THE CARDIAC STIMULANT ACTION OF FATTY ACIDS IN MAMMALIAN HEART

C.T. CHOPDE, D.M. BRAHAMANKAR AND A.K. DORLE

Department of Pharmaceutical Sciences,
Nagpur University Campus, Amraoti Road, Nagpur-440010

Summary: The long chain fatty acids exhibited positive inotropic and positive chronotropic effects on Langendorff isolated heart and open chest rabbit heart preparations. Propranolol, guanethidine, reserpinization and adrenalectomy failed to block the fatty acid-induced cardiac stimulation on rabbit heart.

Key words: fatty acids, propranolol, cardiac stimulation, guanethidine, reserpinization, adrenalectomy

INTRODUCTION

The heart utilizes free fatty acids more preferentially over glucose for oxidation (16,19). There is a marked increase in the uptake of free fatty acid during cardiac stimulation (12) which is also accompanied by depletion of myocardial store of triglycerides (10), while the glycogen content remains unaltered (15). The decrease in the myocardial uptake of fatty acids by beta receptor blockade without affecting glucose, pyruvate and lactate utilization (7, 12) further shows the probable relationship between FFA metabolism and the beta adrenergic regulation of cardiac function. The recent studies exploring the cardiac stimulant actions of fatty acids *per se* (3) reveal the importance of FFA in contractile mechanism of heart. However, in these studies the effects of FFA were tested on perfused frog heart which differs functionally from that of mammals (14). Therefore, further investigations are required to confirm the cardiac stimulatory properties of fatty acids in mamals. Some reports demonstrate cardiac depressant actions of continuous perfusion of fatty acids in concentrations of 3.6 mM or above on islated rabbit heart (8), however, the quantities in the perfusate appear to be much higher than those noted to produce stimulation of the frog heart. In fact the higher concentrations of fatty acids were found to cause cardiac depression in frog heart (3). This presentation, therefore, explores the influence of fatty acids at the optimal concentration on mammalian heart.

MATERIALS AND METHODS

Open Chest Rabbit Heart Preparation: Healthy rabbits of either sex weighing between 1.5—2 *kg* were anaesthetized by injecting urethane, 2 *g/kg* intraperitoneally. The rabbits had been heparinized before anaesthesia by injecting 200 *I.U/kg* through marginal ear vein. The

trachea was canulated for artificial respiration. Animals were maintained on artificial respiration with the help of respiration pump (INCO Miniature Pump MA 201). The heart was exposed and pericardium was removed and the left ventricular contractions were recorded on smoked paper by attaching one end of the thread to the pin at the apex of the ventricles of the heart and the other end to the Starling's heart lever. The increased tension was represented by downward deflection. The hert rates were counted as number of heart beats per min at the maximum reponses induced by the materials.

The saturated fatty acids namely lauric, myristic, palmitic, stearic and unsaturated fatty acids such as oliec and linoleic were injected (0.05 to 1 *ml/kg* - from 0.5 - 6 mM solution) through marginal ear vein and responses were recorded. Separate groups of ten rabbits in each were employed for each fatty acid. Similar experiments were also performed in two separate groups (five rabbits in each) of acute bilateral adrenalectomized and also in reserpinized (1 *mg/kg* of reserpine i.p. in two divided two doses on two successive days) rabbits.

In separate groups of five rabbits the effect of (i) propranolol (0.5 *mg/kg*) and (ii) guanethidine (5 *mg/kg*) on responses to fatty acids was studied. Time interval of twenty min was allowed between the administration of these agents and that of fatty acids.

Langendorff Isolated Rabbit Heart: Hearts were perfused by the technique of Langendorff (11). Rabbits of either sex (1.5 - 2 *kg*) were decapitated 5 min after intravenus injection of sodium heparin (200 *I.U./kg*) and the hearts were rapidly transferred to 0.9% NaCl at 0°C. After the heart had been freed from extraneous tissue it was mounted on a cannula at the aorta. Hearts were perfused with Krebs-Ringer (NaCl 5.5 *g*, KCl 0.35 *g*, $MgSO_4$ 7 H_20 0.11 *g*, $CaCl_2$ 0.28 *g*, KH_2PO_4 0.16 *g*, $NaHCO_3$ 2.1 *g*, Glucose 2.0 *g* per litre) gassed with 0_2+CO_2 (95 : 5). The pH of the perfusing solution was 7.4. The temperature of the perfusion fluid was maintained at 37±0.5°C. The perfusion was accomplished by maintaining a constant hydrostatic pressure (47.8 mm Hg) on the heart. Cardiac contractions were recorded by Starling heart lever. Heart rates (beats/min) were measured at maximum responses induced by the drugs.

