

## Original Research

## Ventricular Arrhythmias and Antioxidative Medication: Experimental Study

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**Introduction:** Reperfusion arrhythmias could be due to free radicals, while contraction excitation feedback is the cause of arrhythmias generated by blood pressure elevation (BPE). The aim of this study was to test the antiarrhythmic effects of an antioxidant (vitamin C [vit C], 1.5 g), an iron-binding agent (deferoxamine [Def], 1 g), and their combination in an experimental model of arrhythmia based on these 2 mechanisms.

**Methods:** Thirty anaesthetised sheep were divided into 4 groups, depending on the infused agent: saline (8 sheep), combination of vit C and Def (8), Def (6), and vit C (8). Induction of ventricular arrhythmias was attempted in all animals using both ischaemia-reperfusion (phase I) and a combination of ischaemia and BPE (phase II). In all cases ischaemia was caused by ligating the left anterior descending coronary artery distally to the origin of the 1st diagonal artery, while reperfusion was achieved by releasing the ligation 45 min later. BPE was achieved by obstructing the ascending aorta or by administering intravenous metaraminol. All agents were infused intravenously for 15 min and their administration was started 30 min after the first ligation. Phases I and II lasted 50 and 20 min, respectively.

**Results:** Ventricular tachycardia/fibrillation (VT/VF) was induced in all animals in the control group (8/8) and in the Def group (6/6). VT/VF appeared in 6/8 of the animals in the vit C group (75%) and in only 3/8 of the animals in the combination therapy group (37.5%). The difference between the combination and control groups was statistically significant ( $p < 0.03$ ).

**Conclusions:** The intravenous administration of vit C and Def in combination protects against VT/VF induced by ischaemia-reperfusion and/or BPE. Administration of Def alone does not appear to help, while the action of vit C alone is not clear.

**E**xperimental and clinical data have shown that acute myocardial ischaemia can cause sudden death.<sup>1,2</sup> Fatal ventricular arrhythmias arise during both ischaemia<sup>3</sup> and the reperfusion that follows its resolution (“reperfusion damage”).<sup>4,5</sup> The precise pathophysiological mechanism of arrhythmogenesis in ischaemia-reperfusion injury is not clear. Free oxygen radicals probably play an important role, while in a number of experimental studies these arrhythmias were improved by the administration of various free radical

scavengers or antioxidative agents, namely those that neutralise reactive oxygen species.<sup>5</sup> However, the results of other studies were negative.<sup>6</sup> One of the reasons for these conflicting results is the fact that antioxidants, in the presence of metallic ions such as iron, can act as pro-oxidants (Fenton reaction). In such a case a large number of iron ions could be released during ischaemia, over-saturating the serum’s iron-binding capacity.<sup>7</sup> Thus, it would be reasonable to suppose that the administration of iron-binding agents at the same time as antioxi-

dants might be essential for the prevention of reperfusion arrhythmias.

Another possible mechanism of arrhythmogenesis is the phenomenon of mechano-electrical interaction, or contraction-excitation feedback, which refers to changes in the electrophysiological properties of the myocardium caused by pressure or volume loading.<sup>8-10</sup> Acute pressure loading of the left or right ventricle through mechanical or pharmaceutical means, apart from causing electrophysiological changes, also facilitates the development of ventricular arrhythmias, while conversely a reduction in blood pressure exerts an antiarrhythmic effect.<sup>11-13</sup>

In the present experimental study, we investigated whether the combination of an antioxidant (ascorbic acid) with an iron-binding agent (deferoxamine) could reduce the susceptibility of the myocardium to ventricular arrhythmias caused by ischaemia-reperfusion syndrome, or by ischaemia combined with blood pressure elevation.

## Methods

A total of 42 sheep, aged 10 to 24 months and weighing 15 to 25 kg, were used for the experiments. The sheep was chosen for this study because it is a large animal, easy to handle, and shows similar anatomy of the coronary arteries, and more generally of all blood vessels, to the human.<sup>14,15</sup> It does not develop native collateral coronary vessels after coronary artery occlusion.<sup>16,17</sup> In addition, it is very easy to induce ventricular arrhythmias, mainly persistent ventricular tachycardia or resistant ventricular fibrillation, using minimal manipulation of its heart.<sup>14</sup>

In all animals open thoracotomy was performed under general anaesthesia and a two-phase arrhythmological protocol was applied. The first phase was based on the induction of ischaemia-reperfusion. In the second phase, ischaemia was combined with blood pressure elevation in all the animals that survived the first phase.

