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## A Subtype of $\alpha_1$ Adrenoceptor Mediates Depression of Conduction in Purkinje Fibers Exposed to Halothane

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**Background:** An action of epinephrine at  $\alpha$  adrenoceptors has been reported to slow conduction in Purkinje fibers exposed to halothane. In Purkinje fibers one pharmacologically distinguishable  $\alpha_1$ -adrenoceptor subtype ( $\alpha_{1B}$ ) sensitive to the noncompetitive antagonist chloroethylclonidine mediates decreases in automaticity. Another  $\alpha_1$  subtype ( $\alpha_{1A}$ ), sensitive to the competitive antagonist WB4101, increases spontaneous rate and action potential duration by a mechanism thought to involve hydrolysis of membrane phosphoinositides by phospholipase C. This study examined the dose-response relation and receptor-effector mechanisms underlying depression of conduction in canine Purkinje fibers by epinephrine with halothane.

**Methods:** Conduction velocity was determined *in vitro* by measuring the conduction time between action potentials recorded from two Purkinje fibers located about 6 mm apart along the length of free running portions of the ventricular conduction system, the false tendons. Velocity was evaluated at 1-min intervals during trials of rapid exposure to different agonists in groups of 6-12 preparations.

**Results:** Epinephrine (0.2-5.0  $\mu$ M) transiently decreased Purkinje conduction velocity in a dose-related manner by as much as 33% (at 5  $\mu$ M epinephrine with 0.86 mM (2.8%) halothane).

Velocity decreased by 5% ( $P \leq 0.01$ ) at an epinephrine concentration similar to "just-threshold" dysrhythmogenic plasma epinephrine concentrations (0.2  $\mu$ M epinephrine with 0.46 mM halothane) reported in halothane-anesthetized dogs. The decreases of conduction velocity were blocked by prazosin but not by metoprolol, were produced by phenylephrine but not by clonidine, and were antagonized by equimolar (0.5  $\mu$ M) concentrations of WB4101 more so ( $P \leq 0.01$ ) than by chloroethylclonidine. WB4101 (0.1  $\mu$ M) produced 87% inhibition of the response to 0.2  $\mu$ M epinephrine after chloroethylclonidine pretreatment, indicating mediation by the  $\alpha_{1A}$  subtype. Other agonists linked to cardiac phospholipase C activation, including endothelin 1 (40 nM) and the muscarinic agonist carbamylcholine (1 mM), also decreased conduction velocity in fibers exposed to halothane.

**Conclusions:** Clinically relevant concentrations of epinephrine transiently depress conduction in Purkinje fibers exposed to halothane by activating cardiac  $\alpha_1$  adrenoceptors, largely but not exclusively the WB4101-sensitive  $\alpha_{1A}$  subtype, reportedly coupled to stimulation of phospholipase C and generation of the second messengers diacylglycerol and inositol trisphosphate. Anesthetic potentiation of cardiac  $\alpha_1$ -adrenoceptor effects may contribute to the generation of halothane-epinephrine dysrhythmias by abnormally slowing conduction and facilitating reentry. (Key words: Anesthetics, volatile: halothane. Heart: conduction. Heart, dysrhythmias: Purkinje fibers. Pharmacology: endothelin. Sympathetic nervous system:  $\alpha$ -adrenergic agonists. Sympathetic nervous system, catecholamines: epinephrine.)

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kinje fiber automaticity by activating a subtype of  $\alpha_1$  receptor ( $\alpha_{1B}$ ) sensitive to the alkylating agent chloroethylclonidine (CEC) leading to stimulation of the  $\text{Na}^+ - \text{K}^+$  electrogenic pump.<sup>7,10-12</sup> High concentrations of catecholamines ( $>10^{-6}$  M, in the presence of propranolol) increase spontaneous rate and Purkinje action potential duration<sup>7,8</sup> by activating a subtype of  $\alpha_1$  receptor ( $\alpha_{1A}$ ) sensitive to the competitive antagonist WB4101. Both  $\alpha_1$ -receptor subtypes appear to be linked to stimulation of phospholipase C (PLC) and hydrolysis of sarcolemmal phosphoinositides in cardiac tissues.<sup>7,13-18</sup> A different  $\alpha$  adrenoceptor-mediated action on Purkinje fibers was suggested by Reynolds and Chiz,<sup>19</sup> who first reported that epinephrine ( $4.5 \mu\text{M}$ ) markedly potentiated the slowing of conduction produced by halothane in Purkinje fibers in a manner blocked by phentolamine but not by propranolol. Studies from this laboratory<sup>20</sup> confirmed that a high ( $5 \mu\text{M}$ ) concentration of epinephrine, which alone did not depress conduction, transiently depressed conduction velocity in Purkinje fibers exposed to halothane and that this "abnormal" depression of conduction also occurs to a smaller degree with the less "sensitizing" anesthetic isoflurane.

