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Effects of verapamil on atrial fibrillation and its electrophysiological determinants in dogs

Agnès Bénardeau[a,c], Samir Fareh[a], Stanley Nattel[a,b,*]

[a]*Department of Medicine and Research Center, Montreal Heart Institute and University of Montreal, Montreal, Quebec, Canada*
[b]*Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada*
[c]*Hoffman-La Roche Pharmaceuticals, Basel, Switzerland*

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Abstract

Background: Atrial tachycardia-induced remodeling promotes the occurrence and maintenance of atrial fibrillation (AF) and decreases L-type Ca^{2+} current. There is also a clinical suggestion that acute L-type Ca^{2} channel blockade can promote AF, consistent with an AF promoting effect of Ca^{2+} channel inhibition. **Methods:** To evaluate the potential mechanisms of AF promotion by Ca^{2+} channel blockers, we administered verapamil to morphine–chloralose anesthetized dogs. Diltiazem was used as a comparison drug and autonomic blockade with atropine and nadolol was applied in some experiments. Epicardial mapping with 240 epicardial electrodes was used to evaluate activation during AF. **Results:** Verapamil caused AF promotion in six dogs, increasing mean duration of AF induced by burst pacing, from 8±4 s (mean±S.E.) to 95±39 s ($P<0.01$ vs. control) at a loading dose of 0.1 mg/kg and 228±101 s ($P<0.0005$ vs. control) at a dose of 0.2 mg/kg. Underlying electrophysiological mechanisms were studied in detail in five additional dogs under control conditions and in the presence of the higher dose of verapamil. In these experiments, verapamil shortened mean effective refractory period (ERP) from 122±5 to 114±4 ms ($P<0.02$) at a cycle length of 300 ms, decreased ERP heterogeneity (from 15±1 to 10±1%, $P<0.05$), heterogeneously accelerated atrial conduction and decreased the cycle length of AF (94±4 to 84±3 ms, $P<0.005$). Diltiazem did not affect ERP, AF cycle length or AF duration, but produced conduction acceleration similar to that caused by verapamil ($n=5$). In the presence of autonomic blockade, verapamil failed to promote AF and increased, rather than decreasing, refractoriness. Neither verapamil nor diltiazem affected atrial conduction in the presence of autonomic blockade. Epicardial mapping suggested that verapamil promoted AF by increasing the number of simultaneous wavefronts reflected by separate zones of reactivation in each cycle. **Conclusions:** Verapamil promotes AF in normal dogs by promoting multiple circuit reentry, an effect dependent on intact autonomic tone and not shared by diltiazem.

Keywords: Arrhythmia (mechanisms); Ion channels; Mapping; Remodeling

1. Introduction

Atrial fibrillation (AF) is presently the most common sustained cardiac arrhythmia in clinical practice, and its treatment is less than optimal in many cases [1]. Antiarrhythmic drugs improve sinus rhythm maintenance, but at the expense of a variety of potential adverse effects, of which one of the most worrisome is ventricular proarrhythmia [2]. An alternative approach is to leave patients in AF, but to control the ventricular response and to prevent thromboembolic complications with oral anticoagulants [3]. Among the drugs used to control the ventricular response rate are inhibitors of L-type Ca^{2+} current such as diltiazem and verapamil. There is, however, clinical evidence that Ca^{2+} channel blockers such as verapamil and diltiazem may promote AF maintenance [4]. Two recent experimental studies also suggest that the Ca^{2+} channel blocker verapamil may promote experimental AF. Lee et al. showed that verapamil administration during a 7–42 day period of atrial tachycardia and at the time of a subsequent electrophysiological study fails to prevent tachycardia-induced remodeling and promotes AF mainte-

*Corresponding author. Tel.: +1-514-376-3330, ext. 3990; fax: +1-514-376-1355.
E-mail address: nattel@icm.umontreal.ca (S. Nattel).

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nance during the electrophysiological study [5]. Friedman et al. showed that verapamil promotes AF maintenance in anesthetized dogs, an effect that can be prevented by β-adrenoceptor blockade [6]. Neither study evaluated the electrophysiological mechanisms potentially underlying verapamil's apparent AF promoting action. The present study was designed to (1) determine whether verapamil increases AF duration in the anesthetized dog in a dose related fashion; (2) evaluate potential underlying mechanisms by measuring atrial effective refractory period (ERP) and conduction velocity, as well as by studying changes in activation during AF with the use of atrial epicardial mapping; and (3) determine whether the Ca^{2+} channel blocker diltiazem shares verapamil's AF promoting potential.

2. Methods

2.1. Animal preparation

Twenty-five mongrel dogs (27±1 kg) were studied in four groups: (1) Group 1: a dose–response group ($n=6$), in which the effects of verapamil on AF duration were studied at two dose levels (0.1 and 0.2 mg/kg loading doses, followed by maintenance infusions of 0.5 and 1.5 μg/kg/min infusions, respectively); (2) Group 2: a detailed electrophysiology group ($n=5$), in which the electrophysiological effects of verapamil were studied at a single, high loading dose (0.2 mg/kg, followed by 1.5 μg/kg/min); (3) Group 3: a diltiazem group ($n=5$), in which the effects of diltiazem (0.8 mg/kg, followed by 15 μg/kg/min) were studied; and (4) Group 4: an autonomic blockade group, in which the effects of verapamil ($n=5$) and diltiazem ($n=4$) were studied in the presence of pharmacological blockade of cardiac β-adrenergic and muscarinic cholinergic receptors.

Animal handling procedures followed the guidelines of the Canadian Council on Animal Care, and were approved by the institutional animal research ethics committee. On the study day, dogs were anesthetized with morphine (2 mg/kg s.c.) and α-chloralose (120 mg/kg i.v. load, 29.3 mg/kg h^{-1}) and ventilated to maintain physiological arterial blood gases (pH 7.38–7.45, SaO_2>90%). Body temperature was maintained at 37°C and a femoral artery and both femoral veins were cannulated for arterial blood pressure monitoring and drug administration. A median sternotomy was performed and bipolar PTFE coated stainless electrodes hooked into right and left atrial appendages for stimulation and recording. The surface ECG was monitored and epicardial mapping arrays sewn to the epicardial surfaces of both atria.

