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Volatile Anesthetics Attenuate Sympathomimetic Actions on the Guinea Pig SA Node

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The authors examined and compared the direct effects of three volatile anesthetic agents and three sympathomimetic agonists on transmembrane action potential (AP) characteristics and automaticity of sinoatrial (SA) nodal pacemaker cells. SA nodal tissue was isolated from guinea pig hearts and suffused *in vitro* with oxygenated Krebs-Ringer solution. Electrophysiologic variables measured were: amplitude of the AP, slopes of phase 4 and of phase 0 of the AP, AP duration, and spontaneous sinus rate. The authors found that 1 and 2 MAC equivalents of each anesthetic, 0.8 and 1.6 vol % halothane, 1.4 and 2.8 vol % isoflurane, and 1.7 and 3.4 vol % enflurane similarly depressed the slopes of phase 4 and 0 of the AP, prolonged AP duration, and slowed the sinus rate at 1 and 2 MAC equivalents. Isoproterenol, 0.25 μM, and epinephrine, 50 μM, maximally enhanced the slopes of phase 4 and 0 of the AP, shortened AP duration, and increased the sinus rate, but phenylephrine, 50 μM, only moderately increased the slope of phase 4 and the sinus rate. Each of the three anesthetics caused baseline depressions of phase 4 and phase 0 slopes and of automaticity of SA nodal cells; the fall in sinus rate was counteracted, but was not reversed maximally by increasing the concentrations of isoproterenol, epinephrine, or phenylephrine. Regression analyses of linearly transformed data showed that each of the anesthetics similarly depressed basal sinus rate, so that changes in rate produced with isoproterenol and epinephrine were not different from those observed with beta agonists in the absence of anesthetics. This study suggests that halothane, isoflurane, and enflurane act in a noncompetitive way to blunt the increases in sinus rate produced with adrenergic agonists. Because the sinus rate lowering effect could not be competitively overridden by adrenergic agonists, our study supports the view that the volatile anesthetics probably interfere with intermediate or multiple steps in the mechanism of SA nodal pacemaker activity. (Key words: Anesthetics, volatile: halothane, isoflurane, enflurane. Animal: guinea pig. Heart: sinoatrial node. Membrane: electrophysiology; transmembrane action potential. Sympathetic nervous system: epinephrine; isoproterenol; phenylephrine.)

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ADMINISTRATION OF GENERAL ANESTHETICS to intact animals and humans has been associated with increases, decreases, or no change in heart rate.[1,2] These diverse findings probably reflect the many variables, such as different intrinsic rates, differential afferent information, and altered reflex effects, which cannot be adequately controlled *in vivo*. More specific information on the direct action of volatile anesthetics on altering intrinsic pacemaker function has come from studies on the isolated atrium and SA node.[3–6] In a previous report from our laboratory, we demonstrated that halothane, isoflurane, and enflurane each similarly decreased sinus rate, the slopes of phase 4 and phase 0 of the action potential (AP), and the AP duration at concentrations equivalent to 1 and 2 MAC (minimum alveolar concentration);[6] increasing the extracellular concentration of calcium from 2.5 to 5 mM counteracted, but did not completely reverse, the negative chronotropic effects of each anesthetic.

In the present study, we have extended previous *in vitro* experiments to examine the effects of each of three volatile anesthetic agents, halothane, isoflurane, and enflurane, on altering the AP characteristics and chronotropic responses of the SA node to direct alpha- and beta-adrenergic stimulation using isoproterenol, epinephrine, and phenylephrine. We chose these agonists because they display a range of beta and alpha receptor agonist properties. The nature of the antagonism, *e.g.*, competitive *versus* noncompetitive, was suggested by comparing linearized agonist-sinus rate response curves during exposure to each anesthetic. Our aim was to determine if the volatile anesthetics might alter SA nodal pacemaker activity by competitive antagonism of adrenergic receptor sites or by other less defined mechanisms.

Materials and Methods

SA nodal tissue was isolated from 60 anesthetized, adult guinea pig hearts and suffused as described previously by us.[6,7] The suffusate solution contained (in mM): Na^+ 137; K^+ 5.9; Ca^{++} 2.5; Mg^{++} 1.2; Cl^- 134; HCO_3^- 15.5; $H_2PO_4^-$ 1.2; and glucose 11.5. A suffusion flow rate of 15 ml/min was maintained *via* a constant volume pump (Masterflex, Cole-Palmer Co.) and the organ bath temperature was kept at 37 ± 0.1° C. To attain a stable *p*H of 7.4, a P_{CO_2} of about 40 mmHg, and

a P_{O_2} greater than 400 mmHg, the solution was equilibrated with 3% CO_2 in O_2; bath samples were checked frequently for stability of *p*H, P_{CO_2}, and P_{O_2} with a blood gas analyzer (Radiometer Corp., ABL 2). Intracellular potentials were measured with ultrafine glass microelectrodes that were filled with 3M KCl and had tip impedances from 50–60 Megaohms. Microelectrodes were made from 1 mm OD borosilicate glass with a microelectrode puller (David Kopf Instruments, Model 700C). Transmembrane potentials were detected with an electrometer (WP Instruments, Model 707) and a storage oscilloscope (Tektronix Corp., R5113). All data were recorded on a FM tape recorder (Tandberg Instruments, Model 100) and displayed on a digital memory oscilloscope (Nicolet Instruments Corp., Explorer II).

