Anesthesiology
82:1274–1282, 1995
© 1995 American Society of Anesthesiologists, Inc.
J. B. Lippincott Company, Philadelphia

Effects of Propofol on Ca^{2+} Regulation by Malignant Hyperthermia-susceptible Muscle Membranes

Bradley R. Fruen, Ph.D., James R. Mickelson, Ph.D.,† Timothy J. Roghair, B.S.,‡ Lynn A. Litterer, B.S.,§ Charles F. Louis, Ph.D.‖*

Background: **The effects of inhalation anesthetics on Ca^{2+} regulation in malignant hyperthermia–susceptible skeletal muscle are considered to be responsible for triggering malignant hyperthermia. The intravenous anesthetic propofol does not trigger malignant hyperthermia in susceptible patients or experimental animals, suggesting that there are important differences between the effects of propofol and the effects of inhalation anesthetics on Ca^{2+} regulation in malignant hyperthermia–susceptible muscle. Understanding these differences may help to clarify the mechanisms responsible for triggering malignant hyperthermia.**

Methods: **To investigate the effects of propofol on Ca^{2+} regulation by malignant hyperthermia–susceptible skeletal muscle, we determined its effects on the membrane channels and pumps that control myoplasmic Ca^{2+} concentrations: the sarcoplasmic reticulum ryanodine receptor, the transverse tubule dihydropyridine receptor, and the sarcoplasmic reticulum Ca^{2+}–adenosine triphosphatase (Ca^{2+}-ATPase). Terminal cisternae-derived sarcoplasmic reticulum vesicles enriched in the junctional proteins of the sarcoplasmic reticulum and the transverse tubule membranes were isolated from the muscle of malignant hyperthermia–susceptible and normal pigs. Ca^{2+} flux, Ca^{2+}-ATPase, and ligand binding measurements on these isolated vesicle preparations were performed in the presence of varying propofol concentrations.**

Results: **Propofol (10–500 μM) had no effect on ryanodine receptor–mediated Ca^{2+} efflux from muscle membrane vesicles. Propofol (1–100 μM) also had no effect on sarcoplasmic reticulum vesicle [^{3}H]ryanodine binding, whereas higher concentrations (200–300 μM) slightly inhibited [^{3}H]ryanodine binding. Binding of the dihydropyridine receptor Ca^{2+} channel blocker [^{3}H]PN200–110 to these preparations was inhibited by propofol (10–300 μM). Ca^{2+}-ATPase activity was stimulated by 10–100 μM propofol but was inhibited by higher concentrations. In all cases, the effects of propofol on malignant hyperthermia–susceptible and normal membrane preparations were similar.**

Conclusions: **In contrast to malignant hyperthermia–triggering inhalation anesthetics, propofol does not stimulate malignant hyperthermia–susceptible or normal ryanodine receptor channel activity, even at >100 times clinical concentrations. Effects on dihydropyridine receptor and Ca^{2+}-ATPase function, however, are similar to the effects of inhalation anesthetics and require much lower concentrations of propofol. These findings, demonstrating that propofol does not activate ryanodine receptor Ca^{2+} channels, suggest a plausible explanation for why propofol does not trigger malignant hyperthermia in susceptible persons. (Key words: Anesthetics, intravenous: propofol. Hyperthermia: malignant. Muscle, skeletal: calcium channels; sarcoplasmic reticulum. Receptors: ryanodine.)**

Ca^{2+} CONCENTRATIONS in skeletal muscle are regulated by channels and pumps located in the transverse tubule and sarcoplasmic reticulum (SR) membranes. During muscle contraction, dihydropyridine receptor (DHPR) Ca^{2+} channels in the transverse tubule undergo a conformational change in response to surface membrane depolarization that, in turn, effects the opening of ryanodine receptor (RYR) Ca^{2+} channels in the SR.[1,2] Ca^{2+} released to the myoplasm through RYR channels is subsequently resequestered to the SR by the action of the Ca^{2+}–adenosine triphosphatase (Ca^{2+}-ATPase) pump, allowing the relaxation of muscle.

There is now general agreement that skeletal muscle Ca^{2+} regulation is altered in the pharmacogenetic disease malignant hyperthermia (MH).[3] On exposure to inhalation anesthetics, MH-susceptible patients exhibit increased myoplasmic Ca^{2+}, muscle contracture, and accelerated metabolism.[3] Linkage of MH to mutations in the gene encoding the skeletal muscle RYR in a porcine model of MH[4] and in certain human families[5,6] has provided further evidence that defects in skeletal muscle Ca^{2+} regulation are responsible for MH suscep-

* Research Associate, Department of Veterinary PathoBiology.

† Assistant Professor, Department of Veterinary PathoBiology.

‡ Junior Scientist, Department of Veterinary PathoBiology.

§ Scientist, Department of Veterinary PathoBiology.

‖ Professor, Department of Veterinary PathoBiology and Department of Biochemistry.

Received from the Department of Veterinary PathoBiology and the Department of Biochemistry, University of Minnesota, St. Paul, Minnesota. Submitted for publication August 17, 1994. Accepted for publication February 6, 1995. Supported by National Institutes of Health grant GM-31382.

Address reprint requests to Dr. Fruen: Department of Veterinary PathoBiology, University of Minnesota, 1988 Fitch Avenue, Room 295, St. Paul, Minnesota 55108.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024

tibility. Nevertheless, the mechanisms by which certain anesthetics act to trigger an MH episode in susceptible patients are less clear.

Previous biochemical studies indicate that the interaction of anesthetics with the SR RYR may play an important role in triggering MH.[7–9] For example, we have shown that clinical concentrations of halothane, isoflurane, and enflurane increased the rate of RYR-mediated Ca^{2+} efflux from porcine SR.[8] This effect of inhalation anesthetics of RYR activity was more pronounced on MH-susceptible than on normal muscle membrane preparations, consistent with an inherent defect in the RYR channel protein in porcine MH.[8] In contrast, the effects of inhalation anesthetics of DHPR and Ca^{2+}-ATPase function cannot explain the ability of these agents to increase myoplasmic Ca^{2+} and trigger MH. Thus, dihydropyridine binding to MH-susceptible and normal muscle membranes was inhibited by clinical concentrations of inhalation anesthetics,[10] whereas Ca^{2+} uptake by MH-susceptible and normal preparations was stimulated by clinical concentrations of these agents and inhibited by greater concentrations.[8] Existing evidence therefore points to the activation of RYR Ca^{2+} channels being a primary mechanism by which inhalation anesthetics act to trigger an MH episode.