After recording normal contractions of heart on smoked paper, the graded doses (0.1 to 1.0 *ml* from 0.01 mM or 0.4 mM solution) of fatty acids were injected via the coronary inflow canula and the responses were recorded. Effects of fatty acids were also tested after prior treatment with 0.4 *ml* of 1 mM aqueous solution of propranolol.

The four millimolar solutions of salts of fatty acids were prepared by dispersing suitable amount of fatty acids in water by sonication (vibronix), adjusting the pH to 7.4 with sodium hydroxide and resonicating the mixture.

RESULTS

Open chest rabbit heart: All the fatty acids exhibited positive inotropic and positive chronotropic effects (Fig. 1) on rabbit heart (Table I). The stimulant action was, however, noted only after 10±3 min and the maximum force of contraction could be observed after about 30±5

TABLE I: Effect of fatty acid on rate of contraction of open chest heart of rabbit
Av. beats per min.

Fatty acid 4 *mM*	*Dose ml/kg*									
	.05 *ml*		0.1 *ml*		0.2 *ml*		0.4 *ml*		0.6 *ml*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Oleic	160.8±5.2	171.6±2.6	161.2±6.8	177.7±4.6	160.3±4.8	190.3±2.9	162.2±5.0	216.2±3.6	162.0±6.0	220.2±4.9
Linoleic	165.3±2.8	177.0±3.5	167.5±3.0	186,7±7.2	165.0±3.2	193.2±4.0	166.4±3.0	218.7±4.5	164.6±3.2	221.0±5.2
Lauric	159.7±6.0	169.1±5.2	160.5±5.6	175.2±4.3	160.6±5.8	185.4±6.1	161.3±5.4	206.3±3.9	159.2±5.8	199.3±3.7
Myristic	160.5±5.6	173.1±4.6	160.7±6.8	178.7±2.5	162.2±4.9	188.3±3.0	160.5±5.3	200.5±8.4	163.7±2.5	206.2±3.0
Palmitic	155.2±7.8	168.7±6.3	158.3±5.3	179.7±3.5	154.8±6.8	189.4±5.9	156.4±6.9	213.4±6.4	155.3±5.3	214.4±4.8
Stearic	163.4±3.4	178.3±7.8	160.6±5.0	180.6±4.5	162.2±4.0	197.0±1.8	164.3±4.0	223.2±5.1	162.4±4.1	318.6±4.5

±Standard error of the mean of five observations.
The differences between control and experimental values in all the cases were found to be significant ($P<0.05$).

TABLE II: Effect of fatty acids on rate of contractions of isolated heart of rabbit.
Av. beats per min.

	Dose							
	0.2 *ml*		0.4 *ml*		0.6 *ml*		0.6 *ml*	
	Control	*Treated*	*Control*	*Treated*	*Control*	*Treated*	*Control*	*Treated*
Oleic	147.4±5.7	157.6±4.3	147.2±5.2	182.4±2.6	149.0±5.5	189.1±4.6	148.2±4.9	190.2±3.5
Linoliec	145.2±4.6	159.4±6.5	146.4±4.4	176.2±3.5	145.2±5.1	179.6±3.6	147.4±3.9	183.4±2.9
Lauric	157.5±1.8	169.2±5.2	155.4±2.3	187.2±4.3	158.0±2.4	192.2±3.5	156.5±2.5	192.3±3.0
Myristic	150.3±2.3	164.0±4.6	150.2±1.8	180.2±4.0	153.0±1.6	189.2±4.0	153.4±2.0	192.0±1.0
Palmitic	148.4±3.8	166.2±2.0	150.3±4.5	189.2±2.0	150.4±4.3	193.2±3.8	150.4±2.6	196.2±3.8
Stearic	155.3±2.0	174.9±3.5	155.0±2.5	192.4±1.8	157.3±2.6	202.0±3.9	155.5±3.1	201.6±5.4