The following action potential parameters were measured: rate of rise of phase 4 and of phase 0 (in mV/s), action potential duration at 50% of absolute AP amplitude (APD_{50}, in ms), absolute amplitude of the action potential (AAP, in mV), and intrinsic sinus rate, which was derived from the phase 0 slope interval. Primary pacemaker cells were identified by the following criteria: 1) a maximum diastolic potential of −55 to −65 mV; 2) a spontaneous phase 4 slow diastolic depolarization with a smooth transition to phase 0; 3) absence of a plateau phase; and 4) an AP overshoot less than 10 mV. SA nodal rate measurements were made from primary pacemaker cells, and, occasionally, from subsidiary pacemaker cells because of pacemaker shift. Only cells identified as primary pacemaker cells were used to record AP characteristics. Continuous impalement of individual cells was attempted during the various experimental maneuvers. Electrophysiologic characteristics were recorded in each preparation during suffusion with a given sympathomimetic agonist and with exposure to a given anesthetic. Because the strength of atrial tissue contractions increased with suffusion of an agonist, it was not always possible to maintain a continuous impalement of one cell, so that additional cells had to be impaled in order to complete the dose-response protocol.

The fractions (in vol %) of anesthetics, which were delivered in a total gas flow of 4 l/min, were: halothane (Draeger vaporizer) 0.8% and 1.6%, isoflurane (Ohio vaporizer) 1.4% and 2.8%, and enflurane (Ohio vaporizer) 1.7% and 3.4%. Bath concentrations of anesthetics were measured by gas chromatography with a flame ionization detector, as described previously for halothane.[8] Bath concentrations corresponding to the above fractions for each anesthetic were: halothane 0.2 and 0.4 mM; isoflurane 0.4 and 0.7 mM; and enflurane 0.5 and 0.9 mM. The range of concentrations of sympathomimetics were: 0.001–0.25 μM isoproterenol hydrochloride; 0.25–50 μM epinephrine bitartrate; and 0.5–50 μM phenylephrine hydrochloride. These agents were prepared daily from frozen stock and added to 50-cc aliquots of oxygenated solution for suffusion. To inhibit oxidative degradation of catecholamines, EDTA (ethylenediaminetetraacetic acid, 99%) was added to the suffusate to a concentration of 50 μM. This concentration of EDTA did not alter basal SA nodal rates compared with EDTA-free suffusate (N = 5).

Protocol

SA nodal tissue was allowed to equilibrate in the bath for 60 min before recordings were made. Any given SA nodal tissue preparation was exposed to a combination of only one agonist (isoproterenol, epinephrine, or phenylephrine) and one anesthetic (halothane, isoflurane, or enflurane). In the first part of each experiment, sinus rate responses to increasing doses of one of three randomly chosen sympathomimetic agonists was determined in the absence of anesthetic. In the second part, the same dose-response series was repeated at a lower and at a higher concentration of one of the three randomly chosen anesthetics. Total anesthetic exposure time was about 2 h of the approximately 4-h experiment. Each anesthetic was equilibrated for 20 min in the reservoir before being introduced into the tissue bath. AP characteristics and sinus rates were recorded, for later analysis, 20 min after introduction of the anesthetic to the bath and 2 min after suffusion with the agonist.

Statistical Analysis

Five SA nodal preparations made up the mean for each of nine experimental conditions (three anesthetics by three sympathomimetics). Data points during drug-free controls, maximal sympathetic stimulation, and two levels of anesthetics were subjected to analysis of variance. Following a significant F-test, the differences among mean sinus rate responses and action potential characteristics were assessed using least significant difference (LSD) tests. Dose-sinus rate response curves with sympathomimetic agents in the presence and absence of two levels of an anesthetic were first maximally linearized by polynomial transformation (H-P 98820A Statistical Library). This was done by applying equations of the general form, $y = ax^2 + bx + c$, which yielded the smallest sum of squares for each agonist alone, and again for data obtained at the two anesthetic levels. Regression lines were compared for differences in slopes and y-intercepts by F-tests. In addition, linear regression of double reciprocal plots (1/sinus rate *vs.* 1/drug dose) were compared to suggest the classical drug-receptor type of interaction,[9] *i.e.*, competitive

