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Effect of physostigmine on ventricular fibrillation and myocardial glycogen in hypothermic dogs

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Summary

1. Physostigmine (0·1 mg/kg i.v.) given at 37° C and 25° C rectal temperatures, completely protected the hypothermic dog heart against ventricular fibrillation.

2. Pentolinium, atropine, vagotomy and reserpine did not significantly alter the incidence of ventricular fibrillation.

3. The incidence of ventricular fibrillation under hypothermia could be significantly increased by ligating the anterior descending branch of the left coronary artery. The incidence of ventricular fibrillation in coronary ligated hypothermic dogs was reduced to half by physostigmine pretreatment.

4. Hypothermia produced ventricular glycogen depletion and physostigmine prevented ventricular glycogenolysis under hypothermia. However, in the normothermic state physostigmine itself produced a significant decrease in cardiac glycogen.

5. The relation between the antifibrillatory and antiglycogenolytic effects of physostigmine under hypothermia are discussed.

Introduction

During the last two decades hypothermia has been widely used as an aid to surgery or other forms of treatment (Blair, 1964). However, one of the inevitable sequelae of deepening hypothermia is ventricular fibrillation and the primary cause of this fibrillation has not yet been ascertained. The physiological and biochemical aspects of hypothermia and the possible causes of ventricular fibrillation have been reviewed by several authors (Brown, 1956 ; Kayser, 1957 ; Lewis, 1961 ; Vandam & Burnap ; 1959). Several known anti-arrhythmic agents including antihistaminics, antimalarials and local anaesthetics have been screened for their protective value against fibrillation during hypothermia (Angelakos & Hegnauer, 1959 ; Covino, Wright & Charleson, 1955), but none of these studies has resulted in any great success. However, Anand, Malhotra, Singh, Pundlik & Chhina (1958) reported that there was a significant reduction in the acetylcholine and glutathione content of dog heart under hypothermia, and Malhotra, Anand, Singh & Das (1960) subsequently showed that intravenous infusion of acetylcholine during progressive hypothermia in dogs could markedly reduce the incidence of ventricular fibrillation. Montgomery, Prevedal & Swan (1954) have reported that intravenous and intracoronary administration of prostigmine could respectively prevent hypothermic ventricular fibrillation partially, or completely, but Pokrovskii & Bensman (1960) and Saxena (1963) could not confirm these results.

Wynn (1954) has shown that utilization of glucose is impaired under hypothermia. The glycogen content of both liver (Fuhrman & Fuhrman, 1963) and skeletal muscle (Bernstein, 1962) has been shown to be depleted under hypothermia. Histochemical studies have indicated that hypothermia also reduces the glycogen content of heart muscle (Fisher, Fedor & Fisher, 1957). As our previous studies indicated a possible action of acetylcholine in the prevention of hypothermic ventricular fibrillation and as acetylcholine has been reported to have an antiglycogenolytic effect under certain circumstances (Vincent & Ellis, 1963), it was thought worthwhile to study the effect of physostigmine on ventricular fibrillation and myocardial glycogen concentrations under hypothermia. The effects of other procedures which disturb the balance of the autonomic nervous system (treatment with pentolinium, atropine, reserpine and vagotomy) have also been studied.

Methods

Experiments were conducted on healthy mongrel dogs weighing 8·5–18·5 kg. The animals were anaesthetized with pentobarbitone sodium (35 mg/kg) given intraperitoneally. Carotid arterial pressure and respiration were recorded using a mercury manometer and a Brodie tambour respectively (1 mmHg≡1·333 mbar). Lead II of the electrocardiogram was recorded using a Fukuda electrocardiograph. Deep rectal temperature was recorded using an electric thermometer. Hypothermia was induced by surface cooling using salted ice packs. Forced ventilation was always instituted with a respiratory pump when the respiration was very much depressed (usually at 28°–30° C). The following experiments were conducted. With every set of experiments appropriate untreated controls were studied. All drugs were administered intravenously.

