Multiple Agents Potentiate α₁-Adrenoceptor–induced Conduction Depression in Canine Cardiac Purkinje Fibers

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Background: Halothane more so than isoflurane potentiates an α₁-adrenoceptor (α₁-AR)-mediated action of epinephrine that abnormally slows conduction in Purkinje fibers and may facilitate reentrant arrhythmias. This adverse drug interaction was further evaluated by examining conduction responses to epinephrine in combination with thiopental and propofol, which "sensitize" or reduce the dose of epinephrine required to induce arrhythmias in the heart, and with etomidate, which does not, and responses to epinephrine with verapamil, lidocaine, and L-palmitoyl carnitine, a potential ischemic metabolite.

Methods: Action potentials and conduction times were measured in vitro using two microelectrodes in groups of canine Purkinje fibers stimulated at 150 pulses/min. Conduction was evaluated each minute after exposure to 5 μM epinephrine (or phenylephrine) alone or with the test drugs. Changes in the rate of phase 0 depolarization (V_{max}) and the electrotonic spread of intracellular current were measured during exposure to epinephrine with octanol to evaluate the role of inhibition of active and passive (intercellular coupling) membrane properties in the transient depression of conduction velocity.

Results: Lidocaine (20 μM) and octanol (0.2 mM) potentiated α₁-AR-induced conduction depression like halothane (0.4 mM), with maximum depression at 3–5 min of agonist exposure, no decrease of V_{max}, and little accentuation at a rapid (250 vs. 150 pulses/min) stimulation rate. Thiopental (95 μM), propofol (50 μM), and verapamil (2 μM) similarly potentiated epinephrine responses, whereas etomidate (10 μM) did not. Between groups, the decrease of velocity induced by epinephrine in the presence of (10 μM) L-palmitoyl carnitine (−18%) was significantly greater than that resulting from epinephrine alone (−6%; 0.05 ≤ P ≤ 0.10). Current injection experiments were consistent with marked transient inhibition of cell-to-cell coupling correlating with α₁-AR conduction depression in fibers exposed to octanol.

Conclusions: Anesthetic "sensitization" to the arrhythmogenic effects of catecholamines may be a special case of a more general phenomenon by which not only some anesthetics and antiarrhythmic drugs but also possible ischemic fatty acid metabolites potentiate conduction depression due to acute α₁-AR–mediated cell-to-cell uncoupling. (Key words: Anesthetics; electrophysiology; ion channel regulation; gap junctions, sodium channels.)

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PREVIOUS studies have shown that halothane, more so than isoflurane, potentiates an α₁-adrenoceptor (α₁-AR)-mediated action of epinephrine that transiently depresses conduction in canine cardiac Purkinje fibers by a mechanism that may involve inhibition of Na channel activity, cell-to-cell coupling, or both.[1-4] The aim of this study was to evaluate the scope of this interaction between epinephrine and other drugs on conduction velocity in the Purkinje model.

The actions of epinephrine with halothane were first compared at two stimulation rates with those of epinephrine with octanol, a normal-alcohol anesthetic that "uncouples" or inhibits excitatory current transfer across the gap junctional channels between myocytes,[5] and those with lidocaine, which produces modest rate-dependent depression of Na channels without cell-to-cell

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uncoupling.[6] Because lidocaine, which antagonizes halo-thane–epinephrine dysrhythmias,[7] also potentiated epinephrine-induced conduction depression, we further examined the actions of epinephrine with etomidate as a control agent that does not "sensitize" the heart to the arrhythmogenic effects of epinephrine,[8,9] compared with its actions with propofol and thiopental, two "sensitizing" anesthetics.[8,10] To evaluate the possibility that β- or α_1-AR mediated enhancement of L-type Ca channel activity might contribute to conduction slowing by increasing intracellular Ca^{2+} and thereby gap junctional resistance,[11] we examined the actions of epinephrine on conduction in the presence of verapamil, which also antagonizes halothane–epinephrine arrhythmias.[12] Finally, because the arrhythmogenic effects of α_1-AR activation in acute myocardial ischemia may be enhanced by accumulation of amphiphatic fatty acid metabolites known to produce cell-to-cell uncoupling,[13,14] we evaluated the actions of epinephrine in combination with L-palmitoyl carnitine, a synthetic long-chain acylcarnitine (LCA). The results indicate that both volatile and intravenous sensitizing anesthetics potentiate α_1-AR–induced conduction depression in canine Purkinje fibers and that the same proarrhythmic interaction occurs with other lipophilic drugs (lidocaine and verapamil, but not etomidate) as well as with an endogenous fatty acid metabolite (LCA) that contributes to ischemic arrhythmias.

