromatographic method as described (15).

Statistical analysis was made by using Tukey's HSD (honestly significant difference, ANOVA) procedure.

Results

Results are shown in Table 1 as % of total FFA content. As shown, the levels of all FFAs studied were found to be decreased but unsaturated oleic acid (CI8:1) levels were significantly increased in both heart and liver tissues from the animals treated with halothane compared with those of control animals.

Discussion

Although the mechanism of action of volatile anesthetics has not been known in detail, it has been suggested that some of the halothane-induced cellular changes are primarily modulated by FFAs (14,16). It has been suggested that FA modulation of ion channels may have important implications for general muscle physiology and skeletal muscle disorders like malignant hyperthermia (16). In fact, evidence is accumulating that in MH, generalized structural alterations exist within all muscle membranes and in those of non-muscle cells (5-8). We think that in as much as MH is most often triggered by some volatile anesthetics including halothane under some conditions influencing membrane stability (17,18) and, FA modulation may have impli-

Table 1. Mcan±SD values of free fatty acid compositions (%of total FFA content) of heart and liver tissues from 7 guinea pigs exposed to halothane and from 7 control animals.

Groups	12:0	14:0	16:0	18:0	18:1
1	2.8±1.2	4.8±2.0	28.7±7.6	41.0±4.9	14.4±3.1
2	2.0+1.9	0.74±0.26	21.9±6.6	32.3±8.6	35.2±2.1
3	3.2±2.8	4.6±3.8	25.2±11.6	41.1±7.0	16.6±9.7
4	1.2±1.7	0.9±0.1	25.7±2.2	I9.7±7.2	45.2±9.3
			Statistical analysis		
1-2	n.s.	p<0.0005	p<0.05	p<0.025	p<0.0005
3-4	p<0.05	p<0.025	n.s.	p<0.0005	p<0.0005

n.s.:p>0.05

Groups: 1: Liver control, 2: Liver halothane, 3: Heart control, 4: Heart halothane.

12:0 Laurie acid, 14:0 Myristic acid, 16:0 Palmitic acid, 18:0 Stearic acid, 18:1 Oleic acid.

cations in these events, it is important to investigate the effect of halothane on the FA metabolism not only in skeletal muscles but also in some other muscles and tissues.

As seen from Table 1, there arc important differences between the levels of some FFA levels in the heart and liver tissues from halothanc-trcatcd and control animals. In this regard, the amount of unsaturated oleic acid was found to be significantly higher in the halothane group compared with control group. This change may be of importance in the mechanism of action of halothane and in the MH triggered by volatile anesthetics.

It is possible that increased concentration of oleic acid after halothanc-treatment may alter membrane structure of the cells of affected tissues and may increase membrane fluidity. This hypothesis is in agreement with some researchers' previous suggestion that halothane-induced Ca2+ release and Na+ currents arc modulated by FAs and, FA modulation of ion currents may have important implications for general muscle physiology and some muscle diseases including MH (16). This is coincidental with Fletcher's suggestion that the greater sensitivity of the patients with MH to halothane can be explained by elevated production of FAs, in particular, of unsaturated FAs in the skeletal muscle tissues exposed to halothane (13,19). As schematized by Seewald ct al. (3), halothane and possibly other volatile anesthetics alter membrane properties by increasing unsaturated FA levels within the tissues affected and this change then lead to intracellular elevation of ions, especially Ca2+ and Na+ releases. These changes cause very important alterations in some cellular functions such as muscle contraction, nerve impulse transmission etc., some of which are thought to be the basis of the mechanism of action of halothane and of MH triggered by volatile anesthetics.

To sum up, our results show that halothane causes elevated concentration of oleic acid (possibly, of some other unsaturated FAs not studied in this study) in the heart and liver tissues as previously established in skeletal muscle.

