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Molsidomine prevents post-ischaemic ventricular fibrillation in dogs

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1 Forty anaesthetized dogs were subjected to left circumflex coronary artery ligation followed by reperfusion. Molsidomine was randomly administered to 20 dogs (50 $\mu g\,kg^{-1}$ as an i.v. bolus – 15 min prior to coronary occlusion – followed by an infusion of 0.05 $\mu g\,kg^{-1}\,min^{-1}$). Standard electrocardiographic leads 2 and 3 were continuously recorded to measure ST segment and $\Delta R\%$ changes and to document both the number of ventricular premature beats and the onset of ventricular fibrillation; aortic pressure and cardiac output were measured; thromboxane B_2 plasma levels, platelet aggregation produced by ADP, and molsidomine plasma levels were determined before and at 10, 30 and 75 min after the start of the drug protocol.

2 Molsidomine protected the treated animals from early (10 min) post-ischaemic ventricular fibrillation (0 of 20 vs 6 of 20, $P = 0.0202$), reduced the incidence of overall post-occlusion ventricular fibrillation (3 of 20 vs 10 of 20, $P = 0.0407$) and improved the total survival rate ($P = 0.0067$).

3 In molsidomine treated dogs: mean aortic pressure and the rate-pressure product were lowered 10 min after the start of the drug; immediate post-occlusion (3 min) ST segment changes (0.82 ± 0.52 vs 1.52 ± 0.78 mV, $P < 0.025$) and $\Delta R\%$ changes (37 ± 50 vs $90 \pm 84\%$, $P < 0.025$) were less marked; the number of ventricular premature beats was lowered and finally, a progressive decline of platelet aggregation produced by ADP was achieved after 75 min of drug infusion.

4 These results were obtained in the presence of mean plasma levels of molsidomine ranging from 20 to 28 $ng\,ml^{-1}$.

5 The time-action curve of the antifibrillatory effect of molsidomine parallels those at the level of post-ischaemic electrocardiographic changes.

Introduction

Molsidomine (N-ethoxycarbonyl-3-morpholine-sydnonimine) is an effective long-acting antianginal drug (Guerchicoff *et al.*, 1978; Majid *et al.*, 1980; Detry *et al.*, 1981; Baudry, 1982; Balakumaran *et al.*, 1983; Balestrini *et al.*, 1984), which possesses similar haemodynamic properties to those of nitrates (Guerchicoff *et al.*, 1978; Majid *et al.*, 1980; Detry *et al.*, 1981). In addition, from one randomized trial it was concluded that molsidomine may be a useful adjunct to β-adrenoceptor blocking drugs in patients with coronary artery disease (Balestrini *et al.*, 1984). This synergistic action is comparable to that previously demonstrated for long-acting nitrates (Russek, 1968). Other potentially useful pharmacological properties of molsidomine have been described in the dog. These include: a coronary collateral vasodilator action in hearts with coronary insufficiency (Hirata & Kikuchi, 1970), improved endocardial blood flow (Berdeaux *et al.*, 1978), a reduction of both occlusive coronary artery thrombosis and myocardial ischaemia (Fiedler, 1982), decreased infarct size after coronary artery ligation (Fiedler *et al.*, 1982), and an anti-ischaemic effect following controlled reduction of coronary perfusion as assessed by ST segment changes in multiple unipolar epicardial leads (Nitz & Martorana, 1985). Finally, a reduction in the incidence of post-

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reperfusion ventricular fibrillation in a non-controlled study was observed after left anterior descending coronary artery ligation in 8 dogs (Nitz & Martorana, 1985). Whether molsidomine is capable of preventing post-occlusion ventricular fibrillation and of reducing the mortality rate in acute coronary artery occlusion models, though of potentially clinical relevance, has not been previously ascertained.

Therefore, the potency of molsidomine at preventing post-occlusion ventricular fibrillation and at improving the outcome in dogs was investigated. For this purpose a canine model left circumflex coronary artery ligation and continuous standard electrocardiographic recordings was devised in order both to control factors of variability which may affect the significance of the results and to obtain clinically applicable parameters (Jouve *et al.*, 1985, 1986a,b).

