SHORT COMMUNICATION

EFFECT OF KREBS CYCLE METABOLITES ON FROG HEART

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Summary: All the Krebs metabolites except pyruvate, lactate, acetate and succinate reduced the force and rate of myocardial contractions and also decreased cardiac output in frog. Succinate on the contrary was found to augment the rate and force of heart. The cardiac stimulation produced by epinephrine was reduced by fumarate, malate, oxaloacetate and alpha-oxoglutarate, whereas transaconitate and citrate produced only a slight inhibition. Pyruvate, lactate, acetate and succinate did not alter cardiac response to epinephrine.

Key words: Krebs metabolites heart intermediary metabolites

INTRODUCTION

Several workers have viewed the inhibitory effect of carbohydrate intermediary metabolites such as fumarate, oxaloacetate and citrate on cardiac function, except succinate which in small doses showed some cardiac stimulation (4). When tested for the antiarrhythmic action in electrically induced arrhythmia on Langendorff cat heart preparation it was noticed that except succinate all other metabolites, like acetate, pyruvate, fumarate, malate, citrate and lactate showed adverse effects on rhythm and rate of heart (2). Cascarano *et al* (1), however, reported that the combinations of Krebs metabolites such as fumarate, malate and glutamate or oxaloacetate and alpha-oxoglutarate together with 5 mM glucose in the perfusing medium could promote the physiologic performance of the anoxic heart. It was, therefore, thought desirable to reinvestigate the influence of Krebs metabolites *per se* on the functional activity of the frog heart.

MATERIALS AND METHODS

Rana tigrina weighing 200 to 300 *g* was decapitated and pithed. The heart was exposed, the right aorta was ligated while into the left a polyethylene cannula was inserted. The Ringer's solution as modified by Clark (Nacl 6.5 *g*, KCl 0.14 *g*, $CaCl_2$ 0.12 *g*, NaH_2PO_4 0.01 *g*, $NaHCO_3$ 0.2 *g*, glucose 2.0 g, distilled water to 1 litre) was perfused through the vena cava at a constant hydrostatic pressure of 47.8 mm Hg. Heart contractions were recorded by Starling heart lever. Frog heart was perfused with Krebs metabolites in Ringer (1 mM) for thirty minutes. The pH of the perfusing solution was 7.4. Heart rate (heart beats/min) and cardiac output (measured as outflow ml/min, through a cannula inserted into aorta) were measured at 10 minutes interval during the course of perfusion and were compared with control (perfused with plain Ringer solution only). Ten frogs were employed for the perfusion of each metabolite.

The cardiac responses to epinephrine (0.05 μg) were also recorded during continuous perfusion of heart with Krebs metabolites and compared with the control responses.

RESULTS AND DISCUSSION

Fumarate, malate, alpha-oxoglutarate, citrate and transaconitate were found to reduce the force and rate of myocardial contractions and also decreased cardiac output (Fig. 1, Table I). The negative inotropic effect produced by them was most significant as compared to the negative chronotropic effect. The cardiac depressant effects were observed only after 10 minutes, the ma-