Methods

The experimental protocols in this study were approved by the Animal Care Committee of the Medical College of Wisconsin. Adult mongrel dogs were anesthetized by mask with isoflurane, intubated, and killed within 10–15 min during isoflurane anesthesia by rapid excision of the heart. Purkinje fiber strands were dissected from either ventricle, mounted to the floor of a 2-ml tissue chamber, and superfused at 5–6 ml/min with 37°C Tyrode's solution equilibrated with 97% O_2–3% CO_2. The superfusate contained 137 mM NaCl, 4.0 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.9 mM NaH_2PO_4, 16 mM $NaHCO_3$, 5.5 mM dextrose, and 44 μM sodium EDTA, added to limit catecholamine oxidation. The fibers were stimulated orthodromically from one end at 150 pulses/min, except where indicated, using 2-ms constant current square wave pulses applied *via* bipolar platinum wire electrodes. Action potentials were simultaneously recorded using two glass intracellular microelectrodes located 3–8 mm apart and at least 2 mm distant from the

stimulation site. The signals were amplified and analyzed by standard laboratory methods.[1,2] The maximum rate of phase 0 depolarization (V_{max}) was determined by electronic differentiation of the upstroke of the action potential. In some instances the action potential upstroke was sampled at 100,000 Hz, and the maximum voltage change per unit time was determined using the stored values. Interelectrode conduction time was measured between action potential upstrokes monitored on a digital storage oscilloscope, and conduction velocity was calculated from the constant interelectrode distance and measured times.

The actions of 5 μM epinephrine HCl (Adrenaline Chloride; Parke-Davis, Morris Plains, NJ) or L-phenylephrine HCl (Phenylephrine; Sigma, St. Louis, MO) on Purkinje conduction velocity were evaluated by rapidly switching to solutions containing the adrenergic agonist and timing the entry of the solution into the tissue chamber. Measurements of action potential characteristics and conduction velocities were generally obtained under drug-free control conditions, after 30-min exposure to test drugs, at each minute during trials of exposure to epinephrine and/or phenylephrine in a randomized sequence of trials in the absence or presence of the test agent, and 15 min after washout of the agonist. Only trials in which both microelectrode impalements were maintained continuously during catecholamine exposure were accepted.

A preliminary study of responses to 5 μM epinephrine at each of two pacing rates (150 and 250 pulses/min), in combination with 0.4 mM (approximately 1.36 vol%) halothane, 0.2 mM octanol, and 20 μM lidocaine was performed in a single group of six preparations to permit direct within-group comparisons of the simultaneous changes of conduction velocity and V_{max} produced by epinephrine with each anesthetic. Six 5-min trials of epinephrine exposure were performed in each preparation, including testing at two pacing rates with each of three anesthetics in a balanced randomized manner. Responses to 5 μM phenylephrine and epinephrine were also determined in three separate anesthetic groups (0.4 mM halothane, 0.2 mM octanol, or 20 μM lidocaine, eight preparations each) to exclude possible crossover effects between agents (failure of anesthetic washout) and to examine potentiation of catecholamine responses with each anesthetic relative to the response without that agent. Halothane concentrations were measured from samples drawn directly from the tissue bath using gas chromatography (0.40 ± 0.02 mM). Octanol (capryl alcohol, 99%, Sigma) was prepared as a 4.5-mM saturated

solution in Tyrode's solution and diluted to a concentration of 0.2 mM. Lidocaine HCl was diluted to 20 μM in Tyrode's from a 2% stock solution (Xylocaine-MPF; AstraZeneca, Westborough, MA). In a similar manner, velocity changes during 5 min of exposure to 5 μM epinephrine alone, epinephrine with 0.4 mM halothane, and epinephrine with 2 μM verapamil (Sigma) were compared within another group of seven preparations. These fibers were stimulated at 150 pulses/min, and 60 min of Tyrode's superfusion was used to washout verapamil between randomized epinephrine trials.

Responses of conduction velocity to 5 μM epinephrine with 95 μM thiopental (Pentothal 2.5%; Abbott Laboratories, North Chicago, IL) and 50 μM propofol (Diprivan 1%; AstraZeneca Pharmaceuticals, Wilmington, DE) were studied in separate groups at one pacing rate (100 pulses/min) in the presence of 0.2 μM propranolol to limit loss of impalements resulting from increased contractility during catecholamine exposure. Each group included three trials of epinephrine exposure of 8-min duration: once without anesthetic, once with 10 μM etomidate (Amidate 2 mg/ml in 35% propylene glycol, Abbott Laboratories) and once with thiopental or propofol. The trials were performed in randomized order with 30-min periods of exposure to the intravenous agents diluted in Tyrode's solution before the catecholamine trial and 45-min anesthetic washout.