The first objective of this study was to determine the epinephrine dose-response relation for the interaction with halothane on Purkinje fiber conduction velocity. In addition, we evaluated the receptor mechanisms modulating conduction by using the selective  $\alpha_1$ -adrenoceptor subtype antagonists WB4101 and CEC and other agonists (endothelin and carbamylcholine) known to activate similar G-protein-linked receptor-effector pathways leading to phosphoinositide hydrolysis in cardiac tissues.<sup>21</sup>

## Materials and Methods

The experimental protocols were approved by the Animal Care Committee of the Medical College of Wisconsin. Purkinje fibers were obtained from the hearts of adult mongrel dogs killed during halothane anesthesia. The preparations were dissected from the free running intracavitary portions of the conduction system connecting the main bundle branches to the papillary muscles. Small branches were divided 2-3 mm away from the main false tendon and segments measuring 6-12 mm in length were pinned to the floor of a 2-ml tissue chamber and superfused at 5-7 ml/min (time constant approximately 20 s) with  $37^\circ\text{C}$  Tyrode's solution equilibrated with 97%  $\text{O}_2$ -3%  $\text{CO}_2$ . The super-

fusate contained the following (millimolar): NaCl 137, KCl 4.0,  $\text{CaCl}_2$  1.8,  $\text{MgCl}_2$  0.5,  $\text{NaH}_2\text{PO}_4$  0.9,  $\text{NaHCO}_3$  16, and dextrose 5.5 and  $50 \mu\text{M}$  Na ethylenediamine tetraacetic acid to limit catecholamine oxidation. All preparations were stimulated orthodromically at 150 beats/min with bipolar platinum wire electrodes and twice-threshold square-wave pulses 2 ms in duration. Action potentials were obtained from two fibers 3-8 mm apart and at least 2 mm from the stimulation site by using glass microelectrodes and intracellular amplifiers. Only drug trials in which both cell impalements were maintained for 10-20 min were accepted. Every effort was made to maintain continuous impalements throughout each sequence of experimental interventions without rezeroing the amplifiers by withdrawal into the superfusate. The action potential signals were monitored on oscilloscopes, sampled by analogue-to-digital conversion, stored, and analyzed with a computer by standard methods for this laboratory.<sup>20</sup> The rate of phase 0 depolarization was measured by electronic differentiation and a peak and hold detector. Conduction velocity was calculated from the time between the phase 0 upstrokes of the action potentials measured at rapid sweep with a digital oscilloscope and conduction distance measured at the end of each experiment with calibrated dividers.

The dose-response relation for the actions of epinephrine on conduction velocity of fibers exposed to halothane was determined at two anesthetic concentrations within a group of ten preparations in a balanced randomized sequence of trials. Solutions with and without L-epinephrine HCl, added as fractions of 1 ml stock solution (1 mg/ml, Adrenalin Chloride, Parke-Davis, Morris Plains, NJ) to measured volumes of superfusate, were preequilibrated with halothane from a single vaporizer. The tissues were first exposed to halothane<sup>22</sup> for at least 20 min to obtain equilibrium. The anesthetic concentrations in the tissue bath were sampled before and after the drug trials, measured by gas chromatography and are reported with each group. The drug trials were conducted by rapidly switching between solutions and timing the trial from entry of the solution into the tissue chamber. Our preliminary studies under these conditions<sup>20</sup> indicated that epinephrine transiently decreased velocity, reaching minimum values within 3-5 min and dissipating by 10-12 min, despite continuing exposure to both halothane and epinephrine. Therefore the conduction velocities and action potential characteristics were measured at 1-min intervals during 5-min trials of epinephrine ex-

posure and at least 15 min of epinephrine-free superfusion was used for return to baseline between trials. The preparations were exposed, in randomized order, to trials of 0.2, 1, 2, and 5  $\mu\text{M}$  epinephrine at each of two anesthetic concentrations. These epinephrine concentrations correspond approximately to reported plasma values associated with "just-threshold" epinephrine doses in halothane (0.2  $\mu\text{M}$ , 40–50 ng/ml) and pentobarbital (1.5  $\mu\text{M}$ , about 300 ng/ml)–anesthetized dogs<sup>23–25</sup> and to about three times that which might occur (5  $\mu\text{M}$ , 1 mg/l) at equilibrium in the plasma after intravenous injection of 1 mg epinephrine in the perioperative setting.

Receptor mechanisms modulating Purkinje fiber conduction in the presence of halothane were studied in groups of 6–12 preparations. The changes in conduction velocity in response to 5  $\mu\text{M}$  epinephrine with halothane were first determined before and then after 30 min exposure to 1  $\mu\text{M}$  DL-metoprolol (Sigma, St. Louis, MO) or 1  $\mu\text{M}$  prazosin HCl (Pfizer, New York, NY). The responses to 5  $\mu\text{M}$  L-phenylephrine HCl (Sigma) and 5  $\mu\text{M}$  clonidine HCl (Sigma) were evaluated in the absence and presence of halothane in two groups to investigate the potentiation of  $\alpha_1$ -adrenergic effects by halothane and to exclude a possible effect of  $\alpha_2$ -adrenergic activation.<sup>26</sup> The roles of the two pharmacologically distinguishable  $\alpha_1$ -adrenoceptor subtypes in modulating conduction with halothane were evaluated by establishing control responses to trials of 0.2, 1.0 and 5  $\mu\text{M}$  epinephrine, in randomized order, in the presence of 0.2  $\mu\text{M}$  DL propranolol HCl (Sigma). One half of this group was then exposed for 30 min to 0.5  $\mu\text{M}$  WB4101 (Research Biochemicals, Natick, MA), and the others were exposed to 0.5  $\mu\text{M}$  CEC (Research Biochemicals). The epinephrine trials were then repeated in the presence of the antagonists to compare their effects on conduction depression at equimolar concentrations. Additional preparations were pretreated for 30 min with CEC (0.5  $\mu\text{M}$ ) to attenuate responses ( $\alpha_{1B}$ ) sensitive to the alkylating agent and washed for 1 h to reduce the potential<sup>7</sup> for competition by CEC at the  $\alpha_{1A}$ -receptor. These preparations were thereafter studied to determine the degree of inhibition of conduction changes by a lower "more selective" concentration (0.1  $\mu\text{M}$ ) of the  $\alpha_{1A}$  antagonist WB4101 as used by others in this model.<sup>7,8</sup> The actions of epinephrine, angiotensin II, carbamylcholine, and endothelin 1 (Sigma) on conduction in fibers exposed to halothane were determined in two other groups of false tendons.

