Vol. 186, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1992 Pages 595-600

VOLATILE ANESTHETICS SELECTIVELY ALTER [^{3}H]RYANODINE BINDING TO SKELETAL AND CARDIAC RYANODINE RECEPTORS[1]

Timothy J. Connelly[a], Roque-El Hayek[a,b], Ben F. Rusy[a] and Roberto Coronado[b]

Departments of [a]Anesthesiology and [b]Physiology,
University of Wisconsin-Madison,
Madison, Wisconsin 53792

Received June 1, 1992

SUMMARY. The effect of clinical concentrations of volatile anesthetics on ryanodine receptors of cardiac and skeletal muscle sarcoplasmic reticulum was evaluated using [^{3}H]ryanodine binding. At 2 volume percent, halothane and enflurane stimulated binding to cardiac SR by 238% and 204%, respectively, while isoflurane had no effect. In contrast, halothane and enflurane had no effect on [^{3}H]ryanodine binding to skeletal ryanodine receptors, while isoflurane produced a significant stimulation. These results suggest that volatile anesthetics interact in a site-specific manner with ryanodine receptors of cardiac or skeletal muscle to effect Ca^{2+} release-channel gating.

Halothane, isoflurane and enflurane are halogenated inhalational anesthetics that depress myocardial contractility (1). A preponderance of evidence suggests that these agents restrict the availability of Ca^{2+} for excitation-contraction coupling (2). This alteration may result from an impairment of the Ca^{2+} uptake and release by the SR, although a specific target has not been identified. Ca^{2+} release from the SR of cardiac and skeletal muscle is controlled by the ryanodine receptor, a 550 kDa protein that binds the plant alkaloid ryanodine at nanomolar concentrations (3). Since the ryanodine binding site is formed when the channel is in the open conformational state, [^{3}H]ryanodine binding activity has been widely used as

[1] Supported by International Anesthesia Research Society, NIH (GM 36852), American Heart Association, and Muscular Dystrophy Association.

Abbreviations: SR, sarcoplasmic reticulum; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)N,N,N',N-tetraacetic acid; Pipes, piperazine-N,N'-bis-(2-ethanesulfonic acid).

0006-291X/92 $4.00

a powerful index of channel activity (4,5). The purpose of the present study was to use [^{3}H]ryanodine binding to investigate the effect of the volatile anesthetics on the conformational states of the SR Ca^{2+} release channel of cardiac and skeletal SR.

EXPERIMENTAL PROCEDURES

Isolation of ryanodine receptors. With institutional approval, adult swine were anesthetized with sublethal doses of thiobarbiturate and euthanized by exsanguination. Skeletal muscle was harvested from the vastus and trapezius muscles and immediately frozen in liquid nitrogen. The entire heart was excised, rapidly dissected free of connective tissue and frozen in liquid nitrogen. Skeletal and cardiac muscle was then stored at -80°C for later use. Heavy SR was prepared from skeletal muscle and ventricular free wall by a differential and sucrose gradient method (6) in the presence of protease inhibitors.

[^{3}H] ryanodine binding. All assays were performed in 800 µl Teflon-capped vials (Kimax, Owens-Illinois) for 120 minutes at 37°C using 50-75 µg of SR protein in the presence of 7 nM [^{3}H]ryanodine (60 mCi/mmole-DuPont New England Nuclear, Boston, MA). Incubation medium consisted of 750 µl of buffer (Na Pipes 10mM; KCl 0.2 M, pH 7.2), equilibrated with N_2 or a N_2-anesthetic mixture delivered to a reservoir by a calibrated vaporizer through scinterred glass. Isoflurane and enflurane were obtained commercially (Anaquest, Madison, WI) and thymol-free halothane was generously provided by Halocarbon Laboratories (Augusta, NC). Free Ca^{2+} was 10^{-5} M in all experiments, except in that addressing the calcium dependence of ryanodine binding. The stability constants of Fabiato (7) were used to prepare $CaCl_2$-EGTA (1 mM) buffers to set free [Ca^{2+}] at the desired value. All assays were completed in quadruplicate. At the end of the incubation period, 24 samples were filtered simultanously through Whatman GF/C filters using a Brandel Cell Harvester (Brandel, Gaithersberg, MD) and counted by liquid scintillation. The specific binding was calculated as the difference between the binding in the abscence (total binding) and the presence of (nonspecific binding) 100 µM unlabelled ryanodine.

Measurement of anesthetic concentrations. At the end of the incubation period, but before filtering, 25 µl was aspirated from the head space gas (approximately 50 µl) in each vial with a gas-tight syringe. The concentration was measured by gas chromatography (Varian Model 3100, Sugarland, TX) with a flame ionization detector and referenced to calibration gases commercially prepared in N_2 (Scott Medical Products, Plumsteadville, PA). The mean anesthetic concentration for quadruplicate samples was used for that set. The coefficient of variation for gas chromatographic measurements of standard gases was 10% over the course of this study.