The intravenous anesthetic propofol appears to be a safe, nontriggering alternative to the inhalation anesthetics in the management of the MH-susceptible patient. Clinical reports indicate that MH-susceptible patients undergoing propofol anesthesia showed no signs of an MH response.[11–13] Similarly, propofol did not induce contractures during *in vitro* testing of MH-susceptible muscle[13,14] or trigger an MH episode in susceptible pigs.[15–17] To understand better how the effects of propofol on Ca^{2+} regulation by MH-susceptible muscle may differ from the effects of MH-triggering inhalation anesthetics, we have investigated the effects of propofol on MH-susceptible and normal RYR, DHPR, and Ca^{2+}-ATPase function. Our results reveal differences between the effect of propofol and the previously described effects of inhalation anesthetics on RYR function that support the hypothesis that the activation of RYR Ca^{2+} channels by inhalation anesthetics is a primary mechanism for triggering MH in pigs and some susceptible patients.

Materials and Methods

Animals and Drugs

Pigs (30–45 kg) were obtained from the University of Minnesota Experimental Farm where they are part of a swine herd maintained for studies of the inheritance of MH susceptibility. The two breeds of pig used in this study were a Pietrain herd that is homozygous for the MH-susceptibility mutation on the basis of its *ryr-1* genotype (cytosine at base pair 1843 determined by the polymerase chain reaction diagnostic procedure of Fujii *et al.*[4]), and a Yorkshire herd that is homozygous normal (thymidine at base pair 1843). Animals were killed by intravenous administration of Na thiamylal (15 mg/ml; Parke-Davis, Morris Plains, NJ) followed by euthanasia solution (Schering-Plough, Kenilworth, NJ), in adherence with a protocol approved by the Institutional Animal Care Committee of the University of Minnesota. Pure propofol was generously provided by Zeneca Pharmaceuticals (Wilmington, DE). Propofol was stored in the dark, at 22°C, under N_2, in Teflon-sealed Reacti-vials (Pierce Chemical, Rockford, IL). Propofol stock solutions were prepared fresh daily in dimethyl sulfoxide. Concentrations of the dimethyl sulfoxide solvent were held constant through all treatments at ≤2%. These concentrations of dimethyl sulfoxide had no significant effect on Ca^{2+} flux or radioligand binding. $^{45}Ca^{2+}$ and [^{3}H]ryanodine were purchased from du Pont–New England Nuclear (Boston, MA). [^{3}H]PN200–110 was purchased from Amersham (Arlington Heights, IL).

Membrane Preparation

SR vesicle preparations that are enriched in the junctional membrane proteins of the SR and the transverse tubule[18] were prepared from the longissimus dorsi muscle. In brief, the 2,600*g* supernatant of a muscle homogenate was centrifuged at 10,000*g* for 30 min. The resulting pellet was resuspended in 0.6 M KCl, 10% sucrose, 10 mM Tris-2-(N-morpholino)-ethanesulfonic acid (*p*H 6.8), incubated on ice for 1 h, and centrifuged at 100,000*g*. The pellets were resuspended in 10% sucrose, 0.4 M KCl, and 10 mM piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES) (*p*H 6.8) and placed on discontinuous sucrose density gradients containing 0.4 M KCl and 10 mM PIPES, *p*H 6.8, in all layers. Membranes that banded at the 36–45% interface after 5 h centrifugation at 85,000*g* were collected, frozen in liquid N_2, and stored at −70°C. All isolation buffers contained 0.1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml aprotinin to inhibit proteolysis.

$^{45}Ca^{2+}$ Efflux

SR vesicles (10–15 mg protein/ml) were passively loaded with $^{45}Ca^{2+}$ to a concentration of 20–40 nmol/

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024

mg by incubation for 2 h at 22°C in 0.1 M KCl, 10 mM PIPES (adjusted to *p*H 6.5 with KOH) and 2 mM $CaCl_2$ (containing 0.1 mCi/ml $^{45}CaCl_2$). $^{45}Ca^{2+}$-loaded vesicles (2 μl) were placed on the side of a polystyrene tube containing 200 μl of Ca^{2+} release medium (100 mM KCl, 10 mM PIPES, *p*H 6.5, and a $CaCl_2$–ethyleneglycol-bis-(β-aminoethyl ether)N,N,N′,N′-tetraacetic acid (EGTA) buffer set to give 6 μM ionized Ca^{2+}). Nonspecific efflux was measured in a release medium containing 10 mM EGTA, 10 mM $MgCl_2$, and 10 μM ruthenium red. Ca^{2+} efflux was initiated with rapid mixing and stopped after 1 s (timed with a metronome) by the addition of 20 μl of a Ca^{2+} release-inhibiting medium (10 mM $MgCl_2$, 10 mM EGTA, 100 mM KCl, 10 μM ruthenium red, and 10 mM PIPES, *p*H 6.5), followed by rapid filtration onto 0.45-μm filters (Millipore, Bedford, MA) and washing with ice-cold release-inhibiting medium. Experiments were performed at *p*H 6.5 to slow the rate of Ca^{2+}-induced Ca^{2+} efflux, allowing for accurate measurements of first-order rate constants[19] in the presence of micromolar Ca^{2+}.

Radioligand Binding

[^{3}H]Ryanodine binding was measured at equilibrium essentially as described previously[20] in media containing 0.2 mg SR protein/ml, 0.1 M KCl, 10 mM PIPES (adjusted to *p*H 7.0 with Tris), 0.5 mM phenylmethylsulfonyl fluoride, 100 nM [^{3}H]ryanodine, and a $CaCl_2$-EGTA buffer set to give 6 μM ionized Ca^{2+}. After 16-h incubations at 22°C, membranes were filtered (GF/B filters, Whatman, Hillsboro, OR) and washed with 8 ml ice-cold 0.1 M KCl, 10 mM PIPES buffer (*p*H 7.0). Nonspecific binding was measured in the presence of 20 μM nonradioactive ryanodine and was unaffected by propofol (1–300 μM).