Methods

Surgical preparation

A constant time schedule was maintained during all phases of this investigation. Anaesthesia was induced in 40 mongrel dogs of either sex (9 to 14 kg) by intramuscular injection of levomepromazine ($0.5\,mg\,kg^{-1}$) followed 30 min later by intravenous administration of sodium pentobarbitone ($10\,mg\,kg^{-1}$). After endotracheal intubation, ventilation was maintained (with 1:1 oxygen and nitrous oxide) using a Logic 5 respirator with respiratory rate adjusted to 20 strokes min^{-1} and a tidal volume of $20\,ml\,kg^{-1}$. Pancuronium bromide ($0.1\,mg\,kg^{-1}$) and atropine sulphate ($0.01\,mg\,kg^{-1}$) were injected intravenously. A catheter inserted into a peripheral vein was used for drug or saline administration. A left thoracotomy was performed at the level of the fourth intercostal space. The pericardium was incised parallel to the phrenic nerve and raised to form a cradle in which the heart was supported. A catheter (20 g, 38 mm) was introduced at the level of the anterior aspect of the ascending aorta. This catheter was connected to an Elema-Schönander transducer (0–300 mmHg) using a Vygon bolt extensor (tested at $40\,kg\,cm^{-1}$) for aortic pressure recording and blood sampling. Arterial gases and pH were determined after thoracotomy and several times thereafter and remained in the normal range ($90 < Pa_{O_2} < 160$ mmHg; $25 < Pa_{CO_2} < 35$ mmHg; $7.38 < pH < 7.44$). A Swan-Ganz catheter (5F) was advanced through the superior vena cava to the pulmonary artery for determination of cardiac output which was obtained in duplicate using 5 ml cold saline injections and a model 9538 A Edwards Laboratories computer. The left circumflex coronary artery was prepared for occlusion after careful dissection at a site 1 to 5 mm away from its origin using a braided thread (Teflene 2.0) mounted on a half round-curve needle (Archimed 2.0). Acute coronary occlusion was performed in these animals 25 to 30 min after left thoracotomy, that is 30 to 35 min after the induction of intravenous anaesthesia.

Electrocardiographic monitoring

The dogs were restrained in a fixed position (Hill, 1968). Needle limb electrodes were immediately inserted within 1 min of pentobarbitone anaesthesia and a standard frontal 6 lead electrocardiogram recorded on a jet-ink electrocardiograph (Elema-Schönander: Mingograf 81) which allowed a paper speed of 2.5 to 1000 mm s^{-1}. Continuous electrocardiographic recording of standard leads 1, 2 and 3 was performed. Moreover, a contourographic display of lead 2 was obtained (Torresani *et al.*, 1983). This was aimed at providing a synoptic view of electrocardiographic changes and documenting the stability of the recording. R wave amplitude in mm (10 mm = 1 mV) was measured from the isoelectric line to the zenith of R wave ($\Delta R\%$ changes from baseline values). ST segment elevation in mV was measured perpendicular to the isoelectric line and 60 ms after the beginning of R wave or the nadir of Q wave when present. These measurements were performed at a paper speed of 100 mm s^{-1} every 60 s throughout the experiments starting 1 min before ligation. Each data point represented a mean of 5 to 10 consecutive beats to negate respiratory influences (David *et al.*, 1982). Both R wave and ST segment were measured in all recorded leads. However, to facilitate clinical correlation, R wave and ST segment amplitude data were only presented from leads 2 and 3 (mean data).

Post-occlusion arrhythmias were monitored electrocardiographically with continuous recordings from 0 to 60 min after left circumflex coronary artery occlusion. Animals which survived 60 min post-occlusion were considered to be survivors of early phase ventricular arrhythmias. After that time, reperfusion was achieved by cutting the thread around the previously occluded artery. A 20 min post-reperfusion electrocardiographic monitoring was performed: dogs which survived were considered to be occlusion-reperfusion survivors.

Haemodynamic monitoring

Aortic pressure was continuously recorded in all experiments. Cardiac output was determined in the control state, before and 5 min after left circumflex coronary artery occlusion and at 30 and 60 min thereafter. Standard formulae were used to calculate systemic vascular resistance and rate-pressure product.

Platelet studies

Platelet-rich plasma (PRP) was obtained by centrifugation of citrated blood (0.011 M) at 100 *g* for 10 min. Platelets were counted. Aggregation of adjusted PRP using the photometric method (Born, 1962) was studied in the presence of adenosine 5'-pyrophosphate (ADP, final concentration 2.5 μM). The intensity of platelet aggregation was measured before and 10 min after the start of the drug protocol and 15 and 60 min after left circumflex coronary artery occlusion.

The plasma levels of thromboxane B_2 (TXB_2), a stable metabolite of thromboxane A_2, were investigated by means of a sensitive radioimmunoassay technique as previously described (Jouve *et al.*, 1984). Blood samples were drawn from the Vygon bolt extensor connected to the aortic catheter and collected in EDTA (0.034 M, 0.01 vol./1 vol.) containing tubes. After immediate centrifugation (4000 *g* for 10 min at 4°C) the resulting plasma samples were frozen until further processing. Dosages from the collected plasma samples were ascertained before and 10 min after the start of drug protocol and 15 and 60 min post-occlusion, respectively.

Experimental protocol

Forty unidentified 2 ml vials containing either 4 mg of molsidomine ($n = 20$) or the vehicle alone ($n = 20$) had been prepared previously by Hoechst Laboratory, Paris, France and randomly coded. Thus, the study was conducted blind until the results had been collected. Fifteen to 20 min after the induction of anaesthesia, i.e. at the end of the surgical preparation, the contents of the vials were diluted in 200 ml of 0.9% saline. Then, a bolus of 2.5 ml kg^{-1} i.e. 50 μg kg^{-1} of molsidomine, when present, was started by use of a precalibrated infusion pump. The infusion was continued throughout the experiment.