The findings are reported as values of the mean  $\pm$  SEM. All values obtained within an experimental group were evaluated by repeated-measures analysis of variance and means at specific times were compared using Waller-Duncan's least significant difference method.<sup>27</sup> A probability level of 0.05 or less was considered significant.

## Results

Figure 1 illustrates the dose-response relation for the conduction velocity at the time (3 min) of maximum epinephrine effect at two halothane concentrations. Halothane alone decreased conduction velocity ( $P \leq 0.05$ ) from a mean drug free control value of  $2.21 \pm 0.10$  m/s by 5% and 11% at the low (0.46 mM) and high (0.86 mM) concentrations, respectively. 5  $\mu\text{M}$  epinephrine with 0.86 mM halothane decreased velocity by 33% (to  $1.31 \pm 0.05$  m/s) of the value with halothane ( $1.97 \pm 0.08$  m/s) alone. Significant depression of conduction ( $-5\%$  relative to halothane alone,  $P \leq 0.01$ ) was observed even at the lowest (0.2  $\mu\text{M}$ ) epinephrine and halothane (0.46 mM) doses. The depression of conduction was larger ( $P \leq 0.05$ ) at high

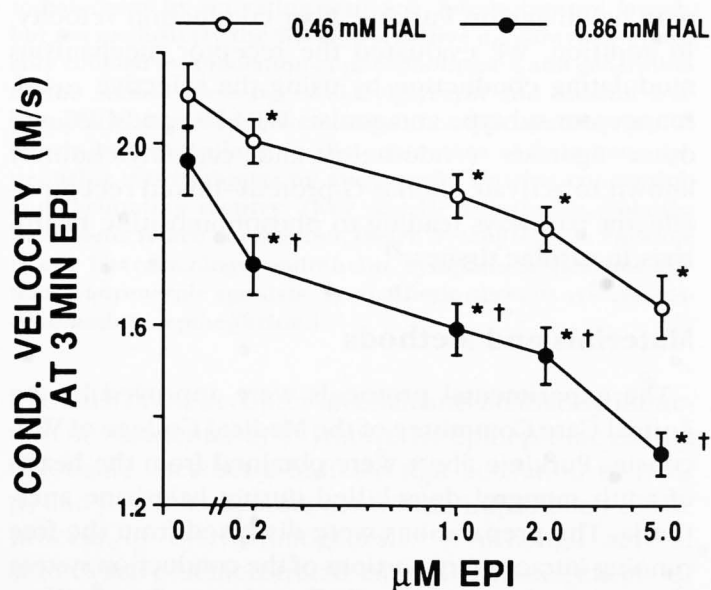


Fig. 1. Dose-related effects of epinephrine (EPI) on conduction velocity (mean  $\pm$  SEM, eight preparations) in Purkinje fibers exposed to 0.46 mM (1.5%) and 0.86 mM (2.8%) halothane (HAL). Values at 0 EPI represent controls with halothane alone; values at each epinephrine concentration represent those at the time (3 min) of maximum effect of epinephrine on velocity. \* $P \leq 0.01$  versus halothane alone (at 0 epinephrine). † $P \leq 0.01$  versus epinephrine at lower halothane concentration.

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compared to low epinephrine doses at each halothane concentration and larger ( $P \leq 0.05$ ) at high compared to lower halothane concentration at each epinephrine dose. The depression of conduction on exposure to epinephrine was not associated with significant reductions of the rate of phase 0 depolarization or action potential amplitude (data not shown).

Figure 2 shows the changes of conduction velocity on exposure to 5  $\mu\text{M}$  epinephrine with 0.45 mm halothane before (top) and after (bottom) treatment with prazosin and metoprolol. The depression of conduction by epinephrine with halothane was completely abolished by 1  $\mu\text{M}$  prazosin but remained in the presence of 1  $\mu\text{M}$  metoprolol. The relative roles of  $\alpha_1$  and  $\alpha_2$  adrenoceptors in the interaction on conduction was evaluated in two groups with the  $\alpha$ -agonists phenylephrine and clonidine. As shown in figure 3 (top), phenylephrine (5  $\mu\text{M}$ ) in the absence of halothane slightly decreased ( $-2\%$ ) conduction velocity at 4–5 min of exposure ( $P \leq 0.05$ ) relative to the preceding