TABLE 1. Electrophysiological Effects of Sympathomimetic Amines on SA Nodal Cells

Agonist (μM)	AAP (mV)	Phase 4 Slope (mV/s)	Phase 0 Slope (mV/s)	APD_{50} (ms)	SA Rate (APs/min)
ISOP 0	78 ± 2	175 ± 12	3610 ± 250	70 ± 3	267 ± 6
ISOP 0.25	81 ± 2 ns	296 ± 21†	6430 ± 320*	53 ± 3†	387 ± 7‡
EPI 0	77 ± 3	184 ± 23	3640 ± 530	75 ± 3	262 ± 5
EPI 50	78 ± 3 ns	283 ± 24†	6530 ± 1130*	56 ± 2†	377 ± 7‡
PHE 0	73 ± 1	160 ± 11	3020 ± 320	76 ± 2	273 ± 7
PHE 50	76 ± 3 ns	215 ± 20*	3950 ± 830 ns	70 ± 3*	316 ± 11†

Values are means ± SEM; N = six cells in six preparations for each agonist group. AAP = absolute amplitude of action potential; APD_{50} = duration of action potential at 50% AAP; ISOP = isoproterenol; EPI = epinephrine; PHE = phenylephrine; ns = nonsignificant.
* $P < .05$; † $P < .01$; ‡ $P < .001$ *versus* agonist controls.

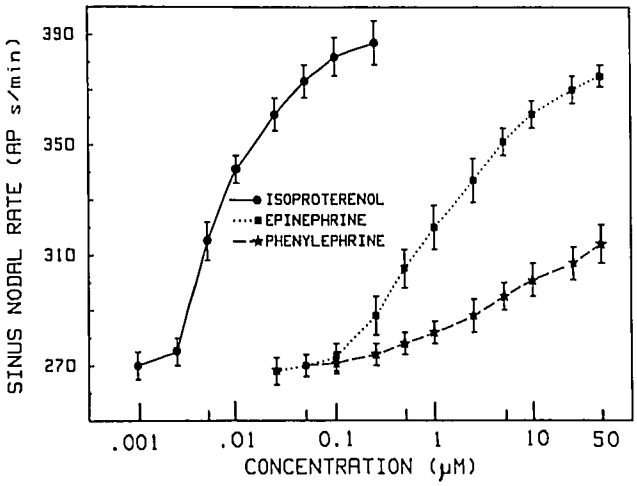

FIG. 1. Comparison of positive chronotropic effects of sympathomimetic amines on SA nodal pacemaker cells. Sinus rate was determined from phase 0 intervals.

versus noncompetitive, of anesthetics during treatment with agonists. All values are means ± standard error of the means (SEM). Probability (P) levels of 0.05 or smaller were used to indicate statistical significance.

Results

Table 1 shows the maximal effects of isoproterenol, epinephrine, and phenylephrine on transmembrane AP characteristics of SA nodal cells. Compared with controls, the slopes of phase 4 were significantly increased by 70, 54, and 34%, and the slopes of phase 0 were significantly increased by 78, 79, and 31%, with isoproterenol, epinephrine, and phenylephrine, respectively. These changes were associated with increases in SA nodal pacemaker rates (APs/min) of 45, 44, and 16%, compared with controls. APD_{50} was significantly shortened by each of the agonists. Figure 1 compares the relative responses of the three agonists on increasing sinus rate. Maximal sinus rates with isoproterenol and epinephrine were similar ($P > .05$), and were greater than the maximal response with phenylephrine ($P < .001$). Isoproterenol was about 100 times more potent than epinephrine on the SA node.

Table 2 displays the effect of each of two levels of halothane, isoflurane, and enflurane on SA nodal action potentials. Compared with controls, the slopes of phase 4 were significantly decreased by 25, 18, and 25%, and the slopes of phase 0 were significantly decreased by 30, 31, and 31%, with the higher of the two concentrations of halothane, isoflurane, and enflurane, respectively; sinus rate decreased concomitantly by 18, 12, and 4%. APD_{50} was prolonged significantly by both concentrations of these anesthetics.

Figure 2 compares transmembrane APs of a single SA nodal cell during exposure to enflurane with and without epinephrine; changes in SA nodal rates were proportional to changes in the slopes of phase 4 and 0 and inversely proportional to APD_{50}. Figures 3–5 show the rate responses to log-dose concentrations of isoproterenol, epinephrine, and phenylephrine before and during exposure to enflurane. Similar results were observed with halothane and isoflurane, and are not illustrated. Each anesthetic caused a basal reduction in the sinus rate that paralleled the increase produced by isoproterenol and epinephrine. Sinus rate changes produced by phenylephrine alone were small. Each anesthetic inhibited the small increase in rate due to phenylephrine as shown for enflurane in figure 5.

