Broad Sensitivity of Rodent Arrhythmia Models to Class I, II, III, and IV Antiarrhythmic Agents (42909)

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Abstract. **To determine specificity of rodent models of arrhythmia for different Vaughan Williams classes of antiarrhythmic drugs, we tested 17 drugs from the four classes in one *in vitro* and four *in vivo* models. In the *mouse chloroform-induced ventricular fibrillation model* and in the *guinea pig ouabain-induced arrhythmia model*, drugs of classes I (amefalone, aprindine, lidocaine, mexiletine, phenytoin, procainamide, or quinidine), II (metoprolol or propranolol), and IV (bepridil) were active. Class III drugs (bretylium, clofilium, or melperone did not suppress ouabain arrhythmias, but were active in the mouse chloroform model. In the *rat coronary artery ligation model*, disopyramide (class I), amefalone and melperone significantly ($P < 0.05$) reduced the number of extrasystoles. Propranolol, sotalol, and verapamil (class IV) were less effective. In the *rat coronary artery ligation/reperfusion model*, all four classes of antiarrhythmic agents were active *in vitro* (isolated heart) and *in vivo* (anesthetized rat). Thus, one model of automaticity, the guinea pig ouabain model, detected class I, II, and IV drugs, whereas another automaticity model, the mouse chloroform model, also detected class III agents. The model of reentry induced by ischemia plus reperfusion (rat coronary artery ligation reperfusion) can be recommended as a screen for new antiarrhythmic agents based on its sensitivity to all four classes of antiarrhythmic drugs. The Vaughan Williams class of an antiarrhythmic agent must be determined, however, by additional mechanism studies.** [P.S.E.B.M. 1989, Vol 191]

Numerous authors have pointed to the need for small animal models of cardiac arrhythmia that may be of use in screening for new antiarrhythmic agents. Problems with large animal models, particularly dogs, were cited by Clark *et al.* (1) as "time, money, and the amount of compound needed" and prompted their development of the coronary artery - ligated rat model. Curtis *et al.* (2) in their excellent review of rat ischemia-reperfusion models noted that the rat could be recommended for its lack of effective coronary collaterals, low cost, quantitative assessment of arrhythmias, availability of clinically relevant concomitant diseases, extensive biochemical data, and similarities to larger animals in drug responses.

Although there have been many studies of small animal models of cardiac arrhythmias (see references in (3)), most reports deal with model methodology and characterization (1) and relatively few consider the model's ability to discriminate among antiarrhythmic agents of different mechanistic classes, or whether all agents of a given class are equally effective in a given model (2, 4). Curtis *et al.* (2) noted that, besides the usual antiarrhythmic agents of the four Vaughan Williams classes, a variety of other agents have been tested for efficacy in rat arrhythmia models. These include arachidonic acid metabolites (proarrhythmic effects), prostaglandins, nonsteroidal anti-inflammatory agents, actinomycin, cycloheximide, creatine phosphate, adenosine, naloxone, meptazinol, a serotonin antagonist, an antihistamine, nitroglycerin, and atropine. Procedures such as dietary fatty acid modification, vagotomy, sympathectomy, and adrenalectomy have also been investigated (see (2) for references). In a comprehensive review of methods for detecting antiarrhythmic activity, Winslow (3) provided some data on sensitivity of some models to different classes of reference agents. In a study of isoproterenol-induced sudden death in heavy rats, Johnson *et al.* (5) showed that pretreatment with class I agents (quinidine, procainamide, and phenytoin), the class II agent, propranolol, the class III agent, clofilium, and the class IV agent, verapamil, reduced

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the incidence of ventricular fibrillation (VF) and death. Thus, this model was responsive to antiarrhythmic agents of all classes, although it was reported that the class III compound, bretylium, even at twice the milligram per kilogram clofilium dose, was ineffective. Nolte *et al.* (6) have described a guinea pig isolated heart model of mechanically induced arrhythmias that was sensitive to class I, but not class IV agents. Gerbsch and Hagen (7) showed that certain class I, II, and IV drugs were effective in suppressing both ventricular tachycardia (VT) and VF in a rat coronary artery ligation model.