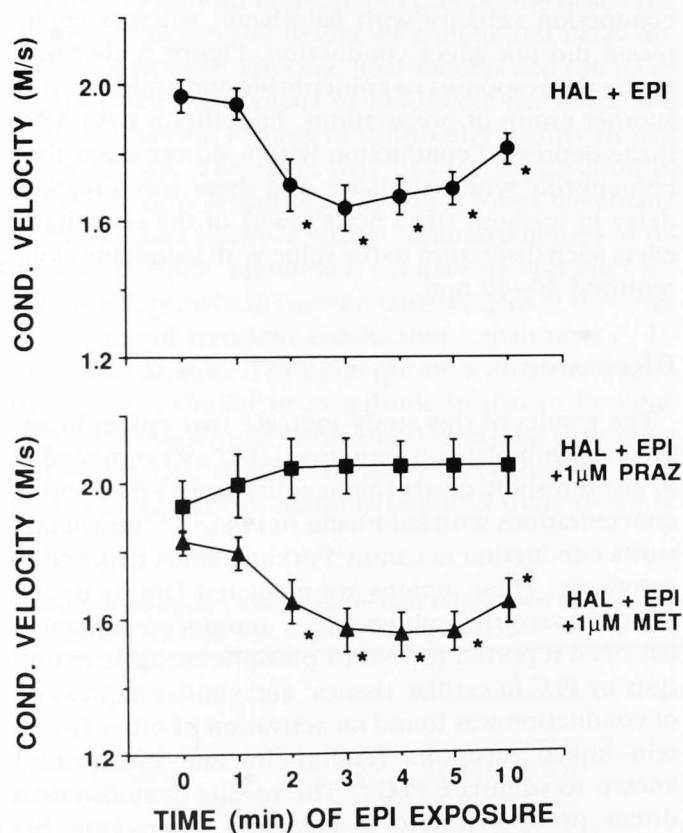


Fig. 2. Time-dependent effects of 5  $\mu\text{M}$  epinephrine (EPI) with 0.45 mm halothane (HAL) on conduction velocity before (ten preparations) (top) and after (five preparations each) (bottom)  $\alpha_1$ - and  $\beta_1$ -adrenergic blockade. PRAZ = prazosin; MET = metoprolol. \* $P \leq 0.01$  versus halothane alone (at time 0).

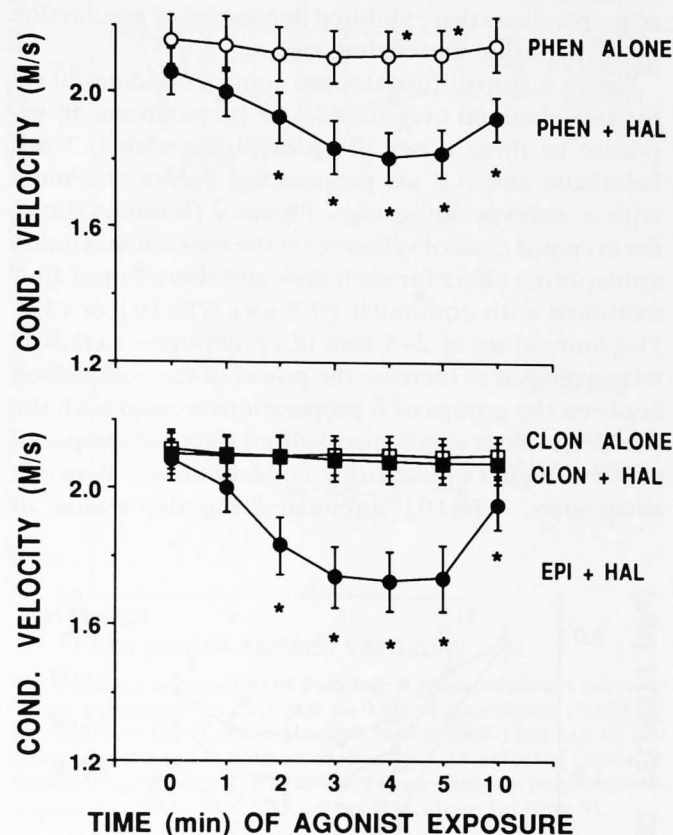


Fig. 3. Time-dependent effects of  $\alpha_1$ - (12 preparations) (top) and  $\alpha_2$ - (8 preparations) (bottom) adrenergic agonists alone and with 0.4 mm halothane (HAL) on conduction velocity. PHEN = 5  $\mu\text{M}$  phenylephrine; CLON = 5  $\mu\text{M}$  clonidine; EPI = 5  $\mu\text{M}$  epinephrine. \* $P \leq 0.05$  versus value at time 0 (without or with HAL) just before agonist exposure.

drug free control value (time 0). Halothane alone (0.4 mm) decreased velocity ( $P \leq 0.01$ ) to  $2.06 \pm 0.07$  m/s (at time 0) from a preceding control value of  $2.14 \pm 0.08$  m/s. The actions of phenylephrine were potentiated in the presence of halothane. The  $\alpha_1$  agonist produced a larger ( $P \leq 0.01$ ) decrease of velocity at maximum effect with halothane ( $-0.25 \pm 0.04$  m/s at 4 min of exposure, about  $-12\%$  vs. halothane at time 0) than in the absence of halothane ( $-0.05 \pm 0.01$  m/s at 4 min,  $-2\%$  vs. time 0). The mean velocity was significantly less at 4 min of phenylephrine exposure with halothane ( $1.81 \pm 0.07$  m/s) than without halothane ( $2.11 \pm 0.08$  m/s) largely because of the accentuated transient effect of  $\alpha_1$  activation in the presence of anesthetic. In contrast, the  $\alpha_2$  agonist clonidine, as shown in figure 3 (bottom), did not depress conduction velocity without or with halothane in a different group

of preparations that exhibited depression of conduction by epinephrine with halothane.

