Br. J. Pharmac. (1972), **44**, 397–403.

Studies on the effect of physostigmine on experimental cardiac arrhythmias in dogs

P. K. DAS AND S. K. BHATTACHARYA

Department of Pharmacology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India

Summary

1. Experimental cardiac arrhythmias were produced in dogs anaesthetized with pentobarbitone. Ventricular arrhythmias were induced by strophanthin-K, light petroleum plus adrenaline or coronary ligation procedures. Atrial flutter was induced by an injury-stimulation technique. The acetylcholine and glycogen concentrations of the atria and ventricles were estimated.

2. Physostigmine pretreatment (0·1 mg/kg) significantly reduced the incidence of ventricular arrhythmias after myocardial ischaemia but had no effect on any of the other arrhythmias.

3. Physostigmine markedly increased the acetylcholine concentrations of atria and ventricles in control dogs, to nearly the same extent. Physostigmine had no effect on ventricular acetylcholine concentrations in dogs treated with strophanthin-K and light petroleum plus adrenaline but in the coronary ligation group it caused a significant increase in the acetylcholine concentrations of both atria and ventricles, and of atrial acetylcholine only in the injury-stimulation group.

4. All the arrhythmias produced marked glycogenolysis of both the atria and the ventricles, to nearly the same extent. Although physostigmine produced marked glycogenolysis in the control dogs it significantly inhibited cardiac glycogenolysis after light petroleum plus adrenaline, atrial glycogenolysis after strophanthin-K-induced arrhythmias and ventricular glycogenolysis after myocardial ischaemia.

5. There appears to be a possible correlation between the increase in the acetylcholine concentration of the ventricles and anti-arrhythmic actions of physostigmine, but there is a less clear correlation between changes in the glycogen concentration of ventricles and the anti-arrhythmic action.

Introduction

Many problems concerning the initiation and maintenance of cardiac arrhythmias have still to be solved (Vaughan-Williams, 1964). A relation between the presence of choline esters or of vagal stimulation and the likelihood of atrial ectopic rhythms have been reported by many workers (Burn, Vaughan-Williams & Walker, 1955; Nahum & Hoff, 1940; West, Turner & Loomis, 1954). However, similar arrhythmias have not been produced in the ventricles and this is compatible with the reported insensitivity of the ventricular myocardium to acetylcholine (Hoffman & Suck-

ling, 1953). Burn (1957) has discussed the differential effects of acetylcholine on atrium and ventricle. However, Malhotra, Anand, Singh & Das (1960) showed that acetylcholine infusion could prevent ventricular fibrillation under hypothermia. Das & Sinha (1972) reported that intravenous physostigmine prevented ventricular fibrillation and antagonized cardiac glycogenolysis under hypothermia in dogs. There appeared to be some relationship between the antifibrillatory and antiglycogenolytic effects of physostigmine under hypothermic conditions.

Encouraged by these observations, it was thought worthwhile to study the effect of physostigmine on different types of experimental cardiac arrhythmias under normothermic conditions. The present work was undertaken to determine the effect of different cardiac arrhythmias on cardiac acetylcholine and glycogen concentrations and the effect of physostigmine pretreatment. Arrhythmias were induced by four methods: two were drug-induced and the other two were induced by mechanical methods. Out of these, three produced ventricular arrhythmias and one an atrial arrhythmia.

Methods

Mongrel dogs, of either sex weighing 10–24 kg were used. They were anaesthetized with pentobarbitone sodium (35 mg/kg i.p.). Lead II of the electrocardiogram, heart rate and mean arterial pressure (1 mmHg≡1·333 mbar) were recorded in all experiments. Cardiac arrhythmias were induced by the following methods:

1. Strophanthin-K-induced ventricular arrhythmias (Mosey & Tyler, 1954): strophanthin-K (Strophosid) was injected (0·2 mg/kg i.v.).

2. Light petroleum and adrenaline-induced ventricular arrhythmias (Arora & Madan, 1955): light petroleum 0·1 ml/kg was given intratracheally and 15 s later adrenaline hydrochloride (60 μg/kg i.v.) was injected.

3. Coronary ligation-induced ventricular arrhythmias (Harris, 1950): the anterior descending branch of the left coronary artery was ligated 4 mm from its origin.

4. Injury-stimulation-induced atrial flutter (Rosenblueth & Garcia Ramos, 1947): the area of the sino-atrial node was crushed mechanically and the right atrium was stimulated for 1 min with rectangular, 20 V pulses at a frequency of 20 Hz using an electronic stimulator.