In order to evaluate the plasma levels of molsidomine achieved with this protocol, plasma samples were obtained from 20 consecutive animals. The samples were drawn from the Swan-Ganz catheter and collected in heparin (Li Heparin 143 USP units) containing tubes. After immediate centrifugation (4000 *g* for 10 min at 4°C) the resulting plasma samples were frozen until further processing. A high-performance liquid chromatographic method for the analysis of N-ethoxycarbonyl-3-morpholine-sydnonimine compound was used (Dell & Chamberlain, 1978). The drug was extracted from the plasma into 1–2 dichlorethane and the analysis was carried out on a reversed-phase column, the column effluent being monitored by u.v. absorption at 312 nm. The sensitivity of the method ranges from 2 to 3 ng ml^{-1}.

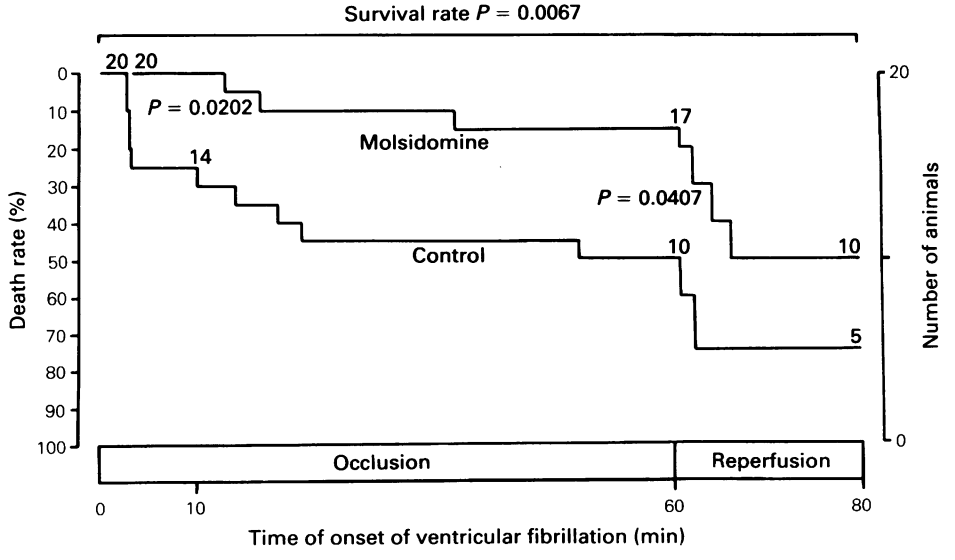

Figure 1 Number and time of onset of ventricular fibrillations after left circumflex coronary artery occlusion are shown in molsidomine-treated and control dogs. *P* values, as calculated with Fisher's exact test, at 10 and 60 min after occlusion are indicated. The survival rate is calculated for the overall occlusion-reperfusion period with the modified Wilcoxon test.

Data analysis

All continuous parameters expressed as mean ± standard deviation (s.d.) were analysed by a one-way analysis of variance. To analyse the effect of molsidomine on the incidence of ventricular fibrillation following coronary artery occlusion periods of time were defined *a priori*, based on data indicating that this arrhythmia peaks during the first 10 min post-occlusion and then decays exponentially (Kaplinsky *et al.*, 1979; Menken *et al.*, 1979). In addition, the effect of treatment on post-reperfusion ventricular fibrillation was also assessed. For these purposes the bilateral Fisher's exact test (Fiegel, 1956) was used at either 10 min or 60 min post-occlusion and at the end of the reperfusion period. Finally, to assess the effect on total survival the modified Wilcoxon test (Burdette & Gehan, 1970), which takes into account the exact date on onset of ventricular fibrillation after the start of experiments, was performed.

Results

Effect of treatment on mortality

The onset of ventricular fibrillation was always coincident with death since electrical defibrillation was not performed. Of the 40 animals used in this randomized study, 13 fibrillated during the 60 min post-occlusion

