HYPOTHERMIA AND ACETYLCHOLINE CONTENT OF DOG HEART

N.K. KHANNA*, P.C. HURKAT** AND T.S. THETI

Department of Pharmacology, J.L.N. Medical College, Ajmer

Summary: The acetylcholine level of dog heart has been determined under hypothermia. It was increased at 28°C, and decreased at 20°C. Further, it was observed that the heart rate and blood pressure decreased with gradual reduction in the body temperature of the dog and ventricular fibrillation occurred in 50% of the animals at 20°C. It is proposed that cholinergic agents may have a role as antifibrillatory agents in hypothermia.

Key words: moderate hypothermia deep hypothermia acetylcholine

INTRODUCTION

Hypothermia has recently been widely employed as an aid to surgery or other forms of treatment (3). However, one of the serious complications of deepening hypothermia is ventricular fibrillation. Anand *et al.* (2) reported that there was a significant reduction in the acetylcholine (ACh) and glutathione content of dog heart under hypothermia. Pokrovskii and Benseman (15) and Saxena (20) reported that intravenous infusion of neostigmine during hypothermia did not reduce the incidence and degree of ventricular fibrillation. However, Malhotra *et al.* (13) reported that intravenous infusion of ACh or physostigmine could markedly reduce the incidence of ventricular fibrillation during progressive hypothermia. These findings were confirmed later by Das and Sinha (5). The present study was undertaken to re-assess these findings.

MATERIALS AND METHODS

Experiments were conducted on healthy mongrel dogs weighing 7.5-16 *kg*. The animals were anaesthetized by intravenous injection of 30 *mg/kg* pentobarbitone sodium. Hypothermia was induced by the central cooling mechanism using the technique of Hurkat *et al.* (6). Rectal temperature was recorded continually, inserting one and a half inch of thermometer into the rectum.

The procedure for extraction of ACh was essentially the same as that of Nachmansohn as described by Anand (1). ACh was assayed on frog rectus abdominis muscle according to Richter and Crossland (19).

Half an hr after the rectal temperature had reached either 28 or 20°C, hearts were excised for ACh estimation. In the control group, hearts were removed for ACh estimation one hr

Present address: *Department of Pharmacology, S.N. Medical College, Jodhpur-342003.
**Laboratory of Cryobiology, Department of Physiology and Biochemistry, J.L.N. Medical College, Ajmer.

after the anaesthesia in groups (a) and one hr after the anaesthesia with complete setting of experiment, but without inducing hypothermia in group (b).

RESULTS

Changes in the cardiac rate, rhythm and blood pressure:

With gradual reduction in the core body temperature of dog to 30°C, 25°C and 20 significant decrease in the heart rate and blood pressure was recorded (Table I).

TABLE I : Heart rate and arterial pressure under hypothermia in dogs.

	Mean values ±S.E.M.			
	37°C	*30°C*	*25°C*	*20°C*
Heart rate	182.2±3.2(a) (6)	—	—	—
(per min)	180.4±4.0(b) (6)	112.4±2.6	65.5±1.9	58.6±1.2
Blood pressure	142.0±3.0(a) (6)	—	—	—
(mm Hg)	140.5±3.4(b)	120.3±3.4	85.6±2.1	50.2±1.6

(a) One hr of anaesthesia.

(b) One hr of anaesthesia with complete settihg up o experiment but without hypothermia.

Figures in parentheses indicate the number of observations.

TABLE II: Myocardial acetylcholine concentrations under hypothermia in dogs.

Group	*Mean ACh content (μg/g) ±S.E.M.* *Right atrium*	*Left atrium*	*Ventricle*
1. Control			
(a) One hr of anaesthesia (12)	5.3±0.6	4.3±0.7	1.9±0.4
(b) One hr of anaesthesia with complete setting up of experiments but without hypothermia (8)	6.0±0.6*	4.5±0.6*	1.9±0.4*
2. Hypothermia			
(a) Rectal temperature : 28°C (6)	7.3±0.14**	6.4±0.65**	2.3±0.22***
(b) Rectal temperature : 20°C (6)	2.3±0.16**	1.9±0.16**	0.5±0.09**

* Comparison with control group la : $P > 0.05$
** Comparison with control group lb : $P < 0.05$
*** Comparison with control group lb : $P > 0.05$

Significance of difference assessed by Student's 't' test.

Figures in parentheses indicate the number of observations.