Anaesthesia was induced with xylazine (0.1-0.2 mg/kg im) and atropine (0.02-0.03 mg/kg im), and a percutaneous venous catheter was placed in the cephalic or auricular vein for administration of pentothal (5-7 mg/kg iv). Endotracheal intubation was then performed, followed by connection of a positive pressure ventilator (Engstrom model 200, Engstrom, Sweden) delivering a mixture of oxygen and air (1:2) at a respiratory rate of 14-16 per minute, inspiratory/expiratory ratio 1:2, ventilation volume 200-300

ml/kg/min, and positive end-expiratory pressure 3-4 cm H<sub>2</sub>O, so that the animal could be maintained on mechanical ventilation throughout the experiment. Electrocardiographic monitoring at a paper speed of 25 mm/s was started once anaesthesia had been achieved. Anaesthesia was maintained with halothane or sevoflurane (0.5-1 vol%), and fentanyl (5 µg/kg) and atracurium (0.6 mg/kg).

An introducer (7 Fr) was placed percutaneously in the right femoral vein, for the infusion of solutions, and another (7 Fr) in the right femoral artery, for continuous blood pressure monitoring and recording. An anterior transverse thoracotomy was performed at the mid-sternal level, followed by transverse section of the pericardium. The anterior descending branch of the left coronary artery was then ligated close to the origin of the first diagonal branch and the ligation was released after 45 minutes. The fifteen-minute iv administration of medication or placebo was started ten minutes before reperfusion, after the animal had been randomised to one of four treatment groups.

## Experimental groups

Twelve of the 42 animals died before any medication was administered and were excluded from the study. Three died during intubation and thoracotomy, while 9 died during the first ligation of the anterior descending artery or a few minutes later. The remaining 30 animals were randomised to one of the following groups, using sealed envelopes: a) controls (C), receiving 500 ml NaCl 0.9% (n=8); b) ascorbic acid + deferoxamine (A+D), given 1 g deferoxamine and 1.5 g ascorbic acid with 500 ml NaCl 0.9% (n=8); c) ascorbic acid (A), given 1.5 g ascorbic acid with 500 ml NaCl 0.9% (n=8); and d) deferoxamine (D), given 1 g deferoxamine with 500 ml NaCl 0.9% (n=6). The antioxidant dosage was chosen on the basis of its ability to protect the animal's myocardium from reperfusion-induced stunning.<sup>18,19</sup>

The animal was monitored for 60 minutes after reperfusion and any ventricular arrhythmias were recorded. One hour and forty-five minutes after the start of the experiment a further attempt was made to induce ventricular arrhythmias, initially with 20 minutes' myocardial ischaemia, in all animals that had survived the first phase. Myocardial ischaemia was again induced via ligation of the anterior descending artery close to the origin of the first diagonal branch. Following this, an attempt was made to induce ven-

tricular arrhythmias using an elevation of blood pressure, arterial or ventricular, achieved either by the administration of metaraminol hydrochloride at a rate of 1 mg/min, or by mechanical stenosis of the ascending aorta. If no ventricular tachycardia or fibrillation was observed within 20 minutes it was considered that the myocardium had not been rendered susceptible to those arrhythmias.

All the animals used in the study were treated in accordance with directive N.1197/81 concerning "Protection of animals", and specifically with article 4 of directive N.2015/92 concerning "Ratification of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes", and with the directives P.D.160/91 concerning "Protection of animals used for experimental or other scientific purposes, in conformance with guideline 86/609/EEC of the Council". The animal experimentation was approved by the regional authority.

### Statistical analysis

The t-test was used for comparisons between groups to detect numerical differences, whereas for qualitative differences the  $\chi^2$  test with Yates' correction was employed. For parameters which showed statistically significant differences a dispersion analysis was performed using analysis of variance. A p-value <0.05 was the criterion of significance throughout.

### Results

Of the 42 sheep used in the study, 30 completed the experimental protocol. Details are given in Table 1.