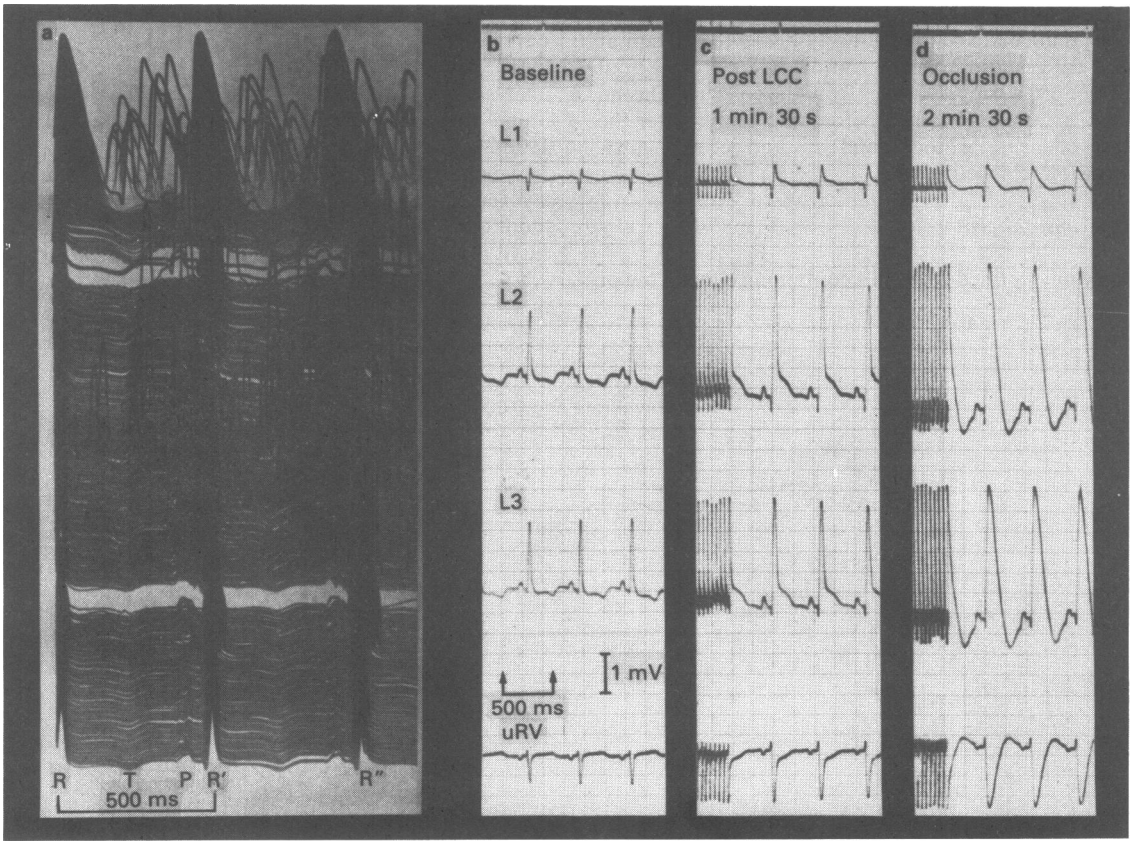

Figure 2 (a) Contourographic display of standard lead 2 in a control dog subjected to left circumflex coronary artery ligation (to be read from below upwards). Note the stability of the electrocardiographic recording. Parasitic spikes may be seen in the middle part due to dissection of the left main trunk. Following left circumflex coronary artery occlusion ST segment elevation appears and R wave amplitude increases: these changes preceded the occurrence of an early phase ventricular fibrillation. R, T, P denote specific electrocardiographic waves. R′ and R″ denote the 2nd and 3rd QRS complex respectively which imbricate in the contourographic display. (b) Leads 1, 2, 3 and unipolar right ventricle (uRV) electrocardiographic recordings at the baseline and (c) 1 min 30 s and (d) 2 min 30 s after left circumflex coronary artery (LCC) occlusion in the same dog.

period and 12 on reperfusion (Figure 1). In the molsidomine-treated group, 3 out of 20 fibrillated during the occlusion period (15%) as compared to 10 in the control group (50%) ($P = 0.0407$, Fisher's exact test). During the first 10 min following left circumflex coronary artery occlusion no animal showed signs of ventricular fibrillation in the treated group as compared to 6 cases in the control group (30%) ($P = 0.0202$, Fisher's exact test). On release ventricular fibrillation ensued in 7 of the 17 remaining molsidomine-treated dogs (41%) and in 5 of the 10 remaining controls (50%) ($P = 0.9614$, Fisher's exact test). A highly significant difference was observed between treated animals and controls in the total survival rate ($P = 0.0067$). Thus, these data show that molsidomine prevented ventricular fibrillation during the entire post-occlusion period and improved outcome in this model after left circumflex coronary artery occlusion and reperfusion.

Electrocardiographic monitoring

A synoptic view of post-ischaemic changes that follow left circumflex coronary artery occlusion are presented in a contourographic display of lead 2 (Figure 2) from a representative control animal and include: a short lasting decrease of R wave amplitude reaching its nadir within 30 s along with an increase in ST segment; an increase in R wave amplitude preceding the onset of early (3 min) post-occlusion ventricular fibrillation.

Post-occlusion ST segment changes are presented in Table 1 in both treated and control animals further subdivided according to the occurrence of ventricular fibrillation during the 60 min post-occlusion period. Significant differences in mean ST segment elevation were present at 3 min post-occlusion. These include: a lower mean ST segment in the 20 molsidomine-treated dogs as compared to the 20 controls (0.82 ± 0.52 vs 1.52 ± 0.78 mV, $P < 0.025$) and a higher intragroup mean ST segment elevation in control animals which further fibrillated (1.98 ± 0.71 vs 1.00 ± 0.52 mV, $P = 0.01$). At 5 min post-occlusion no significant intergroup differences were present. However, intragroup differences were observed between those animals which further fibrillated and those which did not, both in controls (1.90 ± 0.56 vs 1.21 ± 0.53 mV, $P < 0.05$) and in molsidomine-treated dogs (1.97 ± 0.17 vs 0.95 ± 0.65 mV, $P < 0.025$). At 10, 15, 30 and 45 min post-occlusion significant intragroup differences were still present in control animals (Table 1). At 60 min post-occlusion, i.e. just prior to the release, ST segment elevation was 0.53 ± 0.17 in controls and 0.32 ± 0.27 mV in molsidomine-treated dogs (NS).