Studies such as these which attempt to address sensitivity of models to drugs suffer several shortcomings. In some cases only one model and one mechanism of arrhythmogenesis was considered (8). In studies of several models, the number of drugs tested was often limited (3, 4, 8). Class I agents, because of the many examples available, usually dominated the selection. The lack of drugs with "pure" mechanistic profiles limits conclusions about model specificity. Melperone is an example of a compound with multiple relevant mechanisms. Melperone in rabbit bundle of His both increased action potential duration (class III effect) and decreased depolarization rate (V_{max}, class I effect) (9). We have used melperone as a class III agent, since it had a significant effect on action potential duration at half the concentration required for a significant effect on V_{max} (9). Bepridil also has multiple mechanisms. Since it lowered action potential plateau significantly in sheep Purkinje fibers at half the concentration required for a significant effect on V_{max} (10), bepridil has been considered a class IV agent in these studies. It should be noted, however, that bepridil has been reported to cause increases in effective refractory period (class III action) in anesthetized dogs (11). Sotalol is both a class II and class III agent, but its antiarrhythmic effect seems to reside in its class III action (12). A summary of the antiarrhythmic drugs used in this study, and their Vaughan Williams classification (13), appears in Table I.

We present studies in several rodent models, including chemically induced and ischemia/reperfusion-induced arrhythmias and including automaticity and reentry mechanisms. For the rat coronary artery ligation-reperfusion model, we have used both the *in vitro* and *in vivo* versions. For each model we have examined the effect of representatives of the four Vaughan Williams classes of antiarrhythmic agents. To at least partially overcome the problem of the lack of antiarrhythmic drugs with "pure" mechanisms, we have looked in most cases at more than one representative

Table I. Vaughan Williams Classification of Antiarrhythmic Drugs Used in This Study

Class	Action	Examples	Supplier
I: Local anesthetics	Depress fast sodium current	Quinidine SO_4	Sigma Chemical Co., St. Louis, MO
		Procainamide HCl	Sigma Chemical Co.
		Disopyramide PO_4	G. D. Searle & Co., Chicago, IL
		Amefalone	Organon, Oss, Holland
		Aprindine HCl	Eli Lilly & Co., Indianapolis, IN
		Lidocaine HCl	Astra Pharmaceutical Products, Worchester, MA
		Phenytoin sodium	Parke-Davis Division, Warner-Lambert Co., Morris Plains, NJ
		Mexiletine HCl	Boehringer Ingelheim Ltd., Ridgefield, CT
II: β blockers	Block β-adrenergic receptors	Propranolol HCl	Sigma Chemical Co.
		Metoprolol HCl	Ciba Pharmaceutical Co., Summit, NJ
III: Prolongers of action potential duration	Prolong repolarization	Bretylium tosylate	American Critical Care, McGraw, IL
		Clofilium tosylate	Eli Lilly & Co.
		Melperone HCl	Ferrosau, Malmo, Sweden
		Sotalol HCl	Mead Johnson & Co., Evansville, IN
IV: Calcium antagonists	Block slow calcium channel	Nifedepine	Norwich Eaton Pharmaceuticals Inc., Norwich, NY
		Bepridil	Wallace Laboratories, Cranbury, NJ
		Verapamil HCl	Knoll Pharmaceutical Co., Whippany, NJ

Note. The classification of antiarrhythmic drugs is discussed by Reiser and Sullivan (13).

drug in each class. A preliminary report of these results has appeared (14).

Materials and Methods

Animals. Male CD-1 mice and male Hartley strain guinea pigs were purchased from Charles River Breeding Laboratories (Wilmington, MA), Male Sprague-Dawley rats were obtained from Taconic Farms, Inc. (Germantown, NY). Animals were housed under standard laboratory conditions with laboratory chow and water available *ad libitum.*

Chemicals. Reference antiarrhythmic drugs were obtained as gifts from their manufacturers or bought from commercial suppliers (Table I).

Mouse Chloroform Model. The method of Lawson (15) was used. In brief, test compounds in 0.5% (w/v) methylcellulose were administered to groups of 10–20 mice at doses of 3–100 mg/kg ip. Thirty minutes (15 min for lidocaine and procainamide) after drug administration, the mice were placed in a chamber whose atmosphere contained chloroform vapor sufficient to induce anesthesia within 2–3 min and apnea 3–4 min later. The mice were removed from the chamber at onset of apnea and a thoracotomy was performed immediately. A visual assessment of heart rhythm (i.e., normal sinus rhythm or arrhythmia) was promptly made. The 30- or 15-min pretreatment time was chosen based on probe experiments that indicated that these times were appropriate for clearly demonstrating activity with these reference drugs. Drug efficacy was expressed as the percentage of drug-treated animals showing normal sinus rhythm. Treatment effects were tested for significance by the chi-square procedure, with a correction for continuity (16). For selected active compounds, an ED_{50} value was estimated by least squares regression analysis of the log dose-percentage of inhibition curve.