Table 2 shows the occurrence of ventricular arrhythmias in the four groups. The between-group difference concerning the likelihood of developing ventricular tachycardia (VT) or fibrillation (VF) was statistically significant ( $\chi^2=11.134$  with Yates' correction,  $p<0.009$  for 3 degrees of freedom, Figure 1).

**Table 1.** Analysis of results from the animals used in the experimental study.

Groups	n (total 42)	Deaths					Survival	
		Before substance administration		After substance administration				
		Start	1st. ligation	Reperfusion		2nd ligation		↑BP
			≤1 min	>1 min				
Control	8	0	0	0	3	1	4	0
D	6	0	0	1	2	1	2	0
A	8	0	0	0	2	1	3	2
A+D	8	0	0	0	2	0	1	5
Animals that died before substance administration	12	3	9	0	0	0	0	

A – ascorbic acid; D – deferoxamine; ↑BP – blood pressure elevation.

**Table 2.** Comparison of groups in relation to the occurrence of ventricular tachycardia or fibrillation (VT/VF) in different phases of the experiment.

Groups	VT/VF				No VT/VF	Total VT/VF
	1st. ligation	Reperfusion	2nd. ligation	↑BP		
Control	0	3	1	4	0	8/8 (100%)
D	1	2	1	2	0	6/6 (100%)
A	0	2	1	3	2	6/8 (75%)
A+D	0	2	0	1	5	3/8 (37.5%)

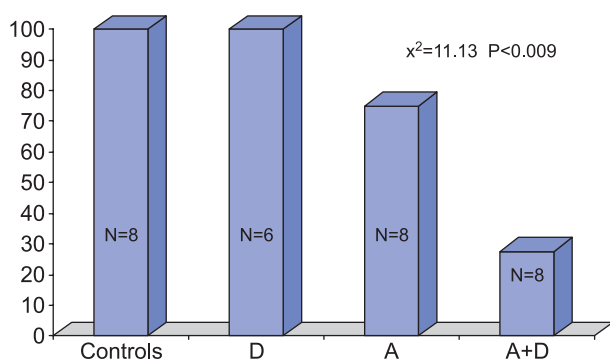
Abbreviations as in Table 1.

The incidence of VT/VF in the A+D group (37.5%) was significantly lower ( $\chi^2=4.564$ ,  $p<0.03$  for one degree of freedom) than in the control group (100%), but the differences from the other two groups were not significant.

Table 3 summarises the values of mean systolic blood pressure (SBP) and mean peak SBP in the four treatment groups, comparing measurements made in the absence of VT/VF with those made when VT/VF occurred. Taking all the groups together, the peak SBP in the 23 animals in which VT/VF was induced was  $163.44 \pm 64.84$  mmHg, whereas in the 7 that exhibited no ventricular arrhythmia peak SBP was  $226.43 \pm 57.15$  mmHg ( $t=2.472$ ,  $p<0.027$ ). The greatest peak values of SBP were seen in the combination therapy (A+D) group, in the set of measurements made while the animals were free of VT/VF ( $185.75 \pm 76.69$  mmHg, Table 3). Thus, the occurrence of malignant arrhythmias cannot be attributed to an excessive increase in blood pressure.

Some ventricular arrhythmia (ventricular extrasystoles, VT or VF) was induced in 69 manoeuvres in the 30 experiments, while in 77 other manoeuvres no ventricular arrhythmia was induced. The mean SBP in the experiments with some kind of arrhythmia ( $125.06 \pm 61.00$  mmHg) was significantly ( $t=1.995$ ,  $p<0.05$ ) greater than in those with no arrhythmias ( $107.61 \pm 41.70$  mmHg).

Table 4 and Figure 1 show the arrhythmogenic effect of the various manoeuvres performed, more specifically the number of manoeuvres that caused no arrhythmia, and the number that caused ventricular extrasystoles, VT or VF. The effect of the various manoeuvres on arrhythmogenesis was inhomogeneous ( $\chi^2=64.98$ ,  $p<0.0005$  for 10 degrees of freedom). The most intensely malignant manoeuvre that caused



**Figure 1.** Percentage incidence of ventricular tachycardia or fibrillation in the four groups. A – ascorbic acid; D – deferoxamine.

