EFFECTS OF METABOLIC SUBSTRATES ON ISOLATED RABBIT ATRIA AT LOW TEMPERATURE

By

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Cold causes profouned depression of the myocardial activity (2) which could be due to alteration in the normal metabolic pathways leading to a lack or excess of certain metabolic substrates. Nakamura *et al.* (3) reported myocardial depressant effect of high concentrations of pyruvate, acetate, butyrate, lactate and succinate on the isolated perfused rat heart. Webb (5) studied the effects of important metabolic substrates on the isolated rabbit atria at 30°C. He observed that all the metabolic substrates studied depressed the atria except citrate which at higher concentrations produced a positive chronotropic effect. The present work was undertaken to study tne effect of some metabolic substrates on the rabbit atria maintained at 25°C and 20°C.

MATERIALS AND METHODS

Fifty four rabbits of either sex weighing between 1 to 2 *kg.* were used in this study. They were killed by a blow on the head and the heart was rapidly removed and kept in continuously oxygenated Tyrode's solution. The atria were dissected from the heart and mounted in an isolated organ both containing 100 *ml.* of Tyrode's solution. Gradual hypothermia was produced by adding ice into the outer jacket of the organ bath. The temperature of organ bath was maintained at 25°C or 20°C by allowing a regulated flow of cold water into the outer Jacket. The atrial contractions were recorded on a smoked kymograph paper.

Molar solutions of metabolic substrates were prepared by dissolving sodium salts of pyruvate, malate, succinate, butyrate, lactate, acetate and citrate in distilled water, and a neutral pH was attained by neutralizing with 10% NaOH. The test solutions of different concentrations (0.0001 M to 0.2M) were prepared from the Molar solutions by diluting with Tyrode's fluid. 100 *ml.* of the test solution was kept in an ice bath till its temperature fell to the desired level. Subsequently the Tyrode's solution of the organ bath was replaced by 100 *ml* of the test solution.

RESULTS

Control atrial contractions were recorded at 25°C or 20°C. Subsequently the Tyrode's solution was replaced by test solution cooled to the same temperature and the effects on rate and

amplitude of contraction were recorded. The magnitude of the effects was expressed as percentage change above or below the control level (Table I)

TABLE I

Effects of metabolic substrates on the rate and amplitude of contraction.

Substrate	*Temp. in °C*	*Mean percentage change in rate/min.*	*Mean percentage change in amplitude*	*Molar concentration*
Sodium pyruvate	25	+15.7	+18.68	0.0001—0.02
	20	— 8.8	— 9.3	0.0001—0.02
Sodium acetate	25	+10.4	—21.7	0.0005—0.001
	25	—20.6	—8.1	0.01 —0.03
	20	+ 5.5	—30.0	0.0005—0.001
	20	—11.1	—22.0	0.01 —0.03
Sodium lactate	25	—4.5	—38.8	0.001 —0.03
	20	+11.5	—29.6	0.001
Sodium butyrate	25	+2.3	—5.1	0.0005—0.01
	20	+26	—2.5	0.001
	20	—12	+37.0	0.01
Sodium citrate	25	+2.6	—11.3	0.0001—0.01
	20	+5.1	—25.6	0.0001—0.01
Sodium succinate	25	+1.1	+4.8	0.005 —0.01
	20	—18.4	—17.9	0.005 —0.01
Sodium malate	25	—5.5	—15.6	0.0001—0.005
	25	+11.5	—13.5	0.02
	20	+36.2	Irregular	0.02

Sodium pyruvate produced a mild positive inotropic and chronotropic effect at 25°C. Positive chronotropic effect was particularly marked with higher concentration (0.02 M) of sodium pyruvate. At a temperature of 20°C. sodium pyruvate depressed atrial activity. Typical effects of pyruvate on isolated atria at 25°C. (upper panel) and at 20°C. (lower panel) are shown in Fig. 1.

Sodium acetate in low concentrations (0.0005—0.001 M) stimulated the rate but hihg concentrations (0.01—03 M) depressed the rate at 25°C as well as at 20°C. The amplitude was invariably reduced with all concentrations of the acetate.