Guinea Pig Ouabain Model. A procedure modified from Sekiya and Vaughan Williams (17) was used. Guinea pigs weighing 450–550 g were anesthetized (urethane, 1.25 g/kg ip), intubated, and respired. After the preparation had stabilized (as judged by continuous blood pressure and electrocardiogram tracings), the infusion (jugular vein) of 75 μg/ml of ouabain octahydrate in saline was started at the rate of 0.1 ml/min. The time of onset of the first ventricular extrasystole and fibrillation was identified. Compounds were administered intravenously (jugular vein) as a slow bolus injection (about 0.5 ml/kg/min) during the 7 min before the start of the ouabain infusion. Mexiletine, metroprolol, and bretylium were dissolved in water. Phenytoin and nifedipine were dissolved in a 50% organic vehicle containing 15.6% glycerin, 5.7% propylene glycol, 11.5% 2-pyrrolidone, 17.2% *N*-(2-hydroxyethyl)-2-pyrrolidone, 5.0% urea, and 10% glucose (all w/v) in water. Other compounds were dissolved in saline. Compound-treated animals (n = 3–5) were compared with vehicle-treated groups (saline, n = 17; water, n = 22; organic, n = 16) for differences in arrhythmia or fibrillation threshold by an unpaired t test.

Rat Coronary Ligation (CAL) model. The method of Marshall *et al.* (18) was used. Rats (250–400 g) were anesthetized (pentobarbital sodium, 50 mg/kg ip), tracheotomized, and respired with room air. A thoracotomy was performed through the left fifth intercostal space (19). The heart was exteriorized and a loose ligature (5-0 silk) was passed through the myocardium under the left coronary artery. Rats with arrhythmias or mean arterial blood pressure <70 mm Hg at the end of the 15-min equilibration period were rejected. Compounds were infused intravenously (femoral vein) in saline over 5 min at 0.2–1 ml/kg/min just before the ligature was tightened or were administered intraperitoneally in 0.5% (w/v) methylcellulose (10 ml/kg) 60 min before the ligature was tightened (15 min before anesthesia). Ventricular extrasystoles occurring during the 30 min following ligation were counted.

Rat Coronary Artery Ligation/Reperfusion (CALR) Models. Compounds were evaluated for *in vitro* antiarrhythmic activity according to a method adapted from Lubbe *et al.* (20). Rats were anesthetized (pentobarbital sodium, 60 mg/kg ip) and given 200 units of heparin sodium/kg in 1 ml/kg 0.9% saline via a femoral vein. The heart was rapidly excised and retrogradely perfused (at approximately 55 mm Hg pressure) with Krebs-bicarbonate saturated with 95% oxygen-5% carbon dioxide gas at 37°C (pH 7.4) with either no compound present (control) or compound present. A loose ligature of 5-0 silk was placed around the left anterior descending coronary artery and the ends of the suture were threaded through a 2-cm piece of PE-280 tubing (3-mm outside diameter). Coronary flow was estimated (volume collection) before and after ligation.

After 35 min of perfusion, the artery was occluded by clamping the suture/tubing tightly against the heart surface with a 25-mm Schwartz forceps. Successful ligation was indicated by a 20% or greater reduction in coronary flow. After a 15-min ligation, the left ventricle was reperfused for 5 min by releasing the clamp. The incidence and time of onset during the 5-min reperfusion of ventricular extrasystole (VES), tachyarrhythmia (VT) (>4 VES), and fibrillation (VF) were noted from the continuous electrocardiogram. At the termination of the experiment, 0.1% Patent Blue V dye was injected into the perfusate and reperfusion of the heart was verified by the presence of uniform staining of both ventricles.

For the *in vivo* evaluation of activity in the CALR rat model (4, 21), rats weighing 350–450 g were given either vehicle (0.5% w/v methylcellulose) in a volume of 10 ml/kg ip or compound in this vehicle. Twenty minutes later, the rats were anesthetized (pentobarbital sodium, 60 mg/kg ip), thoracotomized, and attached

to a ventilator. A carotid artery was catheterized for monitoring blood pressure and a femoral vein was catheterized for compound administration. A loose ligature of 5-0 silk was placed around the left anterior descending coronary artery. Forty-five minutes after compound or vehicle was administered intraperitoneally, the artery was ligated for 5 min and then released as described above. Successful ligation was determined by measuring the decrease in surface temperature of the ventricle with a Bailey (Saddle Brook, NJ) thermalert model TH-8 temperature monitor and a Type MT-4 needle thermometer sensor before and after ligation.

