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Effect of Starvation on Contractility of Lidocaine-Depressed Isolated Rat Atria

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ABSTRACT

The experiments were performed to determine whether lidocaine interferes with the utilization of lipid as source of energy fuel for the contractile process by the isolated rat atria.

Rats were starved for two days in order to inerease the lipid content of the heart. Atria from starved rats were better able to maintain their contractility in the absence of exgenous substrate, and also were more resistant to depression by lidocaine than atria from fed rats. Starvation results in a marked loss of body weight in rats. In contrast to the starved rats, the body weight of fed rats inereased with time. The smaller reduction in contractile activity of atria from the starved rats may suggest that endogenous lipid accumulates during starvation period and is used as an energy source for the contractile process in the face of a lidocaine-induced blockade in glycolysis.

Key Words: Lidocaine, Contractility, Heart, Starvation, Lipid

INTRODUCTION

This study is a continuation of investigation from our previous reports dealing with the mechanism of the cardiac depressant action of inhalation anesthetics (Ko and paradise, 1969a, 1970b, 1970b, 1971b, 1971c, 1972b, 1973a, 1975) and barbiturates (Ko and Yoon, 1980; Ko, 1981; Ko and Paradise, 1983; Ko, 1989) on isolated rat atria and human hearts. Having ruled out interference with the supply of oxygen by halothane as the basis for its depressant action (Paradise and Griffith, 1966), we next focused our attention on glycolysis. That pyruvate in rat and human atria, and acetate, lactate, and fructose in rat atria, could overcome the contractile depression induced by halothane, while glucose was ineffective in rat and human atria, suggested that halothane was blocking an early step in glycolysis (Ko and Paradise, 1969a; Paradise and Ko, 1970; Ko and Paradise, 1970a, 1970b). Since fructose apparently is metabolized via the phosphofructokinase step (Paradise and Ko, 1970; Ko and Paradise, 1970c), the probable sites of halothane blockade were confirmed to; 1) uptake of glucose into the cell; 2) phosphorylation of glucose to glucose-6-phosphate by hexokinase; or 3) conversion of glucose-phosphate to fructose-6-phosphate by glucose phosphate isomerse (Paradise and Ko, 1970).

It is evident that local anesthetic lidocaine also depress the cardiac function (Austen and Moran, 1965; Contantino *et al.*, 1969; De Jong *et al.*, 1973; Kahn *et al.*, 1986, 1990). It has been demonstrated that the cardiac depressant action of lidocaine is at least partly linked to a block at an early step or steps in the glycolytic pathway in the heart, as shown by the abilities of pyruvate, acetate, and fructose, but not glucose, to produce a positive inotropic effect in rat atria depressed by lidocaine (Lim and Kim, 1984; Ko and Sohn, 1986), similar to those from the experiments with inhalation anesthetics (Ko and Paradise, 1969a; Paradise and Ko, 1970) and barbiturates (Ko, 1989).

The purpose of the present study is to deter-

mine whether lidocaine interferes with the utilization of lipid as a source of fuel for the contractile process by the isolated rat atria. It would be of intrest to add fatty acids to the lidocaine-depressed isolated atria and determine whether the force of contraction could be increased, an approach found useful in our previous experimints with other substrates. Fatty acids, however, are insoluble in the saline medium bathing the atria, so they must be coupled to albumin or other protein to remain in solution. The presence of protein, however, prevents the bubbling process for admission of oxygen, since foam results. In additon, it is impossible to be sure which fatty acid should be added, as several are used by the heart in vivo. For the reasons we decided to increase the endogenous supply of lipid in the heart prior to sacrifice by the simple and well-known process of starvation (Evans, 1964). These experiments provide evidence for the view that lidocaine has little or no effect on the utilization of endogenous lipid for contractility by rat atria.

MATERIALS AND METHODS

Male rats weighing 180 to 200g were decapitated, and the atria were removed and suspended in modified Krebs-Ringer bicarbonate glucose medium (Gimeno *et al.*, 1965, 1966, 1969).

The medium was gassed with 95% O_2 -5% CO_2 at pH 7.4 and 30℃. The mechanical activity of rat atria electrically stimulated at a rate of 200 per minute in the medium was determined using a sensitive strain gage as previously described (Gimino *et al.*, 1966; Ko *et al.*, 1969b; Ko and Paraidse, 1973a).

In the experiments with substrate-free medium, the normal Krebs-Ringer bicarbonate glucose medium was changed to substrate-free medium (free of glucose) following the one-hour equilibration

