

Alterations in Muscle Resting Potentials and Electrolytes during Halothane and Cyclopropane Anesthesia

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Halothane was found to depress the resting potential of rat gastrocnemius muscle cells by an average of 6.2 mV and also to prevent the hyperpolarization which can be induced by epinephrine in this muscle. Halothane anesthesia was also associated with a higher muscle intracellular sodium concentration than cyclopropane anesthesia. Cyclopropane, in contrast to halothane, hyperpolarized rat muscle cells by an average of 4.7 mV. Catecholamine depletion partially prevented the hyperpolarization associated with cyclopropane.

In the light of the known effects of halothane and cyclopropane on sodium transport, these data are consistent with the hypotheses that halothane depolarizes muscle cell membrane by blocking the tonic stimulating action of catecholamines on the sodium pump, as well as by decreasing catecholamine release; and that cyclopropane hyperpolarizes the cell membrane by augmenting the pump-stimulating effects of catecholamines and/or by increasing catecholamine release. (Key words: Resting potentials; Muscle; Electrolytes; Halothane; Cyclopropane.)

THE RESTING POTENTIAL of mammalian skeletal muscle has a ouabain-sensitive component responsive to changes in the level of activity of the sodium pump. Application of ouabain, which inhibits the active extrusion of sodium, leads to rapid depolarization to a new lower stable resting potential. The magnitude of the depolarization varies with the particular muscle and ranges from 5 mV in isolated rat diaphragm¹ to 20 mV in rat gastrocnemius muscle *in situ*.²

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A resting potential partially dependent on continuous active extrusion of sodium should be affected by drugs which alter sodium pump activity. In studies to be published elsewhere, we have shown that epinephrine, which stimulates the sodium pump,³ hyperpolarizes rat gastrocnemius muscle cells, and that ouabain blocks this hyperpolarization. In the present paper we explore the effects on resting membrane potential of cyclopropane, an anesthetic which has been reported to stimulate sodium transport,⁴ increase catecholamine release,⁵ and sensitize catecholamine target organs.^{6,7} We compare cyclopropane with halothane, an agent which depresses sodium transport⁴ and is associated with a low level of circulating catecholamines. We also explore the relationship between transcellular ion distribution and the altered resting potentials associated with cyclopropane and halothane anesthesia.

Methods

Male Sprague-Dawley rats weighing 300-400 g were studied. All operations were done under anesthesia, and rats were mechanically ventilated by means of a Harvard rodent respirator through an endotracheal tube with 100 per cent oxygen or 97 per cent oxygen with 3 per cent CO₂, to which the appropriate anesthetic agent was added. Blood pressures and blood gases were measured as previously described.⁸ No significant abnormalities in blood pressure, arterial pH, P_{O₂} or P_{CO₂} were observed in any of the experimental conditions.

In measurements of resting potential each animal served as his own control, the resting potentials of 25 or more cells being measured in the gastrocnemius of one leg during anesthesia and the gastrocnemius of the other leg after the animal, his spinal cord transected at a high cervical level to provide anesthesia and immobility, had recovered from the anesthetic agent. For the measurements, the rat was

placed on a warmed table with the leg immersed in warm mineral oil. Muscle temperatures ranged from 33 to 38 C but remained constant for each rat throughout the experiment. The gastrocnemius muscle was exposed and resting potentials measured as previously described.⁸ Average resting potential for each muscle was calculated as the mean of values from at least 25 cleanly impaled subsurface cells. Differences were analyzed by paired *t* test, each animal serving as his own control.

The following protocols were observed for the resting potential measurements.

HALOTHANE

Rats were maintained on 1.25 per cent halothane for three hours with either a Foregger anesthesia machine or a Fluotec vaporizer calibrated periodically with a Fluothane Monitor. After measurements on one side had been made, the spinal cord was transected and anesthesia discontinued. Control measurements were made after a three-hour recovery period.