Acetylcholine and glycogen concentrations of the left atrial appendix and the anterior surface of the left ventricle were determined. The procedure for extraction of acetylcholine was essentially the same as that of Nachmansohn described by Anand (1952). Acetylcholine was assayed on the frog's rectus abdominis muscle by the technique described by Richter & Crossland (1950). Glycogen was estimated by the phenol-sulphuric acid method (Montgomery, 1957).

Strophanthin-K and coronary ligation arrhythmias were induced 30 min after induction of anaesthesia and the hearts were then removed for chemical analysis 30 min later. Light petroleum plus adrenaline and injury-stimulation arrhythmias were initiated 45 min after induction of anaesthesia and the hearts were removed 15 min later. In the treated group, physostigmine salicylate (0·1 mg/kg i.v.) was given immediately after induction of anaesthesia.

Results

Incidence of arrhythmias

As shown in Table 1, treatment with strophanthin-K and with light petroleum plus adrenaline induced various types of ventricular arrhythmias in ten out of ten animals. Coronary artery ligation produced ventricular arrhythmias in nine out of ten animals. Using the injury-stimulation technique, all animals developed atrial flutter and three of these also developed ventricular fibrillation.

When these four techniques for producing arrhythmias were carried out in animals pretreated with physostigmine, one group, the coronary artery-ligation group, showed a significant reduction in the incidence of ventricular arrhythmias; there were no significant differences in any of the other groups.

The heart rate changes associated with the various arrhythmias are shown in Table 2. In the control groups, there was a significant increase in heart rate associated with the injury-stimulation arrhythmias but there were no significant changes in any of the other groups. Pretreatment with physostigmine reduced the control heart rate in each group but after induction of arrhythmias only the light petroleum plus adrenaline group showed a significantly lower heart rate than in the untreated control groups. Thus the reduction in the incidence of ventricular arrhythmias after physostigmine in the coronary-ligation group was not associated with any change in heart rate.

TABLE 1. *Incidence of cardiac arrhythmias during different experimental procedures*

Groups	No. of animals per group	Ventricular arrhythmias: Extra-systoles	Tachy-cardia	Fibril-lation	Total	χ^2†	*P*	Atrial flutter
1. Strophanthin-K	10	9	7	2	10			—
2. Strophanthin-K+ physostigmine	10	2	4	0	6	2·81	N.S.	—
3. Light petroleum-adrenaline	10	5	3	5	10			—
4. Light petroleum-adrenaline+ physostigmine	10	5	5	6	8	0·55	N.S.	—
5. Coronary ligation	10	3	1	6	9			—
6. Coronary ligation+ physostigmine	10	2	0	1	3	5·20	<0·02*	—
7. Injury-stimulation	10	0	0	3	3			10
8. Injury-stimulation+ physostigmine	10	0	0	0	0	1·57	N.S.	10

* P=0·019 using Fisher's Exact Test. † = with Yates' correction for continuity.

TABLE 2. *Mean heart rate per min* (±S.E.M.) *in different experimental arrhythmias*

Group	Initial	Before induction of arrhythmias	After induction of arrhythmias (terminal)
Strophanthin-K		171·1± 9·81	188·1±18·47
Strophanthin-K+physostigmine	178·0± 8·40	111·2± 9·60†	146·0±15·1
Light petroleum+adrenaline		184·5± 7·93	189·0±23·70
Light petroleum+adrenaline+physostigmine	168·0± 7·70	132·5± 8·48*	110·9±10·6§
Coronary ligation		172·0±11·50	149·5±12·90
Coronary ligation+physostigmine	192·5± 9·60	129·2±12·10†	151·6±14·90
Injury-stimulation		166·5± 9·70	242·0±38·40‡
Injury-stimulation+physostigmine	185·5+10·70	148·5± 8·90*	216·5± 8·50‡

Comparison with heart rate before physostimine: $P<0·05$*, $P<0·005$†. Comparison with heart rate before induction of arrhythmia: $P<0·05$‡. Comparison with terminal rate without physostigmine: $P<0·05$§.

Cardiac glycogen concentrations

Anaesthesia alone for 1 h produced a significant reduction in the glycogen concentration of the ventricles (Table 3). There were no significant differences in the glycogen concentrations of the atria and ventricles between those animals with anaesthesia alone and those in which anaesthesia was combined with thoracotomy. This was a necessary control observation since the strophanthin-K and light petroleum plus adrenaline arrhythmias were induced in closed-chest dogs and the coronary ligation and injury-stimulation arrhythmias were induced in open-chest dogs.