VT/VF was the elevation of SBP after the second ligation. The incidence of VT/VF for this manoeuvre was significantly higher than for ligation ( $\chi^2=12.90$ ,  $p<0.0005$ ), 1 minute after reperfusion ( $\chi^2=4.15$ ,  $p<0.04$ ), up to 105 minutes after reperfusion ( $\chi^2=5.67$ ,  $p<0.02$ ), or after the second ligation ( $\chi^2=5.94$ ,  $p>0.015$ ). The second greatest incidence of VT/VF was seen immediately (up to 1 minute) after reperfusion, but other differences were not statistically significant.

In general, arrhythmias (ventricular extrasystoles, VT, VF) were induced most often by the combination of ligation and blood pressure elevation (82.3%). This percentage was significantly ( $\chi^2=9.90$ ,  $p<0.005$ ) higher than after the first ligation (30.0%), but did not differ significantly from the rates immediately after reperfusion (58.6%), up to 105 minutes after reperfusion (60.9%), or after the second ligation (75.0%) (Figure 2).

## Discussion

The present study investigated whether the combination of an antioxidant (ascorbic acid) with an iron-

**Table 3.** Comparison of measurements of mean systolic blood pressure (SBP) and mean peak SBP in the animal groups in the absence of VT/VF and when VT/VF occurred. N is the total number of measurements made in each state. Once VT/VF was recorded the experiment was stopped; thus, in the VT/VF Yes rows N corresponds to the number of animals in the group.

Group	VT/VF	Mean SBP	Mean peak SBP
Control	No	$98.3 \pm 37.05$ N=30	$136.0 \pm 35.33$ N=8
	Yes	$164.75 \pm 77.38$ N=8	$164.75 \pm 77.38$ N=8
D	No	$105.0 \pm 18.29$ N=20	$111.00 \pm 20.26$ N=6
	Yes	$136.5 \pm 71.48$ N=6	$136.5 \pm 71.48$ N=6
A	No	$114.75 \pm 77.37$ N=34	$151.12 \pm 63.51$ N=8
	Yes	$165.17 \pm 98.80$ N=6	$165.17 \pm 98.80$ N=6
A+D	No	$116.8 \pm 55.72$ N=40	$185.75 \pm 76.69$ N=8
	Yes	$88.33 \pm 44.77$ N=3	$88.33 \pm 44.77$ N=3
Total	No	$109.82 \pm 43.62$ N=124	$148.3 \pm 59.02$ N=30
	Yes	$146.48 \pm 73.16$ N=23	$146.48 \pm 73.16$ N=23

Abbreviations as in Table 1.

**Table 4.** Number of manoeuvres (and percentages) that caused different types of arrhythmia.

Time (T)	No arrhythmia	VES	VT/VF	Total
T=0 min (before 1st. ligation)	30 (100.0)	0	0	30
T=1-45 min (1st. ligation/ischaemia)	21 (70.0)	7 (23.3)	2 (6.7)	30
T=46 min (1st. min of reperfusion)	12 (41.4)	10 (34.5)	7 (24.1)	29
T=47-105 min (reperfusion)	9 (39.1)	10 (43.5)	4 (17.4)	23
T=106-116 min (2nd. ligation/ischaemia)	5 (25.0)	12 (60.0)	3 (15.0)	20
T=116-125 min (2nd. ligation + ↑BP)	3 (17.7)	4 (23.5)	10 (58.8)	17

VES – ventricular extrasystoles. Other abbreviations as in Table 1.

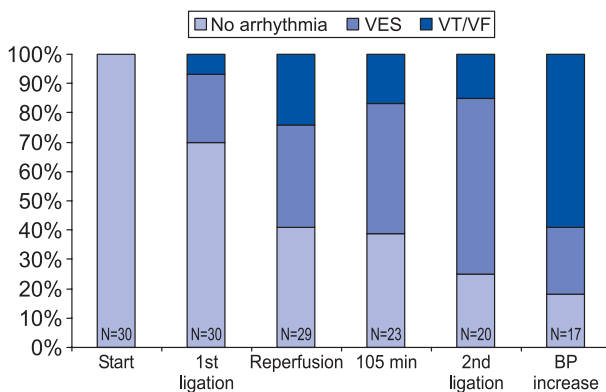
binding agent (deferoxamine), could protect against the myocardium's susceptibility to ventricular arrhythmias caused by ischaemia-reperfusion syndrome or by the combination of ischaemia and blood pressure elevation. The combination of antioxidant and iron-binding agent was chosen because ascorbic acid, under conditions of intense oxidative pressure, can become toxic, and this change can be catalysed by the presence of iron (Fenton reaction). The combination of an antioxidative with an iron-binding agent – and ascorbic acid with deferoxamine in particular – has not been used in any similar study in the international literature, despite the fact that the two agents have each been used in isolation.