Figure 4 (top) illustrates the control conduction velocities obtained over time in 12 preparations on exposure to three doses of epinephrine with 0.7 mM halothane and 0.2  $\mu$ M propranolol before treatment with  $\alpha_1$ -subtype antagonists. Figure 4 (bottom) shows the averaged control velocities at the times of maximum epinephrine effect for each dose and those found after treatment with equimolar (0.5  $\mu$ M) WB4101 or CEC. The four values at 2–5 min of epinephrine exposure were averaged to increase the power of the comparison between the groups of 6 preparations treated with the two drugs. Both antagonists shifted the dose–response curve to higher epinephrine concentrations. Between antagonists, WB4101 attenuated the depression of

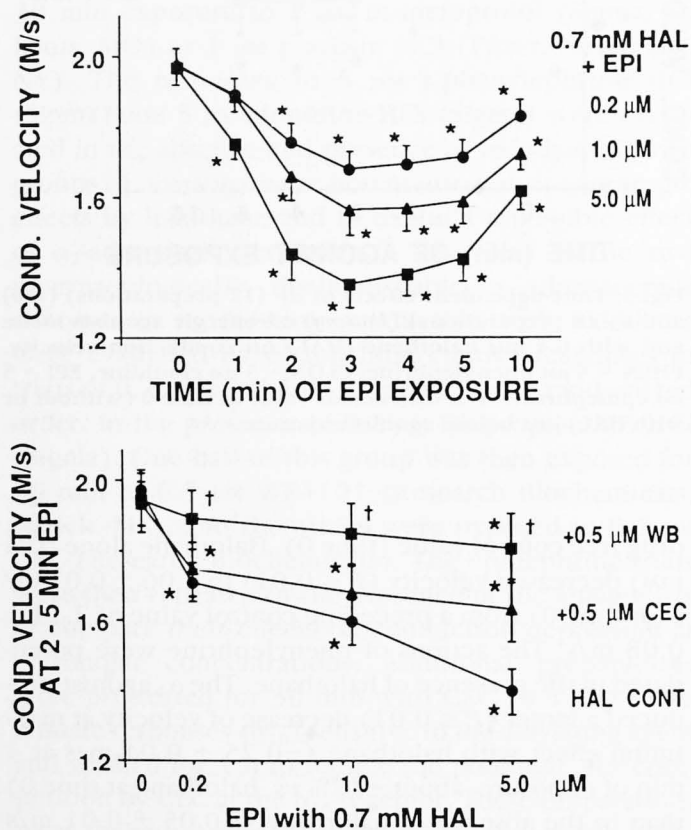


Fig. 4. (Top) Control dose-related depression of conduction velocity (mean  $\pm$  SEM, 12 preparations) by epinephrine with 0.7 mM halothane (HAL) in the presence of 0.2  $\mu$ M propranolol before treatment (6 preparations each) with  $\alpha_1$ -subtype antagonists WB4101 (WB) and chloroethylclonidine (CEC). (Bottom) Dose-response curves for the velocities (mean  $\pm$  SEM of 2nd–5th-min epinephrine [EPI] values) found before (control [CONT]) and in the presence of 0.5  $\mu$ M WB4101 or CEC. \* $P \leq 0.05$  versus HAL alone. † $P \leq 0.01$  between groups treated with WB or CEC.

conduction at each epinephrine dose to a greater degree ( $P \leq 0.01$ ) than CEC, which antagonized the decreases of conduction velocity by only about 40%. Table 1 shows the averaged conduction velocities found at maximum epinephrine effect with halothane in the absence and presence of 0.1  $\mu$ M WB4101 after CEC treatment and washing. Compared to the control group illustrated in figure 4 ( $n = 12$ ), pretreatment with CEC also attenuated ( $P \leq 0.05$ ) the response to 5  $\mu$ M epinephrine (table 1,  $n = 10$ ) with halothane by about 40%. In these preparations after washout of CEC, WB4101 (0.1  $\mu$ M) produced 87% inhibition of the remaining velocity decrease resulting from 0.2  $\mu$ M epinephrine and proportionately less inhibition at higher agonist concentrations in a manner consistent with competitive antagonism of an action on conduction mediated by the  $\alpha_{1A}$ -adrenoceptor subtype.

Figure 5 (top) illustrates the conduction responses to epinephrine, angiotensin and carbamylcholine with halothane in one group of preparations. Both epinephrine and the muscarinic receptor agonist depressed conduction velocity with halothane, whereas angiotensin did not affect conduction. Figure 5 (bottom) shows the responses to epinephrine and endothelin in another group of preparations. Endothelin with halothane depressed conduction with a slower onset than epinephrine with halothane, and there was a marked delay in washout (data not shown) of the endothelin effect such that return to the value with halothane alone required 30–40 min.

## Discussion

The results of this study indicate that epinephrine, at submicromolar concentrations (0.2  $\mu$ M) comparable to just-threshold dysrhythmogenic plasma epinephrine concentrations with halothane *in vivo*,<sup>23–25</sup> transiently slows conduction in canine Purkinje fibers exposed to halothane. These actions are mediated largely by the WB4101-sensitive subtype of  $\alpha_1$  adrenoceptor, which has been reported to lead to phosphoinositide hydrolysis by PLC in cardiac tissues<sup>7</sup> and similar depression of conduction was found on activation of other G-protein-linked receptors (endothelin and muscarinic) known to stimulate PLC.<sup>21</sup> The results demonstrate a direct prodysrhythmic  $\alpha_1$ -mediated interaction between catecholamines and halothane and support the hypothesis<sup>19</sup> that the mechanism generating halothane–epinephrine dysrhythmias may involve abnormal conduction leading to reentry.