The development of arrhythmias was associated with a highly significant reduction in both atrial and ventricular glycogen concentrations in all groups. No obvious correlation could be seen between the presence or absence of atrial irregularities, heart rate and the degree of reduction in atrial glycogen concentration.

Treatment with physostigmine alone reduced the atrial and ventricular glycogen concentrations of control animals. However, after induction of arrhythmias in animals pretreated with physostigmine, there was a significantly smaller reduction in the glycogen concentration of the atria in the strophanthin-K and light petroleum plus adrenaline groups and a significantly smaller reduction in the glycogen concentration of the ventricles in the light petroleum-adrenaline and coronary ligation groups. No obvious correlation could be seen between these changes and the effects of physostigmine on arrhythmias.

Cardiac acetylcholine concentrations

Anaesthesia, with or without thoracotomy, did not produce any significant changes in the acetylcholine concentrations of the atria or ventricles. However, pretreatment with physostigmine alone produced a highly significant, 3-fold increase in the acetylcholine concentrations of both atria and ventricles. Induction of arrhythmias pro-

TABLE 3. *Comparison of the effects of different arrhythmogenic procedures with and without pretreatment with physostigmine, on myocardial glycogen and acetylcholine concentrations*

Groups	*n*	Glycogen (mg/g)		Acetylcholine (μg/g)	
		Atrium Mean±S.E.M.	Ventricle Mean±S.E.M.	Atrium Mean±S.E.M.	Ventricle Mean±S.E.M.
1. *Control*—					
a. Immediately after anaesthesia	7	4·97±0·18	3·72±0·25*	8·53±2·01	1·07±0·24
b. One hour of anaesthesia	18	4·84±0·14	3·02±0·14	5·56±0·29	1·08±0·23
c. One hour of anaesthesia with chest open	12	5·02±0·54	3·04±0·15	5·51±0·35	0·91±0·16
d. Physostigmine	10	1·97±0·25†	1·18±0·19†	19·19±2·04†	2·95±0·30†
2. a. Strophanthin-K	10	1·23±0·21†	0·83±0·15†	9·57±1·00†	1·53±0·28
b. Strophanthin-K+ physostigmine	10	3·42±0·73‡	1·51±0·34	8·90±1·08	1·18±0·17
3. a. Light petroleum+ adrenaline	10	1·03±0·14†	0·48±0·25†	9·26±0·97†	1·57±0·35
b. Light petroleum+adrenaline+physostigmine	10	2·12±0·23§	1·44±0·28‡	6·25±0·14‡	1·46±0·35
4. a. Coronary ligation	10	1·81±0·17†	1·06±0·13†	1·68±0·20†	0·17±0·11†
b. Coronary ligation+ physostigmine	10	2·35±0·21	1·45±0·12‡	5·35±1·07§	0·54±0·07‡
5. a. Injury-stimulation	10	1·87±0·27†	1·66±0·29†	2·19±0·50†	0·43±0·08†
b. Injury-stimulation+ physostigmine	10	2·32±0·40	1·42±0·15	7·40±1·94‡	0·58±0·48

Comparison with control group 1 b: $P<0·05$—*; $P<0·005$—†. Comparison with respective control arrhythmia group: $P<0·05$—‡; $P<0·005$—§. Significance of differences assessed by Student's *t* test.

duced divergent changes in acetylcholine concentrations. On the one hand, in the strophanthin-K and light petroleum plus adrenaline groups there was no change in the acetylcholine concentrations of the ventricles but the acetylcholine concentrations of the atria were significantly increased. On the other hand, in the coronary ligation and injury-stimulation groups there was a significant decrease in the concentrations of both atria and ventricles.

Pretreatment with physostigmine did not produce any consistent pattern of changes in these effects of the various arrhythmias. Physostigmine did not produce any significant changes in the strophanthin-K group although there was some reduction in the concentration of atrial, but not ventricular, acetylcholine in the light petroleum-adrenaline group. In the coronary ligation and injury-stimulation groups there was a significant increase in atrial acetylcholine concentrations but only after coronary ligation was there an increase in the concentration in the ventricles. Since it was only in the coronary-ligated hearts that physostigmine reduced the incidence of arrhythmias, this finding may be of some interest.

Discussion

Cardiac arrhythmias were produced in dogs by four methods. Strophanthin-K, light petroleum plus adrenaline and coronary ligation induced ventricular arrhythmias and the injury-stimulation method induced atrial flutter. However, with the injury-stimulation procedure, three out of the ten control dogs developed ventricular fibrillation in addition to atrial flutter. Two of these experimental arrhythmias were induced in closed-chest dogs and two were induced in open-chest dogs. Pretreatment with physostigmine significantly protected the heart against ventricular arrhythmias induced by myocardial ischaemia. However, there was no significant protection against arrhythmias produced by the other three techniques.