To evaluate conduction changes induced by 5 μM epinephrine in combination with a synthetic LCA (L-palmitoyl carnitine, Sigma), a group of seven fibers were exposed in sequence to 10 μM LCA (30 min), LCA plus 5 μM epinephrine (15 min), and LCA alone (15 min) to allow washout of epinephrine. Because of the marked lipophilicity of LCA, this substance could not be washed out to reverse the order for a within-group comparison. Therefore, the changes due to epinephrine with LCA were compared with responses obtained in a group of seven fibers exposed to epinephrine alone for 8 min at the same rate (150 pulses/min) using a between-group design. The increased contractility of otherwise drug-free fibers on exposure to epinephrine (absent propranolol) limited the number of successful continuous microelectrode impalements after 5–8 min.

A few experiments were performed to evaluate directly the role of cell-to-cell uncoupling in epinephrine-induced conduction depression in the presence of octanol using a three-microelectrode current injection technique.[15] The rate of phase 0 depolarization (V_{max}) was measured by electronic differentiation and by "burst" mode A/D sampling (100,000 Hz) while the elec-

trotonic spread of a constant current hyperpolarizing pulse injected through the intracellular amplifier (World Precision Instruments Duo 773, Sarasota, FL) was simultaneously recorded over a fixed distance. Conduction velocity was measured between the action potential upstroke and the signal from a bipolar electrode located at the far end of the strand. Changes in diastolic threshold current required to elicit an action potential during the rapid velocity changes induced by epinephrine were measured in an analogous experiment under identical conditions using 5-ms intracellular depolarizing pulses.

Data are presented as mean ± SEM, except as indicated. The values obtained within each group were evaluated by repeated-measures analysis of variance, and means were compared using the Waller-Duncan least significant difference method.[16] Values obtained in two groups (epinephrine alone vs. epinephrine with LCA) were compared using analysis of variance and least significant difference methods based on a repeated between- and within-group experimental design.[17]

Results

Figure 1 illustrates the depression of Purkinje fiber conduction velocity by epinephrine with lidocaine, halothane, and octanol. At 150 pulses/min (fig. 1A, time 0 vs. drug-free control), conduction velocity was decreased ($P \leq 0.05$) by octanol (−11%) and halothane (−2.4%) but not by lidocaine (−2.2%), without significant change of V_{max} (< 4% with any agent). At 250 pulses/min (fig. 1B), the decrease of velocity ($P \leq 0.05$) by lidocaine (−6.4%, −0.13 ± 0.04 m/s) was greater ($P \leq 0.05$) than that at the slow rate (−2.2%, −0.05 ± 0.02 m/s), whereas the decreases by octanol (−9%) and halothane (−3%) were similar at both rates, and V_{max} was not different from control. Statistical analyses indicated that only lidocaine was associated with significant rate-dependent conduction slowing. Compared with the anesthetics alone (time 0, figs. 1A and 1B), addition of 5 μM epinephrine substantially decreased conduction velocity with a similar transient time course at each pacing rate in the presence of the three agents. The decreases of velocity at the time (3 min) of maximum slowing by epinephrine were not accompanied by any significant change of V_{max} (data not shown), which averaged approximately 3% above that just before epinephrine exposure. Although lidocaine alone produced slight rate-dependent depression of conduction, the larger decrease of velocity induced by epinephrine with lido-
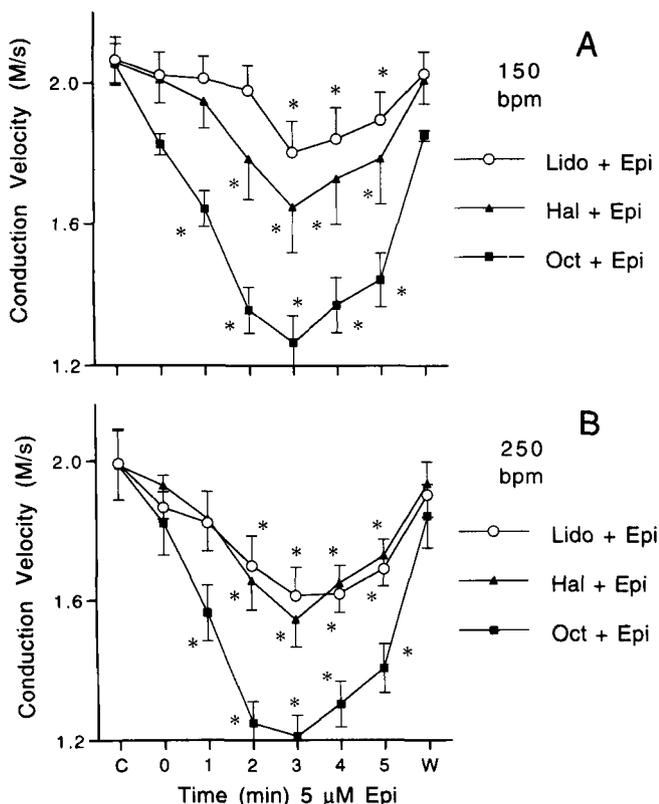
Fig. 1. Time course of actions of 5 μM epinephrine (Epi) on Purkinje fiber conduction velocity (m/s $\pm$ SEM) in the presence of 20 μM lidocaine (Lido), 0.4 mM halothane (Hal), or 0.2 mM octanol (Oct) at pacing rates of (*A*) 150 or (*B*) 250 pulses/min in six preparations. C = drug-free control; time 0 = value just before epinephrine exposure; W = epinephrine washout (anesthetic present). *P $\leq$ 0.05 *verses* time 0 value with each anesthetic.