CYCLOPROPANE

Animals were maintained on 25 per cent cyclopropane, delivered from a Foregger anesthesia machine, for two hours; measurements were made and cord transection performed. A three-hour recovery period was allowed before measurements were repeated on the opposite leg. Eight rats were used in each of the above series of experiments.

EPINEPHRINE DURING HALOTHANE ANESTHESIA

Halothane was administered as above and resting potentials measured after two hours of anesthesia while saline solution was infused through a catheter in the jugular vein at a rate of 0.8 ml/hr. Potentials were then measured during an infusion of epinephrine, 1 mg/l, at the same rate. Four animals were used.

CYCLOPROPANE IN RESERPINIZED ANIMALS

Rats were given 1.5 mg reserpine intraperitoneally on each of two successive days; the experiments were performed on the third day. Resting potentials were measured after two hours of cyclopropane anesthesia, the cord was transected, and measurements were re-

TABLE 1. Effects of Halothane and Cyclopropane on Resting Potential of Rat Gastrocnemius Muscle Cells*

	Control (mV)	Anesthesia (mV)	Change (Anesthesia-Control) (mV)
Halothane	87.9 (1.03)	81.7 (0.82)	-6.2 (0.72)
Cyclopropane	87.2 (0.41)	91.9 (0.84)	+4.7 (0.63)
Cyclopropane (reserpimized animals)	83.1 (1.66)	85.8 (1.16)	+2.8 (1.82)

* Each value represents the mean from eight rats. The value for each animal under each condition was determined from the average of at least 25 cells. Change in resting potential was calculated by subtracting the value for one leg in the anesthetized animal from the value for the opposite leg of the same animal after cord transection and recovery from anesthesia. The upper motor neuron denervation present in the cord-transected control state produces no significant changes in resting potential within the time span of these experiments. Figures in parentheses are standard errors. Differences are significant at the 0.01 level, except in the reserpimized animals, in which the difference is not significant.

peated after a three-hour recovery period. Seven rats were used in the experiment.

In a separate set of experiments, sodium and potassium concentrations were determined in serum and in gastrocnemius muscle. Four rats were anesthetized with halothane and four with cyclopropane in the concentrations used above. The kidneys were removed and approximately 10 μ c Na₂³²SO₄ injected into the inferior vena cava for estimation of extracellular fluid space; the animals were then kept anesthetized for two hours. Previous studies in this laboratory have shown that sulfate space in nephrectomized rats remains constant between 1½ and 6 hours after injection of Na₂³²SO₄. Following the equilibration period a blood sample was drawn from the dorsal aorta and both gastrocnemius muscles removed. Tissue analysis and calculations of ionic distributions were carried out as previously described.⁹

Results

Gastrocnemius muscle resting potentials measured during halothane anesthesia averaged 82

TABLE 2. Electrolyte Distributions during Cyclopropane and Halothane Anesthesia*

	Extracellular Space Calculated as Sulfate Space (Per Cent)	Total Water (l/100 g Fat-free Dry Solids)	[Na] _i (mEq/l)	[Na] _e (mEq/l)	[K] _i (mEq/l)	[K] _e (mEq/l)
Halothane						
Mean	10.34	0.360	141.5	18.0	6.1	149.9
SE	0.336	0.007	1.16	1.24	0.13	6.31
Cyclopropane						
Mean	10.93	0.356	147.6	13.3	5.7	147.0
SE	0.954	0.008	1.06	0.50	0.31	6.55

* Values represent averages from four animals anesthetized with halothane and four with cyclopropane. Only intracellular sodium shows a difference significant at the 0.05 level.

mV, compared with a control value of 88 mV obtained in the awake, cord-transected animal three hours after recovery from anesthesia.† Cyclopropane anesthesia, in contrast, was associated with a resting potential of 91.9 mV, approximately 4 mV above the control level (table 1). The differences were significant at the 0.01 level.

Epinephrine infusion produced no significant change in resting potential in rats anesthetized with halothane. Potentials averaged 89 mV ± 0.81 (SE) during epinephrine infusion, compared with a control value of 88 mV ± 0.85 before epinephrine infusion was begun.