[^{3}H]PN200-110 binding was measured at equilibrium in media containing 10 μg SR membrane protein/ml, 50 mM Tris-Cl buffer (*p*H 7.4), and 0.2 or 2.5 nM [^{3}H]PN200-110, as described.[10] After 45-min incubations at 22°C, vesicles were filtered onto filters (GF/B, Whatman) that had been pretreated for 20 min with 0.5% polyethyleneimine. Filters were washed with 20 ml ice-cold 200 mM choline chloride, 20 mM Tris-Cl buffer (*p*H 7.4). Nonspecific binding was measured in the presence of 1 μM nifedipine.

Ca^{2+}–Adenosine Triphosphatase

Membranes (25 μg protein/ml) were incubated at 22°C in media containing 100 mM KCl, 10 mM PIPES (*p*H 7.0), 10 μM of the Ca^{2+} ionophore A-23187, and 5 mM Mg adenosine triphosphate. Ca^{2+}-ATPase activity was defined as the difference between phosphate liberation in the presence of 100 μM $CaCl_2$ and that in the presence of 1 mM EGTA. $^{45}Ca^{2+}$ uptake was measured in media containing 20 μg SR membrane protein/ml, 100 mM KCl, 10 mM PIPES (*p*H 7.0), 50 mM phosphate, 5 mM Mg adenosine triphosphate, 100 μM $^{45}CaCl$, and 100 μM ruthenium red (to inhibit Ca^{2+} efflux through RYR channels[8]). After 2-min incubations at 22°C, samples were filtered through 0.45-μm membranes (HA, Millipore) and washed with ice-cold KCl/PIPES buffer (*p*H 7.0). Data were corrected for radioactivity accumulated in the absence of Mg adenosine triphosphate.

Analysis of Data

All enzyme activity and ligand binding measurements on each membrane preparation were performed in duplicate and duplicate observations were averaged. Reported values are means obtained from three or four MH-susceptible or three or four normal pigs. Means over all propofol concentrations were analyzed using a one-way analysis of variance. When appropriate, Fisher's least-significant difference test was used to compare effects of individual propofol concentrations with controls (no propofol) to determine the threshold concentration at which propofol produced a significant effect ($P < 0.05$). We have previously demonstrated that the MH-susceptible and normal membrane preparations used in this study do not significantly differ in regard to PN200-110 binding and Ca^{2+}-ATPase activity.[8,10,18] Therefore, analyses of the effect of propofol on [^{3}H]PN200-110 binding and Ca^{2+}-ATPase function are presented as percentages of control activities in the absence of propofol, to emphasize the similarity of effects on MH-susceptible and normal muscle membranes.

Results

Effect of Propofol on Ryanodine Receptor Activity

The effect of propofol on Ca^{2+} efflux from $^{45}Ca^{2+}$-filled SR vesicles was determined in conditions at which RYR-mediated efflux was maximally activated by micromolar Ca^{2+} and in conditions at which RYR-mediated efflux was inhibited by EGTA, Mg^{2+}, and ruthenium red. In this way, effects of propofol on Ca^{2+} efflux could be attributed to effects on RYR channel activity or to effects on other, undefined factors contributing

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024

to membrane vesicle Ca^{2+} permeability (termed nonspecific efflux).

Rate constants of nonspecific Ca^{2+} efflux in the presence of RYR inhibitors were similar for MH-susceptible (fig. 1A) and normal SR vesicles (fig. 1B). In contrast, rate constants of Ca^{2+} efflux in the presence of 6 μM Ca^{2+} were significantly greater for MH-susceptible (fig. 1A) than for normal vesicles (fig. 1B), consistent with the specific defect in RYR-mediated Ca^{2+} efflux in porcine MH.[20,21] Propofol concentrations to 200 μM had no significant effect on Ca^{2+} efflux rate constants from either MH-susceptible or normal vesicles. The greatest propofol concentration examined (500 μM) significantly increased the rate constants of nonspecific Ca^{2+} efflux (1.1 s^{-1} increase for MH-susceptible vesicles, 0.9 s^{-1} increase for normal vesicles). Rate constants of Ca^{2+} efflux in the presence of 6 μM Ca^{2+} were increased by the same magnitude (1.1 s^{-1} increase for MH-susceptible vesicles, 0.8 s^{-1} increase for normal vesicles), so that this increase in Ca^{2+} efflux rate could be attributed entirely to the effect of high propofol concentrations on nonspecific Ca^{2+} efflux. Thus, correcting the data for the effect of propofol on nonspecific Ca^{2+} efflux (fig. 1, insets) showed that RYR-mediated Ca^{2+} efflux was not significantly affected by any concentration of propofol.

RYR activity was directly monitored using [^{3}H]-ryanodine as a specific ligand for the open state of the RYR channel.[22] In the presence of 6 μM Ca^{2+}, MH-susceptible SR vesicle preparations bound significantly more [^{3}H]ryanodine than did normal preparations (fig. 2). Consistent with our Ca^{2+} efflux results, propofol concentrations of 1–100 μM had no effect on [^{3}H]ryanodine binding to MH-susceptible or normal muscle SR vesicles. Concentrations of propofol greater than 100 μM had a small inhibitory effect on [^{3}H]ryanodine binding to MH-susceptible and normal membranes ($p < 0.03$, in the presence of 300 μM propofol).