Recently, both acetylcholine and physostigmine have been shown to prevent the development of ventricular fibrillation in hypothermic dogs (Malhotra *et al.*, 1960; Das & Sinha, 1972), and there appeared to be a correlation between anti-arrhythmic and antiglycogenolytic effects. The present studies further indicate that physostigmine can significantly prevent ventricular arrhythmias induced by myocardial ischaemia without affecting other types of experimental arrhythmias and so cardiac concentrations of acetylcholine and glycogen have been examined for evidence of a similar correlation.

Physostigmine treatment increased the acetylcholine concentrations of the atria and ventricles to nearly the same extent. The increase in the acetylcholine concentration of the ventricles indicates that though the cholinergic innervation of the ventricles is poor (Vaughan-Williams, 1964), some cholinergic activity does exist in the ventricles. In the experimental arrhythmia groups, however, physostigmine had inconsistent effects on cardiac acetylcholine concentrations. It did not increase cardiac acetylcholine concentrations in strophanthin-K or light petroleum plus adrenaline-induced arrhythmias. However, it did significantly increase the acetylcholine concentrations in the ventricles in the coronary ligation-induced arrhythmias and the acetylcholine concentrations of the atria in injury-stimulation arrhythmias. The inconsistency in the effects of physostigmine on cardiac acetylcholine concentrations during different types of cardiac arrhythmias cannot be adequately explained. However, there does seem to be some relationship between physostigmine action and acetylcholine concentration. In closed-chest dogs, where cardiac arrhyth-

mias increased the acetylcholine concentrations of the atria, physostigmine had no effect. But in open-chest dogs, where cardiac arrhythmias reduced the cardiac acetylcholine concentrations, physostigmine seemed effectively to preserve the acetylcholine. Whether these differences are related to acetylcholine release or cholinesterase activity is not known.

All four types of cardiac arrhythmias produced marked cardiac glycogenolysis. The degree of glycogenolysis was not related to the heart rate. The glycogenolytic effect of strophanthin-K agrees with the report of Gvozdjak (1963) who found cardiac glycogenolysis with toxic doses of digitoxin. The present findings with adrenaline are in complete agreement with its well known cardiac glycogenolytic effect (Sutherland & Rall, 1960). Cardiac ischaemia has been reported to cause glycogenolysis in the infarcted area (Rasmussen, Klionsky, Cossman & Allbritten, 1961 ; Brachfeld & Scheur, 1965). Glycogen depletion has also been shown to be the first indication of myocardial ischaemia (Lushnikov, 1962). However, in the present studies, ventricular ischaemia caused glycogenolysis not only of the ventricle but also of the atrium. In addition, atrial flutter caused glycogenolysis of the atrium as well as of the ventricle. Thus it appears that cardiac arrhythmias *per se* in some way result in cardiac glycogenolysis.

Physostigmine produced marked cardiac glycogenolysis in the control dogs confirming our earlier results (Das & Sinha, 1972). On the other hand it significantly inhibited the glycogenolytic effects of strophanthin-K on atria, of light petroleum plus adrenaline on atria and ventricles and of myocardial ischaemia on ventricles. These results are consistent with the antiglycogenolytic effects of acetylcholine against adrenaline and anoxia-induced cardiac glycogenolysis (Vincent & Ellis, 1963) and the antiglycogenolytic effects of physostigmine in hypothermia (Das & Sinha, 1972).

The relationship between the anti-arrhythmic action of physostigmine and its action on ventricular glycogen and acetylcholine concentrations is not completely clear. Physostigmine significantly raised the ventricular glycogen concentration in light petroleum plus adrenaline group without any anti-arrhythmic action while in the coronary ligation group there was both protection against ventricular arrhythmias and a significant rise in the glycogen concentration of the ventricles. However, physostigmine significantly increased the acetylcholine concentration of the ventricles only in the coronary ligation group and it is in this group alone that it showed significant anti-arrhythmic activity. In experiments on hypothermic dogs physostigmine raised the acetylcholine and glycogen concentrations of the ventricles and also showed an antifibrillatory effect (Das & Sinha, 1972 ; Das & Tripathi, 1971 unpublished). Thus it seems that there is a possible correlation between the increase in the acetylcholine concentration of the ventricles and anti-arrhythmic action but a less clear correlation between changes in the glycogen concentration of the ventricles and anti-arrhythmic activity.