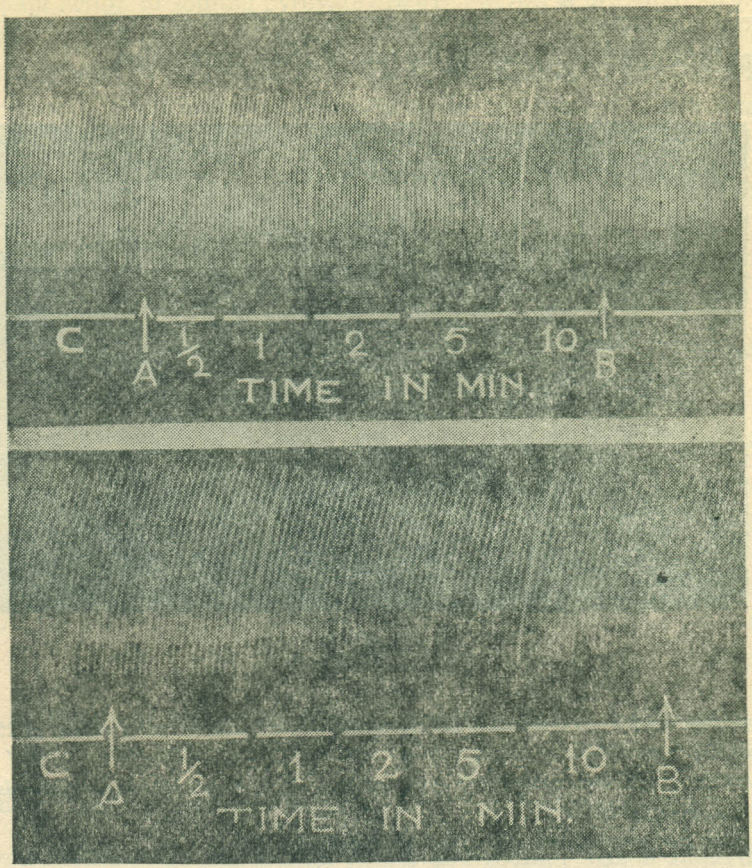

Fig. 1

Kymographic records of the effects of pyruvate (0.0001 *M*) *at* 25°*C* (*upper panel*) *and at* 20°*C* (*lower panel*) *on the contractions of isolated rabbit atrium.*
C, control contractions.
A, pyruvate added and contractions recorded at ½, 1, 2, 5 *and* 10 *minute intervals.*
B, pyruvate washed.
Note that at 25°*C pyruvate increased the amplitude and rate of the atrial contractions. However, at* 20°C *pyruvate depressed the amplitude of atrial contractions.*

Sodum lactate (0.001—0.03 M) consistently depressed the amplitude of artial contractions at 25°C and 20°C. However, at 20°C a positive chronotropic action was observed at a concentration of 0.001 M.

Low concentration of sodium butyrate at 20°C stimulated the rate and depressed the amplitude while high concentration had reverse effect. It showed insignificant effects at 25°C.

Sodium citrate (0.0001—0.01 M) at 25°C and 20°C had a definite inhibitory effect on the amplitude of atrial contraction. In figure 2 are shown the typical effects of citrate (0.005 M) 25°C. There was little effect of citrate on the rate of atrial contraction.

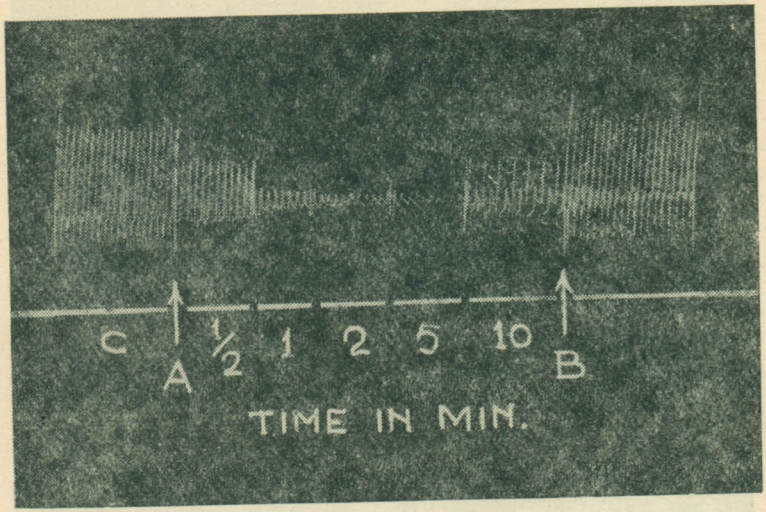

Fig 2

Kymographic record of the effect of citrate (0.005 *M*) *on isolated rabbit atrium at* 25°*C.*
C, control contractions.
A, pyruvate added and contractions recorded at ½, 1, 2, 5 *and* 10 *minute intervals.*
B. Pyruvate washed.
Note that citrate markedly depressed the amplitude.

Sodium succinate, at a temperature of 25°C was found to have an initial negative inotropic effect followed by a mild positive inotropic effect after 10 minutes. The atrial rate was not effected with succcinate at 25°C, however, at a temperature of 20°C it reduced both the rate and the amplitude.

Sodium malate (0.0001—0.005 M) produced a depression in the rate and amplitude of artrial contractions at 25°C. Higher concentration of sodium malate (0.02 M) had a positive chronotropic action on the heart at 25°C which was still more marked at 20°C and was associated with a negative inotropic action. This concentration of malate also induced atrial arrhythmia characterised by inequality of beats.

DISCUSSION

During hypothermia the depressed myocardial activity could be due to a deficiency of substrate (s). External supply of deficient substrate may provide energy through metabolic pathways, or the substrate may have a direct action on the heart.

The stimulant action of sodium pyruvate observed in this study could be due to incorporation of the pyruvate in TCA cycle, thus increasing the available energy for myocardial contraction. Similar stimulant action of pyruvate was observed by Webb (5) in substrate depleted rabbit atria at normal temperature. Such incorporation is likely to be hindered at low temperature. The depressant effect of pyruvate at 20°C suggests some other mechanism of action.

Depressant effects of succinate have also been reported by Forssman and Lindsten (1) on rabbit heart, Salant *et al.* (4) on isolated frog heart and by Webb (5) on rabbit atria. Sodium malate depressed the heart. However, with higher concentrations, an increase in the rate associated with negative inotropic action was observed.

It is possible that the stimulant action of low concentration of acetate, lactate, and butyrate on rate is metabolic.

The inhibitory effect of sodium citrate could be the result of chelation of calcium which is essential for muscular contraction.

It is clear that the pacemaker and contractile mechanism of heart can be independently involved by substrate action.

SUMMARY

1. The effects of metabolic substrates namely pyruvate, malate, succinate, butyrate, lactate, acetate and citrate were studied on isolated rabbit atria at 25°C and 20°C.

2. Sodium pyruvate had a stimulatory effect at 25°C but depressant effect at 20°C.

3. Sodium acetate, sodium lactate, sodium citrate and sodium malate showed a negative inotropic effect. However, some concentrations of these had a positive chronotropic effect. Sodium malate also induced arrhythmia.

4. Sodium succinate depressed the atrial activity at 20°C.

5. Lower concentrations of sodium butyrate increased the rate but depressed the force of atrial contraction, where as higher concentrations depressed the rate and increased the force of contraction at 20°C.

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