Effect of physostigmine, atropine, pentolinium, reserpine and vagotomy on hypothermic ventricular fibrillation

Every dog was subjected to one of the following procedures and the effect on the incidence of ventricular fibrillation studied: cooling was continued until either ventricular fibrillation or asystole developed or rectal temperature dropped to 12° C; physostigmine salicylate (0·1 mg/kg) was given at a rectal temperature of 37° C and again at 25° C; pentolinium bitartrate (0·5 mg/kg) was administered at the same temperatures as physostigmine; atropine sulphate (1·0 mg/kg) was given just before inducing hypothermia, as was section of the right and left vagus; reserpine (0·5 mg/kg) was given 24 h before inducing hypothermia. The dose of physostigmine used has been found to increase the ventricular acetylcholine content from $0{\cdot}28 \pm 0{\cdot}04$ μg/g to $0{\cdot}85 \pm 0{\cdot}15$ μg/g under hypothermia (Das & Tripathi, unpublished).

Effect of physostigmine on hypothermic ischaemic ventricular fibrillation

The incidence of ventricular fibrillation was increased by ligating the anterior descending branch of the left coronary artery under hypothermia. When the rectal temperature reached 24° C, the chest was opened along the left fourth intercostal space and the pericardium was opened. The anterior descending branch of the left coronary artery was identified, freed from adjoining tissues and a thread was passed loosely round the artery, 4 mm from its origin, to facilitate occlusion when desired. The artery was ligated at 23° C rectal temperature. The ligature was removed after

a maximum period of 10 minutes. During this period the rectal temperature dropped to 21 or 21·5° C. Physostigmine salicylate (0·1 mg/kg) was given at 37° C and again at 25° C rectal temperatures. The cooling was continued until either ventricular fibrillation developed or rectal temperature dropped to 17° C.

Effect of hypothermia and physostigmine on cardiac glycogen

Glycogen concentrations of the left atrial appendix and anterior middle portion of the left ventricle were estimated as described by Montgomery (1957). Small pieces (about 0·5 g) of cardiac tissue, including all the layers but excluding any major blood vessels, were quickly cut out and all traces of blood removed from their surfaces, using blotting paper. Tissue glycogen was separated and estimated colorimetrically (with a Leitz photo-electric colorimeter at wave length 490 nm) using phenol-sulphuric acid reagent. Cardiac glycogen concentrations were estimated under the following conditions: (a) normothermic dogs, 1 h after anaesthesia, (b) physostigmine treated normothermic dogs, 1 h after anaesthesia and physostigmine administration, (c) hypothermic dogs, (d) physostigmine treated hypothermic dogs, (e) hypothermic dogs with the anterior descending branch of the left coronary artery ligated (at 23° C), (f) physostigmine treated hypothermic dogs with the anterior descending branch of the left coronary artery ligated (at 23° C). In all hypothermic dogs physostigmine was administered in a dose of 0·1 mg/kg at 37° C and again at 25° C rectal temperatures. All the tissues in the hypothermic dogs were removed at 21° C rectal temperature or earlier if ventricular fibrillation appeared.

Results

Incidence of ventricular fibrillation

The effects of physostigmine, pentolinium, atropine, vagotomy and reserpine on the incidence of ventricular fibrillation under hypothermia are summarized in Table 1. Out of the twenty control dogs 55% developed ventricular fibrillation at a mean rectal temperature of 19·5° C. The rest of the control dogs developed marked bradycardia as the temperature was lowered to 12° C. None of the sixteen dogs which were given physostigmine developed ventricular fibrillation, and in all these dogs the temperature could be lowered to 12° C. Pentolinium, atropine, vagotomy

TABLE 1. *Incidence of ventricular fibrillation (V.F.) in dogs under hypothermia*

	Groups	*n*	Incidence of V.F.	V.F. frequency %	Mean rectal temp. at V.F. (°C)	χ^2 with Yates' correction for continuity	*P*
A.	1. Control	20	11	55	19·5		
	2. Physostigmine	16	0	0	—	10·2	<0·01*
	3. Pentolinium	8	2	25	17·2	1·0	>0·30*
	4. Atropine	8	3	37·5	18·5	0·2	>0·70*
	5. Vagotomy	7	2	28·6	17·4	0·6	>0·50*
	6. Reserpine	9	3	33·3	21·7	0·5	>0·50*
B.	1. Coronary ligation	16	15	93·8	21·2	4·9	<0·05*
	2. Coronary ligation + physostigmine	11	5	45·5	21·0	5·6	<0·02†

* Compared with A-1. † Compared with B-1.

and reserpine had no significant effect on the incidence of hypothermic ventricular fibrillation.