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Reynolds and Chiz<sup>19</sup> originally reported that epinephrine markedly potentiated the conduction slowing by halothane. We previously found that 5  $\mu$ M epinephrine alone produces no or minimal decreases of conduction velocity, but significantly greater transient depression in Purkinje fibers exposed to halothane.<sup>20,22</sup> In the present study we observed marked potentiation by halothane of a small transient decrease of velocity produced by 5  $\mu$ M phenylephrine alone (fig. 3) and suspect that larger pharmacologic concentrations of  $\alpha_1$  agonists transiently depress conduction in the absence of potentiating anesthetic agent. The actions of epinephrine with halothane on conduction (fig. 1) varied from slight transient depression (-5%) with just-threshold concentrations (0.2  $\mu$ M) and 0.46 mM (1.5 vol%) halothane to substantial depression (-33%) at high epinephrine (5  $\mu$ M) and halothane (0.86 mM, 2.8%) doses. The interaction was more dependent on the halothane than epinephrine concentrations because doubling the halothane concentration produced approximately the same transient depression of velocity as a five- to tenfold increase of epinephrine dose. The relation between the degree of conduction delay observed in isolated Purkinje fiber models and the more complicated phenomena of unidirectional block and slow conduction leading to ventricular reentry *in situ* is not known. The latter may involve propagation of premature or postmature responses at lower membrane potentials and intrinsic spatial inhomogeneities in refractory periods, membrane excitability and fiber geometry not present in isolated false tendons.<sup>28</sup> However the degree of transient conduction impairment (-10 to -20%) produced by epinephrine with halothane in this study is similar in magnitude to that in Purkinje fibers cross-superfused with blood from dogs exhibiting intraventricular conduction delay and wide QRS complexes as a result of administration of toxic doses of

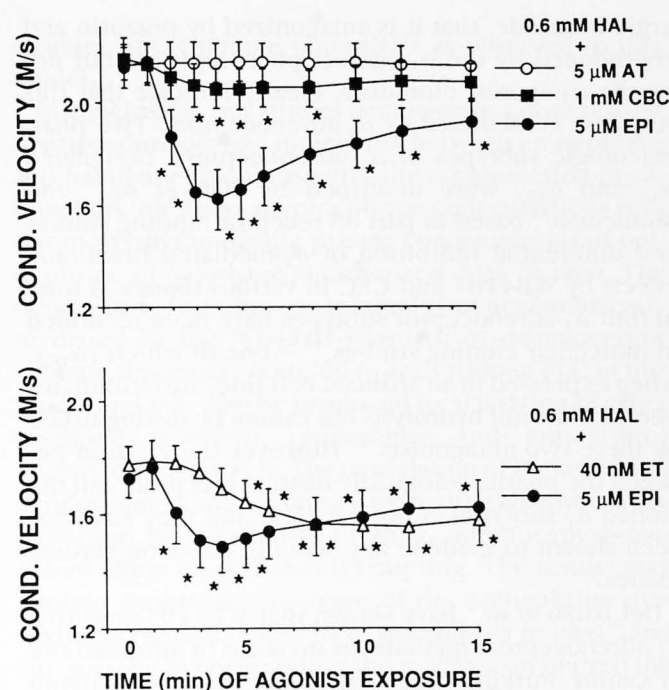


Fig. 5. (Top) Depression of Purkinje fiber conduction velocity in six preparations exposed to 0.6 mM halothane (HAL) by epinephrine (EPI) and carbamylcholine (CBC) but not by angiotensin II (AT). (Bottom) Depression of velocity (mean  $\pm$  SEM) by endothelin 1 (ET) and EPI in another six preparations exposed to HAL. \* $P \leq 0.05$  versus HAL alone (at time 0).

quinidine.<sup>29</sup> Proof that the observed slowing of conduction by epinephrine with halothane is sufficient to facilitate reentry would require mapping activation in the reentrant circuit during onset of the dysrhythmia, localization of the site of unidirectional block and demonstration of sufficient conduction delay to permit reexcitation proximal to the site of block on recovery of excitability.

The findings that the depression of conduction by epinephrine with halothane is not altered by  $\beta_1$ -adren-

Table 1. Inhibition (%) of Conduction Depression due to Epinephrine with 0.6 mM Halothane by the Competitive  $\alpha_1$ -Subtype Antagonist WB4101 (0.1  $\mu$ M) in 10 Preparations

	$\mu$ M EPI		
	0.2	1.0	5.0
Control	-0.135 $\pm$ 0.031*	-0.203 $\pm$ 0.040*	-0.305 $\pm$ 0.044*
+0.1 $\mu$ M WB	-0.017 $\pm$ 0.009	-0.052 $\pm$ 0.008*	-0.104 $\pm$ 0.019*
%	87	75	66

Values are mean  $\pm$  SEM and represent averaged difference from preceding control at the times (4th-7th min) of maximum EPI effect with halothane in the absence and presence of WB4101. All preparations were pretreated with 0.5  $\mu$ M CEC and washed before study in the presence of 0.2  $\mu$ M propranolol.