REFERENCES

ANAND, B. K. (1952). Influence of temperature on vagal inhibition and liberation of acetylcholine in frog heart. *Am. J. Physiol.*, **168**, 218–225.

ARORA, R. B. & MADAN, B. R. (1955). Petroleum ether and adrenaline induced ventricular fibrillation. *University of Rajasthan Studies*, 69–71.

BRACHFELD, N. & SCHEUR, J. (1965). Myocardial glycolysis in the ischaemic state. *Circulation*, **32**, Suppl. II, 57.

BURN, J. H. (1957). Acetylcholine and cardiac fibrillation. *Br. med. Bull.*, **13**, 181–184.

BURN, J. H., VAUGHAN-WILLIAMS, E. M. & WALKER, J. M. (1955). The effects of acetylcholine in the heart-lung preparation including production of auricular fibrillation. *J. Physiol., Lond.*, **120**, 277–293.

DAS, P. K. & SINHA, P. S. (1972). Effect of physostigmine on ventricular fibrillation and myocardial glycogen in hypothermic dogs. *Br. J. Pharmac.*, **44**, 391–396.

GVOZDJAK, J. (1963). Some remarks on the biochemical changes in myocardium after administration of cardiotonic glycosides. *Archs int. Pharmacodyn. Thér.*, **145**, 190–197.

HARRIS, A. S. (1950). Delayed development of ventricular ectopic rhythms following experimental coronary occlusion. *Circulation*, **1**, 1318–1328.

HOFFMAN, B. F. & SUCKLING, E. E. (1953). Cardiac cellular potentials: effect of vagal stimulation and acetylcholine. *Am. J. Physiol.*, **173**, 312–320.

LUSHNIKOV, E. F. (1962). Histochemical study of experimental myocardial infarct. *Arkh. Patol.*, **24**, 55–62.

MALHOTRA, C. L., ANAND, B. K., SINGH, B. & DAS, P. K. (1960). External cardiac excitation as a cause of ventricular fibrillation during hypothermia and its prevention by acetylcholine. *Ind. J. med. Res.*, **48**, 1–10.

MONTGOMERY, R. (1957). Estimation of glycogen. *Archs Biochem. Biophys.*, **67**, 378-386.

MOSEY, L. & TYLER, M. D. (1954). Effect of diphenylhydantoin sodium (Dilantin), procaine hydrochloride, procainamide hydrochloride and quinidine hydrochloride upon ouabain induced ventricular tachycardia in unanaesthetised dogs. *Circulation*, **10**, 65–70.

NAHUM, L. H. & HOFF, H. E. (1940). Production of auricular fibrillation by application of acetyl-β-methylcholine chloride to localised regions on the auricular surface. *Am. J. Physiol.*, **129**, 428.

RASMUSSEN, P., KLIONSKY, B., COSSMAN, E. P. & ALLBRITTEN, F. F. (1961). Elective cardiac arrest. Its effect on myocardial structure and function. *Ann. Surg.*, **154**, 751–768.

RICHTER, D. & CROSSLAND, J. (1950). Quoted by MacIntosh, F. C. & Perry, W. L. M. In: *Methods in Medical Research*, vol. iii, ed. Gerard, W. R., p. 87, Chicago: The Year Book Publishers.

ROSENBLUETH, A. & GARCIA RAMOS, J. (1947). Studies on flutter and fibrillation. II. Influence of artificial obstacle on experimental auricular flutter. *Am. Heart J.*, **33**, 677-684.

SUTHERLAND, E. W. & RALL, T. W. (1960). Relation of adenosine-3′,5′-phosphate to the action of catecholamines. In: *Ciba Foundation Symposium on Adrenergic Mechanisms*, ed. Wolstenholme, G. E. W. & O'Conner, M., pp. 295-304. London: J. A. Churchill.

VAUGHAN-WILLIAMS, E. M. (1964). In: *Pharmacology of Cardiac Function*, ed. Krayer, O., pp. 119–132. Praha: Czechoslovak Medical Press.

VINCENT, N. H. & ELLIS, S. (1963). Inhibitory effect of acetylcholine on glycogenolysis in the isolated guineapig heart. *J. Pharmac. exp. Ther.*, **139**, 60–72.

WEST, T. C., TURNER, L. D. & LOOMIS, T. A. (1954). Effects of acetylcholine on mechanical and electrical properties of isolated rabbit auricle: their relationship to genesis of arrhythmias. *J. Pharmac. exp. Ther.*, **111**, 475–482.

(*Received September* 3, 1969)