+Standard error of the mean of five observations.
The differences between control and experimental values in all the cases were found to be significant ($P<.05$).

min from the time of injection of ,fatty acid. It was, however, note-worthy that the cardiac stimulant effect in this preparation was not as prompt as was observed in perfused frog heart (3). While guanethidine had no effect, propranolol *per se* could induce negative inotropic and nega-

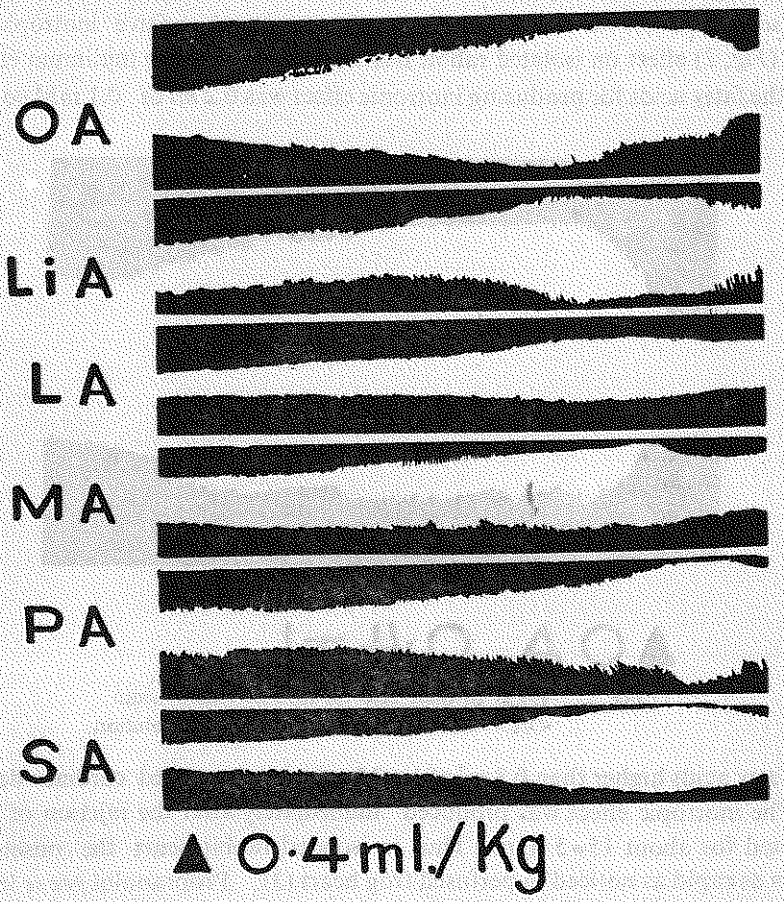

Fig. 1: Stimulant effect of fatty acids (4 mM) 0.4 *ml/kg* on open chest heart of rabbit.
OA — Oleic acid
LiA — Linoleic acid
LA — Lauric acid
MA — Myristic acid
PA — Palmitic acid
SA — Stearic acid

tive chronotropic effect. Treatment with reserpine or bilateral adrenalectomy reduced the rate and force of heart contractions. Neither propranolol, guanethidine nor reserpine could block the typical cardiac stimulatory response to fatty acid. In adrenalectomized rabbits also the fatty acids produced positive inotropic and positive chronotropic effects.

The minimum effective concentration for producing positive inotropic effects seemed to be different for medium long chain fatty acids and long chain fatty acids (0.5 *ml/kg* of 1 mM solution for oleic, linoleic, palmitic, stearic acid and 0.8 *ml/kg* of 1 mM concentration for lauric

and myristic acid). The most effective concentration for all the fatty acids was found to be 0.4 *ml/kg* of 4 mM solution. By administration of higher doses than 0.4 *ml/kg* of 4 mM solution the extent of force of contraction was not further increased.