Effect of Propofol on Dihydropyridine Receptor Activity

To investigate the effect of propofol on DHPR function, the binding of the dihydropyridine Ca^{2+} channel blocker PN200-110 to MH-susceptible and normal SR vesicle preparations was examined. [^{3}H]PN200-110 binding was measured in the presence of 0.2 nM (fig. 3A) and 2.5 nM (fig. 3B) PN200-110. These concentrations were chosen to approximate 1× and 10× the dissociation constant of PN200-110 binding to porcine

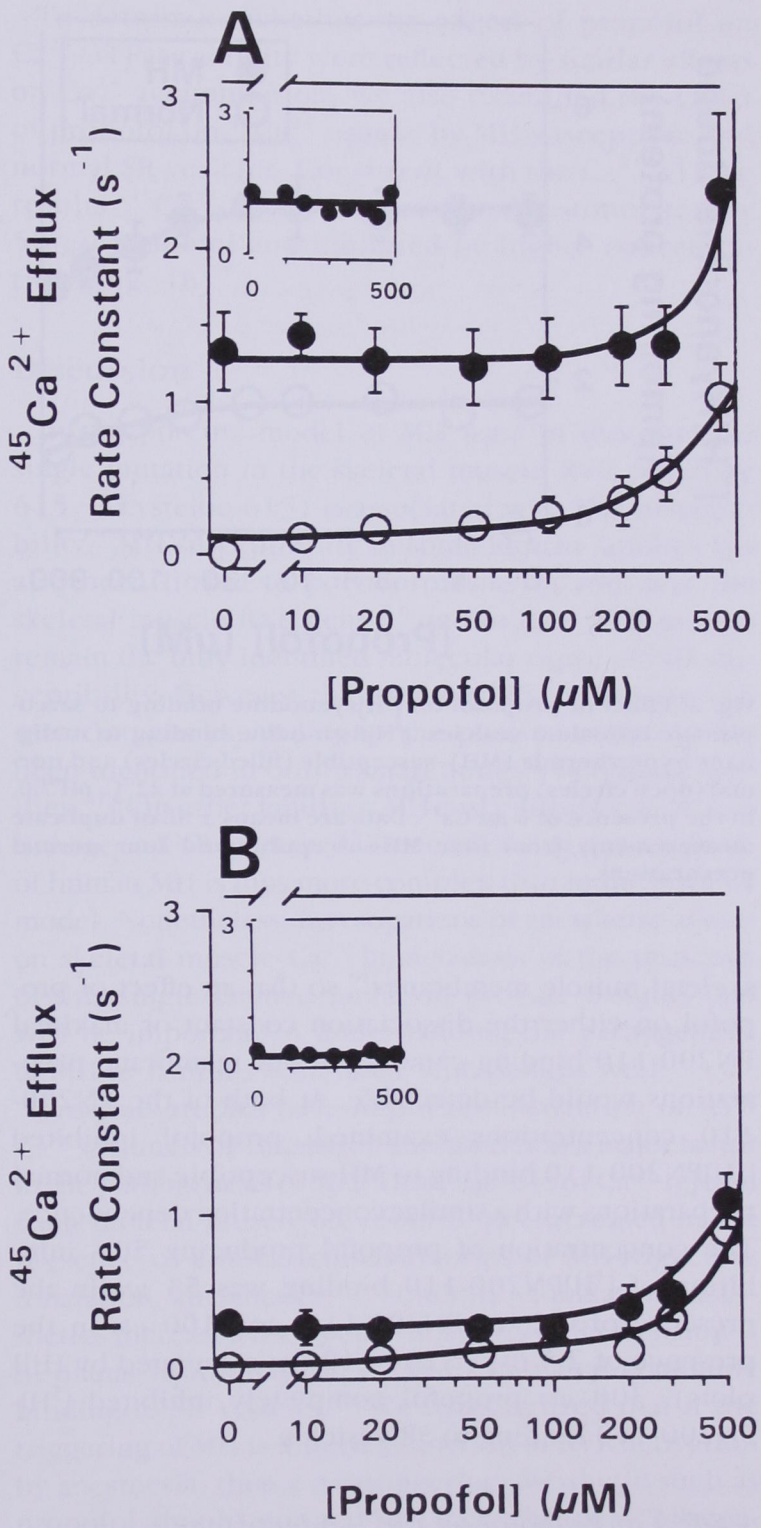


Fig. 1. Effect of propofol on the rate constant of Ca^{2+} efflux from sarcoplasmic reticulum vesicles. Malignant hyperthermia (MH)–susceptible (*A*) and normal (*B*) vesicles were loaded with $^{45}Ca^{2+}$, and Ca^{2+} efflux was measured after dilution into a medium containing various concentrations of propofol and 6 μM Ca^{2+} (filled circles) to activate ryanodine receptor (RYR)–mediated Ca^{2+} efflux, or 10 mM EGTA, 10 mM $MgCl_2$, 10 μM ruthenium red (open circles) to inhibit RYR-mediated Ca^{2+} efflux. (*Insets*) Difference between Ca^{2+} efflux in the presence of 6 μM Ca^{2+} and in the presence of RYR channel inhibitors. Points are means ± SE of duplicate measurements from four MH-susceptible and four normal preparations.

Downloaded from http://asaz.silverchair.com/anesthesiology/article-pdf/82/5/1274/404797/0000542-199505000-00023.pdf by guest on 18 April 2024