Fig. 2. Actions of epinephrine alone, epinephrine with 10 μM etomidate, and epinephrine with either 95 μM thiopental (*A*) or 50 μM propofol (*B*) on Purkinje fiber conduction. Propranolol 0.2 μM present throughout in two groups of eight preparations each. *P $\leq$ 0.05 *verses* time 0 value just before epinephrine exposure without (Epi alone) or with anesthetic.

caine was not particularly accentuated at 250 *versus* 150 ppm (-13.5% *vs.* -10.7% at 3 min, respectively). Similar results were obtained in the three separate single-anesthetic groups, indicating that failure of washout of the different anesthetics between epinephrine trials did not influence the findings. In those groups (data not shown), the depression of velocity by 5 μM epinephrine and phenylephrine was significantly greater in the presence than in the absence of each anesthetic and greater with epinephrine than with phenylephrine.

The actions of epinephrine alone, epinephrine with etomidate, and epinephrine with either thiopental or propofol at 100 pulses/min are shown in figure 2. The decreases of velocity induced by epinephrine were larger ($P \leq 0.05$, from 2 to 7 min) with either thiopental or propofol (-17% and -16% at 4 min, respectively) than the responses to epinephrine alone or with etomidate in each group. Epinephrine alone modestly de-
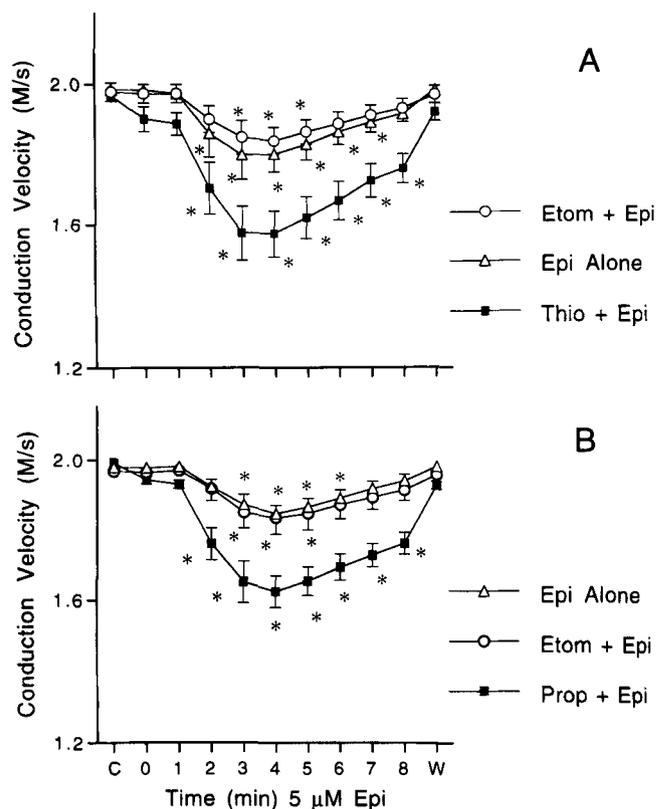
creased ($P \leq 0.05$) velocity in both groups (by -7 to -9% at 4 min *vs.* time 0). V_{max} did not decrease significantly at the time of maximum depression of velocity in either group.

Figure 3A compares responses to epinephrine alone, epinephrine with verapamil, and epinephrine with halothane at 150 pulses/min. At the time (3 min) of maximum slowing, epinephrine alone decreased velocity (by 6%, -0.12 ± 0.10 m/s; $P \leq 0.05$), whereas epinephrine decreased velocity to a greater degree (by 12%, -0.24 ± 0.11 m/s; $P \leq 0.05$) in the presence of verapamil than in its absence.