Post-occlusion $\Delta R\%$ changes are summarized in Table 2. At 3 min post-occlusion, an intergroup difference was observed between control and molsidomine-treated dogs (90 ± 84 vs $37 \pm 50\%$, $P < 0.025$). At the same time $\Delta R\%$ changes were higher in controls which further fibrillated (127 ± 63 vs $44 \pm 45\%$, $P < 0.01$). The same holds true at 5 min post-occlusion in the control group (Table 2). After that time neither intra nor intergroup differences persisted.

The total number of ventricular premature beats in the control group during the post-occlusion period was 2915 as compared to 1865 in the molsidomine-treated group. The number of ventricular premature beats per minute per dog during the early (0–10 min) post-occlusion phase was 4.24 ± 3.6 in molsidomine-treated dogs as compared to 14.34 ± 4.28 in controls ($P < 0.02$). After this period and until 60 min post-

Table 1 ST segment elevation (mV) during the left circumflex coronary artery occlusion period in dogs

		Time post-occlusion (min)						
		3	*5*	*10*	*15*	*30*	*45*	*60*
Vehicle-treated								
Total		1.52 ± 0.78	1.44 ± 0.63	1.34 ± 0.61	1.07 ± 0.38	0.74 ± 0.27	0.64 ± 0.31	0.53 ± 0.17
With VF		1.98 ± 0.71	1.9 ± 0.56	2.08 ± 0.35	1.50 ± 0.42	1.50 ± 0	1.50 ± 0	—
	$P <$	0.01	0.05	0.01	0.05	0.01	0.01	
Without VF		1.00 ± 0.52	1.21 ± 0.53	1.05 ± 0.41	0.94 ± 0.26	0.66 ± 0.12	0.55 ± 0.16	0.53 ± 0.17
Molsidomine-treated								
Total		0.82 ± 0.52^{A}	1.11 ± 0.70	1.03 ± 0.84	0.85 ± 0.84	0.58 ± 0.54	0.40 ± 0.28	0.32 ± 0.27
With VF		1.27 ± 0.39	1.97 ± 0.17	1.53 ± 0.63	0.95 ± 0.25	1.20 ± 0	—	—
	$P <$	NS	0.025	NS	NS	NS		
Without VF		0.80 ± 0.51	0.95 ± 0.65	0.94 ± 0.84	0.84 ± 0.88	0.55 ± 0.53	0.40 ± 0.28	0.32 ± 0.27

VF = ventricular fibrillation during the post-occlusion period.
Values are mean ± s.d.
$^{A}P < 0.025$ molsidomine vs vehicle.

Table 2 ΔR% changes during the left circumflex coronary artery occlusion period in dogs

		Time post-occlusion (min)						
		3	*5*	*10*	*15*	*30*	*45*	*60*
Vehicle-treated								
Total		90 ± 84	51 ± 39	58 ± 52	68 ± 57	34 ± 50	22 ± 46	22 ± 48
With VF		127 ± 63	81 ± 25	92 ± 58	109 ± 52	80 ± 0	20 ± 0	—
	$P<$	0.01	0.05	NS	NS	NS	NS	
Without VF		44 ± 45	36 ± 36	42 ± 39	55 ± 52	29 ± 50	22 ± 48	22 ± 48
Molsidomine-treated								
Total		37 ± 50[A]	48 ± 61	64 ± 74	41 ± 62	37 ± 70	21 ± 42	9 ± 24
With VF		85 ± 47	65 ± 68	75 ± 74	0 ± 0	0 ± 0	—	—
	$P<$	NS	NS	NS	NS	NS		
Without VF		35 ± 50	46 ± 59	63 ± 74	46 ± 63	39 ± 71	21 ± 42	9 ± 24

VF = ventricular fibrillation during the post-occlusion period.
Values are mean ± s.d.
[A] $P<0.025$, molsidomine vs vehicle.

occlusion the total number of ventricular premature beats was 1903 in controls and 1272 in the molsidomine groups ($P<0.02$). On release an increased number of ventricular premature beats was noted. However, no significant difference was noted between the remaining control and molsidomine-treated dogs.

Haemodynamics

Baseline heart rate, mean aortic pressure, cardiac output, systemic vascular resistance and rate-pressure product were not significantly different in controls as compared to molsidomine-treated dogs (Table 3). After 10 min infusion of molsidomine, mean aortic pressure and rate-pressure product significantly declined in the presence of a virtually unchanged mean heart rate. A non-significant fall (15.5%) in cardiac output was noted as a consequence of molsidomine infusion. Following 5 min of left circumflex coronary artery occlusion, mean aortic pressure and cardiac output significantly declined in both control and molsidomine groups. In addition, a significant reduction of rate-pressure product remained at significantly lower levels as compared to baseline values. However, no significant intergroup difference was noted. Finally, the haemodynamic parameters did not show further changes during the following 30 min.