For both the *in vitro* and *in vivo* CALR rat models, arrhythmia scores were calculated based on the sum of factors for the occurrence during the 5-min reperfusion period of ventricular arrhythmias, i.e., VES, VT, and VF. The summed factors were the product of a severity parameter arbitrarily assigned as 1, 3, and 6 for occurrences of VES, VT, and VF, respectively. The severity parameter was multiplied by 10 if onset was from 0 to 60 sec after reperfusion; 8 if onset was from 61 to 120 sec; 6 if onset was from 121 to 180 sec; 4 if onset was from 181 to 240 sec; 2 if onset was from 241 to 300 sec; and 0 if onset did not occur by 300 sec. Development of VES, VT, and VF within the first 60 sec of reperfusion would result in an arrhythmia score of 100, whereas lack of development of arrhythmias within 300 sec of reperfusion would result in a score of 0. Results are also expressed as percentage of protection from VF. Statistical significance of treatment effects was determined by two sample sum of ranks test (Mann-Whitney) for differences between the mean arrhythmia scores of untreated (control) and treated hearts at the 95% confidence level.

Results

Mouse Chloroform-Induced Arrhythmia Model. In 42 control groups of 10 mice each dosed with 10 mg/kg of 0.5% methylcellulose, mean (±SD) protection from fibrillation was 13 ± 8% (Table II). Among the class I agents tested, lidocaine, procainamide, and quinidine were clearly active, providing dose-dependent protection from fibrillation that exceeded 60% as the maximum effect observed. Phenytoin sodium was active at 100 and 200 mg/kg ip and at 300 mg/kg protected 53% (mean of three experiments). Mexiletine

Table II. Effects of Antiarrhythmic Agents in the Mouse Chloroform-Induced Arrhythmia Model

Vaughan Williams class	Compound	No. of mice	Dose (mg/kg)	% Protection	ED_{50} (mg/kg ip)
Control	0.5% Methylcellulose	420	10 (ml/kg)	13 ± 8[a]	
I	Lidocaine HCl	15	30	13	63
		15	56	47*	
		12	100	75*	
	Mexiletine HCl	20	25	30*	>50
		20	50	40*	
	Phenytoin sodium	10	100	40*	>200
		10	200	40*	
		30	300	53*	
	Procainamide HCl	15	130	60*	114
		15	300	60*	
		15	350	80*	
	Quinidine SO_4	15	40	7	79
		15	54	20	
		55	100	73*	
II	Propranolol HCl	60	40	97*	<40
III	Bretylium tosylate	20	3	45*	4
		20	10	65*	
		20	30	75*	
	Clofilium tosylate	20	3	30	8
		20	10	55*	
		20	30	80*	
	Sotalol HCl	20	3	45*	4
		20	10	60*	
		20	30	75*	
	Melperone HCl	10	30	60*	<30
IV	Bepridil	10	50	30	<100
		40	100	80*	
	Verapamil HCl	15	30	13	<67
		15	45	7	
		15	67	62*	

Note. Male mice (10–20 per group; higher numbers indicate multiple experiments) were dosed intraperitoneally with compounds in 0.5% (w/v) methylcellulose (10 ml/kg) 30 min before exposure to chloroform. Lidocaine and procainamide were administered 15 min before chloroform.
[a] Mean ± SD. * Significantly ($P < 0.05$) different from the control group value by continuity-corrected chi-square analysis.

provided a maximum of 40% protection at nontoxic doses. The class II drug propranolol was used as a positive reference agent in this work and so was assayed in 44 groups of mice (n = 10/group), but only at one dose (40 mg/kg ip) chosen to protect approximately 80% of mice. This dose provided 97% protection. At this dose, over 10 times the intraperitoneal ED_{50} (3), membrane stabilization (class I) as well as β blockade (class II) effects would be expected. The class III drugs, bretylium, clofilium, and sotalol, and afforded dose-dependent protection from VF. The dose-response curves of these three agents was practically identical and each drug produced approximately 80% maximum protection (at the 30 mg/kg ip dose). The class IV compound, bepridil, also gave a maximum 80% protection from VF at the high dose of 100 mg/kg ip. At 50 mg/kg of bepridil gave only 30% protection. Verapamil was inactive, except at the high dose of 67 mg/kg ip, where it was also toxic (2 of 15 mice died during 30-min pretreatment period). A summary of ED_{50} values from the mouse chloroform model is given in Table II. Intraperitoneal ED_{50} values for class I agents all exceeded 50 mg/kg. The class III drugs were much more potent in this model, generally having ED_{50} values less than 10 mg/kg ip. These relative potencies apply only to the 30-min dosing interval studied. Other pretreatment times might yield different potencies.