Table 3 compares y-intercepts and slopes of sinus rate responses generated by polynomial transformation and regression analyses of the three agonists during exposure to each anesthetic. Each anesthetic agent altered

TABLE 2. Electrophysiological Effects of Volatile Anesthetics on SA Nodal Cells

Anesthetic (mM)	AAP (mV)	Phase 4 Slope (mV/s)	Phase 0 Slope (mV/s)	APD_{50} (ms)	SA Rate (APs/min)
HAL 0	72 ± 2	195 ± 10	3060 ± 210	74 ± 3	263 ± 10
HAL 0.2	70 ± 1 ns	175 ± 12†	2450 ± 230†	80 ± 3*	241 ± 8*
HAL 0.4	68 ± 2 ns	147 ± 22†	2160 ± 120†	88 ± 3†	216 ± 6†
ISOF 0	78 ± 3	176 ± 12	3260 ± 320	74 ± 2	274 ± 4
ISOF 0.4	76 ± 2 ns	153 ± 13*	2770 ± 330*	78 ± 2*	262 ± 5*
ISOF 0.7	72 ± 1 ns	145 ± 14†	2240 ± 410*	84 ± 3*	241 ± 5†
ENF 0	78 ± 2	153 ± 12	3950 ± 660	74 ± 3	250 ± 6
ENF 0.5	76 ± 3 ns	135 ± 5†	3080 ± 320*	81 ± 3*	235 ± 8*
ENF 0.9	76 ± 1 ns	114 ± 5†	2720 ± 340†	86 ± 4†	218 ± 7†

Values are means ± SEM; N = six cells in six preparations for each anesthetic group. AAP = absolute amplitude of action potential; APD_{50} = duration of action potential at 50% AAP; HAL = halothane; ISOF = isoflurane; ENF = enflurane; ns = nonsignificant.
* $P < .05$; † $P < .01$; ‡ $P < .001$ *versus* anesthetic controls.

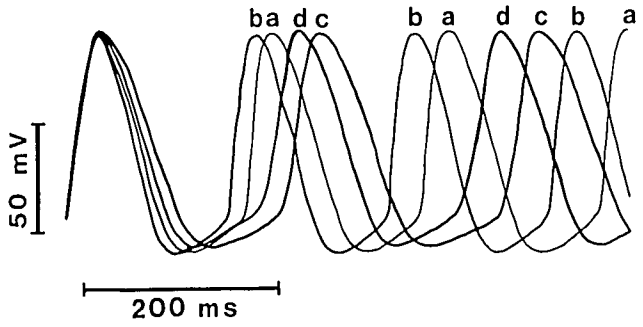

FIG. 2. Transmembrane APs recorded from a single SA nodal cell during control (a) and during exposure to 25 μM epinephrine (b), enflurane 3.4 vol% (c), and both epinephrine and enflurane (d). Phase 4 slopes (mV/s): a = 174; b = 290; c = 112; d = 130. Phase 0 slopes (mV/s): a = 3420; b = 5120; c = 2480; d = 2820. AP intervals (ms): a = 190; b = 175; c = 230; d = 260. Individual tracings were superimposed on an X-Y plotter and retraced.

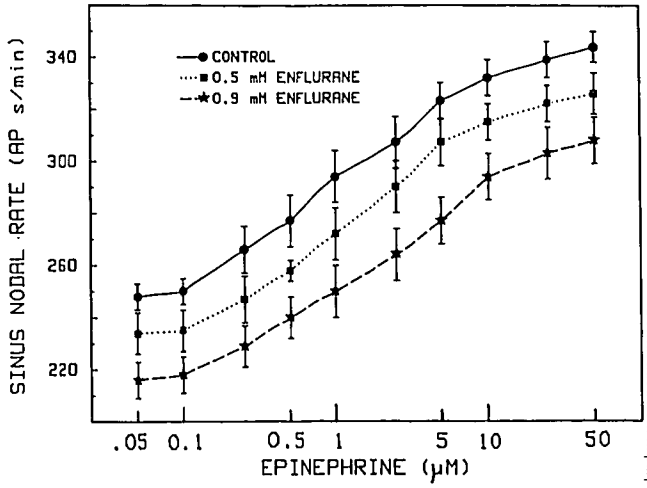

FIG. 4. Antagonism by enflurane of the epinephrine-sinus rate response.