Platelet studies

No significant change was observed in platelet count either in the control or the molsidomine groups during the occlusion period. In addition, no significant change occurred post-occlusion in the intensity of platelet aggregation to 2.5 μM ADP in controls. By contrast, in molsidomine-treated dogs a progressive decline in the intensity of platelet aggregation during the occlusion period was observed which reached significance at 60 min post-occlusion (Figure 3). On the other hand, no significant change occurred in TXB_2 plasma levels either in control or in molsidomine treated groups (Figure 4).

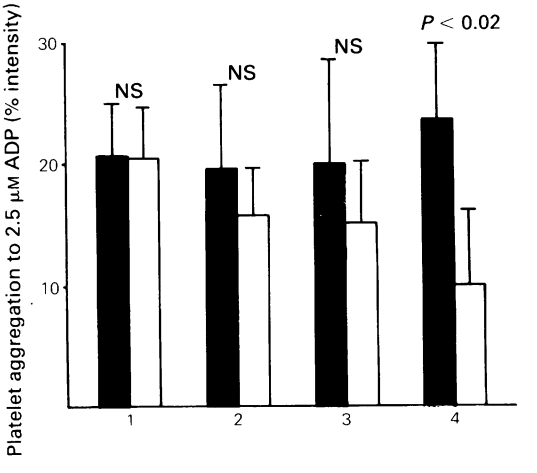

Figure 3 Intensity of platelet aggregation to 2.5 μM ADP measured just before (1), at 10 (2), 30 (3), and 75 (4) min after the start of the drug protocol. (3) and (4) are respectively 15 and 60 min after left circumflex coronary artery occlusion. Stippled columns represent mean control responses and open columns represent mean responses of platelets from molsidomine-treated dogs. Vertical lines indicate s.d.

Table 3 Summary of haemodynamic parameters in both vehicle (controls, $n = 20$)- and molsidomine-treated dogs ($n = 20$)

	Heart rate (beats min^{-1})		*Mean arterial pressure* (mmHg)		*Cardiac output* (l min^{-1})		*Systemic vascular resistance* (dynes s cm^{-5})		*Rate-pressure product* (mmHg beat min^{-1})	
	Controls	*Molsidomine*	*Controls*	*Molsidomine*	*Controls*	*Molsidomine*	*Controls*	*Molsidomine*	*Controls*	*Molsidomine*
Baseline (t_{-10})	148 ± 19	140 ± 17	101 ± 8	101 ± 9	2.28 ± 0.67	2.16 ± 0.53	3858 ± 814	3922 ± 816	14971 ± 1528	13972 ± 1180
10 min after bolus (t_{-5})	149 ± 14	146 ± 13	100 ± 7	88 ± 6^{B,D}	2.27 ± 0.75	1.87 ± 0.58	3710 ± 780	4050 ± 1176	14449 ± 1495	12656 ± 1193^{A,D}
5 min after LCC occlusion (t_5)	146 ± 9	148 ± 19	80 ± 8^{C}	76 ± 9^{C}	1.62 ± 0.49^{B}	1.33 ± 0.46^{C}	4088 ± 1549	4804 ± 1458	11653 ± 1109^{B}	11097 ± 1640^{B}
30 min after LCC occlusion (t_{30})	138 ± 10	136 ± 10	77 ± 7^{C}	78 ± 5^{C}	1.59 ± 0.32^{B}	1.43 ± 0.49^{B}	3983 ± 599	4916 ± 1755	10886 ± 1363^{C}	11296 ± 1004^{B}
60 min after LCC occlusion (t_{60})	136 ± 12	131 ± 16	78 ± 8^{C}	80 ± 5^{C}	1.59 ± 0.48^{B}	1.42 ± 0.47^{B}	4009 ± 565	4856 ± 1497	10705 ± 1755^{C}	10800 ± 2100^{B}

Data are mean ± s.d. $^{A}P < 0.05$, $^{B}P < 0.025$, $^{C}P < 0.01$ vs baseline values and $^{D}P < 0.05$ vs control values at the same time. LCC: left circumflex coronary artery.

Figure 4 Aortic thromboxane B_2 plasma levels measured just before (1), at 10 (2), 30 (3) and 75 (4) min after the start of the drug protocol. (3) and (4) are respectively 15 and 60 min after left circumflex coronary artery occlusion. Stippled columns represent mean control values and open columns represent mean levels in molsidomine-treated dogs. Vertical lines indicate s.d.

Molsidomine plasma levels

Of the 20 dogs in which plasma samples had been collected for determining the dosage of molsidomine, 9 had received the drug. The plasma levels were, respectively, 28 ± 16, 24 ± 14 and 20 ± 6 ng ml^{-1} at 10, 45 and 75 min after the start of molsidomine infusion.