Guinea Pig Ouabain-Induced Arrhythmia Model. The intravenous doses of ouabain causing the first arrhythmia for saline, water, and organic vehicle control groups were 128 ± 7, 127 ± 5, and 125 ± 6 μg/kg, respectively (mean ± SEM for 17, 22, and 16 animals, respectively). The corresponding intravenous doses of ouabain causing VF were 193 ± 8, 185 ± 7, and 187 ± 7 μg/kg, respectively. Class I agents were all effective in raising the threshold doses of ouabain required to produce the first arrhythmia and fibrillation in guinea pigs (Fig. 1). In terms of magnitude of the percentage of increase in ouabain threshold dose, aprindine was the most effective class I drug studied, increasing arrhythmia threshold 90% and fibrillation threshold 70% at 5 mg/kg iv. Aprindine was also the most potent of the class I drugs. Analogous to its profile in the mouse chloroform model, procainamide was active, but its activity was not strongly dose dependent. Arrhythmia threshold was increased approximately 40% at all tested doses of procainamide from 1 to 40 mg/kg iv. Increases of fibrillation thresholds at the 10 and 40 mg/kg iv doses were actually less than those observed at 1 and 5.6 mg/kg. In general, the class I drugs increased the threshold dose for the first extrasystole more than that for fibrillation. The active 20 mg/kg iv dose of phenytoin and the lower two doses of procainamide, however, elevated both thresholds equally. The class II drugs, propranolol and metoprolol, were active and, along with bepridil (class IV), and aprindine, gave significant increases of thresholds at or below 5 mg/kg iv (Fig. 1).

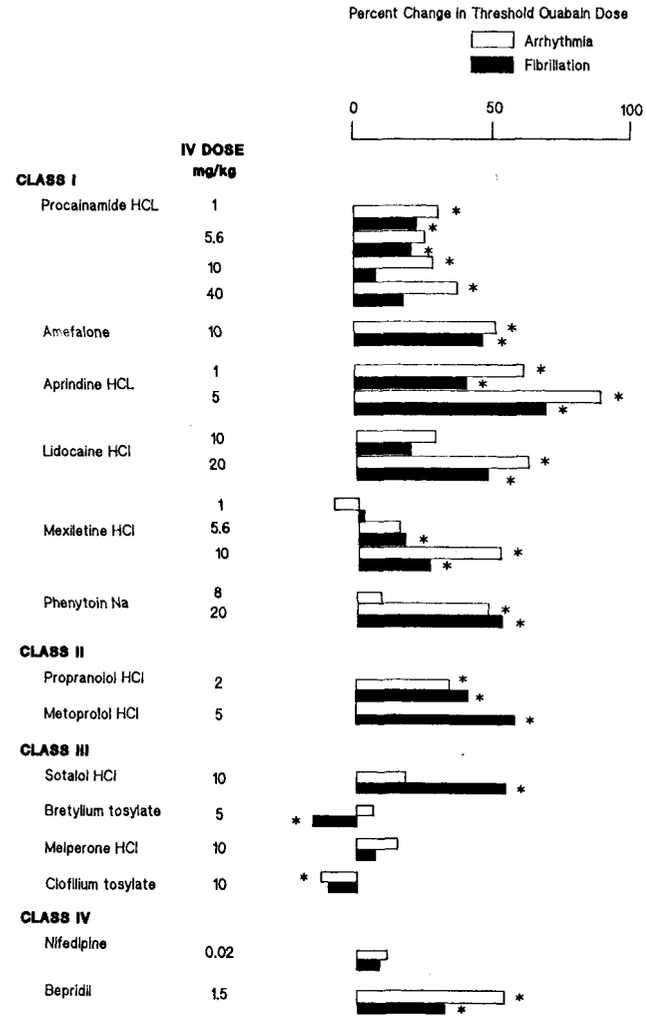

Figure 1. Effects of intravenous antiarrhythmic drugs on the intravenous ouabain dose required to induce the first arrhythmia (open bars) and VF (solid bars) in the anesthetized guinea pig. Mean percentage of changes from the corresponding control group value are shown for groups of three to five guinea pigs per dose (n = 2 for 5 mg/kg aprindine HCl). *Significant difference from control group value ($P < 0.05$).

Metoprolol selectively increased fibrillation threshold. It did not change the dose of ouabain at which the first arrhythmia occurred. Among the class III drugs, only sotalol, perhaps because of its class II component, was active and, like metoprolol, showed a preference for protecting from fibrillation, although it also nonsignificantly raised arrhythmia threshold. Neither bretylium nor clofilium were active (Fig. 1). The inactivity of melperone is consistent with our considering this agent to be class III rather than class I. The strong dihydropyridine-type calcium antagonist, nifedipine, was also inactive, but the other class IV agent, bepridil, significantly raised both thresholds at 1.5 mg/kg, making it as potent as the β blockers (Fig. 1).