The preceding studies were performed using a randomized sequence of epinephrine trials without and with each agent within the groups to minimize potential time-dependent effects. Figure 3B compares conduction changes between groups exposed only once to epinephrine at 150 pulses/min, either alone (without any prior drug exposure except isoflurane for animal sacrifice) or
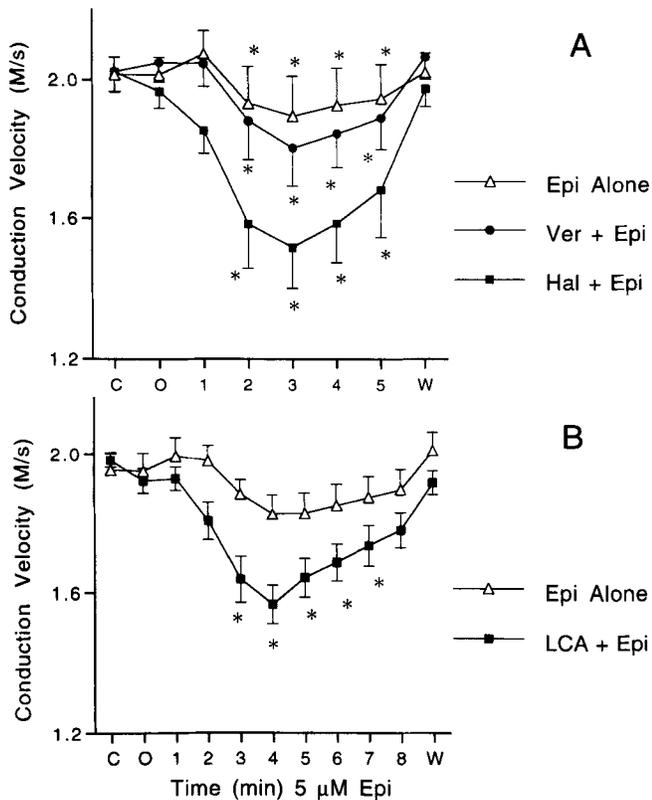
Fig. 3. (*A*) Actions of epinephrine alone, epinephrine with 0.2 μM verapamil, and epinephrine with 0.4 mM halothane in one group of six preparations. *P ≤ 0.05 *verses* time 0 value just before epinephrine exposure. (*B*) The time course of velocity changes in two separate groups (n = 7 each) on exposure to 5 μM epinephrine alone (unfilled triangles) or 5 μM epinephrine in the presence of 10 μM L-palmitoyl carnitine (LCA). *P ≤ 0.05 *verses* mean value at same time of epinephrine exposure without LCA between groups.

with continuous exposure to L-palmitoyl carnitine beginning 20 min before the catecholamine trial. Epinephrine alone did not significantly decrease conduction velocity (−6.4% at 4 min, 0.05 ≤ P ≤ 0.10), whereas epinephrine with the LCA decreased velocity (P ≤ 0.01) by −18% at 4 min. The velocity at 4 min of epinephrine exposure was lower (P ≤ 0.05) with LCA (1.57 ± 0.05 m/s) compared to without LCA (1.82 ± 0.06 m/s), indicating significant potentiation, whereas again V_{max} did not decrease significantly.

Figure 4 illustrates a technique used to evaluate the role of changes in cell-to-cell coupling during the conduction slowing produced by 10 μM epinephrine with 0.2 mM octanol in the presence of 0.5 μM propranolol. Figure 4A illustrates a Purkinje strand, approximately 370 μm in diameter and 10 mm in length, stimulated extracellularly at its left end at 100 pulses/min. The

leftmost intracellular microelectrode (Me_1) was used to inject a constant current (160 nA, 100-ms duration) intracellular hyperpolarizing pulse during the diastolic interval after every 10th stimulated action potential. A middle (Me_2, 250 μm to the right) and third microelectrode (Me_3, 750 μm further to the right) recorded the action potential and relatively large (Δ1) and smaller (Δ2) voltage deflections resulting from the injected pulse. An increase of resistance ("R" in fig. 4A) or decrease of conductance ("S" in fig. 4A), to longitudinal current flow would increase the ratio of the voltage deflections recorded close to (Δ1) and distant from (Δ2) the site of current injection. Velocity was measured using activation times at Me_3 and at a bipolar electrode at the right end of the strand.