RESULTS

Anesthetics were delivered as a gas using a calibrated vaporizer into the [^{3}H]ryanodine binding reaction mixture. The protocol (see Methods)

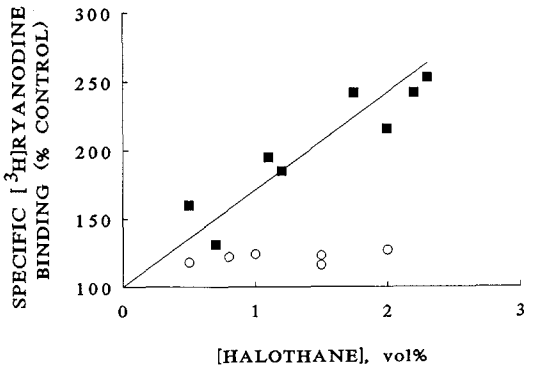

Figure 1. Dose-response effect of halothane on cardiac (squares) and skeletal (circles) [^{3}H]ryanodine binding, plotted as percent of control binding (no halothane). The solid line represents a linear regression of the data shown (r=0.92). Each data point the mean of four separate assays.

ensured that anesthetics would be equilibrated with the SR-containing aqueous phase in the same way as would occur when tissue is exposed to an anesthetic *in situ*. Fig. 1 (filled squares) shows that halothane caused a dose-dependent stimulation of [^{3}H]ryanodine binding to cardiac SR. Stimulation was linear over the clinical concentration range of 0.5 to 2 volume percent, which corresponds to an aqueous phase concentration of 0.25 to 1 mM halothane at room temperature. Actual concentrations at the end of the incubation period were verified by gas chromatography. The stimulatory effect of halothane was specific for the cardiac ryanodine receptor inasmuch as halothane had little or no effect on [^{3}H]ryanodine binding to ryanodine receptors of skeletal muscle assayed in a similar manner (open circles). In separate experiments (not shown), halothane also had no effect on [^{3}H]ryanodine binding to brain ryanodine receptors assayed in rabbit brain microsomes (8). Thus the effect was specific for the cardiac isoform in spite of the fact that cardiac and brain ryanodine receptors are structurally homologous (9).

Fig. 2 compares the effect of halothane with that of two other commonly used anesthetics, the stereoisomers isoflurane and enflurane. The three anesthetics were tested at a concentration of 2 volume percent separately on cardiac (left) or skeletal ryanodine receptors (right). Both halothane and enflurane caused a significant stimulation of [^{3}H]ryanodine binding to the cardiac receptor while isoflurane had no effect. On the other hand, isoflurane, but not halothane or enflurane, stimulated [^{3}H]ryanodine binding to the skeletal ryanodine receptor. Thus there was a remarkable

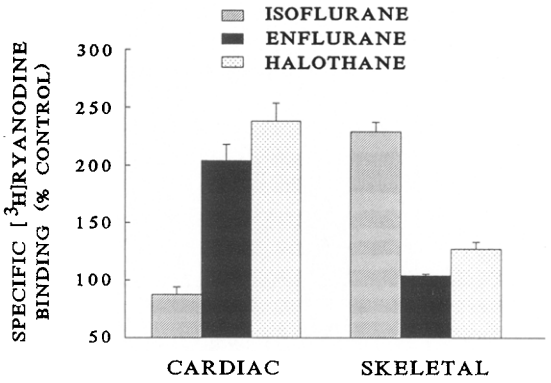

Figure 2. Effect of isoflurane, enflurane and halothane (2 vol%) on [^{3}H]ryanodine binding to cardiac (left) and skeletal (right) SR vesicles, plotted as a percent of control binding (no anesthetic). Each bar represents the mean of two experiments, each completed in quadruplicate assays. Error bars represent the standard deviations of duplicate experiments for each anesthetic.

selectivity of each anesthetic for either cardiac or skeletal ryanodine receptors. Since [^{3}H]ryanodine binding varies with free Ca^{2+} (10), we investigated the Ca^{2+}-dependence of the stimulation produced by halothane in cardiac SR. Fig. 3 shows the Ca^{2+}-dependence of [^{3}H]ryanodine binding in the abscence (triangles) or presence (circles) of 2 volume percent anesthetic. In controls, the Ca^{2+}-dependence was bell-shaped with a maximum at approximately 10 μM. Halothane stimulated binding over the entire range of free Ca^{2+} without producing a change in the overall shape of the curve.

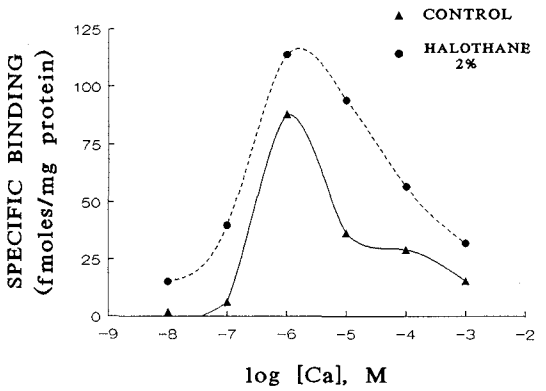

Figure 3. Calcium dependence of [^{3}H]ryanodine binding in the abscence (triangles) and presence (circles) of 2% halothane. Each data point represents a single experiment performed with quadruplicate assays.