Rat CAL Model. Class I drugs and the class III agent melperone were active in suppressing the number of extrasystoles and in protecting rats from VF in the

CAL model (Table III). Although the class II agent propranolol at 30 mg/kg ip decreased VES count 50% (not significant), it did not prevent VF in three rats. Sotalol at 30 mg/kg ip was also ineffective, despite being active in the mouse chloroform model at one tenth this intraperitoneal dose. The class IV drug, verapamil, at 3 mg/kg ip was inactive. Disopyramide and melperone at intravenous doses of 10 mg/kg were the most effective agents in suppressing extrasystoles (to 1% of control group values).

Rat CALR Models. Representatives of all four Vaughan Williams classes were active in the *in vitro* rat CALR model. Concentration-dependent suppression of arrhythmias was provided by quinidine and melperone, propranolol, clofilium, and verapamil (Fig. 2). Sotalol was active, but its concentration-response curve was U shaped.

In the *in vivo* rat CALR model, active representatives of all four Vaughan Williams classes were found (Table IV). Complete protection from VF was afforded by the class I drugs, lidocaine and quinidine, the class II drug, propranolol, the class III agents, bretylium and melperone, and the class IV compound, verapamil. Lesser, but statistically significant, activity was obtained with clofilium and sotalol.

Discussion

Sudden cardiac death has been characterized as the number one public health problem in the United States and other developed countries. The half million annual victims of sudden cardiac death in the Untied States are mostly victims of cardiac arrhythmias, with ventricular tachycardia and fibrillation being the events preceding death. Although there are numerous antiarrhythmic drugs of various mechanisms available for clinical use, only the β blockers have been shown to decrease mortality. As a group, available drugs suffer from poor efficacy or failure to sustain efficacy, and an array of side effects serious enough to preclude use or to cause discontinuance despite efficacy. Thus, the search for better antiarrhythmic drugs has a firm basis in clinical need.

The preclinical phase of new drug discovery involves, of course, testing in animal models. Selection of the best animal model is complicated in this area not only by questions of relevance to the human disease state, but also by the fundamental mechanistic differences between compounds, all of which are labeled "antiarrhythmic." Part of the solution to the former problem lies in assessing new drug candidates in a variety of models. Baum *et al.* (22) noted in their study of various canine arrhythmia models that "in all probability, no single procedure can be expected to predict efficacy in all types of clinical arrhythmias" and that "clinical arrhythmias are the complex result of many factors . . . and it would indeed be surprising if a single chemical would be highly effective in antagonizing all types." Results from profiling a drug development candidate in several animal models could also be better interpreted if there were an understanding of what types of drugs affect the various models.

We have addressed the latter point by examining how some common rodent arrhythmia models respond to antiarrhythmic agents of the four Vaughan Williams

Table III. Effects of Antiarrhythmic Agents in the Anesthetized Rat CAL Model of Ventricular Arrhythmias

Treatment	Route	Dose (mg/kg)	Ventricular extrasystoles		No. with VF/ no. tested
			Count/ 30 min	% of control	
Saline 0.9%	iv	1–5 (ml/kg)	3208 ± 553	100	6/11
Methylcellulose 0.5%	ip	10 (ml/kg)	2396 ± 403	100	4/7
Class I agents					
Amefalone	iv	5	446 ± 345**	14	0/6
Amefalone	ip	30	223 ± 139**	9	0/3
Disopyramide PO_4	iv	10	44 ± 24*	1	0/4
Class II agent					
Propranolol HCl	ip	30	1201 ± 70	50	3/3
Class III agents					
Melperone HCl	iv	10	27 ± 13*	1	0/3
Sotalol HCl	ip	30	2114 ± 378	88	3/3
Class IV agent					
Verapamil HCl	ip	3	1791 ± 408	75	3/3

Note. Treatments were given either intravenously to anesthetized rats just before ligation of the left descending coronary artery, or intraperitoneally 15 min before anesthesia and 60 min before ligation. *VF* (>3-sec loss of sinus rhythm with blood pressure <15 mm Hg). *, ** Significantly different from the corresponding intravenously (saline) and intraperitoneally (methylcellulose) control group value by a *t* test at $P < 0.05$ and $P < 0.01$, respectively. Count values are mean ± SEM for the number of rats in the last column.