Figure 4B illustrates the simultaneous changes of conduction velocity (θ), active (phase 0-V_{max}) and passive (conductance "S") membrane properties induced by epinephrine with octanol, as a percentage of the value (time 0 with octanol) just before epinephrine exposure. The maximum slowing by epinephrine (−25% at 3 min) corresponded in time with greatest reduction of conductance "S" (−60% at 3 min), whereas V_{max} tended to increase throughout epinephrine exposure. Linear cable theory[18] predicts that the change of the square of conduction velocity ($θ^2$) is directly proportional to the change in V_{max} and inversely proportional to the sum of external (r_o) and internal longitudinal resistance (r_i): $θ^2 = V_{max}/(r_o + r_i)$. The solid line in figure 4C represents a least squares fit ($r^2 = 0.77$; $P ≤ 1 \times 10^5$) between the normalized values of $θ^2$ and V_{maxf}/R from the experimental data, assuming no change of r_o and that r_i is approximated by the ratio of the voltage deflections (R = Δ1/Δ2 in fig. 4A). The dotted lines in figure 4C show good correlation between the decrease of $θ^2$ and the decrease of conductance "S," but no correlation between the change of the square of velocity and V_{maxf}. The ratio of voltage deflections might not reflect an increase of intercellular resistance by epinephrine if nonjunctional membrane resistance decreased and some current crossed the cell membrane rather than flowing through intercellular gap junctions. We reasoned that if membrane resistance decreased, a greater depolarizing current would be required to deflect the membrane potential to threshold. This possibility was examined in another preparation by simultaneously measuring velocity and intracellular diastolic threshold current under identical conditions. As shown in figure 4D, the decrease of conduction velocity by epinephrine correlated with a decrease in diastolic threshold current, as if the depolarizing current required
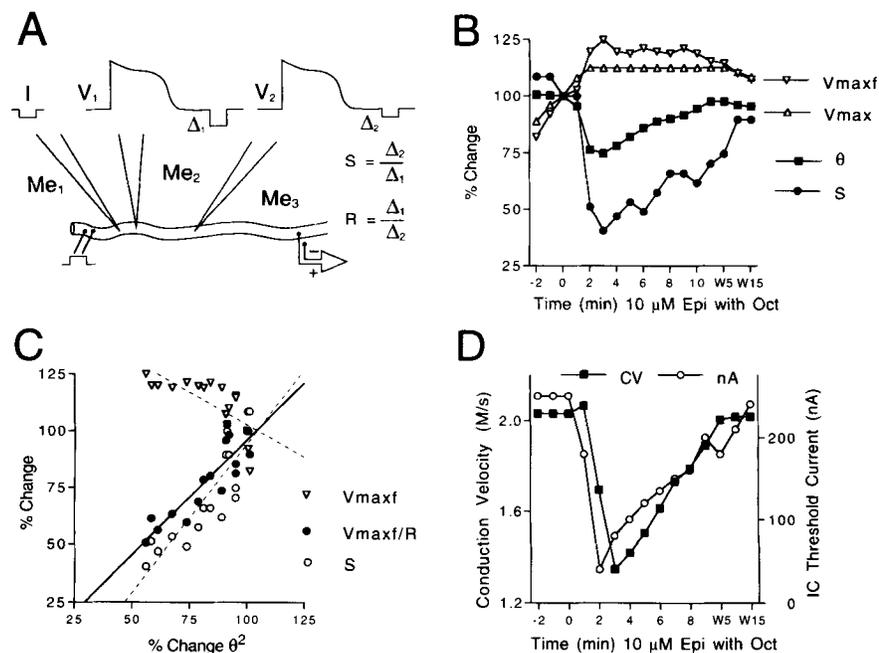
Fig. 4. Current injection experiments. (A) A Purkinje strand impaled by three microelectrodes near one end used for current passing (Me$_1$) and recording electrotonically conducted voltage pulses (ΔV_1 and ΔV_2) as discussed in the text. (B) The time course of epinephrine-induced simultaneous changes in conduction velocity (θ), linear conductance (S in fig. 4A) and rates of phase 0 depolarization by electronic differentiation (V$_{max}$) and rapid A/D sampling (V$_{maxf}$) in one experiment as a percentage of the control (time 0 value) just before exposure to epinephrine with 0.2 mM octanol. (C) Solid black line = linear least squares fit to the equation $\theta^2 = V_{maxf}/R$ using the experimental data from (B); dotted lines = linear correlation between changes in the active (V$_{maxf}$) and passive (conductance S) components of the approximate cable equation. (D) Correlation between decreases of conduction velocity and intracellular (IC) diastolic threshold current in another Purkinje preparation exposed to epinephrine with 0.2 mM octanol.

to elicit an action potential were reduced to that of a single uncoupled cell. Thus, the transient negative dromotropic effects of epinephrine on Purkinje fibers seem to largely result from cell-to-cell uncoupling rather than from decreases in V$_{max}$ or membrane resistance.

Discussion

This study demonstrates that thiopental and propofol, which "sensitize" the heart to the arrhythmogenic effects of epinephrine in the canine model,[8,10] potentiate α_1-AR-induced depression of conduction in isolated canine Purkinje fibers, just like halothane.[1,2] These results and the negative findings with etomidate[8,9] support the hypothesis that the phenomenon of anesthetic sensitization to the arrhythmogenic effects of catecholamines may involve potentiation of an α_1-AR-mediated action that facilitates generation of reentrant arrhythmias by abnormally slowing conduction. Similarly enhanced α_1-AR-induced conduction changes were found with two other uncoupling agents, a normal alcohol anesthetic (octanol) and a potential endogenous ischemic metabolite (an LCA) not known to exhibit any anesthetic properties. However, similar potentiation of α_1-AR-induced conduction slowing was also found with two antiarrhythmic agents (lidocaine and verapamil) that antagonize generation of halothane-epinephrine dysrhythmias.[7,12] Finally, using a current injection technique, we

found that α_1-AR activation produces marked decrease of the electrotonic spread of intercellular current consistent with an electrophysiologic mechanism of acute cell-to-cell uncoupling underlying α_1-AR conduction depression.