The finding that the peak SBP achieved in the animals in which VT/VF was not induced was significantly higher than in those who exhibited those arrhythmias indicates that the VT/VF was not due to the effect of the increased SBP. Moreover, this would be expected, since the SBP was elevated in an attempt

to induce VT/VF and in those animals where this did not occur the SBP continued to be elevated further. The blood pressure elevation in our study was achieved by administration of metaraminol, which could have been directly responsible for causing arrhythmias. However, its effect is known to be exerted, albeit not entirely, via the alpha-adrenergic receptors. Furthermore, Sideris et al<sup>10</sup> showed that a reduction in blood pressure via exsanguination in animals with ventricular arrhythmias caused by an increase in pressure resulted in suppression of the arrhythmias, despite the continuation of metaraminol administration. While the peak SBP was lower in the animals in which VT/VF was induced than in those in which it was not, the SBP during manoeuvres that caused any kind of ventricular arrhythmia was higher compared to those that did not cause any arrhythmia, as would be expected from the known fact that a pressure increase has an arrhythmogenic effect on the ventricles.<sup>10-13,20-26</sup> Finally, the combination therapy group (A+D) was more resistant to the induction of VT/VF, despite having higher values of arrhythmogenic stimuli (higher peak SBP) and the same provocative manoeuvres as in the other groups (ligation, release of ligation, second ligation). We conclude that the combination of the two drugs offers protection against malignant arrhythmias after coronary artery occlusion, removal of the occlusion, and reperfusion.

### Comparison with other studies

Clinical studies of vitamin C administration have produced conflicting findings concerning its role in cardiac diseases.<sup>27-33</sup> Experimental studies have also yielded conflicting results. In ischaemia-reperfusion models in the rat heart some studies found a benefit whereas others failed to show any benefit from free radical scavengers in reperfusion arrhythmias.<sup>34,35</sup> Only a few stud-



**Figure 2.** Percentages without arrhythmia, with ventricular extrasystoles (VES), or with ventricular tachycardia or fibrillation (VT/VF) after the various manoeuvres, regardless of substance administration. BP – blood pressure.

ies examined the role of free radicals in reperfusion arrhythmias in the dog heart. Although agents that remove free radicals or inhibit their formation probably protect the dog heart from post-ischaemic systolic dysfunction, the occurrence of fatal reperfusion arrhythmias in those studies was so sporadic that it was difficult to evaluate the role of free radicals,<sup>18,19,36-41</sup> while the presence of a significant collateral circulation in those animals protected the ventricles from fatal arrhythmias.<sup>42</sup> The oxygen scavengers that were used in those experimental studies in order to examine whether they would reduce the incidence of VF due to reperfusion were superoxide dismutase (SOD), glutathione peroxidase (GP), and Catalase (CAT), which achieve capture of the electrons from oxygen to form water, bypassing the stages of very toxic radicals. In contrast, the systems that generate free radicals increase to the maximum the susceptibility of the heart to arrhythmias.<sup>43</sup> Other oxygen scavengers are N-acetylcysteine, and vitamins (or their derivatives) such as ascorbic acid, alpha-tocopherol, and beta-carotene, allopurinol, methionine, mannitol, and deferoxamine.<sup>44-49</sup> Deferoxamine reacts with free iron ions and has been shown to reduce the ischaemic damage in rabbit hearts.<sup>50</sup> In dogs given deferoxamine during the induction of ischaemia, an improvement was seen in cardiac systolic function after reperfusion damage, compared to dogs in the control group.<sup>51</sup>

Summing up, a large number of studies, as reported above, aimed to reduce the damage to the myocardium during reperfusion, and to reduce the incidence of ventricular arrhythmias caused by ischaemia-reperfusion syndrome, using antioxidative substances; however, the results were often conflicting. One of the reasons for this inconsistency of findings in the various models of ischaemia-reperfusion could be the fact that antioxidants, in the presence of metal ions such as iron, can exert a pro-oxidative effect (Fenton reaction). In such a case a large number of iron ions could be released during ischaemia, sufficient to over-saturate the serum's iron-binding capacity.<sup>7</sup>