In order to confirm the results of physostigmine treatment, the control incidence of ventricular fibrillation under hypothermia was increased by producing myocardial ischaemia. Coronary ligation under hypothermia significantly increased the incidence from 55% to 93·8%. Physostigmine now significantly reduced it from 93·8% to 45·5%.

Heart rate changes

Physostigmine, pentolinium, atropine and vagotomy did not produce any significant change in the heart rate at different temperature levels as compared to the controls. There was, however, significant bradycardia in the reserpine treated group at 37° C and 30° C as compared to the control group, but there was no significant difference at 25° C and below. The mean heart rates (±S.D.) in the reserpine treated group at 37° C, 30° C and 25° C were 135·7±8·6, 87·7±5·8 and 60·6±4·7 as compared to the corresponding heart rates in the control series of 171·9±12·8, 109·4±8·1 and 69·7±5·8, respectively.

Blood pressure changes

Neither physostigmine nor vagotomy significantly altered the mean arterial pressures as compared to the controls (Table 2). Mean arterial pressure was lowest in the reserpine treated group between rectal temperatures of 37° C and 20° C. The arterial pressures in the pentolinium and atropine treated groups were significantly lower than the control group, but were much higher than in the reserpine treated group. It was interesting to note that there were no significant differences in the mean arterial pressures between any of the groups at 15° C.

TABLE 2. *Mean arterial pressure (mmHg±S.D.) under hypothermia*

Groups	*n*	37° C	30° C	25° C	20° C	15° C
1. Control	20	146·5±10·8	126·4±10·7	90·8±6·5	54·8±5·0	14·5±4·1
2. Physostigmine	16	141·2± 8·5	110·6± 9·1	93·0±7·1	56·7±5·3	13·7±3·8
3. Pentolinium	8	121·6± 7·9	92·8± 5·7	56·8±3·7	23·6±2·8	12·5±3·6
4. Atropine	8	129·2± 8·8	106·2± 6·5	63·2±4·4	33·8±2·7	17·7±4·2
5. Vagotomy	7	150·6±11·2	127·6± 9·8	110·0±8·1	65·0±6·2	13·3±5·9
6. Reserpine	9	86·5± 5·2	61·8± 4·1	50·5±3·9	33·7±2·1	13·2±4·1

TABLE 3. *Glycogen concentration of the atrium and ventricle of the dog, expressed in mg/g wet tissue*

Groups	No. of expts.	Atrium Mean±S.D.	Atrium *P*	Ventricle Mean±S.D.	Ventricle *P*
1. Normothermia	11	4·64±0·858	—	4·56±1·289	—
2. Normothermia and physostigmine	11	3·79±0·975	<0·05*	2·76±1·204	<0·01*
3. Hypothermia	14	4·04±1·531	>0·10*	2·00±0·921	<0·001*
4. Hypothermia and physostigmine	10	4·26±1·272	>0·10* >0·50†	4·10±1·357	>0·50* <0·001†
5. Hypothermia and coronary artery ligated	10	5·21±1·702	>0·50* >0·10†	2·03±0·500	<0·001* >0·50†
6. Hypothermia with physostigmine and coronary artery ligated	11	5·30±1·775	>0·50‡ >0·10*	4·42±1·546	<0·001‡ >0·10*

* Comparison with Group 1. † Comparison with Group 3. ‡ Comparison with Group 5. Significance of difference between pairs of means estimated by Student's *t* test.