The findings are limited to a simplified model of linear conduction in isolated Purkinje fibers not exhibiting arrhythmias under these experimental conditions. Additional factors present *in vivo* that might contribute to induction of reentrant arrhythmias by epinephrine include hepatic K$^+$ ion release with transient hyperkalemia to the range of 6-8 mEq/l,[19] sudden changes in heart rate, cardiac autonomic efferent activity,[20,21] ventricular volume, and intracellular calcium transients,[22-25] as well as differential effects of α_1- and β-AR activation on myocardial as opposed to Purkinje fiber repolarization and conduction.[26,27] Unlike this Purkinje model of enhanced α_1-AR responsiveness, anesthetic sensitization *in vivo* requires synergistic effects mediated by β-AR activation.[28] The latter component could lead to triggering of an early premature impulse that then induces reentry in a setting of increased heterogeneity of conduction and refractory characteristics resulting from simultaneous α_1- and β-AR activation. Antiarrhythmic agents that further decrease inward Na$^+$ and/or Ca^{2+} current, like lidocaine and verapamil, may abolish reentry by converting unidirectional to bidirectional block.[29] Thus, the findings that these antiarrhythmic agents also

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potentiate epinephrine-induced conduction depression does not necessarily exclude the possibility that α_1-AR-mediated depression of conduction contributes importantly to the phenomenon of anesthetic sensitization to the arrhythmogenic effects of epinephrine.

The findings with halothane, octanol, and lidocaine indicate that α_1-AR-induced conduction slowing is similar to that produced by the uncoupling agent octanol, which slowed conduction without reducing V_{max}, and differs from that produced by lidocaine, which induced modest rate-dependent conduction slowing not present with either halothane or octanol. Additional studies would be required to fully characterize the rate dependence of the interaction between epinephrine and the potentiating agents on conduction velocity and V_{max}. Nevertheless, the large transient decreases of velocity induced by epinephrine with halothane and octanol were not synergistically increased by stimulation at 250 versus 150 pulses/min. The findings with lidocaine argue against a contribution of α_1-AR-induced conduction depression to anesthetic sensitization to epinephrine-induced arrhythmias. However, the potentiation by propofol and thiopental, but not etomidate, used at concentrations similar to those at which these agents alter the electromechanical properties of isolated cardiac tissues,[30-34] again supports a possible role of this action in anesthetic sensitization. Potentiation of epinephrine-induced conduction depression by verapamil suggests that neither α_1- or β-AR-mediated increases in Ca^{2+} ion flux via the L-type Ca channels[35] underlie the transient negative dromotropic effects of epinephrine on Purkinje fibers. Finally, the results with an LCA reported to enhance α_1-AR responsiveness in ischemia and depress both Na channels and cell-to-cell coupling[14,36] suggests that potentiation of α_1-AR-induced conduction depression by sensitizing anesthetics may represent a special case of a more general mechanism of α_1-adrenergic regulation of conduction rather than only a peculiar drug interaction. It is not known whether significant potentiation occurs on activation of other G-protein coupled receptors, as suggested by findings that endothelin and carbamylcholine also transiently depress conduction in Purkinje fibers exposed to halothane.[2]

Recent studies demonstrating synergistic depression of active Na^+ current generation in guinea pig myocytes by the α_1-agonist methoxamine and halothane suggest that the electrophysiologic mechanism underlying α_1-AR-mediated depression of conduction with anesthetics could involve modulation of Na channel activity.[3,4] The signal transduction pathway linking α_1-AR activation to

inhibition of Na channels in myocytes seems to involve a pertussis toxin sensitive G protein and protein kinase C.[4,37,38] Thus, the interaction between sensitizing agents and epinephrine on conduction could involve a cellular mechanism that potentiates signal transduction at the level of the α_1-AR receptor, G protein, phospholipase, and/or protein kinase C, leading to downregulation of Na channels as one possible effector. LCAs may contribute to enhanced catecholamine-induced generation of inositol trisphosphate by phospholipase C.[13,39] More recent studies suggest that anesthetics (benzodiazepines) can potentiate the function of specific α_1-AR receptor subtypes by allosterically interacting with interhelical sites that form a salt-bridge triggering mechanism for activation by α_1-agonists.[40] However, there is no evidence that α_1-AR activation slows conduction in the canine myocardium exposed to halothane as it does in Purkinje fibers,[27] and the findings that the relatively large decreases of conduction velocity induced by α_1-AR activation with the potentiating agents are not accompanied by decreases in V_{max}, an indirect indicator of peak inward Na^+ current,[18] argues against a role of Na channel inhibition in the Purkinje model.