Langendorff Isolated Rabbit Heart: All the fatty acids produced positive inotropic and positive chronotropic (*Fig.* 2) effect on isolated rabbit heart (Table II). The minimum effective concentration of all the fatty acids for producing inotropic effect was 0.2 *ml* of 0.1 mM solution. When

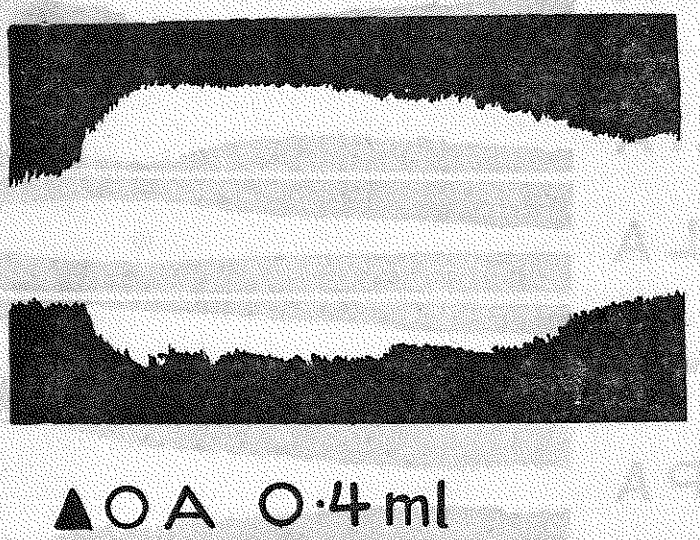

Fig. 2: Effect of oleic acid on isolated rabbit heatr.
OA — Oleic acid 0.4 *ml* of 1.0 mM solution.

the doses of fatty acids higher than 0.8 *ml* of 1 mM solution were tried cardiac depressant action was seen. The inotropic effect was produced immediately after the injetion of fatty acid and the effect lasted for about 15±5 min. Thus in isolated rabbit heart the onset of action after fatty acid was as rapid as noticed in perfused frog heart (3). Propranolol *per se* induced negative inotropic and negative chronotropic effect, however, it failed to change the usual response to fatty acid on isolated rabbit heart.

DISCUSSION

The present results demonstrating cardiac stimulation by long chain fatty acids in open chest heart as well as isolated heart preparation of rabbit confirm the cardiac stimulatory property of fatty acids in mammals. Whereas the amphibian heart exhibited more profound increase in inotropic response than chronotropic one (3), the mammalian heart showed significant increases in both inotropic as well as chronotropic response. The isolated rabbit heart exhibited prompt response as seen previously in the frog heart (3). On the other hand the induction of cardiac stimulatory response was late in open chest intact rabbit heart preparation. This may be

due to the fact that in the intact system, plasma protein would readily sequester large fraction of the administered fatty acids through binding and limit their direct uptake by the cardiac cells.

Since reserpinization, adrenalectomy or guanethidine treatment failed to alter the cardiac stimulatory phenomenon usually noted with fatty acids the possible involvement of catecholamines during the fatty acid actions on heart seems remote. The majority of adrenergic receptors in the heart are believed to be of beta type (1) and further both mechanical and metabolic changes after catecholamines have been attributed to the cardiac β-adrenergic receptors (2, 4, 17). Sutherland *et al.* (18) have suggested the association of beta receptors to adenyl cyclase which is substantiated by the observations that the treatment with beta blocking agents results in elimination of both metabolic and mechanical effects of beta receptor stimulation (13). The attempts that were made to discover the influence of cardiac beta receptor stimulation on extraction of lipid or cabohydrate metabolic substrates (6) reflect the association of the beta receptor with lipid metabolism. It was revealed that the beta receptor blockade induced marked decrease in myocardial uptake of free fatty acids without affecting glucose, pyruvate and lactate utilization (12, 15). Our previous findings in frogs (3) as well as present findings in mammals afford direct evidence indicating the importance of fatty acids in regulating the cardiac function. However, in earlier (3) as well as the present finding, propranolol which specifically blocks the myocardial utilization of FFA (7) failed to block the fatty acid response. Similarly the metabolic inhibitors like dinitrophenol and malonate could not interfere in FFA induced cardiac response (3) which makes it difficult to attribute this phenomenon to the metabolic role of fatty acids. It may be possible that FFA might have the metabolic role independent of oxidative phosphorylation. The exchange of electrolytes was found to be enhanced in presence of fatty acids (3). Whether FFA should interact with cardiac cell membrane to facilitate the transfer of substrates and electrolytes remains obscure at this stage. As a matter of fact fatty acids represent the category of surfactants and a number of surfactants have been reported to increase membrane permeability (5, 9).