Of the six dogs exposed to deep hypothermia three (50%) developed ventricular fibrillation at a mean rectal temperature of 20°C (Fig. 1). The rest of the dogs developed marked bradycardia as the temperature was lowered.

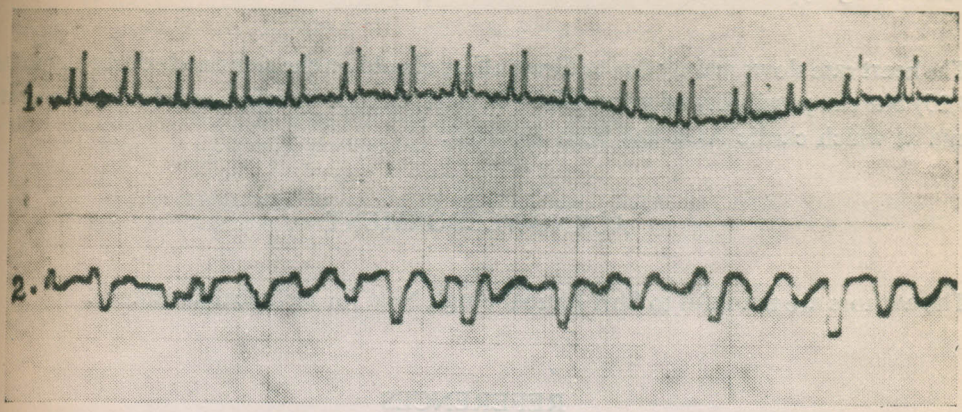

Fig. 1: Effect of hypothermia on the electrocardiogram (Lead II) of a dog.
1 : Control before hypothermia.
2 : Ventricular fibrillation on deep hypothermia (20°C),

Myocardial ACh levels:

The effect of hypothermia on cardiac ACh is summarized in Table II. There was a significant rise in atrial ACh levels and an insignificant increase in ventricular ACh concentration under hypothermia at 28°C. At 20°C a significant decrease in atrial and ventricular ACh concentrations was observed.

DISCUSSION

The present results indicate that ACh concentrations in the heart are increased during moderate hypothermia (rectal temperature: 28°C) and reduced during deep hypothermia (rectal temperature: 20°C). The endogenous production of ACh and noradrenaline, which may act either in concert or antagonism, is essential for the physiological functions of the heart (7). Employing various experimental procedures and designs it has been suggested that increased sympathogenesis in the heart would simultaneously also stimulate the parasympathetic activity in an attempt to maintain homeostasis (8,9,10,11,12,22,23,24). During hypothermia increase in sympathetic activity of the myocardium together with catecholamine release has been reported (16, 21). It, therefore, appears that to maintain internal homeostasis a compensatory increase in cardiac parasympathetic activity occurs during moderate hypothermia as is evident by elevation of myocardial ACh levels. This response is similar to that seen in rabbits and rats exposed to cold for a short period (8,9,23,24). But during deep hypothermia the synthesis of ACh is depressed with consequent reduction in cardiac ACh levels. Owing to this, internal homeostasis in the heart is disturbed, and it becomes readily vulnerable to cardiac arrythmias. Thus the present finding when considered together with previous observations of similar nature (2) will explain the antifibrillatory effects of infused ACh (13), vagal stimulation (17) and intravenous injection of neostig nine (14) and physostigmine (5) during hypothermia because all these procedures will tend

to prevent the fall in cardiac ACh concentrations. However, in view of the present results, it is difficult to explain why intravenous neostigmine could not prevent the development of ventricular fibrillation during hypothermia as reported earlier (15,20).

The heart rate slows mainly due to direct effect of cold on the sinus node during hypothermia (4,16,18,25) but the possibility of vagal effect, in part, cannot be ruled out during moderate hypothermia at which cardic ACh has been elevated.

ACKNOWLEDGEMENT

The authors are thankful to Shri Bhupal Singh Negi for technical assistance.