DISCUSSION

Contraction studies in isolated myocardial tissue (11-13), skinned myofibrils (14), and isolated myocytes (15-17), have suggested that halothane, the most widely studied anesthetic, decreases the net sequestration of Ca^{2+} by the SR. Exposure of cardiac tissue to halothane leads to myocardial depression and negative inotropy (11-13). Our results are consistent with a direct effect of halothane on the cardiac Ca^{2+} release channel since a dose-dependent stimulation of [^{3}H]ryanodine binding was evident in the clinical range of anesthetic delivered to SR in a gaseous form. The stimulation of binding suggested that halothane increases Ca^{2+} release channel activity. This conclusion is based on the generalized finding that ligands that enhance Ca^{2+} release from the SR also enhance [^{3}H] ryanodine binding, whereas ligands that inhibit Ca^{2+} release also inhibit [^{3}H]ryanodine binding (10). Hence, the open conformational state of the receptor appears to represent the high affinity binding site for ryanodine and binding site density therefore serves as an indicator of the fraction of receptors that are present, at a given ligand concentration, in an open conformation. At this point however, we do not know whether halothane only increases [^{3}H]ryanodine binding site density, as would be required by the interpretation above, or if may also increase [^{3}H]ryanodine binding site affinity. In the latter case, anesthetic effects on [^{3}H]ryanodine binding activity may not be directly related to changes in channel gating. Nevertheless, the increase of cardiac [^{3}H]ryanodine binding produced by halothane and enflurane, but not by isoflurane, is consistent with an alteration of gating because isoflurane causes much less myocardial depression than the other anesthetics (11-13).

The idea that anesthetics may affect Ca^{2+} release channel gating is also supported by the Ca^{2+}-dependence of halothane stimulation. The Ca^{2+} - dependence of [^{3}H]ryanodine binding has been interpreted to reflect the binding of Ca^{2+} to a high affinity and a low affinity site controlling channel gating (10). Thus, Ca^{2+} binding to a high affinity site would increase the open probability of the channel, leading to an increase in the density of sites available for [^{3}H]ryanodine binding. Conversely, Ca^{2+} binding to a low-affinity site would close or inactivate the channel and thus decrease the number of binding sites. Within this scheme, the major effect of halothane appeared to be an increase in the Ca^{2+} sensitivity to both the high and low-affinity Ca^{2+} binding sites controlling channel gating.

The selective interaction of enflurane with cardiac receptors and isoflurane with skeletal receptors is significant since these two anesthetics

are methyl-ethyl ether isomers. These results suggest site-specific interactions of the anesthetics with each receptor type. Skeletal and cardiac ryanodine receptor isoforms are only ≈60% homologous (17), so that different pharmacologic responses to various ligands was not totally unexpected. Yet volatile anesthetics are simple hydrocarbons which have been proposed to act on ion channels through a non-protein mediated interaction. Specifically, volatile anesthetics are classically thought to alter bulk membrane lipid properties in the vicinity of protein channels (18). This explanation seems less plausible, at least for the Ca^{2+} release channel of SR, since enflurane and isoflurane are chemical isomers with similar physical properties. The results presented here may have implications for the mechanism of action of anesthetics in other tissues as well.

REFERENCES

1. Brown BR, Crout JR. (1971) Anesthesiology 34:236-245.
2. Rusy BF, Komai, H. (1987) Anesthesiology 67:745-766.
3. Pessah IN and Zimanyi I. (1991) Mol Pharmacol 39:679-689.
4. Meissner G. (1986) J Biol Chem 261:6300-6306.
5. Mickelson JR, Gallant EM, Litterer LA, Johnson KM, Rempel WE, Louis CL. (1988) J Biol Chem 263:9310-93158.
6. Meissner G. (1983) J Biol Chem 259:2365-2374.
7. Fabiato A. (1983) Am J Physiology 245:C1-C14.
8. McPherson PS, Kim Y-K, Valdivia H, Knudson CM, Takekura H, Franzini-Armstrong C, Coronado R, Campbell KP. (1991) Neuron 7:17-25.
9. Otsu K, Huntington WF, Khanna VK, Zorzato F, Green NM McLennan DH. (1990) J Biol Chem 265(23):13472-13483.
10. Valdivia HH, Hogan K, Coronado R. (1991) Am J Physiol 261:C237-C245.
11. Lynch C, Frazer MJ. (1989) Anesthesiology 70:511-522.
12. Komai H, Rusy BF. (1990) Anesthesiology 72:694-698.
13. Housmans PR, Murat. (1988) Anesthesiology 69:451-463.
14. Su JY, Kerrick WGL. (1978) Pflugers Arch 375:111-117.
15. Wheeler DM, Rice RT, Hansford RG, Lakatta EG. (1988) Anesthesiology 69:578-583.
16. Wilde DW, Knight PR, Sheth N, Williams BS. (1991) Anesthesiology 74:1075-1086.
17. Katsuoka M, Kobayashi K, Ohnishi ST. (1989) Anesthesiology 70:954-960.
18. Franks WP, Lieb WR. (1991) Science 254:427-436.