2.2. Electrophysiological study

The study preparation and instrumentation were as previously described in detail [7,8]. A mapping system was connected to five arrays covering the atrial epicardial surfaces with 240 bipolar electrodes (Fig. 1) as previously described. Signals were filtered (bandwidth of 10–900 Hz), digitized (12-bit resolution and 2-kHz sampling rate), and transmitted into a Silicon Graphics computer for analysis. Activation data were analyzed off line with computer determined peak-amplitude criteria for activation, and data for each electrode were reviewed manually. Atrial ERP (longest coupling interval at which premature electrical extrastimuli failed to capture) and conduction velocity were measured during stimulation at sites in various atrial regions as in previous work [7,8]. Activation maps for conduction measurement were obtained after 60 s at a variety of basic cycle lengths. Conduction velocity was measured with the use of two parallel sets of four electrodes during local pacing in each of four regions: the left atrial appendage, the right atrial appendage, the right superior free wall and the right inferior free wall (Fig. 1). Because of variable contact in the left atrium, complete conduction data were only available at right atrial sites. To measure ERP, a 15 stimulus basic train (2-ms twice threshold current pulses) was followed by a premature extrastimulus at a progressively increasing coupling interval and a 2-s pause to observe the response between trains. The premature coupling interval was increased by 10-ms increments to obtain an initial estimate of the ERP. The measurement was then repeated with 5-ms increments and the resulting value taken as the ERP. In the case of a >10-ms difference between the two measurements, a third measurement with 5-ms steps was obtained and the mean of all three ERP values was used. This protocol was used

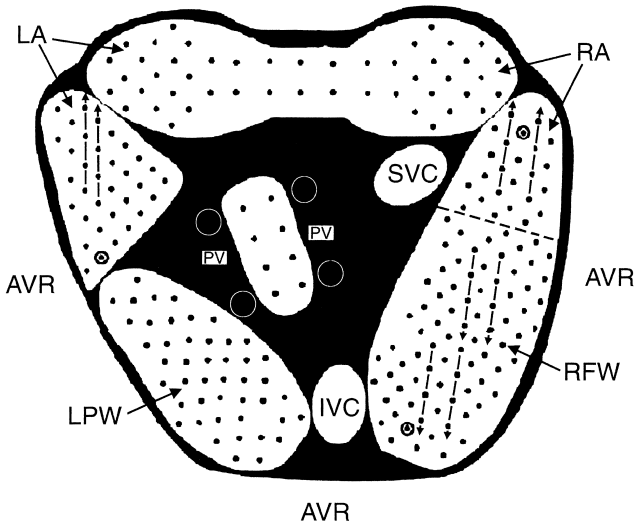

Fig. 1. Schematic of electrode arrays and bipolar electrode sites (dots). Stimulation sites for conduction measurements are indicated by haloed dots. Arrows indicate the series of electrodes used for conduction velocity analysis in each zone. AVR, atrio-ventricular ring; RFW, right free wall; IVC, SVC, inferior, superior vena cava; PV, pulmonary vein; LPW, left posterior wall.

to measure ERPs at cycle lengths of 150, 200, 250, 300 and 350 ms at the right atrial appendage, and to measure ERPs during stimulation at a single pacing cycle length (300 ms) at 23±2 right and left atrial sites.

AF was induced by stimulating the right atrial appendage with 10 Hz, 2 ms stimuli at four times threshold current for 2–10 s. To calculate mean AF duration, AF was induced with burst pacing ten times for AF duration <10 min and twice for AF duration >10 min. AF that lasted >20 min, requiring electrical cardioversion for termination, was considered persistent. A 20-min rest period was allowed before continuing the experiment.

2.3. Pharmacological study

Verapamil and diltiazem were dissolved in 0.9% saline the day of the experiment and were kept at 4°C until 15 min before use. In the first part of the study (Group 1), we tested two intravenous doses of verapamil hydrochloride (Sigma): first dose: a loading infusion of 0.10 mg/kg over 10 min, followed by a maintenance dose of 0.5 μg/kg/min for the time needed to complete electrophysiological measurements (~30–45 min); second dose: load of 0.20 mg/kg, i.v.) followed by a maintenance infusion of 1.5 μg/kg/min. In each case, measurements were begun 10 min after the completion of the loading dose. These doses were based on previously published data showing significant and stable pharmacological actions and plasma concentrations [9]. We then pursued more detailed electrophysiological evaluations with the higher dose only (Group 2 dogs). We then studied a group of dogs receiving diltiazem (Sigma) at the highest dose shown in a previous multiple dose study [9] to be tolerated and produce stable electrophysiological effects in the dog (loading dose 0.8 mg/kg, followed by 15 μg/kg/min maintenance infusion). In a final group (Group 4), the effects of the higher dose of verapamil and of diltiazem were studied in the presence of autonomic blockade produced with nadolol (0.5 mg/kg, followed by an additional 0.25 mg/kg every 2 h) and atropine (1 mg every 2 h).

2.4. Data analysis

The conduction velocity was determined in each region as previously described. The coefficient of variance in ERP was calculated as S.D./mean×100% and used as an index of ERP heterogeneity. The number of sites for ERP determination in each region was equivalent across dogs and between groups, to prevent any selection bias. Statistical comparisons between two groups only were performed by Student's *t* test. Analysis of variance with a range test was used for multiple group comparisons. Average results are given as the mean±S.E.M., and a two-tailed $P<0.05$ was considered statistically significant.

3. Results

3.1. Dose related effects of verapamil

The dose related effects of verapamil observed in Group 1 dogs are summarized in Table 1. Verapamil slightly, but non-significantly, increased sinus cycle length, while causing clear, dose related decreases in arterial pressure. The PR interval was also increased by the drug, with other ECG intervals unaffected. AF duration was clearly increased in a dose related fashion.