the dose-sinus rate relationships of isoproterenol and of epinephrine by significantly shifting the y-intercepts lower without significantly altering the dose-sinus response slopes; *i.e.*, there were no rightward or leftward shifts in the responses of these agonists by any of the three anesthetics. In comparison to its responses with isoproterenol and epinephrine, enflurane did not alter significantly the y-intercept of the phenylephrine-sinus rate response. Each anesthetic decreased significantly the slopes of the phenylephrine-sinus rate relationships. In further analyses (not displayed), a comparison of control and anesthetic double reciprocal plots (1/sinus rate *vs.* 1/agonist concentration) by linear regression, indicated a partial but parallel antagonism by each anesthetic on the sinus rate responses to epinephrine and isoproterenol; this mixed, or noncompetitive, type of interaction was shown by a shift in the y-intercepts with no change in slopes. Table 3 also shows that each anesthetic was more effective in inhibiting the smaller increases in sinus rate with phenylephrine as the concentration of phenylephrine increased; results of this analysis of data are also consistent with a mixed or noncompetitive type of interaction.

Figures 6–8 summarize control and maximal sinus rate responses to the three agonists with two levels of each anesthetic. Both control (lower lines) and maximal agonist effects (upper lines) of isoproterenol and epinephrine were reduced proportionately at each level of enflurane, isoflurane, and halothane. With maximal sinus rate responses to phenylephrine, however, each anesthetic caused a greater than proportionate reduc-

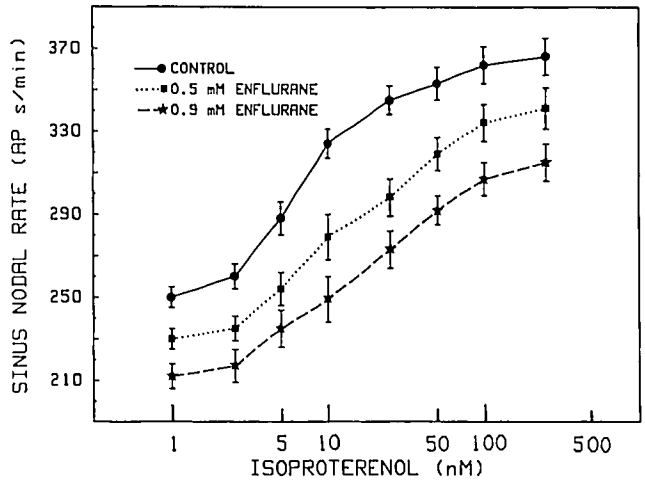

FIG. 3. Antagonism by enflurane of the isoproterenol-sinus rate response. See table 3 for comparison of slopes.

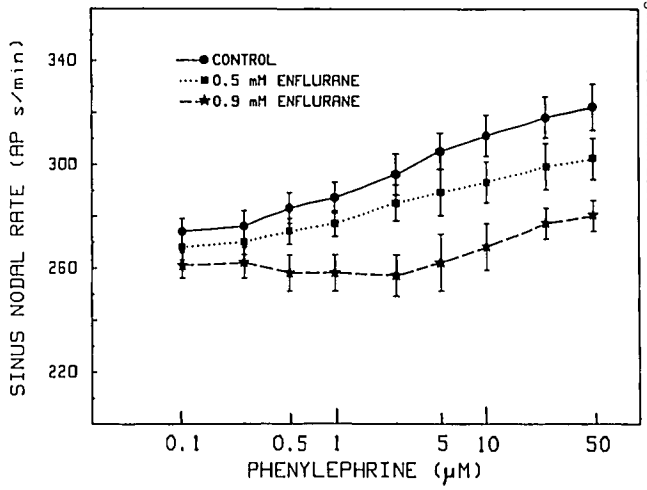

FIG. 5. Antagonism by enflurane of the phenylephrine-sinus rate response.

TABLE 3. Comparison of Y-intercepts and Slopes of Sympathomimetic Dose-response Curves during Exposure to Volatile Anesthetics