REFERENCES

1. Ahlquist, R.P. Study of adrenotropic receptors. *Amer. J. Physiol.*, **153** : 586-599, 1948.
2. Berne, R.M. Effect of epinephrine and norepinephrine on coronary circulation. *Circulation Res.*, **6** : 644-645, 1958.
3. Chopde, C.T., D.M. Brahmankar, A.P. Hardas and A.K. Dorle. Influence of fatty acids on cardiac functions. *Ind. J. Med. Res.*, **61** : 1651-1657, 1973.
4. Clancy, R.L., T.P. Graham Jr., W.J. Powell Jr. and J.P. Gilmore. Inotropic augmentation of myocardial oxygen consumption. *Amer. J. Physiol.*, **212** : 1055-1061, 1967.
5. Feldman, S. and M. Gibaldi. Physiologic surface-active agents and drug absorption: Effect of sodium taurodeoxycholate on salicylate transfer across the everted rat intestien. *J. Pharm. Sciences*, **58** : 425-428, 1969.
6. Glaviano, V.V. and T.N. Masters. The effects of intracoronary norepinephrine on cardiac metabolism before and after beta-adrenergic blockade. *Fed. Proc.*, **26** : 771, 1967.
7. Glaviano, V.V. and T.N. Masters. Effect of norepinephrine on myocardial metabolism before and after beta receptor blockade. *Europ. J. Pharmacol.*, **7** : 135-142, 1969.

8. Hoak, J.C., W.E. Connor, J.W. Eckstein and E.D. Warner. Fatty acid induced thrombosis and death : Mechanism and prevention. *J. Lab. Clin. Med.*, **63** : 791-800, 1964.
9. Kasture, P.V. and D.M. Brahmankar. Influence of surface active agents on transfer of salicylate and salicylamide in everted rat intestine. *Ind. J. Pharm.*, **36** : 3-5, 1974.
10. Kreisberg, R.A. Effect of epinephrine on myocardial triglyceride and free fatty acid utilization. *Am. J. Physiol.*, **210** : 385-389, 1966.
11. Langendorff, O. In "Pharmacological experiments on isolated preparations" by W.L.M. Perry, E and S Livingstone Ltd. page 108-111, 1968.
12. Masters, T.N. and V.V. Glaviano. Effects of dL—propranolol on myocardial free fatty acid and carbohydrate metabolism. *J. Pharmacol. Exp. Ther.*, **167** : 187-193, 1968.
13. Moran, N.C. The development of beta adrenergic blocking drugs: A restrospective and prospective evaluation. *Ann. N.Y. Acad. Sci.*, **139** : 649-660.
14. Penefsky, Z.J. and B.F. Hoffman. Effect of stretch on mechanical and electrical properties. *Am. J. Physiol.*, **204** : 433-438, 1963.
15. Satchell, D.G., E. Freeman and S.V. Edwards. Effects of beta receptor blocking drugs on cardiac metabolism. *J. Biochem. Pharmac.*, **17** : 45-54.
16. Shipp, J.C., L.H. Opie and D. Challoner. Fatty acid and glucose metabolism in the perfused heart. *Nature*, **189** : 1018-1019, 1961.
17. Sonnenblick, E.H., J. Ross Jr., J.W. Covell, G.A. Kaiser and E. Braunwald. Velocity of contraction as a determinant of myocardial oxygen consumption. *Am. J. Physiol.*, **209** : 919-927, 1965.
18. Sutherland, W.E., G.A. Robinson and R.W. Butcher. Some aspects of the biological role of adenosine 3′-5′ monophosphate (cyclic AMP). *Circulation*, **37** : 279-306, 1968.
19. Williamson, J.R. and H.A. Krebs. Acetoacetate as a fuel of respiration in the perfused rat heart. *J. Biochem.*, **80** : 540-547, 1961.