3.2. Electrophysiological mechanisms of AF promotion by verapamil

In order to assess the mechanism by which verapamil promotes AF, we performed epicardial mapping during AF under control conditions and in the presence of the higher dose of verapamil (Group 2 dogs). The general effects of verapamil in Group 2 dogs are shown in Table 2. As in Group 1 dogs, 0.2 mg/kg verapamil slightly (but nonsignificantly) prolonged sinus cycle length, reduced arterial pressure, and significantly increased both PR interval and AF duration. The results of mapping studies are illustrated in Fig. 2, which shows activation during three consecutive cycles under control conditions (Fig. 2A) and in the

Table 1
Dose–response effects of verapamil (Group 1 dogs)[a]

	Control	Verapamil (lower dose)[c]	Verapamil (higher dose)[c]	*P*
n	6	6	6[b]	
Sinus CL (ms)	393±5	424±46	472±45	0.480
Mean arterial BP (mmHg)	104±9	86±9	76±8	0.0004
PR interval (ms)	105±6	124±11	158±12	0.006
QRS duration (ms)	43±2	43±2	43±1	0.990
QTc interval	302±4	293±9	285±7	0.260
AF duration (s)	8±4	95±39	228±101	0.0056

[a] Values are mean±S.E.M. CL, cycle length; BP, blood pressure; AF, atrial fibrillation; NS, not significant.
[b] Each dog was studied under both control and all drug conditions.
[c] Lower dose, 0.1 mg/kg loading dose followed by 0.5 μg/kg/min; Higher dose, 0.2 mg/kg loading dose followed by 1.5 μg/kg/min.

Table 2
Comparison between effects of verapamil (Group 2 dogs) and diltiazem (Group 3)

	Control	Verapamil (0.2 mg/kg, followed by 1.5 μg/kg/min)	*P*	Control	Diltiazem (0.8 mg/kg, followed by 15 μg/kg/min)	*P*
n	5	5[a]	–	5	5[a]	–
Sinus CL (ms)	373±21	451±41	0.113	376±31	393±11	0.656
Mean arterial BP (mmHg)	101±9	86±7	0.0081	89±9	69±11	0.025
PR interval (ms)	92±4	128±4	0.00014	92±4	164±21	0.0101
AF duration (s)	5±2	399±351	0.043	8±5	26±15	0.318

[a] Each dog was studied under control and all drug conditions.

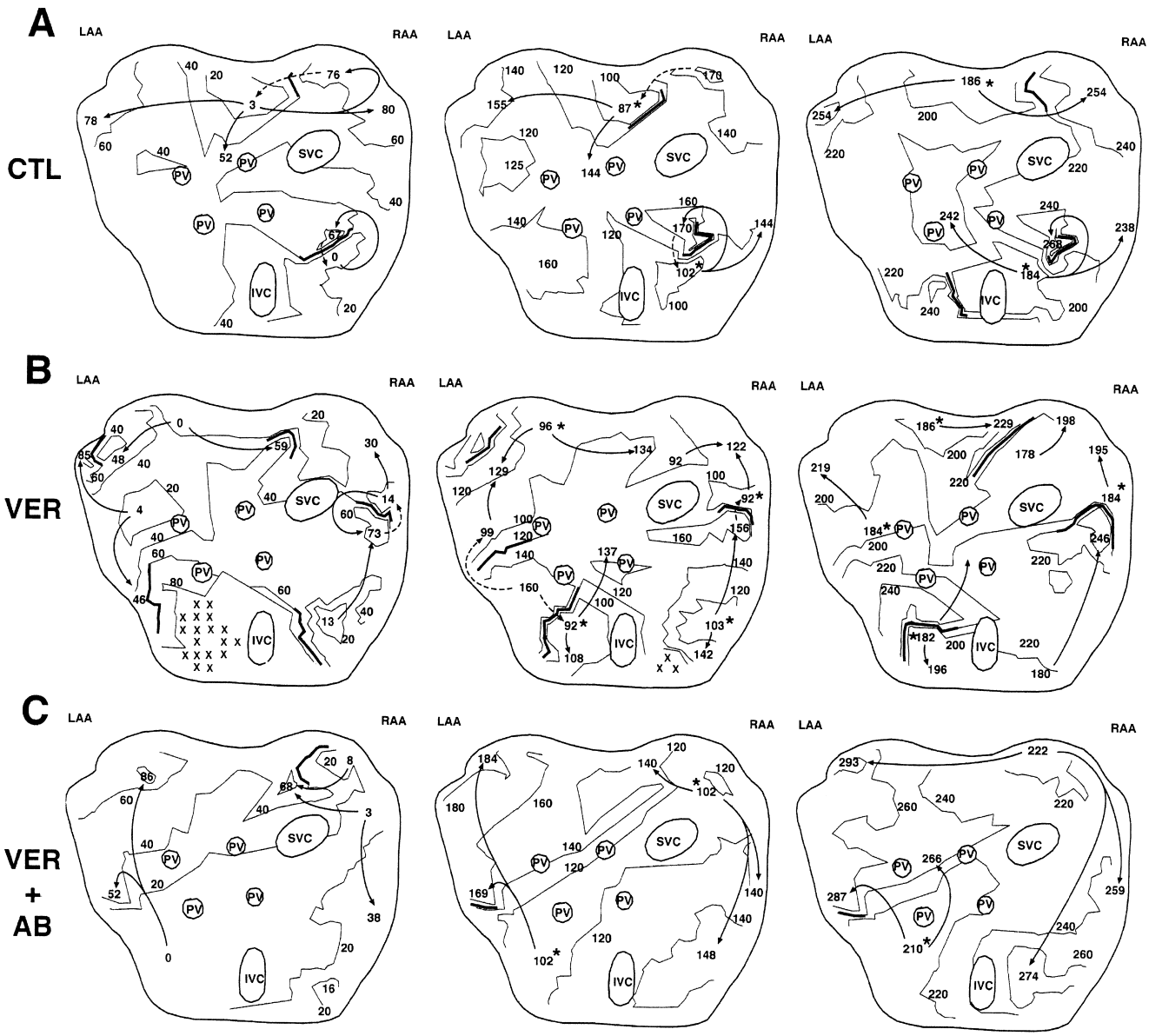

Fig. 2. Activation maps during three consecutive cycles of AF under control conditions (A), in the presence of verapamil, 0.2 mg/kg followed by 1.5 μg/kg/min (B) and in the presence of the same dose of verapamil and autonomic blockade (C). Lines are 20-ms isochrones, arrows are zones of wavefront propagation during a cycle, dashed arrows are possible propagation to reactivate atrial tissue at the beginning of the next cycle and asterisks are zones of reactivation at the beginning of the cycle. Under control conditions, only one or two zones of reactivation are seen per cycle, whereas in the presence of verapamil (autonomic transmission intact) multiple reactivation zones occur (four per cycle in the example shown) and the activation pattern is clearly more complex. In the presence of autonomic blockade, activation patterns in the presence of verapamil resemble those under control conditions.

presence of verapamil (Fig. 2B). Isochrone maps (20-ms) are shown, with the activation times indicated all referenced to the earliest activation in the first cycle. Solid arrows represent the direction of impulse propagation and solid lines, zones of functional block (>40 ms activation time differences between adjacent electrodes). Regions that are not activated within a cycle are designated by ×s. The dashed lines indicate probable paths of reactivation initiating the next cycle. Under control conditions (Fig. 2A), activation is relatively homogeneous, with approximately two discrete zones of reactivation (indicated by the asterisks) per cycle. In the presence of verapamil, activation is more heterogeneous. There are more zones of functional block, more apparent reentry circuits and more zones of reactivation at the beginning of each cycle. Fig. 3A shows mean data for the number of reactivation zones per cycle, based on measurements for three consecutive cycles under each condition in each dog. Verapamil clearly increased the number of reactivation zones considerably, from just over one to almost three/cycle. Thus, verapamil's ability to promote AF is likely related to the promotion of multiple wavelet reentry by decreasing the size and increasing the number of functional reentry circuits during the arrhythmia.