\* $P \leq 0.01$  versus halothane before EPI.

ergic blockade, that it is antagonized by prazosin and reproduced by the  $\alpha_1$  agonist phenylephrine but not by the  $\alpha_2$  agonist clonidine, clearly indicate that this response is mediated by  $\alpha_1$  adrenoceptors. Two pharmacologic subtypes of  $\alpha_1$  adrenoceptors, designated  $\alpha_{1A}$  and  $\alpha_{1B}$ , were described by Han *et al.*<sup>30</sup> and Minneman<sup>31</sup> based in part on receptor binding studies and differential inhibition of  $\alpha_1$ -mediated functional effects by WB4101 and CEC in various tissues. A total of four  $\alpha_1$ -adrenoceptor subtypes have been identified in molecular cloning studies,<sup>32,33</sup> one of which ( $\alpha_{1C}$ ), when expressed in an artificial cell line, also stimulates phosphoinositol hydrolysis but cannot be distinguished by these two antagonists.<sup>32</sup> However the relation between the pharmacologically distinct receptors and the cloned  $\alpha_1$  subtypes is not yet clear and they have not been shown to mediate any specific effect on cardiac tissues.<sup>9</sup>

Del Balzo *et al.*<sup>7</sup> have shown that WB4101-sensitive  $\alpha_1$  adrenoceptors mediate an increase of automaticity in canine Purkinje fibers by a mechanism involving linkage by a pertussis toxin-insensitive G-protein to stimulation of PLC. PLC applied extracellularly also enhances Purkinje automaticity.<sup>14</sup> The increased automaticity and action potential prolongation<sup>8</sup> resulting from  $\alpha_1$ -adrenoceptor activation in Purkinje fibers were antagonized by WB4101 at a concentration (0.1  $\mu\text{M}$ ) that Del Balzo *et al.*<sup>7</sup> reported to effectively (>95%) inhibit norepinephrine stimulation of inositol trisphosphate ( $\text{IP}_3$ ) production by PLC in isolated rat myocytes. The action potential changes probably result from inhibition of outward  $\text{K}^+$  currents<sup>8,10</sup> and have an important influence on abnormal automaticity in ischemic fibers.<sup>34</sup> On the other hand, CEC-sensitive  $\alpha_1$  adrenoceptors were reported to decrease Purkinje fiber automaticity by a mechanism sensitive to pertussis toxin,<sup>7</sup> which leads to activation of the  $\text{Na}^+/\text{K}^+$  electrogenic pump.<sup>12</sup> Del Balzo *et al.*<sup>7</sup> reported that WB4101, but not CEC, antagonized norepinephrine stimulated  $\text{IP}_3$  generation in isolated rat myocytes and suggested that only the  $\alpha_{1A}$ -receptor subtype, linked by a pertussis toxin-insensitive G-protein, stimulated PLC. However, recent contradictory studies in fresh adult rat myocytes indicate that both the  $\alpha_{1A}$  and  $\alpha_{1B}$  subtypes lead to stimulation of PLC<sup>18</sup> and that CEC (100  $\mu\text{M}$ ) typically inhibits epinephrine stimulated  $\text{IP}_3$  generation by about 33%. Our findings of greater antagonism of conduction slowing by equimolar (0.5  $\mu\text{M}$ ) WB4101 than CEC demonstrate that the reduced responses in the presence of the subtype antagonists are not caused

by deterioration of the preparation. The 40% inhibition of velocity decreases resulting from epinephrine with halothane in the two groups pretreated and washed (table 1) or studied in the continued presence of CEC (fig. 4) are consistent with antagonism of a moderate degree of conduction slowing mediated by the  $\alpha_{1B}$  subtype. On the other hand, the substantial (87%) inhibition of velocity changes by a low (0.1  $\mu\text{M}$ ) concentration of WB4101 after pretreatment with CEC suggest that the conduction slowing is probably largely mediated by the same WB4101-sensitive  $\alpha_{1A}$ -adrenoceptor subtype as that reported to increase automaticity and action potential duration in Purkinje fibers.<sup>7,8</sup> These findings are limited in that we can not exclude actions at the other cloned subtypes of  $\alpha_1$  adrenoceptors, which may or may not exist as distinct subtypes in the heart.<sup>18,35</sup> Further studies of the relative densities of  $\alpha_1$ -receptor subtypes in canine Purkinje fibers, the pertussis toxin sensitivity of their coupling G-proteins and their relation to specific isoforms of PLC<sup>36</sup> will be required to better define the  $\alpha_1$  adrenoceptor-effector mechanisms modulating conduction in this tissue.

To evaluate separately the potential role of activation of PLC in modulation of conduction in Purkinje fibers exposed to halothane, we investigated the effects of stimulation of other G-protein-linked receptors that produce phosphoinositide hydrolysis in cardiac tissues. Hilal-Dandan *et al.*<sup>21</sup> compared the effects of several agonists on production of  $\text{IP}_3$  in isolated adult rat myocytes and reported that endothelin 1 was most efficacious, followed by activation of  $\alpha_1$  and muscarinic receptors, whereas angiotensin II was the least effective. Our findings that endothelin and carbamylcholine, but not angiotensin, also transiently decrease conduction velocity in Purkinje fibers exposed to halothane imply that the  $\alpha_1$ -adrenoceptor mechanism mediating depression of conduction by epinephrine probably involves stimulation of PLC regardless of which  $\alpha_1$ -adrenoceptor subtype may mediate this action. The findings suggest a previously unsuspected potential role of hormonal modulation of conduction in Purkinje fibers related to receptor-mediated stimulation of PLC and as yet unknown actions of the intracellular second messengers  $\text{IP}_3$  and diacylglycerol resulting from hydrolysis of sarcolemmal phosphoinositides.