Resting potentials of eight reserpinized rats were lower by about 5 mV ($P < 0.05$) than those of eight normal rats when both were measured three hours after recovery from halothane anesthesia. Anesthesia with cyclopropane did not significantly hyperpolarize the muscle cells in reserpinized rats, as it did in normal animals; however, there was a small and variable hyperpolarization, and the lack of significance was partly due to the increased variability in the reserpinized animals (table 1).

There was a significant difference between sodium distributions in rats anesthetized with halothane and those anesthetized with cyclopropane (table 2). Halothane anesthesia was accompanied by higher intracellular sodium concentrations than cyclopropane. There were

no significant differences between the two agents in total water, extracellular space, extracellular sodium, or potassium distribution.

Discussion

The level of resting membrane potential has a number of determinants. These include the potassium concentration ratio across the cell membrane, the permeability of the membrane to sodium and potassium, and the rate of active sodium transport out of the cell. The relative contribution of each of these variables to resting potential may vary from species to species and tissue to tissue. For example, inhibition of the sodium pump by ouabain has no effect on resting potential in the squid axon,⁹ while in rat gastrocnemius muscle application of ouabain is followed by depolarization from a normal level of 85 mV to between 60 and 70 mV.²

Failure to take into account the differences in the relative contributions of ion distribution, membrane permeability, and metabolic activity to control of resting potential may explain previous conflicting reports of anesthetic effects on resting potentials in various cell types.¹⁰⁻¹² In the present study we deliberately selected a tissue, the rat gastrocnemius muscle, in which the resting potential has a large ouabain-sensitive component presumably dependent on continuous sodium pump activity.

From these considerations we postulated that the administration of cyclopropane, which in relatively low concentrations stimulates active sodium transport in the toad bladder,^{4, 13} should lead to hyperpolarization, and that halothane, which at all concentrations de-

† In a separate set of experiments the administration of pentobarbital, 60 mg/kg, ip, was also associated with depression of resting potential to 82 mV ± 1.59 (SE), compared with a control value of 88 mV ± 1.26 in six cord-transected rats and 91 mV ± 1.49 in six rats subjected to epidural anesthesia with lidocaine.

presses sodium transport,^{4, 23} should result in depolarization. It was further postulated that prior depletion of catecholamines, which reverses the cyclopropane-induced stimulation of sodium transport,¹⁴ should similarly reverse the effect of cyclopropane on resting membrane potential.

The experiments reported herein support these hypotheses; they demonstrate that the administration of cyclopropane or halothane is associated with consistent changes in resting membrane potential which are parallel to the known effects of these agents on sodium transport and to their effects on catecholamine activity. The difference between intracellular sodium concentrations in the two types of anesthesia also supports the hypothesis of contrasting effects on sodium transport.

The data presented are also consistent with the hypothesis that the observed changes in resting potential are secondary to changes in catecholamine output and/or in catecholamine effects at the target organ. Thus, halothane, which depresses catecholamine release, depolarizes the membrane, as does the catecholamine-depleting drug, reserpine. In a separate study,²⁵ we have shown that after recovery from anesthesia cord-transected animals show consistent hyperpolarization of about 7 mV during epinephrine infusion, compared with control measurements obtained during infusion of saline solution. Halothane anesthesia completely blocks this response to epinephrine. Conversely, cyclopropane hyperpolarizes the membrane in normal animals and fails to produce significant hyperpolarization in reserpinized animals. The slight hyperpolarization that was observed may have been due to incomplete catecholamine depletion. The results are consistent with either of two cyclopropane-catecholamine interactions proposed by others, namely, stimulation of catecholamine release⁵ or sensitization of target organs.^{6, 7}

That cyclopropane effects on resting potential would be mediated by catecholamines had been predicted from the catecholamine-dependent effect of this agent on sodium extrusion in the toad bladder.¹⁴ It appears that halothane also may act on resting potential indirectly, by blocking the hyperpolarization associated with catecholamines. This is an unexpected finding, and represents a new instance

of halothane interaction with β -adrenergic functions.

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