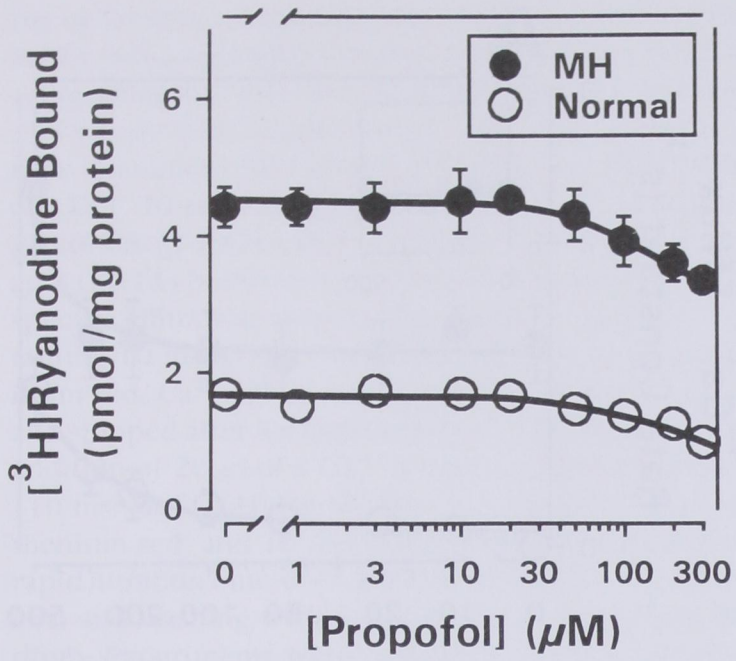
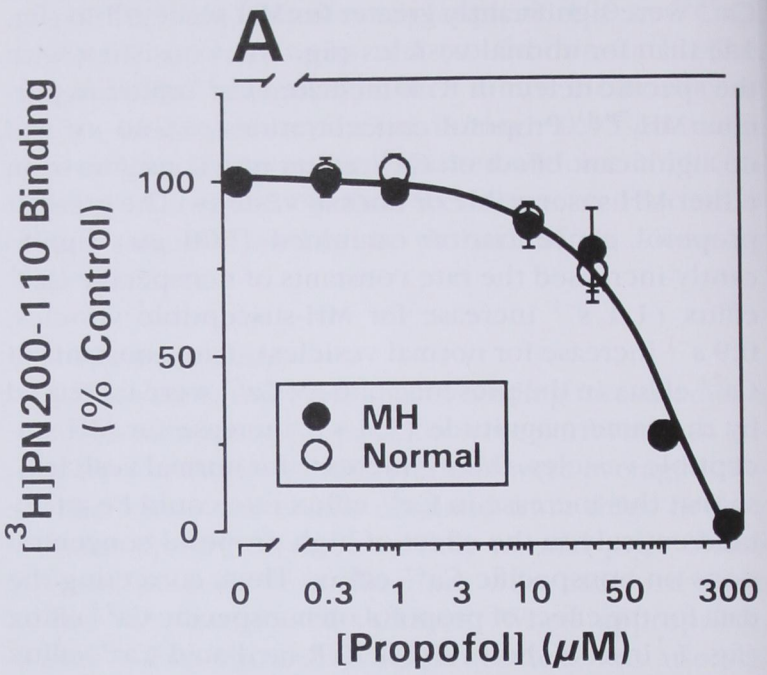


Fig. 2. Effect of propofol on [^{3}H]ryanodine binding to sarcoplasmic reticulum vesicles. [^{3}H]Ryanodine binding to malignant hyperthermia (MH)–susceptible (filled circles) and normal (open circles) preparations was measured at 22°C, *p*H 7.0, in the presence of 6 μM Ca^{2+}. Data are means ± SE of duplicate measurements from four MH-susceptible and four normal preparations.

skeletal muscle membranes[10] so that an effect of propofol on either the dissociation constant or maximal PN200-110 binding capacity of our membrane preparations would be detectable. At both of the PN200-110 concentrations examined, propofol inhibited [^{3}H]PN200-110 binding to MH-susceptible and normal preparations with a similar concentration dependence. The concentration of propofol producing 50% inhibition of [^{3}H]PN200-110 binding was 53 μM in the presence of 0.2 nM PN200-110, and 100 μM in the presence of 2.5 nM PN200-110 (as calculated by Hill plots); 300 μM propofol completely inhibited [^{3}H]-PN200-110 binding to SR vesicles.

Effect of Propofol on Ca^{2+}–Adenosine Triphosphatase Activity

The effect of propofol on the Ca^{2+}-ATPase activity of MH-susceptible and normal SR vesicle preparations was biphasic (fig. 4A). Ca^{2+}-ATPase activity was stimulated by 10–100 μM propofol with maximal stimulation at 50 μM propofol. Stimulation of MH-susceptible and normal preparations did not differ (178 ± 21% *vs.* 183 ± 16%, respectively, in the presence of 50 μM propofol). Stimulation of Ca^{2+}-ATPase activity was progressively decreased at propofol concentrations greater than 100 μM, and in the presence of 300 μM propofol was decreased to 75% of control activity in the absence of propofol.



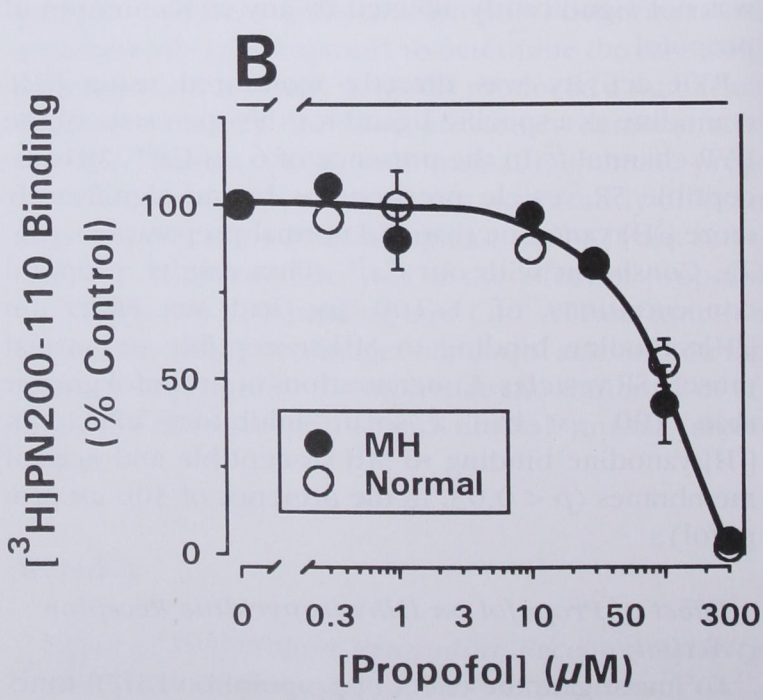


Fig. 3. Effect of propofol on [^{3}H]PN200-110 binding to sarcoplasmic reticulum vesicles. [^{3}H]PN200-110 binding to malignant hyperthermia (MH)–susceptible (filled circles) and normal (open circles) preparations was determined at 22°C, *p*H 7.4, in the presence of 0.2 nM (*A*) or 2.5 nM (*B*) PN200-110. Measurements of control [^{3}H]PN200-110 binding in the absence of propofol were 3.09 ± 0.19 pmol/mg (*A*) and 6.64 ± 0.57 pmol/mg (*B*). Data are means ± SE of duplicate measurements from three MH-susceptible and three normal preparations.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024