Anesthetic (mM)	y-int (±95% CI)	$P_y <$	Slope	$P_{slope} <$	R^2	Number
Isoproterenol 0–0.25 μM						
Halothane 0	270 (±24)	—	18.0	—	0.92	7
Halothane 0.2	234 (±7)	.05	17.1	ns	0.99	7
Halothane 0.4	212 (±7)	.01	16.1	ns	0.99	7
Isoflurane 0	294 (±28)	—	18.9	—	0.86	7
Isoflurane 0.4	261 (±12)	ns	17.5	ns	0.98	7
Isoflurane 0.7	225 (±12)	.01	17.1	ns	0.98	7
Enflurane 0	251 (±27)	—	18.9	—	0.87	7
Enflurane 0.5	220 (±13)	ns	18.5	ns	0.98	7
Enflurane 0.9	201 (±10)	.05	17.2	ns	0.98	7
Epinephrine 0–50 μM						
Halothane 0	267 (±22)	—	14.6	—	0.91	9
Halothane 0.2	234 (±12)	.05	14.9	ns	0.98	9
Halothane 0.4	204 (±11)	.01	15.1	ns	0.98	9
Isoflurane 0	274 (±13)	—	13.4	—	0.96	9
Isoflurane 0.4	250 (±5)	.05	12.4	ns	0.99	9
Isoflurane 0.7	227 (±4)	.01	12.3	ns	0.99	9
Enflurane 0	242 (±9)	—	12.2	—	0.98	9
Enflurane 0.5	224 (±10)	.05	12.3	ns	0.98	9
Enflurane 0.9	205 (±6)	.01	12.0	ns	0.99	9
Phenylephrine 0–50 μM						
Halothane 0	262 (±8)	—	4.6	—	0.93	8
Halothane 0.2	240 (±5)	.05	2.5	.05	0.66	8
Halothane 0.4	209 (±5)	.01	−1.1	.05	0.12	8
Isoflurane 0	269 (±11)	—	6.9	—	0.99	8
Isoflurane 0.4	260 (±6)	ns	3.8	.05	0.96	8
Isoflurane 0.7	250 (±3)	.05	1.1	.01	0.28	8
Enflurane 0	268 (±11)	—	6.9	—	0.99	8
Enflurane 0.5	267 (±7)	ns	4.3	.05	0.96	8
Enflurane 0.9	251 (±6)	ns	3.0	.01	0.70	8

Data analyzed by best-fit linear regression analyses after polynomial transformation of sympathomimetic doses; y-int (±95% CI) = y-intercept (APs/min) ± 95% confidence intervals; P_y = significance of y-intercepts, anesthetics *versus* control; P_{slope} = significance of slopes, anesthetics *versus* control; R^2 = square of the regression coefficient. Numbers refer to rate-agonist coordinates for each anesthetic group representing data from five SA tissue preparations (overall N = 45).

tion in sinus rate. There were no significant differences in control and maximal agonist sinus rates among the enflurane, isoflurane, and halothane groups.

Discussion

This study confirms our previous report[6] that showed that halothane, isoflurane, and enflurane similarly reduce SA nodal pacemaker rate and the slopes of phase 4 and of phase 0 of the action potential. This study shows additionally that the increases in SA nodal rate and AP slopes produced by increasing concentrations of isoproterenol and of epinephrine can be proportionately but incompletely antagonized by each of these anesthetics, and that these anesthetics are effective in attenuating the smaller increases in SA nodal rate produced with phenylephrine.

Depression of the spontaneous sinus rate by these anesthetics was found to be proportional at all concentrations of isoproterenol and epinephrine. Because the

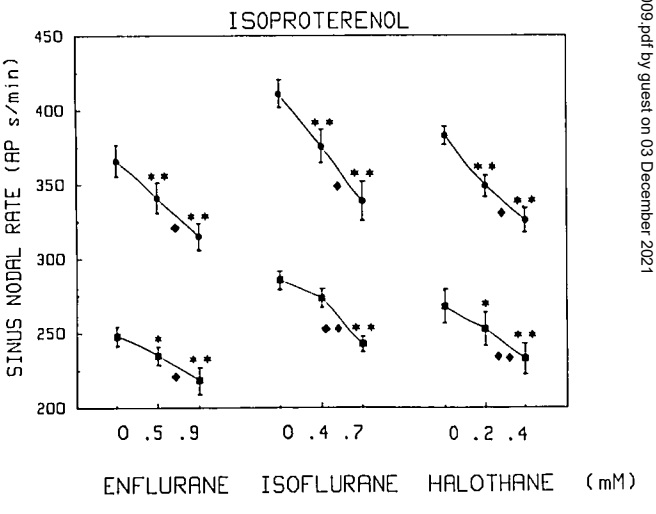

FIG. 6. Comparison of control (lower lines) and maximal (upper lines) sinus rate responses to isoproterenol during suffusion with two levels of each of three anesthetics. *$P < .05$; **$P < .01$ *versus* anesthetic control; ▪$P < .05$, ▪▪$P < .01$ low *versus* high level of anesthetic.