Discussion

The results of this randomized study demonstrate that molsidomine prevents ventricular fibrillation following left circumflex coronary artery occlusion in the anaesthetized dog and improves overall occlusion-reperfusion survival in this model. These effects appear to follow the antifibrillatory action of the drug during the early (0–10 min) post-occlusion period. The time-action curve of this early antifibrillatory effect of molsidomine parallels those at the level of post-ischaemic electrocardiographic changes which are lowered and those against ventricular premature beats which are less in treated as compared to control dogs. These results need to be considered with regard to the experimental setup, taking into account the relevance of standard electrocardiographic changes as an index of pathophysiological precursors of life-threatening ventricular arrhythmias (Kaplinsky *et al.*, 1979; Menken *et al.*, 1979; David *et al.*, 1982; Jouve *et al.*, 1986a) and the reported effects of molsidomine in improving directly or indirectly the oxygen supply in ischaemic areas of the left ventricle (Hirata & Kikuchi, 1970; Berdeaux *et al.*, 1978; Fiedler, 1982; Fiedler *et al.*, 1982; Nitz & Martorana, 1985; Kukovetz &

Holzman, 1985). In contrast, the antiplatelet effects of the drug do not seem to contribute importantly to these effects of the drug.

Experimental design

Experiments in preventing sudden death are fraught with factors of variability. These include: the type and level of anaesthesia (Duchêne-Marullaz *et al.*, 1982), the latency from thoracotomy to ligation (Hiatt *et al.*, 1973), the level of coronary occlusion (Allen & Laadt, 1950), the time in which the occlusion is achieved (Harris, 1950) and the degree of native collateral development (Meesmann *et al.*, 1976).

A deep surgical anaesthesia was obtained with a low dose of sodium pentobarbitone since intramuscular pre-anaesthesia with levomepromazine was used (Geary *et al.*, 1981). The small amount of atropine was aimed at counteracting baroreceptor reflex-mediated bradycardia following activation of mechanical receptors in the infero-lateral wall of the left ventricle (Thames *et al.*, 1978). As a result of this anaesthetic protocol, high levels of heart rate were prevented (Manders & Vatner, 1976). Indeed, heart rate on occlusion was similar to that observed in conscious dogs sedated with morphine (Starnes *et al.*, 1982). Finally, the occlusion was performed on a 1:1 oxygen and nitrous oxide mixture which has been shown to have no depressant effect on left ventricular performance and cardiac output (Van Trigt *et al.*, 1984). The constant time schedule, the occlusion of the left circumflex coronary artery at its origin, thus including all branches running to the lateral and inferior wall of the left ventricle (Moore, 1930), and finally the randomization were designed to mitigate factors of variability. As a result of this procedure the observed 50% incidence of ventricular fibrillation in the control group accords with the 54.12% (92 of 170 control dogs) observed previously after left circumflex coronary artery occlusion in anaesthetized dogs (Jouve *et al.*, 1986a,b). In addition, this figure is similar to the 53% incidence of ventricular fibrillation obtained after left circumflex coronary artery occlusion in conscious dogs (Starnes *et al.*, 1982).

Electrocardiography

Since anaesthesia was used high quality standard multiple electrocardiographic recordings (Hill, 1968; David *et al.*, 1982) were obtained throughout the experiments. The stability of recordings has been controlled by contourography as displayed in Figure 2. These recordings allowed the measurement of ST segment and R wave changes in those electrocardiographic leads which directly explore the acute ischaemic area. Delayed ST segment and $\Delta R\%$ changes were observed in molsidomine-treated dogs. Although there is some controversy as to the sensitivity and specificity of ST segment and R wave changes for either localization or quantification of myocardial ischaemia (Franz *et al.*, 1984; Kent *et al.*, 1973), it has been known for a long time that acute myocardial infarction patients with higher ST segment elevation exhibit more ectopic activity (Nielsen, 1973). Furthermore, in patients with variant angina the higher the elevation of ST segment during an ischaemic attack, the greater the risk of sudden death during the follow-up (Miller *et al.*, 1982; Previtali *et al.*, 1983). In addition, in a study using multiple simultaneous leads (X,Y,Z,2,V5) in the dog it was observed that an increase of 25% or more in the sum of R wave amplitudes after coronary occlusion accurately predicted the occurrence of malignant arrhythmias including ventricular fibrillation (David *et al.*, 1982). Finally, the left circumflex coronary artery occlusion canine model, using ST segment and $\Delta R\%$ changes at 3 and 5 min post-occlusion allowed the prediction of ventricular fibrillation with a high sensitivity and a good specificity (Jouve *et al.*, 1986a,b). Thus, the delayed ST segment elevation and $\Delta R\%$ changes observed in molsidomine treated-dogs might reflect an anti-ischaemic effect of the drug, accounting for both the antifibrillatory action and the reduced number of ventricular premature beats observed in the present investigation. This interpretation fits with previous experiments in which a coronary collateral vasodilator action in hearts with coronary insufficiency (Hirata & Kikuchi, 1970), improved endocardial flow (Berdeaux *et al.*, 1978), decreased infarct size after coronary artery ligation (Fiedler *et al.*, 1982), and a significant anti-ischaemic effect after left anterior descending coronary artery controlled reduction of perfusion, as assessed by ST segment changes in multiple unipolar leads (Nitz & Martorana, 1985), have been documented with molsidomine. These results are also in accordance with clinical data showing that molsidomine reduces electrocardiographic signs of myocardial ischaemia in patients with coronary heart disease undergoing ergometric testing (Detry *et al.*, 1981).