Cardiac glycogen concentrations

The effects of hypothermia and physostigmine on cardiac glycogen are summarized in Table 3. Physostigmine, in normothermic animals, was found significantly to lower the cardiac glycogen concentration, but the decrease was greater in the ventricles (39·4%) than in the atria (18·3%). There was a marked reduction (more than 50%) in the glycogen concentration of the ventricles under hypothermia. There was, however, no significant difference in the glycogen concentrations of the atria of hypothermic and normothermic animals. Physostigmine treated animals did not show any reduction in the glycogen concentration of the ventricles under hypothermia. It appeared, therefore, that though physostigmine itself reduced the cardiac concentration of glycogen in normothermic animals it prevented ventricular glycogenolysis under hypothermia. There was no difference between the findings of the two hypothermic groups—with or without coronary ligation.

Discussion

Physostigmine has been found effectively to prevent hypothermic ventricular fibrillation in dogs. The results are consistent with the antifibrillatory effects of infused acetylcholine (Malhotra *et al.*, 1960), vagal stimulation (Riberi, 1956) and intravenous injection of prostigmine (Montgomery *et al.*, 1954) in hypothermia. The antifibrillatory effect of physostigmine seems to be related to prevention of the fall in cardiac acetylcholine concentration which occurs in hypothermia (Anand *et al.*, 1958).

The antifibrillatory effect of physostigmine is apparently not related to changes in heart rate. Atropine and vagotomy also did not increase heart rate at any temperature, indicating a low level of cardio-vagal tone which has not been altered by hypothermia. This can be correlated with failure of vagotomy and atropine to influence the incidence of ventricular fibrillation. The results confirm the earlier report of Riberi (1956) who showed that bilateral cervical vagotomy had no effect on the incidence of ventricular fibrillation under hypothermia.

Reserpine reduced heart rate but like pentolinium, did not decrease the incidence of ventricular fibrillation, indicating that sympathetic activity is probably not a factor responsible for hypothermic ventricular fibrillation. α-Adrenoceptor antagonists have also been found to be unable to prevent ventricular fibrillation under hypothermia (Covino, Charleson & D'Amato, 1954). Verma, Gillis & Melville (1963) found that reserpine reduced the incidence of hypothermic ventricular fibrillation but, because of interaction with thyroid activity, concluded that the effect was not related to reduction in cardiac catecholamines.

The antifibrillatory effect of physostigmine is apparently not related to changes in blood pressure. Reserpine, pentolinium and atropine, which did reduce blood pressure, did not change the incidence of ventricular fibrillation.

Hypothermia was found to lower markedly the ventricular glycogen concentration and treatment with physostigmine prevented this, even though physostigmine alone lowered cardiac glycogen concentration in normothermic dogs. Edwards, Tuluy, Ruber, Seigel & Bing (1954) have shown that under hypothermia the heart maintains a relatively high oxygen consumption as compared to the rest of the body and they suggested that during hypothermia the heart is unable to convert aerobic metabolism into useful work and thus suffers from tissue anoxia. Under anaerobic

conditions and ischaemia the heart uses up glycogen (Reeves, 1963). In addition, under hypothermia the body is unable to synthesize glycogen from glucose (Fuhrman & Fuhrman, 1963). Thus, ventricular glycogenolysis under hypothermia may be due to enhanced breakdown as a result of anoxia on the one hand and reduced synthesis on the other. Acetylcholine inhibits cardiac glycogenolysis by adrenaline and theophylline and partially inhibits that caused by anoxia (Vincent & Ellis, 1963). The prevention of ventricular glycogenolysis under hypothermia with physostigmine treatment might be related to metabolic effects of acetylcholine. Thus it seems possible that there is a relationship between the antifibrillatory and antiglycogenolytic effects of physostigmine. However, no information is yet available with other agents which might show whether prevention of glycogenolysis is always associated with reduction in ventricular fibrillation under hypothermia but there is evidence that enhancement of cardiac cholinergic activity may play a significant role in the prevention of the arrhythmia under hypothermia.

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