In order to explore further the electrophysiological mechanisms underlying verapamil's AF promoting action, we assessed drug-induced changes in atrial refractoriness and conduction properties. ERP at the right atrial appendix is shown as a function of cycle length in Fig. 4A. Verapamil tended to decrease ERP slightly but not significantly at long cycle lengths, with no change at short cycle lengths. Fig. 4B shows mean ERP data from all sites at a cycle length of 300 ms, and indicates that verapamil slightly but significantly decreased ERP. Verapamil-induced changes in AF cycle length are shown in Fig. 4C, which indicates that the drug significantly decreased the mean cycle length during AF, consistent with the ERP changes. The drug's effects on ERP at a cycle length of 300 ms are further analyzed in Fig. 5. Verapamil did not alter sig-

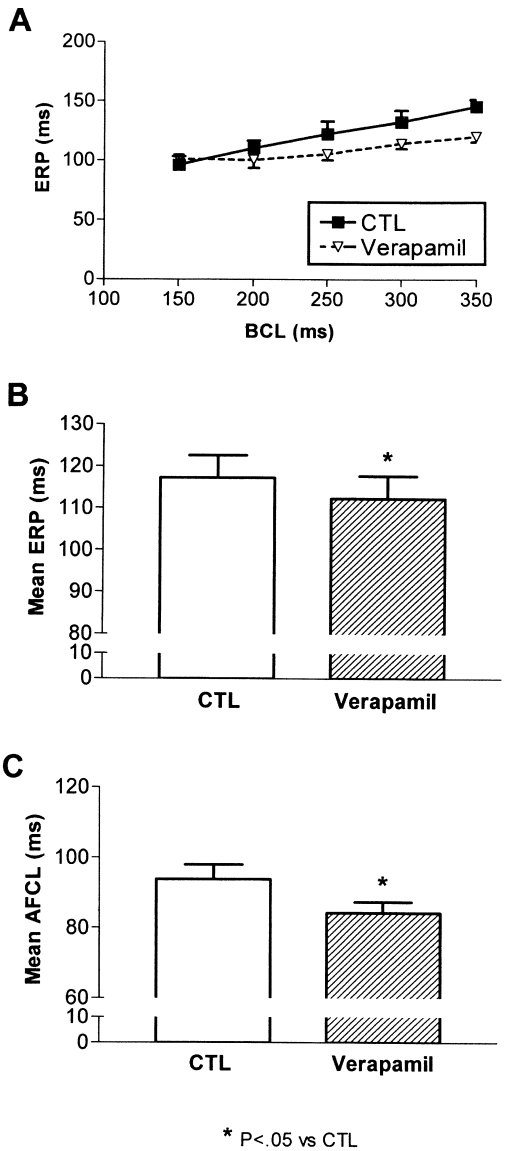

Fig. 4. Effects of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min) in the presence of intact autonomic tone on (A) ERP in the right atrial appendage; (B) mean ERP at all sites tested; and (C) AF cycle length.

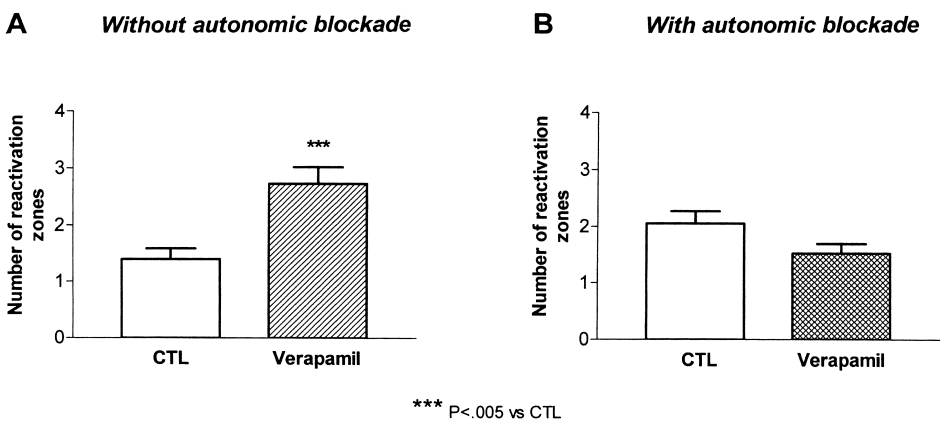

Fig. 3. Effects of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min) on the number of reactivation zones calculated on the basis of activation maps during AF in the absence (A) or presence (B) of autonomic blockade.