REFERENCES

1. Rothman .IF, Lcnard J. Membrane asymmetry.The nature of membrane asymmetry provides clues to the puzzle of how membranes are assembled. Science 1977:195:743-53.
2. Zakim S, Vessey DA. The importance of phospholipid-protein interactions for regulation of the activities of the membrane-bound enzymes. In: Herman RH, ed. Principles of metabolic control in mammalian systems. New York, London, Plenum Press, 1980:337-71.
3. Seewald MJ, Cichinger H M, Iaizzo PA. Malignant hyperthermia: an altered phospholipid and fatty acid composition in muscle membranes. Acta Anaesth Scand 1991;35:380-6.
4. Yoshida T, Okabawahi H, Kamara H, Ucda 1. Saturable and unsaturable binding of volatile anesthetic enflurane with model lipid vesicle membranes. Biochim Biophys Acta 1989;9:287-93.
5. Iaizzo PA, Lehman-Horn F, Taylor SR, Gallant EM. Malignant hyperthermia: effects of halothane on the surface membrane. Muscle Nerve 1989;12:173-9.
6. Klip A, Mills GB, Britt BA, Elliott ME. Halothane-dependent release of intracellular Ca2+ in blood cells in malignant hyperthermia. Am J Physiol 1990; 258: C495-C503.
7. O'Brein PJ, Kalow BI, Brown BD et al. Porcine malignant hyperthermia susceptibility: halothane-induced increase in cytoplasmic free calcium in lymphocytes. Am J Vet Res 1989;50:131-5.
8. Ohnishi ST, Ohnishi T. Halothane induced disorder of red cell membranes of subjects susceptible to malignant hyperthermia. Cell BiochemFunct 1988;6:257-61.
9. Pessah IN, Lynch C, Gronert GA. Complex pharmacology of malignant hyperthermia. Anesthesiology I996;84:I275-9.
10. Wapplcr F, Rocwer N, Kochling A. ct al. Effects of serotonin2 receptor agonist DOI on skeletal muscle specimens from malignant hyperthermia-susceptible patients. Anesthesiology 1996;84:1280-7.
11 .Rosenberg H, Fletcher JE. Side effects and a rational approach to selection. In: Azar I, ed. Muscle Relaxants. New York, Marcel Dekker Inc., 1987:115-48.
12.Akata T, Boyle WA. Dual actions of halothane on intracellular calcium stores of vascular smooth muscle. Anesthesiology 1996;84:580-595.
13.Fletcher JE, Mayerberger S, Trippolits L. et al. Fatly acid markedly lower the threshold for halothane-induced calcium release from the terminal sistcrna in human and porcine normal and malignant hyperthermia susceptible skeletal muscle. Life Sci 1991;49:1651-7.
H.Flctcher JE, Jiang MS, Tripolitis L. et al. Interactions in red blood cells between fatty acids and either snake venom cardiotoxin or halothane. Toxicon 1990;28:657-67.
15.Bondia EM, Castellot A1, Lopez MC, Rivero M: Determination of plasma fatty acid composition in neonates gas chromatography. J Chromatogr B 1994;658:369-74.

lô.Fletcher JE. Wieland S.I, Bcccli J. ct ul. Modulation of Ca2+ and Na+ channel function in skeletal muscle by fatty acids: Excitation-contraction coupling in skeletal, cardiac, and smooth muscle, Frank GB. et al (éd.). New York, Plenum Press. 1992:329-31.

17. Groncrt GA, Mott J. Lee J. Aetiology of malignant hyperthermia. Brit J Anaesth 1988;60:253-67.

18. Eichinger M, Seewald M, laizzo PA. Membrane phospholipid concentrations in cardiac and skeletal muscles from normal and malignant hyperthermia susceptible pigs. In: Proceedings of EAP Seminar, No. 36, Netherlands, Pudoc Wageningen, 1989.

9.Fletcher JE, Tripolitis L, Erwin K. et al. Fatly acid modulate calcium-induced calcium release from skeletal muscle heavy sarcoplasmic reticulum fractions: implications for malignant hyperthermia. Biochcm Cell Biol 1990;68:l 195-201.