This study provides the first direct evidence that α_1-AR-induced depression of conduction by epinephrine with a potentiating agent (octanol) in Purkinje fibers is correlated with changes in passive electrical properties (the intracellular diastolic threshold and linear conductance "S") measured as shown in figure 4. These results are consistent with a recent report by De Mello[41] demonstrating that phenylephrine alone depresses cell-to-cell coupling in isolated paired canine myocytes by a protein kinase C-dependent mechanism and that α_1-AR activation partially antagonizes the usual increase of gap junctional conductance between ventricular myocytes resulting from the β-adrenergic effects of catecholamines. Theoretically, gap junctional uncoupling, without change of Na^+ conductance, can increase V_{max} and produce greater reduction of velocity before conduction block than inhibition of the peak inward Na^+ current underlying cardiac excitability.[29] Experimentally, Jalife et al.[5] found that uncoupling by heptanol simultaneously reduced Purkinje conduction velocity and intracellular current threshold without reducing V_{max}. Similarly, we found that α_1-AR activation produced marked depression of Purkinje velocity and the current threshold with octanol, without reducing V_{max}. Thus, it seems likely that transient α_1-AR-induced protein kinase C-dependent regulation of gap junctional coupling contributes substantially to the depression of conduction

produced by epinephrine with the potentiating agents identified. However, it may not be possible to definitively assess the relative roles of gap junctional *versus* Na channel inhibition in α_1-AR-induced conduction depression in multicellular preparations because marked uncoupling, by reducing the "electrical load" on individual cells, may mask simultaneous depression of peak inward Na^+ current and reduce the reliability of V_{max} as an indicator of cardiac excitability.[5]

The results do not identify one specific mechanism as predominantly responsible for the enhancement of α_1-AR conduction changes by the diverse potentiating agents identified in this study. The underlying mechanisms may involve both direct (membrane level) and indirect effects (on α_1-AR signal transduction and intracellular regulatory kinases) of the potentiating agents on at least two effector channels (the Na and gap junctional channels), which together interact in a complex nonlinear manner to determine conduction velocity. Assuming constant efficacy of receptor coupling to regulation of the effectors, potentiation may represent direct effects on either or both the Na and gap junctional channels that reduce transmembrane and intercellular currents (the "safety factor" for conduction[29]) closer to a critical limit such that further receptor-mediated downregulation of the same channels manifests as a "larger" depression of velocity with the potentiating agent. Although none of the agents studied substantially depresses interventricular conduction *in vivo*, all except perhaps etomidate inhibit cardiac Na channel activity. Halothane, octanol, and LCA inhibit cardiac gap junctions,[14,42] whereas the actions of isoflurane, thiopental, propofol, and etomidate on these channels are unknown. Lidocaine and verapamil do not alter gap junctional resistance.[6] The findings suggest that α_1-AR activation may produce relatively greater depression of conduction in the presence of: (1) greater basal depression by the potentiating agents (as with octanol and lidocaine (at 250 ppm), which alone decreased velocity approximately 10 and 6%, respectively); and (2) agents that depress the gap junctions (octanol, halothane, LCA) more so than those that do not (lidocaine, verapamil). Other than in anesthetic sensitization, transient α_1-AR-induced conduction depression may possibly contribute to the arrhythmogenic effects of catecholamines in clinical settings of marked reduction of Na channel availability, as in hyperkalemia or with potent class IC antiarrhythmic drugs, and in ischemia,[43] in which membrane depolarization and gap junctional depression by intracellular Ca^{2+} ions and LCA also contribute to abnormal slowing of conduc-

tion. Conversely, α_1-AR-mediated depression of conduction could also abolish reentry in the presence of specific electrophysiologic substrates by preventing reexcitation at a site of unidirectional conduction block.

In conclusion, both volatile and intravenous anesthetics that sensitize the heart to the arrhythmogenic effects of epinephrine potentiate α_1-AR-induced transient negative dromotropic effects in canine Purkinje fibers. Similar potentiation is observed with a variety of lipophilic drugs (lidocaine and verapamil but not etomidate) and a potential endogenous ischemic metabolite (LCA), which suggests that α_1-AR activation may modulate ventricular cardiac conduction in a wider setting of pathophysiologic circumstances. The electrophysiologic mechanism responsible for the depression of conduction by epinephrine with potentiating agents may involve α_1-AR-mediated protein kinase C–dependent downregulation of cell-to-cell coupling at the gap junctions, perhaps masking simultaneous inhibition of Na channel activity. Additional studies of gap junctional regulation in paired myocytes would be required to determine whether the interaction between sensitizing anesthetics and the effects of α_1-AR activation on conduction results from additive or synergistic depression of cell-to-cell coupling in cardiac tissues.

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