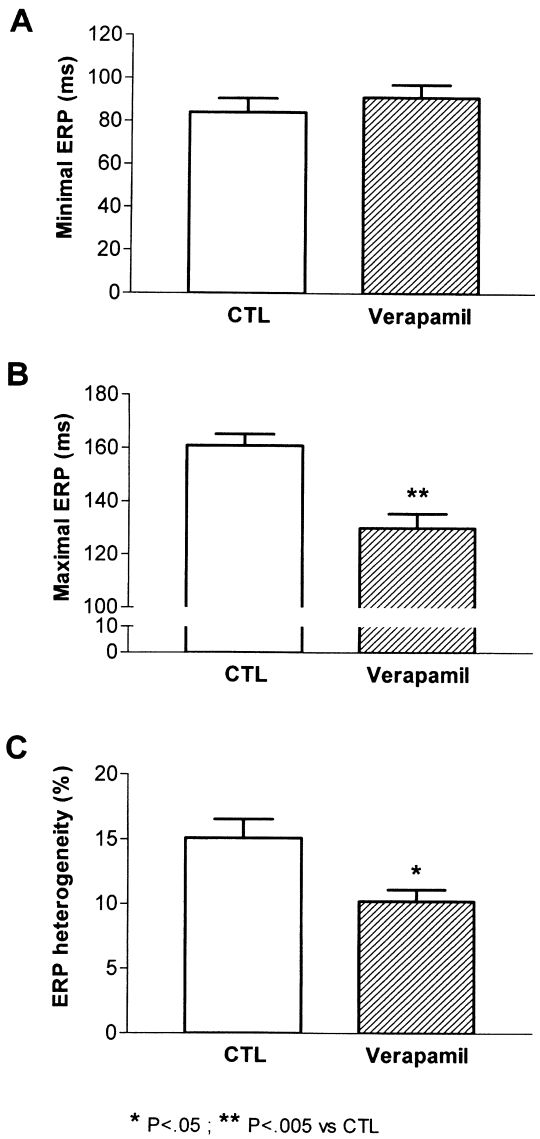

Fig. 5. Effects of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min) in the presence of intact autonomic tone on (A) minimal ERP value at a cycle length of 300 ms in each dog; (B) maximal ERP in each dog; and (C) ERP heterogeneity calculated for each condition in each dog as the standard deviation of all ERP values divided by the mean ERP value times 100%.

nificantly the shortest ERP value for all sites in each dog (Fig. 5A), but substantially decreased the maximum value (Fig. 5B). Since verapamil decreased longer ERP values without significantly affecting short ERPs, the drug significantly reduced ERP heterogeneity (Fig. 5C).

Verapamil produced spatially variable changes in conduction. The drug resulted in faster conduction in the right atrial superior and posterior free walls, but did not change conduction speed in the right atrial appendage (Fig. 6left). When all observations in the right atrium were combined, verapamil reduced ERP from 122±5 to 114±4 ms and increased conduction velocity from 90±2 to 99±3 cm/s ($P<0.02$ for each). Because of the offsetting changes in ERP and conduction, mean reentrant wavelength was unaffected by the drug (11.0±0.6 cm before and 11.3±0.5 cm after verapamil administration).

3.3. Effects of diltiazem on atrial electrophysiology and AF

To determine whether diltiazem shares verapamil's actions on atrial electrophysiology and arrhythmias, we studied the effects of the drug in Group 3 dogs. Like high dose verapamil, diltiazem decreased arterial pressure and increased the PR interval (Table 2). Unlike verapamil, diltiazem did not significantly alter AF duration. Diltiazem did not alter atrial ERP, which averaged 112±2 ms at all sites (cycle length 300 ms) before diltiazem and 116±3 ms after the drug. Similarly, AF cycle length was not affected by diltiazem (94±3 ms control, 92±3 ms drug, P=NS). Diltiazem did produce significant increases in conduction velocity, with a spatial distribution similar to those caused by verapamil (Fig. 6, right). Thus, despite similar effects on blood pressure, PR interval and intra-atrial conduction, diltiazem did not share verapamil's ability to decrease atrial ERP or AF cycle length and did not promote AF.

3.4. Effects of verapamil and diltiazem in the presence of autonomic blockade

In order to evaluate effects of verapamil and diltiazem in the absence of autonomic reflexes, we studied Group 4 dogs in the presence of β-adrenergic and muscarinic receptor blockade. In the presence of autonomic blockade, verapamil reduced arterial pressure and increased the PR interval, but did not affect AF duration (Table 3, Fig. 7A). Typical activation maps for AF in the presence of verapamil and autonomic blockade are shown in Fig. 2C, and indicate that under these conditions activation was relatively homogeneous and there were few reactivation zones per cycle. A quantitative analysis is shown in Fig. 3B, and indicates that in the presence of autonomic blockade verapamil did not alter the number of reactivation zones per cycle of AF. In contrast to its effects in the presence of intact autonomic reflexes, verapamil significantly increased AF cycle length in the presence of autonomic blockade (Fig. 7B) and slightly but significantly increased ERP (Fig. 7C) without affecting ERP heterogeneity (Fig. 7D). Unlike the effects of both verapamil and diltiazem in the presence of intact autonomic reflexes, conduction velocity was unaffected by verapamil administration in the presence of autonomic blockade (Fig. 8left). These results indicate an important role for autonomic reflexes in mediating the electrophysiological changes observed after verapamil administration. Diltiazem produced qualitatively similar changes to verapamil in sinus cycle length, arterial pressure and PR interval in the presence of autonomic blockade (Table 3), and similarly failed to alter AF duration. Changes in conduction velocity after diltiazem administra-