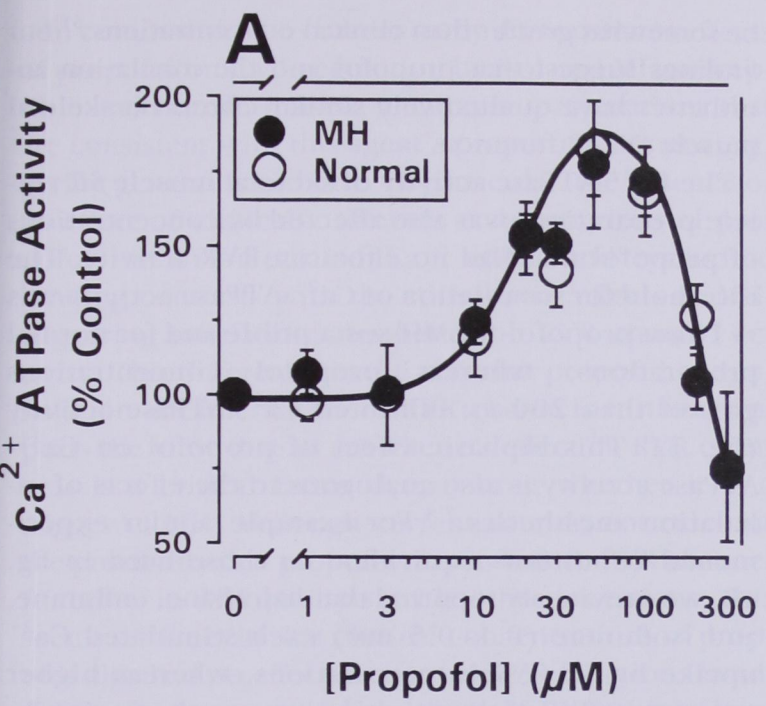


Fig. 4. (*A*) Effect of propofol on the Ca^{2+}–adenosine triphosphatase (Ca^{2+}-ATPase) activity of sarcoplasmic reticulum vesicles. The Ca^{2+}-ATPase activity of malignant hyperthermia (MH)–susceptible (filled circles) and normal (open circles) preparations was measured at 22°C, *p*H 7.0. (*B*) Effect of propofol on $^{45}Ca^{2+}$ uptake by skeletal muscle membrane vesicles. ATP-dependent Ca^{2+} uptake by MH-susceptible and normal preparations was measured at 22°C, *p*H 7.0, in the presence of 100 μM ruthenium red. Control activities in the absence of propofol were 0.28 ± 0.03 μmol inorganic phosphate · mg protein^{-1} · min^{-1} (*A*) and 0.59 ± 0.07 μmol Ca^{2+} · mg protein^{-1} · min^{-1} (*B*). All points represent means ± SE of duplicate measurements from three MH-susceptible and three normal preparations.

To determine whether the effects of propofol on Ca^{2+}-ATPase activity were reflected by similar effects on Ca^{2+} accumulation, we also examined the effect of propofol on $^{45}Ca^{2+}$ uptake by MH-susceptible and normal SR vesicles. Consistent with the Ca^{2+}-ATPase results, $^{45}Ca^{2+}$ uptake was maximally stimulated by 50 μM propofol and inhibited by higher concentrations (fig. 4B).

Discussion

In the porcine model of MH used in this study, a single mutation in the skeletal muscle RYR (arginine 615 → cysteine 615) is associated with MH susceptibility.[4] MH susceptibility in some human families has also been linked to polymorphisms in and near the skeletal muscle RYR gene,[5,6] and to date RYR defects remain the only identified molecular cause of MH susceptibility. However, the arginine 615 → cysteine 615 mutation corresponding to the porcine mutation has been identified in only a small number of human families.[23,24] In other families, MH susceptibility shows no linkage to RYR markers.[25] The underlying genetic basis of human MH is thus more complex than in the porcine model. Nonetheless, investigations of anesthetic effects on skeletal muscle Ca^{2+} homeostasis in the presence of this single defined mutation provide insights that will be important in understanding the pathogenesis of MH in families with other mutations as well.

Previous studies have implicated activation of RYR Ca^{2+} channels by inhalation anesthetics as a major factor in the pathogenesis of MH. Thus, the rate of Ca^{2+} release from skeletal muscle SR vesicles was increased in the presence of clinical concentrations of MH-triggering inhalation anesthetics.[7,8] Similarly, inhalation anesthetics increased the open probability of RYR channels in planar lipid bilayers[26–28] and stimulated ryanodine binding to SR vesicles.[29] We hypothesized that if the triggering of MH is indeed a function of RYR activation by anesthesia, then a nontriggering anesthetic such as propofol should not activate RYR channels. Alternatively, effects on other membrane components controlling myoplasmic Ca^{2+} may compensate for effects of this anesthetic on RYR function. We therefore investigated propofol's effects on MH-susceptible and normal RYR, DHPR, and Ca^{2+}-ATPase function.

Our results demonstrate that propofol did not activate RYR Ca^{2+} channels. Thus, RYR-mediated Ca^{2+} efflux from MH-susceptible and from normal SR vesicles was unaffected by 10–500 μM propofol (fig. 1, insets). Sim-

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/407979/0000542-199505000-00023.pdf by guest on 18 April 2024

ilarly, ryanodine binding to MH-susceptible and normal SR was unaffected by 1–100 μM propofol (fig. 2). At higher concentrations of propofol (300 μM), ryanodine binding to MH-susceptible and normal SR vesicles was slightly inhibited. Because ryanodine interacts with the open state of RYR channels,[22] this inhibition of ryanodine binding by high concentrations of propofol might be taken as evidence of an inhibitory effect on RYR channel activity. However, as these same propofol concentrations had no significant effect on RYR-mediated Ca^{2+} efflux (fig. 1, insets), it is unlikely this inhibition of ryanodine binding represents a direct inhibitory action on RYR channel activity. Furthermore, the concentrations of propofol required to inhibit ryanodine binding to our SR vesicle preparations are far higher than relevant clinical concentrations *in vivo*. For example, blood propofol concentrations necessary to produce unconsciousness in laboratory animals were reported to be in the range of 5–20 μM[30]; free propofol concentrations have been estimated to be closer to 1 μM, because a major fraction is bound by plasma proteins.[31] The concentration of propofol required to significantly inhibit SR vesicle ryanodine binding (300 μM) is thus more than 100 times clinical concentrations, indicating that RYR channels are relatively insensitive to propofol. In contrast, γ-aminobutyric acid A chloride channel activity is altered by propofol concentrations less than 1 μM.[32]