REFERENCES

1. Anand, B.K. Influence of temperature on vagal inhibition in liberation of acetylcholine in frog heart. *Am. J. Physiol.*, **168** : 218-225, 1952.
2. Anand, B.K., C.L. Malhotra, B. Singh, P.G. Pundlik and G.S. Chhina. Effect of hypothermia on the acetylcholine and glutathione content of brain and heart of dogs. *Ind. J. Med. Res.*, **46** : 21-25, 1958.
3. Blair, E. *Clinical Hypothermia.* London, McGraw-Hill Book Co., P. 108, 1964.
4. Crismon, J.M. Effect of hypothermia on heart rate, atrial pressure and electrocardiogram of rat. *Arch. Int. Med.*, **74** : 235-243, 1944.
5. Das, P.K. and P.S. Sinha, Effect of physostigmine on ventricular fibrillation and myocardial glycogen in hypothermic dogs. *Br. J. Pharmac.*, **44** : 391-396, 1972.
6. Hurkat, P.C., N. Khalsa, S.N. Gupta and B.B. Matriya. A simple technique for producing hypothermia in dogs. *The Clinician*, **37** : 240-242, 1973.
7. Jokl, E. *Heart and Sport*, Charles C Thomas, U.S.A., I Edn; P. 58, 1964.
8. Khanna, N.K. Effect of cold stress on acetylcholine content of rat myocardium and its modification by atropine. *Jap. J. Pharmac.*, **21** : 425-426, 1971.
9. Khanna, N.K. and B.R. Madan. Effect of beta-adrenergic blocking agents on stress-induced changes in acetylcholine content of rat's myocardium. *Arch. Int. Pharmacodyn.*, **175** : 136-140, 1968.
10. Khanna, N.K. and B.R. Madan. Effect of phencarbamide and propranolol on the acetylcholine concentrations of rat's brain and heart before and after convulsions induced by electroshocks and chemoshocks. *Ann. Ind. Acad. Med. Sci.*, **9** : 113-119, 1973.
11. Khanna, N.K. and P. Pandhi. Effect of treatment with thyroxine on the acetylcholine content of the rat heart. *Jap. J. Pharmac.*, **22** : 261-262, 1972.
12. Khanna, N.K., P. Lauria and V.N. Sharma. Effect of electroshock stress on the glycogen and acetylcholine content of myocardium of dog. *Ind. J. Med. Sci.*, **26** : 380-382. 1972.
13. Malhotra, C. L., B. K. Anand, B. Singh and P. K. Das. External Cardiac excitation as a cause of ventricular fibrillation during hypothermia and its prevention by acetylcholine. *Ind. J. Med. Res.*, **48** : 198-207, 1960.
14. Montgomery, A.V., A.E. Prevedal and H. Swan. Prostigmine inhibition of ventricular fibrillation in the hypothermic dog. *Circulation*, **10** : 721-725, 1954.
15. Pokrovskii, V.M. and V.M. Benseman. In : *The Problem of Acute Hypothermia*, Ed. Starker, P.M. London, Pergman Press, P. 166, 1960.
16. Precht, H., J. Christopherson, H. Hensel and W. Larcher. "*Temperature and Life*", U.S.A. Springer-Verlag, P. 651, 1973.
17. Reberi, A. In : *The Physiology of Induced Hypothermia*, Ed. Dripps, R.D. Washington, National Acad. Sc., National Research Council, P. 347, 1956.
18. Reid, L.C., H.E. Stephenson and J.W. Hinton. Cardiac arrest. *Arch. Surg.*, **64** : 409-420, 1952.

19. Richter, D. and J. Crossland. Quoted by MaçIntosh, F.C. and W.L.M. Perry In : *Methods in Medical Research* Vol. III, Ed. Gerard, W.R. Chicago. The Year Book Publishers, P. 87, 1950.

20. Saxena, J.P. Some observations of antifibrillatory agents on ventricular fibrillation under hypothermia. *Ind. J. Physiol. Pharmac.*, **7** : 235-239, 1963.

21. Schneider, F.H. and C.N. Gillis. Hypothermic potentiation of chronotropic response of isolated atria to sympathetic nerve stimulation. *Am. J. Physiol.*, **211** : 890-895, 1966.

22. Sharma, V.N. and F.S.K. Barar. Restraint stress as it influences the myocardium of rat. *Ind. J. Med. Res.*, **54** : 1102-1107, 1966.

23. Sharma, V.N. and N.S. Parmar. Effect of chlorpromazine, mecamylamine and guanethidine on the cold stress induced changes in myocardial glycogen and acetylcholine content in the rabbits. *Ind. J. Med. Res.*, **55** : 60-72, 1967.

24. Sharma, V.N. and N.K. Khanna. Effect of cold stress on acetylcholine and 5-hydroxytryptamine contents of rat myocardium and its modification by eserine, mecamylamine and reserpine. *Ind. J. Med. Res.*, **57** : 1563-1572, 1969.

25. Tysinger, D.S., J.T. Grace and R. Gollan. The electrocardiogram of dogs surviving 1.5° Centrigrade *Am. Heart J.*, **50** : 816-822, 1955.