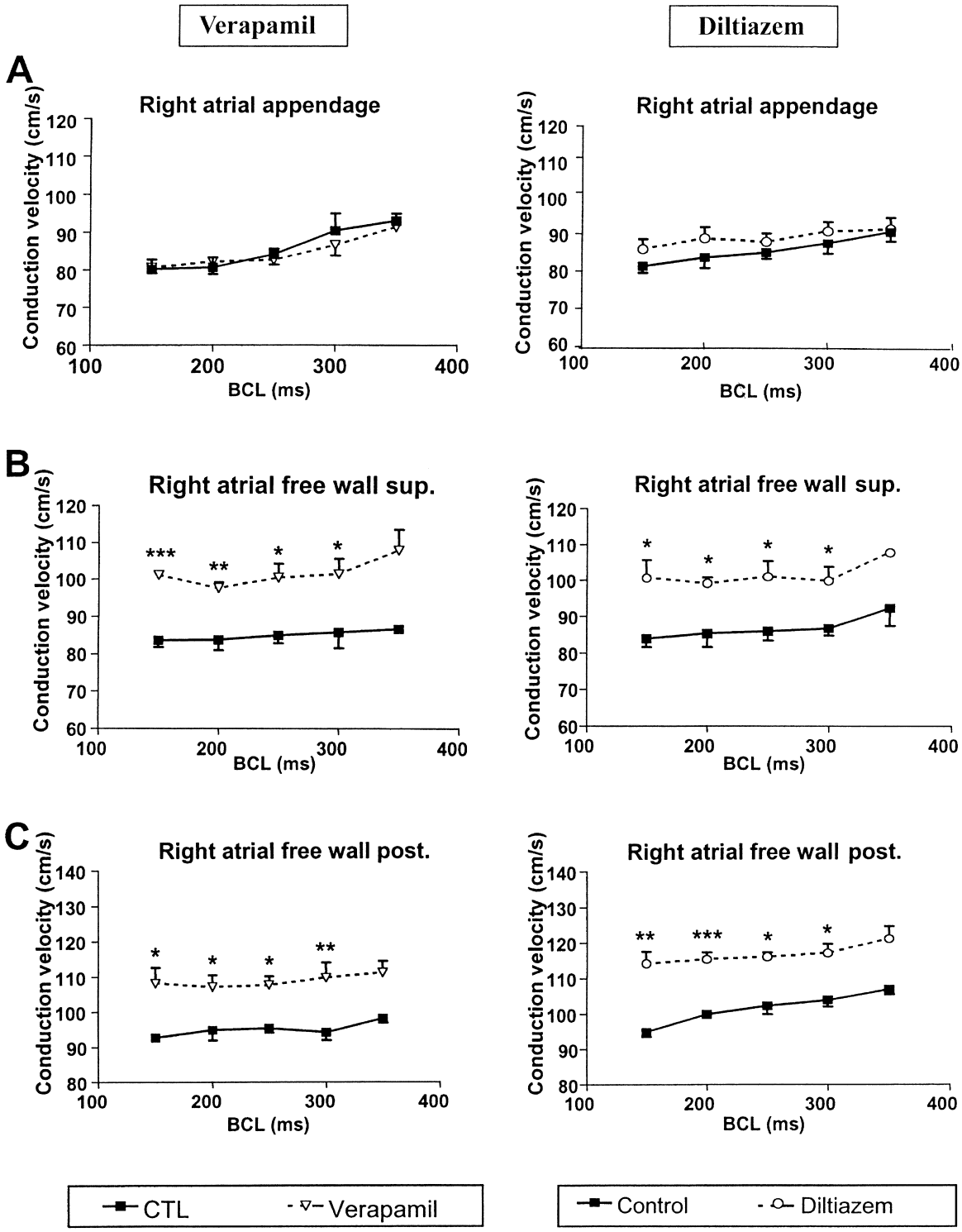

Fig. 6. Effect of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min, left panels) and diltiazem (0.8 mg/kg followed by 15 μg/kg/min, right panels) in the presence of intact autonomic tone on conduction velocity in each of three atrial regions.

tion in the presence of autonomic blockade are shown in Fig. 8 (right panels). As for verapamil, no significant changes in conduction were seen after diltiazem administration when the experiment was conducted in the presence of β-adrenergic and muscarinic blockade.

4. Discussion

We have shown that verapamil promotes the maintenance of AF in normal dogs. The underlying mechanism appears to be a potentiation of multiple circuit reentry

Table 3
Comparison between effects of verapamil and diltiazem in presence of autonomic blockade (Group 4)

	Control	Verapamil (0.2 mg/kg, followed by 1.5 μg/kg/min)	*P*	Control	Diltiazem (0.8 mg/kg, followed by 15 μg/kg/min)	*P*
n	5	5[a]	–	4	4[a]	–
Sinus CL (ms)	448±40	590±46	0.048	585±57	750±23	0.113
Mean arterial BP (mmHg)	92±8	68±10	0.048	91±3	60±4	0.041
PR interval (ms)	108±8	149±15	0.011	115±8	156±22	0.123
AF duration (s)	17±6	15±7	0.80	65±32	33±16	0.54

[a] Each dog was studied under continuous autonomic blockade for both control and drug conditions.

requiring intact autonomic function. Diltiazem did not share the AF promoting action of verapamil in this model.

4.1. Role of L-type Ca^{2+} currents and their blockade in AF

L-type Ca^{2+} current plays an important role in maintaining the plateau of the atrial action potential and changes in Ca^{2+} current are an important contributor to rate-dependent adaptations in atrial action potential duration [10,11]. Atrial tachycardias, including AF, modify atrial properties to promote AF maintenance, with reduced ERP and rate-dependent ERP adaptation being hallmark features [12]. Decreases in L-type Ca^{2+} current seem to be central to these electrophysiological effects of tachycardia-induced remodeling [13]. Ca^{2+} overload due to an increased rate of action potential generation may be important in initiating tachycardia-related remodeling, which may in part be a protective response of cardiomyocytes to prevent damage caused by Ca^{2+} loading [14]. Consistent with this concept, verapamil prevents some of the changes caused by short term (<24 h) atrial tachycardias [15–18]; however, L-type Ca^{2+} channel blockers do not prevent remodeling by atrial tachycardias lasting a week or longer [5,19]. Furthermore,