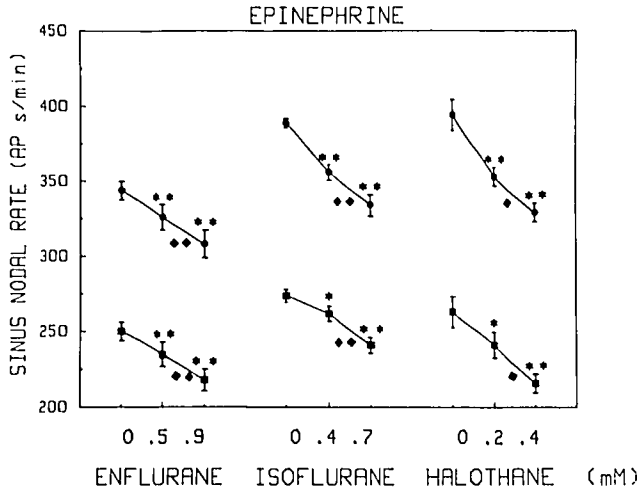

FIG. 7. Comparison of control (lower lines) and maximal (upper lines) sinus rate response to epinephrine during suffusion with two levels of each of three anesthetics.

sinus rate slowing could not be overcome by increasing the concentrations of these beta adrenergic agonists, our study suggests that volatile anesthetics do not exert a major direct inhibitory effect at the specific adrenergic receptors. If we designate the anesthetics as inhibitors in the classical agonist-receptor site interaction, we find a partial or noncompetitive type of antagonism between agonists and anesthetics. This suggests that the volatile anesthetics act on allosteric sites, or at one or more intermediate steps beyond the adrenergic receptor sites of the SA node. It is, moreover, interesting that these volatile anesthetics were more effective in noncompetitively inhibiting the lesser sinus rate effects of

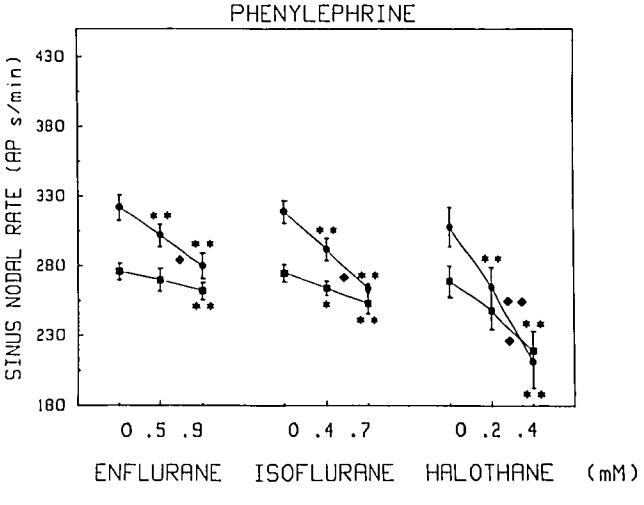

FIG. 8. Comparison of control (lower lines) and maximal (upper lines) sinus rate response to phenylephrine during suffusion with two levels of each of three anesthetics.

phenylephrine at increasing doses. This is in contrast to the proportionate inhibition of sinus rate by each anesthetic observed with isoproterenol and epinephrine, and could represent a different mechanism of antagonism between anesthetics and phenylephrine.

The ionic currents responsible for the pacemaker potential of SA nodal cells have been studied by various voltage clamping techniques.[10-12] The pacemaker potential (phase 4 depolarization) has been suggested to result from a time-dependent fall in potassium conductance in the presence of inward background current.[10-14] The fall in potassium current is believed to lower the membrane potential to the threshold potential at which the action potential is generated. Another important ionic current during phase 4 depolarization of the SA node is the slow inward current. The ions responsible for this current appear to be sodium and calcium, since a decrease in extracellular sodium concentration[14] and a blockade of slow calcium channels by verapamil[6] depress pacemaker activity by decreasing AP overshoot and the slopes of phase 4 and of phase 0. An increase in extracellular calcium causes an increase in pacemaker rate,[6,15] but a calcium concentration greater than 8 mM causes a decrease in heart rate due to excess accumulation of intracellular calcium.[16]

Catecholamines, through activation of the $beta_1$ adrenergic receptors and formation of cyclic AMP,[17] stimulate the slow inward current and induce an increase in upstroke velocity of the SA nodal cell action potential.[18,19] In addition, catecholamines increase potassium outward current and a hyperpolarization-activated inward current.[18] The net effect of these currents is an increase in pacemaker firing. When compared to the effects of epinephrine and isoproterenol, the smaller observed positive chronotropic effect of phenylephrine might be expected, since the SA nodal region has a greater density of $beta_1$ adrenergic receptors and phenylephrine is primarily an $alpha_1$ agonist. However, the effect of phenylephrine on the isolated right guinea pig atria has been shown not to be modified by phentolamine, an alpha adrenergic blocker, but to be inhibited by propranolol or practolol, which are beta adrenergic blockers.[20] The small positive chronotropic effect of phenylephrine may result from a direct action on beta adrenergic receptors,[21,22] as well as from an indirect action secondary to release of norepinephrine.[21,23]