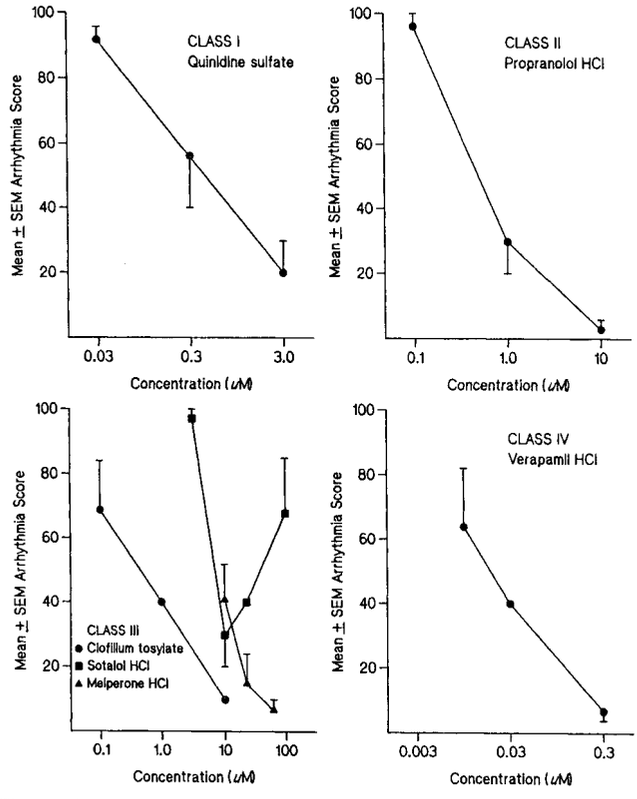

Figure 2. Effects of antiarrhythmic drugs in an *in vitro* coronary artery ligation/reperfusion model of ventricular arrhythmias in the isolated perfused rat heart. Three to five hearts per concentration were used. The arrhythmia score (100 = full protection) is described in Materials and Methods.

Table IV. Effect of Antiarrhythmic Agents Administered Intraperitoneally in the Anesthetized Rat CALR Model of Ventricular Arrhythmias

Treatment	*n*	Dose (mg/kg)	% Protection from VF	Arrhythmia score
Methylcellulose 0.5%	45	10 (ml/kg)	7	79 ± 3
Class I agents				
Lidocaine HCl	3	10	100	40 ± 0*
Phenytoin sodium	5	10	40	69 ± 14
	5	56	60	64 ± 15
	3	100	33	60 ± 11
Quinidine SO_4	3	3	100	49 ± 0*
Class II agent				
Propranolol HCl	3	0.1	33	56 ± 8
	5	1	100	40 ± 0*
	5	3	80	47 ± 7*
	4	10	75	49 ± 9*
Class III agents				
Bretylium tosylate	4	0.1	33	68 ± 14
	3	1	67	60 ± 20
	4	10	100	40 ± 0
	3	30	66	60 ± 20
	3	50	100	13 ± 13*
Clofilium tosylate	4	0.01	0	76 ± 7
	3	0.1	33	64 ± 12
	3	1	67	48 ± 8*
	3	3	67	52 ± 12
	3	10	67	44 ± 4*
Melperone HCl	3	10	0	100 ± 0
	5	20	80	30 ± 19*
	5	30	100	16 ± 10*
Sotalol HCl	5	3	20	69 ± 10
	5	10	40	74 ± 14
	5	20	80	36 ± 18*
Class IV agent				
Verapamil HCl	3	0.01	33	64 ± 14
	3	0.1	33	60 ± 0
	3	1	100	40 ± 0*

Note. Compounds were administered intraperitoneally in methylcellulose 45 min before ligation of the left descending coronary artery for 5 min, followed by reperfusion. Arrhythmias were monitored in the 5 min following reperfusion and scored as described in Materials and Methods. Scores are given as mean ± SEM. Significantly different from the vehicle group score at $P < 0.05$ by the Mann-Whitney sum of rank test.

classes. Our results in the four *in vivo* and one *in vitro* models with the 17 drugs studied are summarized in Table V. Our results in the mouse chloroform model concur with those summarized by Winslow (3). This model is sensitive to agents of all four Vaughan Williams classes. We extend the Winslow data by showing that the class III compounds, bretylium, clofilium, melperone, and sotalol, are not only quite effective in this model, but also more potent than the tested class I drugs. Although the mouse chloroform model has features of simplicity and economy, the uncertainty of what is being called an arrhythmia, the heavy influence of elevated sympathetic tone, and lack of discrimination by this model are disadvantages. The last point may be the most important defect, since any bradycardia agent, CNS and respiratory depressants, and α-adrenolytic agents will appear active.