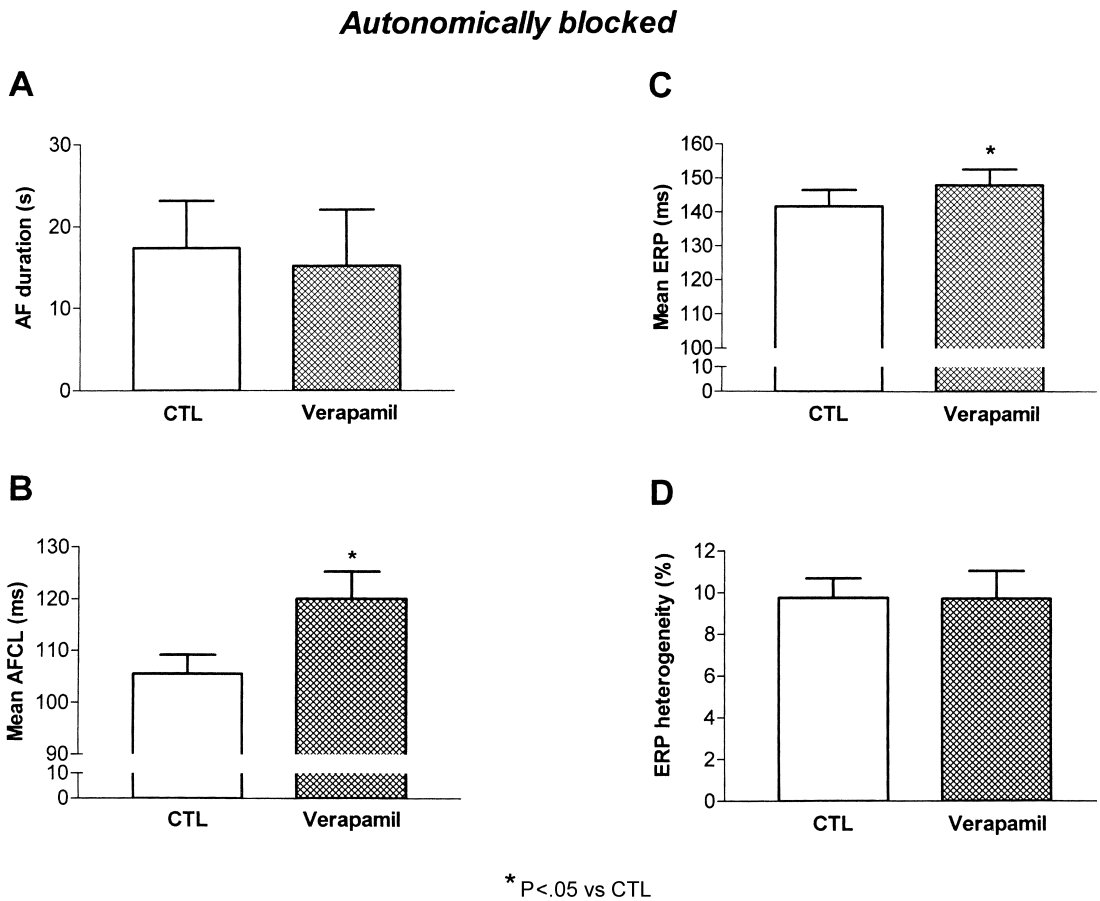

Fig. 7. Effects of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min) in the presence of autonomic blockade on (A) AF duration; (B) AF cycle length; (C) mean ERP at all atrial sites; and (D) ERP heterogeneity.

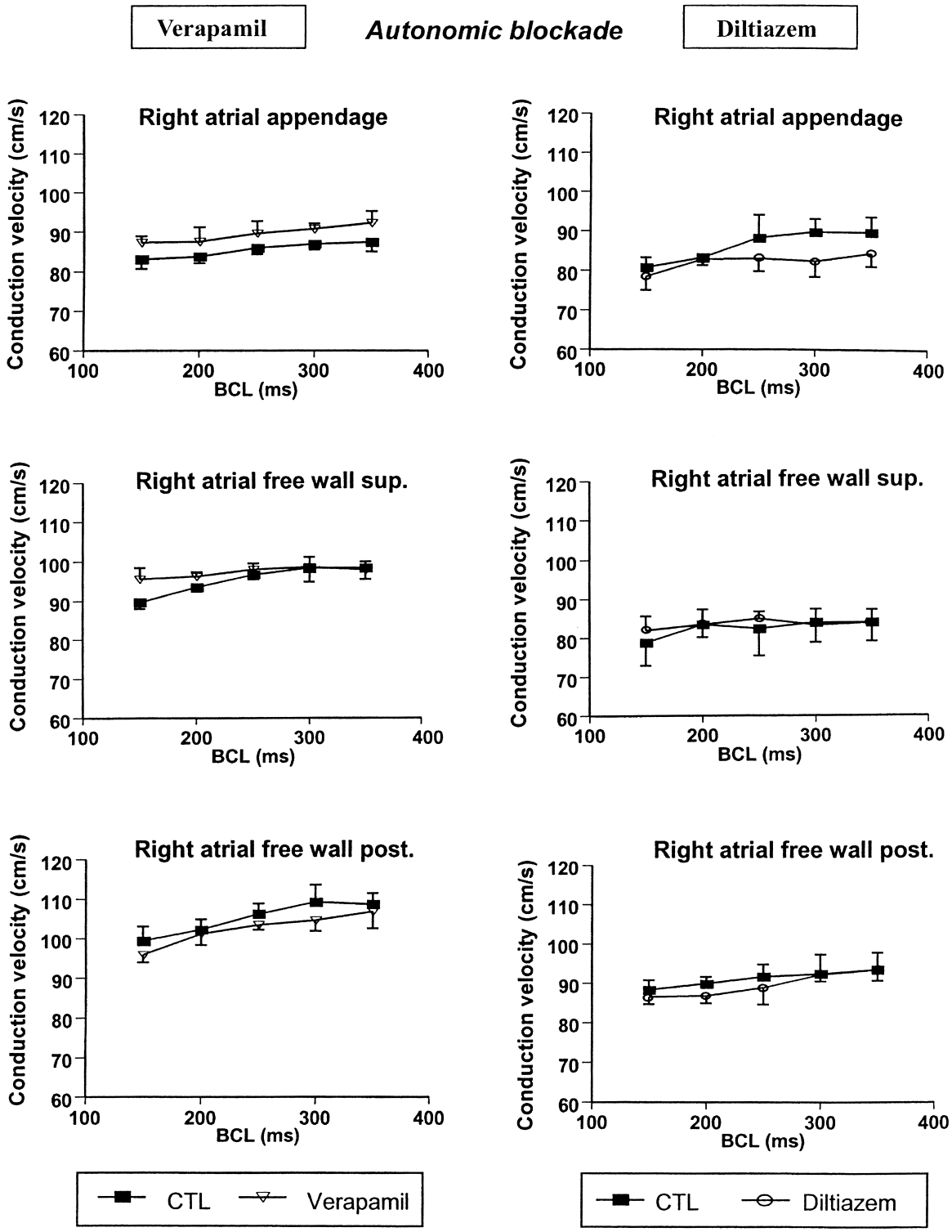

Fig. 8. Effect of verapamil (0.2 mg/kg followed by 1.5 μg/kg/min, left panels) and diltiazem (0.8 mg/kg followed by 15 μg/kg/min, right panels) in the presence of autonomic blockade on conduction velocity in each of three atrial regions.

dogs maintained on verapamil up to and during electrophysiological study after a 7-day or greater period of atrial tachycardia have more sustained AF than control dogs [5].