The effects of molsidomine are prominent in the first few minutes following left circumflex coronary artery occlusion. Since it is well known that the incidence of ventricular fibrillation peaks during the first few minutes after myocardial ischaemia and then decays exponentially (Kaplinsky *et al.*, 1979), it seems reasonable to conclude that the absence of ventricular fibrillation during the first 10 min post-occlusion in treated dogs might be related to the anti-ischaemic effect of the drug.

Haemodynamics

The main change observed is that molsidomine sig-

nificantly reduced the rate-pressure product after 10 min infusion and prior to left circumflex coronary artery occlusion. The rate-pressure product reflects myocardial oxygen consumption (Gobel *et al.*, 1978). In fact, as reported in previous experiments this effect was associated with an oxygen sparing action of the drug (Hirata & Kikuchi, 1970; Berdeaux *et al.*, 1978; Fiedler, 1982). In particular, a normalization of ST segment displacements in unipolar epicardial leads has been found to parallel the decrease of preload in a canine model of ischaemia using a dosage of the drug similar to that administered in this investigation (Nitz & Martorana, 1985). Indeed, molsidomine is known to possess similar haemodynamic properties to those of nitrates (Majid *et al.*, 1980). In addition, its active metabolite SIN-1 which carries a free nitroso group relaxes vascular smooth muscle by direct stimulation of guanylate cyclase (Kukovetz & Holzmann, 1985). As in the case of nitrates these effects may participate in the anti-ischaemic action of the drug (Vatner & Heyndrickx, 1975). Further studies are, however, necessary to clarify the relationship between the anti-ischaemic, antifibrillatory and left ventricular haemodynamic effects of molsidomine since these issues were not specifically investigated here. It was, in fact, decided not to interfere mechanically with the vigorously beating heart *in situ* when dealing with a protocol aimed at assessing the antifibrillatory effect of a drug. The 15.5% fall in cardiac output was not significant. Since it has been documented by Fiedler (1982) that no depressant effect of molsidomine on left ventricular haemodynamics follows a $0.10\,mg\,kg^{-1}$ bolus dose of the drug, the non-significant effect of molsidomine on cardiac output presented here might be ascribed to its well known action on preload (Nitz & Martorana, 1985).

Platelet studies

Neither left circumflex coronary artery occlusion nor molsidomine modified aortic TXB_2 plasma levels. Although it has been pointed out that the changes in TXB_2 plasma levels following acute myocardial ischaemia are best seen in the venous blood draining away from the ischaemic zone (Coker *et al.*, 1981; Walinsky *et al.*, 1984), the involvement of thromboxane A_2 in the arrhythmias occurring during acute myocardial ischaemia is controversial (Coker *et al.*, 1982; Burke *et al.*, 1982). Our results do not support the view that the aortic thromboxane plasma content may play a contributory role to post-reperfusion ventricular arrhythmias. In addition, aortic TXB_2 plasma content was not decreased by molsidomine although its haemodynamically effective metabolite SIN-1 has been shown to inhibit thromboxane A_2 formation *in vitro* (Block *et al.*, 1982). On the other hand, an antiplatelet effect of molsidomine was observed, as assessed by the intensity of platelet aggregation to $2.5\,\mu M$ ADP. Even so, this effect observed in another study (Darius *et al.*, 1982) was only relevant after 75 min infusion of the drug. Thus, it seems that the antiplatelet effects of molsidomine do not contribute to its early antifibrillatory action following acute ischaemia.

Antifibrillatory effect

In a previous study dealing with left anterior descending coronary artery occlusion in 20 dogs, molsidomine was found to reduce the incidence of post-reperfusion ventricular fibrillation (Nitz & Martorana, 1985). However, in that study no episode of ventricular fibrillation was observed on occlusion, a fact which is in keeping with the 7% incidence of ventricular fibrillation obtained after occlusion of the left anterior coronary artery in a large group of 98 consecutive dogs (Balke *et al.*, 1981). Thus, the antifibrillatory action after reperfusion observed in 8 molsidomine-treated dogs might be ascribed to the limitation of the infarct size (Hale *et al.*, 1984). Our data indicate that molsidomine prevents ventricular fibrillation following a massive ischaemic insult. However, no reduction of post-reperfusion ventricular fibrillation was observed. Although this could result either from methodological differences or from the scant number of animals at the moment of the reperfusion, this point deserves further investigation. Indeed, at the moment of reperfusion treated animals and controls were not comparable as they were at the beginning of the experiments. This further complicates the applicability of statistical analysis along with the interpretation of the result.

The incidence of post-ischaemic ventricular fibrillation in this randomized study was reduced in the presence of molsidomine plasma levels similar to the peak achieved in humans after a single oral dose of the drug (Dell & Chamberlain, 1978). Our data lend some support to the hypothesis that interfering with acute ischaemic myocardial insults may prevent the onset of life-threatening ventricular arrhythmias. Further insight along these lines may be fruitful and deserves attention in relation to predisposing pharmacological strategies aimed at preventing sudden death.

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