How volatile anesthetics cause depression of the slow inward current in the SA node is unclear. In partially depolarized (26 mM K^+) ventricular cells of the guinea pig, the maximal rate of rise of the slow AP has been used as measure of peak inward current to study effects of volatile anesthetics.[24-26] These slow APs are characterized by a small resting membrane potential (−55 to −65 mV), absence of a plateau phase, a spontaneous phase 4

diastolic depolarization, an AP overshoot less than 10 mV, and a slow rate of rise of phase 0 for these cells. Halothane,[24] enflurane,[25] and isoflurane[26] have been found to cause dose-dependent depressions of the slow AP upstroke (slow channel dependent) and of contractility. But isoflurane causes a lesser depression of the slow AP upstroke and a lesser maximal tension development than does halothane.[26] We have reported that halothane, isoflurane, and enflurane similarly depress the phase 0 slope of the SA nodal AP.[6] Moreover, we found that the concomitant slowing of pacemaker rate could not be completely reversed by increasing the extracellular calcium concentration. We suggested that it is unlikely that these anesthetics exert their effects solely on the calcium entry mechanism, since increasing calcium did not override the depression of pacemaker rate by the anesthetics.

Although normal Purkinje fibers do not exhibit pacemaker potential, variably depolarized ischemic Purkinje fibers exhibit abnormal automaticity that is characterized by enhanced spontaneous diastolic depolarization.[27] In this study, we found that halothane decreased the rate of slow diastolic depolarization that originates in the depolarized ischemic Purkinje fibers. We suggested that the action of halothane is mediated by both calcium and sodium slow channel blockade. Support for this hypothesis are the reports that halothane can reduce the myocardial cell slow inward current, carried mostly by calcium ions,[28] and the myocardial sodium current.[29]

Volatile anesthetics have been suggested to depress ionic channel conductivity by altering the fluidity of the sarcolemma.[30] We reported recently that halothane causes dose-dependent decreases in intracellular free Ca^{2+} (measured by a decrease in aequorin light emission) and twitch tension in cat papillary muscle.[31] Anesthetics may also interfere with ATPase activity at the contractile apparatus.[32,33]

In the present study, we found that the volatile anesthetics blunted the increases in the slopes of phase 4 and phase 0 produced by sympathomimetic amines. However, our comparisons of the agonist-sinus automaticity relationship in the absence and presence of anesthetics suggest that it is unlikely that volatile anesthetics simply inhibit the occupation of adrenergic receptors by catecholamines. Volatile anesthetics may decrease basal sinus automaticity to produce an increase in the threshold for the stimulatory effects of catecholamines *via* occupation of adrenergic receptors. Anesthetics could also allosterically affect adrenergic receptors or alter their lateral mobility.[9]

We found that all three anesthetics caused dose-dependent increases in APD_{50} of the SA nodal cell. This is opposite to the observations for halothane[24] and enflurane,[25] but similar to that noted for isoflurane,[26] in the partially depolarized ventricular muscle fiber. Prolongation of AP duration by volatile anesthetics suggests that they may also affect membrane conductance for potassium. Activation of the potassium outward current and inactivation of the slow inward current determine the duration of the AP, and activation of potassium channels is stimulated by free intracellular calcium.[13] Because anesthetics appear to depress the slow inward current, the effect on potassium conductance could be indirect through depression of calcium influx, or direct through an effect on the cell membrane or at intracellular sites. Differences between slow APs in depolarized ventricular cells and in SA nodal cells could be due to differences in the properties of the slow channel of pacemaker cells compared with ventricular cells.[14] For example, verapamil or D600-induced decreases in membrane conductance could not be overcome by increased extracellular calcium or by isoproterenol in the pacemaker region, as compared to the response in ventricular myocardium.[34]

In summary, our study indicates that halothane, isoflurane, and enflurane similarly depress the positive SA nodal chronotropic effects of beta adrenergic stimulation. The nature of this interaction appears not to be competitive, because enhanced beta adrenergic stimulation could not reverse the depression in sinus rate caused by the volatile anesthetics. Moreover, the negative chronotropic effect of the volatile anesthetics may noncompetitively inhibit the moderate direct or indirect effects of phenylephrine.

Clinically, our *in vitro* studies suggest that a sinus tachycardia secondary to enhanced beta receptor activity during sympathetic stimulation may be dose-dependently attenuated by any of the commonly used volatile anesthetics. Conversely, adrenergic stimulation may antagonize, but not completely reverse, the negative chronotropic effects of the anesthetics. In the intact human, other factors, such as differential actions of volatile anesthetics on altering baroreflex activity, cardiac contractility, and basal sympathetic *versus* parasympathetic tone,[1,2] interact to modify the responses observed in the isolated and denervated heart.

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