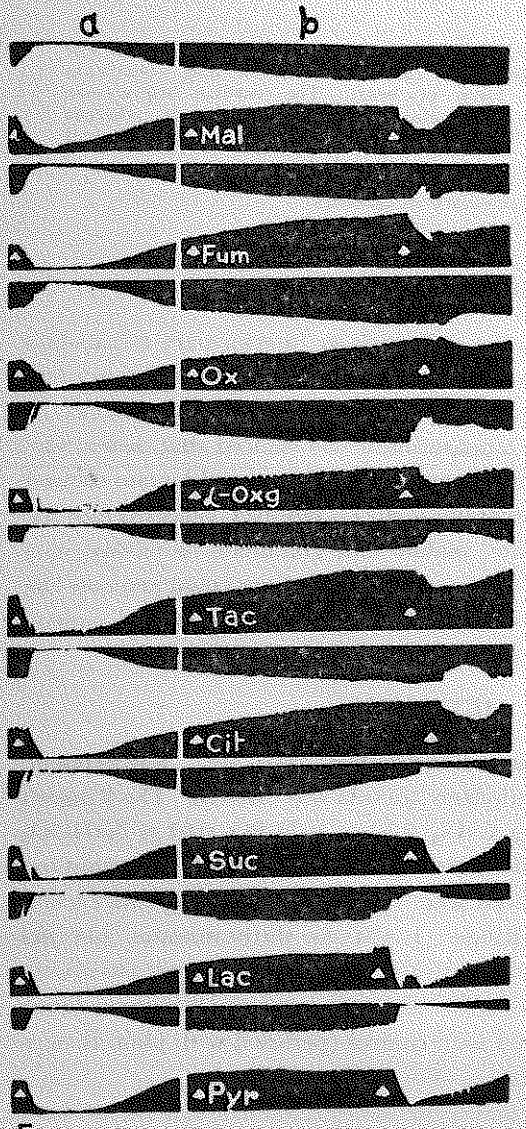

Fig. 1: Effect of epinephrine on frog heart during continuous perfusion with Krebs metabolites (1 mM)

a — Control responses to epinephrine (0.05 μg)
b — Response to epinephrine after 20 minutes of perfusion with Krebs metabolites.
E — Epinephrine
Mal — Malate
Ox — Oxaloacetate
Tac — Transaconitate
Suc — Succinate
Pyr — Pyruvate
Fum — Fumarate
Oxg — Alpha-oxoglutarate
Cit — Citrate
Lac — Lactate

ximal inhibition was noted at 20-30 minutes of their perfusion. Pyruvate, lactate and acetate had no adverse effect on cardiac activity, succinate, on the contrary was found to augment the rate and force of heart. The phenomenon as reduced cardiac activity in presence of some of the Krebs metabolites was further substantiated by the fact that the usual cardiac stimulatory response to epinephrine was reduced (Fig. 1) by malate, fumarate oxaloacetate and alpha-oxoglutarate after 20 minutes of their perfusion, whereas tranaconitate and citrate had only slight inhibitory action after 20-30 minutes of their perfusion. Succinate, acetate, pyruvate and lactate did not cause any change in the cardiac stimulant action of epinephrine.

TABLE I : Mean percentage change in heart rate (beats/min) and cardiac output (volume ml/min) at 30 minutes of Krebs metabolites perfusion

Metabolite 1 *mM*	*Percentage change in*	
	Heart rate	*Cardiac output*
Fumarate	13.8 ± 2.1*	51.5 ± 4.5*
Malate	26.7 ± 3.8*	62.0 ± 6.7*
Oxaloacetate	11.7 ± 0.9*	29.2 ± 1.9*
Alpha-oxoglutarate	17.8 ± 1.8*	30.5 ± 4.2*
Citrate	10.0 ± 1.5*	11.5 ± 2.6*
Transaconitate	10.0 ± 1.5*	26.1 ± 2.1*
Succinate	16.6 ± 3.4*	34·7 ± 0.8*
Acetate	3.0 ± 0.5	1.0 ± 0.27
Pyruvate	2.8 ± 1.0	4.7 ± 1.3
Lactate	4.2 ± 1.6	2.2 ± 1.5

All the figures indicate negative change except in case of succinate which showed augmentation of heart rate and cardiac output.

* These changes were found to be significant ($P<0.05$).

± Standard error of mean of five observation.

The above observations are in accordance with those of Tripod *et al.* (4) who demonstrated cardiac inhibition by the same Krebs metabolites in rabbits. The mechanism of the adverse effect produced by various intermediary metabolites of Krebs cycle excepting some like pyruvate, lactate, acetate and succinate on heart is intriguing since they are associated with the energy generating reactions. Miyaji and Sacki (2) have demonstrated the stimulatory action of succinate on heart. Our observations with regard to the action of succinate on heart are also similar. A considerable improvement was demonstrated in the physiological performance of the perfused anaerobic rat heart by increasing the concentration of glucose from 5 to 40 mM in the perfusing medium (3) or the anaerobic perfusion conducted with 20 mM glucose combined with fumarate+malate+glutamate or oxaloacetate+alpha-oxoglutarate. However these situations

were found to implicate the accumulation of succinate many fold higher than in hearts perfused with 20 mM glucose alone and it is suggested that succinate itself being cardiac stimulatory as reported by some workers (2,4) could have been responsible for improvement of cardiac activity during the perfusion of several Krebs metabolites in combination.

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