Propofol (10–300 μM) inhibited the binding of the DHPR ligand PN200-110 to MH-susceptible and normal SR vesicle preparations (fig. 3). The concentrations of propofol required to inhibit PN200-110 binding (10–30 μM) were an order of magnitude less than those required to inhibit ryanodine binding, indicating that DHPR channels are more sensitive to propofol than RYR channels. That inhibition of PN200-110 binding was similar at saturating and subsaturating PN200-110 concentrations suggests that the major effect of propofol was to reduce the maximal dihydropyridine binding capacity of our SR vesicle preparations. This effect of propofol on PN200-110 binding is thus comparable to previously described effects of inhalation anesthetics. For example, we have previously shown that halothane, enflurane, and isoflurane (0.1–2.0 mM) also inhibited skeletal muscle membrane PN200-110 binding capacity.[10] Similarly, Lamb *et al.* reported corresponding reductions in DHPR-mediated charge movement and Ca^{2+} current in the presence of halothane.[33] Although the concentrations of propofol required to significantly inhibit PN200-110 binding may be somewhat greater than clinical concentrations,[31] our findings suggest that propofol and the innalation anesthetics have qualitatively similar effects on skeletal muscle DHPR function.

The Ca^{2+}-ATPase activity of skeletal muscle SR vesicle preparations was also affected by concentrations of propofol that had no effect on RYR activity. The threshold for stimulation of Ca^{2+}-ATPase activity was ~10 μM propofol for MH-susceptible and for normal preparations, whereas propofol concentrations greater than 200 μM inhibited Ca^{2+}-ATPase activity (fig. 3). This biphasic effect of propofol on Ca^{2+}-ATPase activity is also analogous to the effects of inhalation anesthetics.[7,8] For example, under experimental conditions equivalent to those used in fig. 4B, we previously reported that halothane, enflurane, and isoflurane (0.1–0.5 mM) each stimulated Ca^{2+} uptake by SR vesicle preparations, whereas higher concentrations of these inhalation anesthetics inhibited Ca^{2+} uptake.[8] Considering the differences in the structures and physical properties of propofol and the inhalation anesthetics, it is perhaps remarkable that these anesthetics have similar effects on Ca^{2+}-ATPase and DHPR function. Of interest, a recent report suggests that the stimulation of Ca^{2+}-ATPase activity by various general anesthetics may reflect the dissociation of oligomeric Ca^{2+}-ATPase enzyme complexes in the SR membrane.[34]

A comparison of our results with those of previous investigations of the effects of propofol on cardiac muscle Ca^{2+} regulation suggests that propofol may have similar effects on cardiac and skeletal muscle Ca^{2+} channels. For example, Puttick and Terrar[35] and Takahashi *et al.*[36] reported that propofol inhibited DHPR-mediated (L-type) Ca^{2+} currents in guinea pig ventricular myocytes. The concentrations of propofol that produced ~50% inhibition of L-type Ca^{2+} currents (50–100 μM).[35,36] are equivalent to concentrations that produced 50% inhibition of PN200-110 binding to skeletal muscle membrane vesicles (fig. 3). These authors also indirectly examined the effect of propofol on cardiac SR function by analyzing Ca^{2+}-activated tail currents. Their observations indicated that propofol had little or no effect on Ca^{2+} release from cardiac SR.[35,37] Cook and Housmans reached a similar conclusion based on their observation that exposure to ryanodine did not influence the negative inotropic effect of propofol on isolated ventricular myocardium.[38] Thus, cardiac and skeletal muscle RYR Ca^{2+} channels may be similarly insensitive to propofol.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024

In conclusion, the current study demonstrates effects of propofol on Ca^{2+} regulation by isolated MH-susceptible and normal muscle membrane preparations that are consistent with this agent's properties as a nontriggering general anesthetic. In particular, the absence of an effect of clinically relevant concentrations of propofol on RYR-mediated Ca^{2+} efflux and ryanodine binding contrasts with the stimulatory effects of MH-triggering inhalation anesthetics on RYR channel activity. This difference in the way that propofol and inhalation anesthetics affect RYR channels further supports the view that an anesthetic's effect on RYR channels is a critical factor determining whether it is a trigger of MH. Although current data do not rule out the possibility that propofol may influence RYR channel function in MH patients with other mutations, this seems unlikely in that clinical concentrations of propofol also had no effect on normal RYR function. In addition, the apparent insensitivity of RYR channels to propofol suggests that activation of these intracellular Ca^{2+} channels is not a fundamental property of all general anesthetics, but rather reflects a more specific interaction between inhalation anesthetics and RYR channels.

References

1. Rios E, Pizarro G: Voltage sensor of excitation-contraction coupling in skeletal muscle. Physiol Rev 71:849–908, 1991

2. Coronado R, Morrissette J, Sukhareva M, Vaughan DM: Structure and function of ryanodine receptors. Am J Physiol 266:C1485–C1504, 1994

3. MacLennan DH, Phillips MS: Malignant hyperthermia. Science 256:789–794, 1992

4. Fujii J, Otsu K, Zorzato F, DeLeon S, Khanna VK, Weiler JE, O'Brien P, MacLennan DH: Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253:448–451, 1991

5. McCarthy TV, Healy JMS, Heffron JJA, Lehane M, Deufel T, Lehmann-Horn F, Farrall M, Johnson K: Localization of the malignant hyperthermia susceptibility locus to human chromosome 19q12–13.2. Nature 343:562–564, 1990

6. MacLennan DH, Duff C, Zorzato F, Fujii J, Phillips M, Korneluk RG, Frodis W, Britt BA, Worton RG: Ryanodine receptor gene is a candidate for predisposition to malignant hyperthermia. Nature 343: 559–561, 1990

7. Nelson TE, Sweo T: Ca^{2+} uptake and Ca^{2+} release by skeletal muscle sarcoplasmic reticulum: Differing sensitivity to inhalation anesthetics. ANESTHESIOLOGY 69:571–577, 1988