The guinea pig ouabain model was primarily responsive to class I agents, although bepridil was also active. Winslow (31) emphasized that only agents with strong membrane stabilizing actions are active in this model. Our findings that the class III drugs are not active in the guinea pig, confers on this model the clearest selectivity of those studied. The moderate activity detected with sotalol agrees with the similar finding of Singh and Vaughan Williams (23). Although it has been argued that the guinea pig ouabain model offers no advantages over the mouse chloroform model, our finding a selective efficacy of class III drugs in the latter, but not in the former, model may make the guinea pig model of use as a quick way of determining whether a compound has a prominent class III effect in the whole animal.

Our limited data in the rat CAL model suggests that it responds best to strong class I agents. The activity of melperone could be a reflection of the class I effects of this compound. The inactivity of sotalol and of verapamil suggests a bias against class III and class IV drugs. Winslow (3), however, reported that verapamil and, more clearly, nifedipine, did suppress extrasystoles in this model. A major problem encountered in the rat CAL model is the variability of the postocclusion ar-

Table V. Efficacy Summary of Antiarrhythmic Drugs in Rodent Arrhythmia Models

Agent	Mouse chloroform	Guinea pig ouabain	Rat CAL	Rat CALR	
				In vitro	*In vivo*
Class I					
Quinidine SO_4	***			***	****
Procainamide HCl	**	**			
Disopyramide PO_4			****		
Amefalone		***	****		
Aprindine HCl		****			
Lidocaine HCl	***	***			****
Phenytoin sodium	*	***			*
Mexiletine HCl	**	***			
Class II					
Propranolol HCl	***	**	*	***	***
Metoprolol HCl		**			
Class III					
Bretylium tosylate	****	0			***
Clofilium tosylate	****	0		***	**
Melperone HCl	***	0	****	**	***
Sotalol HCl	****	*	0	*	**
Class IV					
Nifedepine		0			
Bepridil	**	****			
Verapamil HCl	*		0	****	****

Note. Activity is summarized from other tables and figures as follows: 0 = no activity; * = some activity; ** = moderate activity; *** = strong activity; **** = clear, dose-dependent activity or potency.

rhythmias, made more severe by the fact that each animal cannot serve as its own control.

The recognition of reperfusion as a separate arrhythmogenic component elevates the importance of the rat CALR models. Manning and Hearse (4) noted that only with the realization that reperfusion arrhythmias are distinct from ischemia arrhythmias and that reperfusion arrhythmias are encountered in man has their study increased. These authors pointed out that "whilst the pharmacology of ischaemia-induced arrhythmias has been extensively studied and documented, relatively little is known about the effects of various drugs on reperfusion-induced arrhythmias." Our data suggest that compounds of all four Vaughan Williams classes are active in both the *in vitro* and *in vivo* versions of this model and that there is good agreement between the two versions. Our finding of propranolol's activity does not agree with several studies in dog and cat which showed that propranolol was not effective in suppressing reperfusion arrhythmias. Propranolol has not, however, been studied in a rat reperfusion model, and there may be species differences. There seems to be a difference, for example, between rat and dog or pig in the efficacy of class I agents on reperfusion arrhythmias (23). Although class I drugs are generally effective in the rat, they are not clearly antiarrhythmic in dog and pig reperfusion arrhythmia models.

Manning and Hearse (4) note that "confusion exists over the ability of agents which prolong action potential duration to reduce reperfusion-induced arrhythmias." These authors concluded that the case for utility of class III drugs against reperfusion arrhythmias remains unproven. Curtis *et al.* (2) echoed this uncertainty about sensitivity of rat reperfusion models to class III agents, stating "inhibitors of the fast inward current (class I agents) and inhibitors of the slow inward current (class IV agents) appear to be particularly effective antiarrhythmic in rats against both ischaemia- and reperfusion-induced arrhythmias. The effectiveness of class II and class III agents is questionable." Our data clearly show that several class III drugs are active in the *in vitro* and *in vivo* CALR models.

It may be concluded that a variety of models detect activity of antiarrhythmic drugs of all Vaughan Williams classes. Only the guinea pig ouabain model is strongly biased against class III agents. The CALR rat models can be recommended on the basis of convenience and economy, broad sensitivity, and lack of bias. It is worth reemphasizing, however, that the uncertain connection between animal models of arrhythmia and clinically observed arrhythmias makes it important to assess potential new drugs in a variety of models, including chemically induced arrhythmias, those due to ischemia, and those due to reperfusion. The CALR rat model can be used to identify a new antiarrhythmic agent of any Vaughan Williams class, but further work, either *in vitro* electrophysiology or isolated heart studies (24), is necessary to uneqivocally assign a mechanism.

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