Given the fact that reduced Ca^{2+} current seems to be important in the AF-promoting atrial action potential changes caused by atrial tachycardia-induced remodeling [13], it would not be surprising for Ca^{2+} channel blockers like verapamil to promote AF maintenance. The results of the present study do show that verapamil promotes AF; however, a number of our findings argue against the simple

notion that this action of verapamil is due to a mimicking of the changes caused by atrial tachycardia. First, atrial tachycardia-induced remodeling causes substantial decreases in atrial ERP [12,13], whereas the decreases caused by verapamil in the present paper were relatively modest. Second, increased atrial ERP heterogeneity is a prominent AF-promoting feature of tachycardia-induced remodeling in the dog [20,21], whereas ERP heterogeneity was reduced by verapamil. Third, if verapamil's AF-promoting effects were due to L-type Ca^{2+} channel blockade alone, one would have expected them to be enhanced by β-adrenergic blockade, since the latter reduces L-type Ca^{2+} current; however, the opposite result (an elimination of AF promotion) was seen in autonomically blocked dogs. Finally, if Ca^{2+} channel blockade was the primary mechanism of verapamil's AF-promoting properties, one might have expected to have observed similar phenomena with diltiazem, particularly at doses (like the ones we used) that cause comparable degrees of PR interval prolongation.

4.2. Mechanism of verapamil's AF promoting properties

Verapamil promoted AF in the present study by increasing the apparent number of reentry circuits during AF and thereby stabilizing multiple circuit reentry. Precisely how verapamil achieved this remains unclear. Although verapamil did decrease atrial ERP, this effect was relatively small during 1:1 atrial pacing, and seems insufficient to explain the significant changes in atrial activation that occurred during AF. Furthermore, verapamil accelerated conduction in several atrial regions, which counteracts the effect of ERP reductions on wavelength, leaving the minimal reentrant circuit size apparently unchanged. It is possible that the regional variability of the conduction speeding effect of verapamil contributed to heterogeneous activation during AF; however, this is unlikely to have been of prime importance, since diltiazem had similar effects on atrial conduction but did not promote AF. The abolition of conduction-speeding by both drugs with autonomic blockade suggests that this action was indirect, quite possibly due to adrenergic enhancement.

We measured atrial ERP and conduction velocity during 1:1 atrial pacing, and it is possible that these do not necessarily reflect the drug's electrophysiological actions at the very rapid rates of AF. Verapamil has complex effects on a variety of K^+ channels [22], as well as rate and voltage dependent blocking actions on L-type Ca^{2+} channels [23]. The K^+-channel inhibiting actions of the drug may explain its effect to prolong ERP in the present study when adrenergic reflexes were absent in autonomically-blocked dogs. The rate dependent effects of verapamil on various K^+ and Ca^{2+} channels, the varying importance of each channel during the action potential plateau at different rates, and varying degrees of adrenergic reflex activity at different heart rates may have resulted in much smaller ERP changes at slower 1:1 rates than during AF. Alternatively, verapamil's actions on atrial activation during AF may have resulted from presently unrecognized actions on impulse propagation during the arrhythmia, or from other at present unrecognized actions.

Verapamil has also been noted to have effects on ventricular fibrillation (VF). In isolated hearts, which by definition lack reflex autonomic responses, verapamil decreases the complexity of fibrillatory activity [24,25]. On the other hand, verapamil increases the frequency of ventricular fibrillatory activity in the in situ heart [26]. These results are consistent with AF activation changes seen respectively in the absence and presence of autonomic blockade in the present study. They suggest that verapamil's effects on AF and VF have some interesting similarities, which warrant more detailed analysis in subsequent work.

Although intact autonomic reflexes were required for verapamil's AF-promoting actions, consistent with previous observations [6], autonomic interactions alone were insufficient to explain the effects of verapamil. Vagal enhancement is unlikely to have played any significant role, since increasing vagal tone substantially increases atrial ERP heterogeneity [27], the opposite of the changes seen with verapamil. Friedman et al. showed that the AF-promoting effects of verapamil are prevented by β-adrenergic receptor blockade, pointing to an important role for sympathetic stimulation [6]. Sympathetic enhancement is likely to have occurred during verapamil infusion, but increased sympathetic outflow itself has not been found to have a significant AF-promoting effect in the normal dog heart [27]. It is therefore likely that intact sympathetic responses are necessary for verapamil's AF-promoting action, but that in themselves sympathetic effects are insufficient to account fully for this property of the drug.

The discrepancy between verapamil and diltiazem in AF-promoting properties is an interesting observation that requires explanation. Unlike verapamil, diltiazem did not decrease ERP or AF cycle length. Of note, diltiazem's effects on atrial conduction were very similar to those of verapamil in both the presence and absence of autonomic tone (Figs. 6 and 8). Diltiazem's lack of AF-promoting potential could be due to a different time and voltage dependent profile of Ca^{2+} channel blockade or to different collateral properties. Further studies to clarify this issue, in both experimental and clinical settings, might be of considerable interest.

4.3. Novel aspects and potential clinical relevance

Verapamil's AF promoting action has been reported clinically [4] and in an experimental model [6]. Neither publication addressed potential underlying electrophysiological mechanisms. Friedman et al. did show that beta-adrenoceptor blockade prevents AF promotion by verapamil, but did not examine electrophysiological changes. In the present study, we show that verapamil promotes AF

by increasing the heterogeneity of activation and the number of apparent reentry zones during the arrhythmia. This observation is consistent with the multiple wavelet mechanism of AF maintenance [28]. On the other hand, despite obtaining careful measurements of properties (like ERP and ERP heterogeneity) usually associated with the stability of multiple circuit reentry, the precise electrophysiological mechanism by which multiple wavelet reentry was promoted remains obscure. It is possible that changes in ERP, ERP heterogeneity, conduction velocity and wavelength during 1:1 pacing do not, in the presence of verapamil, reflect well changes during AF. Alternatively, multiple wavelet reentry may have been promoted by changes in variables that we were unable to study. For example, heterogeneous changes in connexin 40 distribution may be important in tachycardia-remodeling related AF [29]. Although the number of connexins is very unlikely to have changed over the time course of the present experiments, changes in connexin function (or some other aspect of cellular coupling) could have been induced by verapamil during AF and would not have been detected by the methods we used.

Patients with AF are often treated with Ca^{2+} channel blockers to control the ventricular response rate. Our observations raise the question of whether diltiazem might be a better choice for rate control among patients in whom a subsequent cardioversion is considered, since diltiazem did not promote AF maintenance in our dogs. Shenasa et al. did observe AF promoting effects of diltiazem, as well as verapamil, administration in man [4]. Further clinical studies of the effects of verapamil and diltiazem on AF maintenance are warranted, to determine the relative actions of these agents on the tendency of AF to sustain itself. Should our findings be substantiated by such clinical studies, our observations could contribute to providing more effective drug therapy for patients with AF.

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