The electrophysiologic mechanism underlying  $\alpha_1$  adrenoceptor-mediated depression of conduction in Purkinje fibers exposed to halothane is not known. Although action potentials were recorded simultaneously

with conduction slowing, the mechanism of action of halothane on conduction precludes a direct effect on the action potential. The reduction in action potential rate (150 bpm) by halothane tends to mimic the effect of vagal stimulation and is not directly related to the action potential. The "take-off" potential of repolarization (automaticity) increases during vagal stimulation or epinephrine administration. The reduction in action potential amplitude and the increase in the rate of change of the action potential with halothane suggest that the mechanism of action of halothane on conduction is related to the cell coupling mechanism. The reduction in conduction velocity by halothane is not dependent on the concentration of epinephrine (10  $\mu\text{M}$ ) in the cells, although it has been confirmed that changes in conduction velocity would appear to be dependent on the concentration of epinephrine. However, the mechanism of action of epinephrine in increasing conduction velocity is related to the release from  $\text{Na}^+/\text{Ca}^{2+}$  exchange evidence.<sup>44</sup> The relation between the mechanism of action of halothane on conduction and the release from  $\text{Na}^+/\text{Ca}^{2+}$  exchange is not clear. An interaction between the  $\alpha_1$ -adrenoceptor-mediated conduction slowing and the release from  $\text{Na}^+/\text{Ca}^{2+}$  exchange is not clear.

$\alpha_1$ -ADRENERGIC DEPRESSION OF CONDUCTION BY EPINEPHRINE

with conduction times, the requirement to maintain impalements in two fibers over long periods of time precludes accuracy in measurements of maximum diastolic potential. In addition, we used a rapid pacing rate (150 beats/min) similar to that at the onset of halothane-epinephrine dysrhythmias *in vivo*, which tends to minimize drug effects on action potential duration and phase 4 diastolic depolarization. Qualitatively we did not observe any decrease of membrane potential just before the action potential upstroke (the "take-off" potential), as might occur with marked delay of repolarization or spontaneous diastolic depolarization (automaticity), that might explain the velocity decreases during the trials with epinephrine, carbamylcholine or endothelin. As previously reported with epinephrine,<sup>20</sup> the transient decreases of conduction velocity in this study were not accompanied by simultaneous decreases (data not shown) in action potential amplitude or rate of phase 0 depolarization, major determinants of conduction velocity. This relative lack of change in rate of phase 0 depolarization associated with marked depression of conduction by epinephrine with halothane suggests that the mechanism modulating conduction probably involves an action on cell-to-cell coupling,<sup>20,37</sup> rather than or in addition to possible reduction of the peak inward  $\text{Na}^+$  current. Both high concentrations (2–3%) of halothane<sup>38</sup> and phenylephrine (10  $\mu\text{M}$ ) have been reported to uncouple cardiac cells, although the latter preliminary report<sup>26</sup> has not been confirmed. The finding that the conduction changes were not sensitive to  $\beta_1$ -adrenergic blockade would appear to exclude mechanisms of conduction slowing related to cyclic adenosine monophosphate-dependent depression of  $\text{Na}^+$ <sup>39,40</sup> channel current or increased  $\text{Ca}^{2+}$  influx through L-type  $\text{Ca}^{2+}$  channels. However, this finding does not exclude potential mechanisms of cellular uncoupling resulting from increases in intracellular  $\text{Ca}^{2+}$  caused by enhanced  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum or effects on  $\text{Na}^+ - \text{Ca}^{2+}$  exchange.<sup>9,42,43</sup> Finally, there is increasing evidence<sup>44,45</sup> that the conductance of cardiac gap junctions is related to the phosphorylation state of the connexin proteins forming the channels and may be physiologically modulated by cyclic adenosine monophosphate-dependent protein kinases and protein kinase C. An interaction between halothane and  $\alpha_1$  adrenoceptor-mediated effects on processes regulating junctional resistance between Purkinje fibers could slow conduction by increasing the current required for propagation or altering threshold without substantial

changes of membrane potential<sup>37</sup> as observed in this model.

In conclusion, our studies demonstrate a dose-related negative dromotropic interaction between epinephrine and halothane that transiently slows conduction in canine Purkinje fibers at epinephrine concentrations similar to dysrhythmogenic plasma concentrations of epinephrine in halothane-anesthetized dogs *in vivo*. The depression of conduction is largely but not exclusively mediated by the WB4101-sensitive  $\alpha_{1A}$ -adrenoceptor subtype reportedly coupled to activation of PLC in this model and may also be produced by activation of other hormone receptors (endothelin and muscarinic) known to activate PLC. The modulation of Purkinje fiber conduction velocity appears to involve an adverse potentiation by anesthetics of an  $\alpha_1$  adrenoceptor-mediated effect on cell-to-cell coupling. The results may explain progressive increase of the epinephrine dysrhythmia threshold dose by  $\alpha_1$ -antagonists *in vivo*<sup>46</sup> and support the hypothesis that the mechanism underlying generation of ventricular dysrhythmias by epinephrine during halothane anesthesia may involve abnormal conduction and reentry.

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