8. Louis CF, Zualkernan K, Roghair T, Mickelson JR: The effects of volatile anesthetics on calcium regulation by malignant hyperthermia–susceptible sarcoplasmic reticulum. ANESTHESIOLOGY 77: 114–125, 1992

9. Otsu K, Nishida K, Kimura Y, Kuzuya T, Hori M, Kamada T, Tada M: The point mutation arg615→cys in the Ca^{2+} release channel of skeletal sarcoplasmic reticulum is responsible for hypersensitivity to caffeine and halothane in malignant hyperthermia. J Biol Chem 269:9413–9415, 1994

10. Louis CF, Roghair T, Mickelson JR: Volatile anesthetics inhibit dihydropyridine binding to malignant hyperthermia and normal pig skeletal muscle membranes. ANESTHESIOLOGY 80:618–624, 1994

11. Harrison GG: Propofol in malignant hyperthermia (letter). Lancet 337:503, 1991

12. Allen G: Propofol and malignant hyperthermia (letter). Anesth Analg 73:359, 1991

13. McKenzie AJ, Couchman KG, Pollock N: Propofol is a safe anesthetic agent in malignant hyperthermia susceptible patients. Anaesth Intensive Care 20:165–168, 1992

14. Denborough M, Hopkinson KC: Propofol and malignant hyperthermia (letter). Lancet 1:191, 1988

15. Krivosic-Horber R, Reyfort H, Becq MC, Adnet P: Effect of propofol on the malignant hyperthermia susceptible pig model. Br J Anaesth 62:691–693, 1989

16. Raff M, Harrison GG: The screening of propofol in MHS swine. Anesth Analg 68:750–751, 1989

17. Forster PS, Hopkinson KC, Denborough MA: Propofol anaesthesia in malignant hyperpyrexia susceptible swine. Clin Exp Pharmacol Physiol 19:183–186, 1992

18. Mickelson JR, Ervasti JM, Litterer LA, Campbell KP, Louis CF: Skeletal muscle junctional membrane content in pigs with different ryanodine receptor genotypes. Am J Physiol 267:C282–C292, 1994

19. Segal IH: Biochemical Calculations. 2nd edition. New York, John Wiley & Sons, 1976, pp 225–228

20. Mickelson JR, Gallant EM, Litterer LA, Johnson KM, Rempel WE, Louis CF: Abnormal sarcoplasmic reticulum ryanodine receptor in malignant hyperthermia. J Biol Chem 263:9310–9315, 1988

21. Fill M, Coronado R, Mickelson JR, Vilven J, Ma J, Jacobson BA, Louis CF: Abnormal ryanodine receptor channels in malignant hyperthermia. Biophys J 50:471–475, 1990

22. Meissner G, El-Hashem A: Ryanodine as a functional probe of the skeletal muscle sarcoplasmic reticulum Ca^{2+} release channel. Mol Cell Biochem 114:119–123, 1992

23. Gillard E, Otsu K, Fujii J, Britt BA, Worton RG, MacLennan DH: A substitution of arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics 11: 751–755, 1991

24. Hogan K, Couch F, Powers PA, Gregg RG: A cysteine for arginine substitution in the human skeletal muscle calcium release channel cosegregates with malignant hyperthermia. Anesth Analg 75: 441–448, 1992

25. Deufel T, Golla A, Iles D, Meindel A, Meitinger T, Schindelhauer D, DeVries A, Pongratz D, MacLennan DH, Johnson KJ, Lehmann-Horn F: Evidence for genetic heterogeneity of malignant hyperthermia susceptibility. Am J Hum Genet 50:1151–1161, 1992

26. Bull R, Marengo JJ: Calcium-dependent halothane activation of sarcoplasmic reticulum calcium channels from frog skeletal muscle. Am J Physiol 266:C391–C396, 1994

27. Nelson TE: Halothane effects on human malignant hyperthermia skeletal muscle single calcium-release channels in planar lipid bilayers. ANESTHESIOLOGY 76:588–595, 1992

28. Nelson TE, Lin M: Abnormal function of porcine malignant hyperthermia calcium release channel in the absence and presence of halothane. Cell Physiol Biochem 5:10–22, 1995

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/367979/0000542-199505000-00023.pdf by guest on 18 April 2024

29. Connelly TJ, Hayek RE, Rusy BF, Coronado R: Volatile anesthetics selectively alter [^{3}H]ryanodine binding to skeletal and cardiac ryanodine receptors. Biochem Biophys Res Commun 186:595–600, 1992

30. Adam HK, Glen JB, Hoyle PA: Pharmacokinetics in laboratory animals of ICI 35 868: A new IV anesthetic agent. Br J Anaesth 52: 743–746, 1980

31. Franks NP, Lieb WR: Molecular and cellular mechanisms of general anaesthesia. Nature 367:607–614, 1994

32. Hara M, Kai Y, Ikemoto Y: Propofol activates $GABA_A$ receptor-chloride ionophore complex in dissociated hippocampal pyramidal neurons of the rat. ANESTHESIOLOGY 79:781–788, 1993

33. Lamb GD, Hopkinson KC, Denborough MA: Calcium currents and asymmetric charge movement in malignant hyperthermia. Muscle Nerve 12:135–140, 1989

34. Karon BS, Thomas DD: Molecular mechanism of Ca^{2+}-ATPase activation by halothane in sarcoplasmic reticulum. Biochemistry 32: 7503–7511, 1993

35. Puttick RM, Terrar DA: Effects of propofol and enflurane on action potentials, membrane currents and contraction of guinea-pig isolated ventricular myocytes. Br J Pharmacol 107:559–565, 1992

36. Takahashi H, Puttick RM, Terrar DA: The effects of propofol on single calcium channel currents of guinea-pig isolated ventricular myocytes. Br J Pharmacol 111:1147–1153, 1994

37. Puttick RM, Terrar DA: Differential effects of propofol and enflurane on contractions dependent on calcium derived from the sarcoplasmic reticulum of guinea pig isolated papillary muscles. Anesth Analg 77:55–60, 1993

38. Cook DJ, Housmans PR: Mechanism of the negative inotropic effect of propofol in isolated ferret ventricular myocardium. ANESTHESIOLOGY 80:859–871, 1994

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/82/5/1274/647979/0000542-199505000-00023.pdf by guest on 18 April 2024