Blood pressure elevation is known to create an arrhythmogenic substrate. Sideris et al,<sup>10,11</sup> by increasing the arterial pressure of healthy anaesthetised dogs through mechanical or pharmaceutical means, caused the development of ventricular ectopic rhythms; in contrast, reducing the pressure resulted in the suppression of pre-existing ventricular ectopic activity. The same researchers<sup>12</sup> examined the arrhythmogenic effect of acid metabolites on blood pressure in 24 patients, in 13 of whom a pressure increase was induced

by intravenous metaraminol administration. Seven of the latter patients had indications of cardiac disease while the remaining 6 had no cardiac history. The significant increase in SBP caused the appearance of ventricular extrasystoles in 12 of the 13 patients, with the number of ectopic complexes being greater in the patients who had a history of heart disease or palpitations. In the present study the peak SBP was lower in the animals in whom VT/VF was induced than in those who remained free of those arrhythmias. The SBP during manoeuvres that caused some kind of ventricular arrhythmia was higher than in those where no arrhythmia was induced, as would be expected from the known fact that a pressure increase has an arrhythmogenic effect on the ventricles.<sup>10-13,20-26</sup>

### **Clinical implications**

Patients with coronary artery disease and regional disturbances of left ventricular contractility form a group with a high incidence of severe ventricular arrhythmias<sup>52,53</sup> and a high absolute risk of sudden death,<sup>54,55</sup> which is proportional to the degree of reduction in global left ventricular systolic function.<sup>56,57</sup> Oxidative stress is implicated in the pathogenesis of atherosclerosis<sup>58,59</sup> and endothelial dysfunction,<sup>60-62</sup> in pathological platelet aggregation, heart failure, and in many other processes that influence the development of cardiovascular diseases.<sup>63</sup> Vitamin C has been found to improve endothelium-dependent vasodilation in the brachial arteries of patients with diabetes,<sup>64</sup> in smokers,<sup>65</sup> in patients with coronary artery disease,<sup>66,67</sup> and in hypertensives.<sup>68,69</sup> It acts as a first line of defence against oxidative stress in the human body, even though its precise role still remains unclear. As a complement to the numerous antioxidative enzymes, organisms also appear to have available another mechanism of resistance against the damaging effects of reactive oxygen species. This mechanism consists of the binding of free metal ions (e.g. Fe and Cu), as well as free haem and haemoglobin, so as to inhibit their contribution to reactions that cause the creation of free radicals (e.g. Fenton reaction and creation of ferryl species). In the future, oxidative stress will be one of the new therapeutic targets in coronary artery disease and its clinical manifestations.

### **Limitations**

This study had several limitations. A large number of

animals were excluded (12/42). However, the death of these animals occurred before administration of the placebo or other substances. Also, there was no control group in which the second ischaemic episode was not combined with blood pressure elevation, in order to show the effect of the first ischaemia-reperfusion (without hypertension) on the final result. In addition, the use of a mechanical increase in blood pressure by partial regional occlusion of the ascending aorta, rather than using metaraminol, would have eliminated the possible disadvantages from the use of this hypertensive agent. The intravenous administration of therapeutic substances in the experimental model is probably inferior to administration via the coronary sinus, which can cause adequate intracellular concentrations. Also, we do not have data concerning the extent of the infarct and its possible relation with the occurrence of malignant arrhythmias. Finally, no measurements were made in the peripheral blood or tissues of indexes of the antioxidative action of ascorbic acid or its combination with deferoxamine (e.g. concentrations of malonate dialdehyde, or coupled dienes, or nitrotyrosine, or isoprostanes) that would have verified that the dosages and means of administration used indeed had a biological antioxidative effect.

## Conclusions

In the specific experimental model of ischaemia-reperfusion described here, the combination of ascorbic acid and deferoxamine had a protective antiarrhythmic effect compared with a control group. On its own, neither of these two agents has the same protective effect. Susceptibility to malignant arrhythmias could not be attributed to an excessive increase in blood pressure in experiments in which arrhythmia occurred.

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