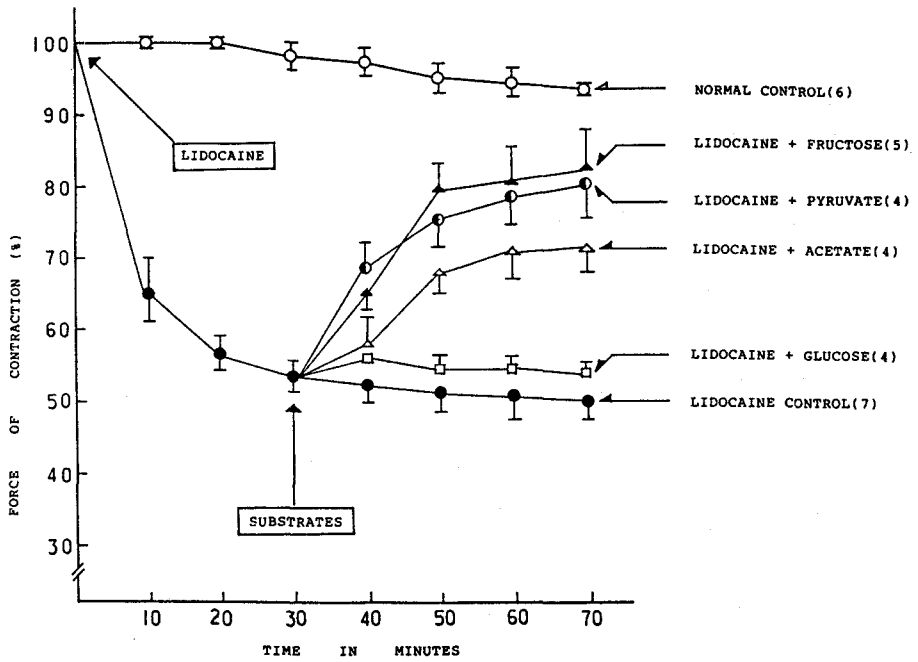

Fig. 1. Effects of substrates on contractility of isolated atria depressed with lidocaine (0.1mM). Substrates were added 30 minutes after the addition of lidocaine (0.1 mM). In this and subsequent figures, lidocaine was added at zero time (following a 60 minutes equilibration period in the normal Krebs-Ringer bicarbonate glucose medium). Vertical bars represent one standard error of the mean. Parentheses represent number of experiments.

period. In the experiments with starvation, rats were starved for one to three days prior to the time they were killed. The atria from the starved rats were followed to beat in the normal Krebs-Ringer bicarbonate glucose medium for a 60 min equilibration period.

RESULTS

Effects of metabolic substrates on atrial contractility depressed with lidocaine

Various substrates were added to the bathing medium 30 minutes after the atria were depressed approximately 50% with 0.1 mM lidocaine. It is evident from Fig. 1 that pyruvate, acetate, and fructose partially restored the force of contraction of isolated rat atria depressed with lidocaine, but additional glucose was without effect. The results are in agreement with the previous reports (Lim and Kim, 1984; Ko and Sohn, 1986).

Effect of starvation on body weight of rats

Figure 2 shows that the body weight of starved rats decreased markedly with time. In contrast to the starved rats, the body weight of fed rats increased with time. It is also evident from the Fig. 2 that the decreased rate in body wight of starved rats were more than two times greater than that of the increased body weight of fed rats.

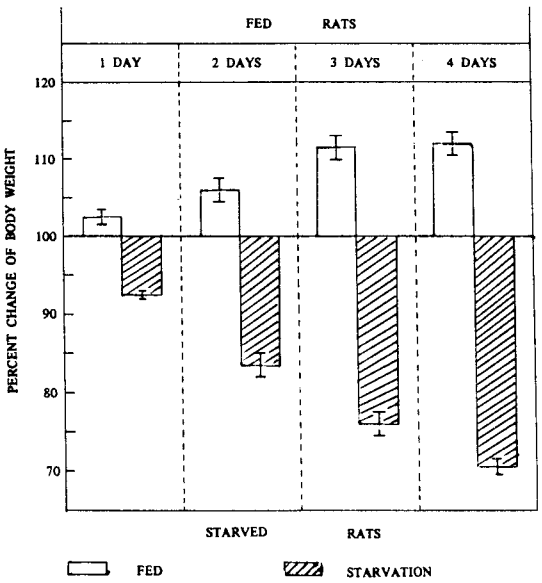

Fig. 2. Percent changes in body weight of fed and starved rats. Vertical lines represent standard error of the mean (10 cases).

Effect of starvation on depression rate of atrial contractility in substrates-free medium

The experiments were performed to determine the importance of endogenous substrates for the contractile force of atria from starved rats by comparing the rate of contractile depression of these atria in substrate-free medium to that of fed atria. After a one-hour equilibration period, the normal Krebs-Ringer bicarbonate glucose (5.5 mM) medium was replaced with substrate-free

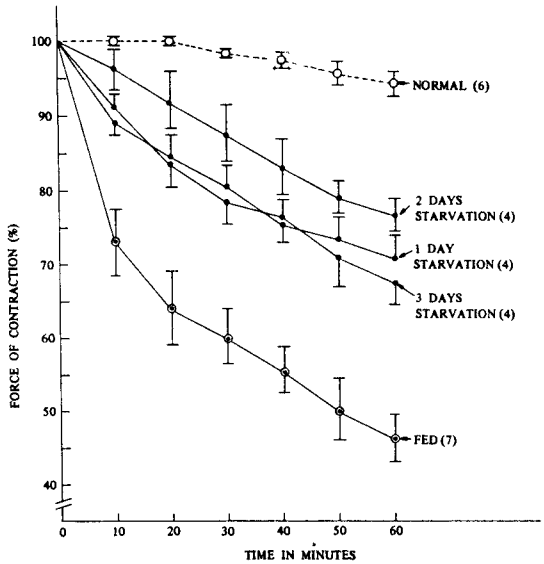

Fig. 3. Effect of starvation on developed tension of isolated atria in substrate-free medium. Normal represnts force of contraction of isolated atria from fed rats in the normal glucose-containing medium. All other curves represent the force of contraction of atria from fed of starved rats incubated in normal goucose medium for one hour, then exposed to the goucose-free medium for an additional 60 minutes. Values in parentheses represent number of experiments. Vertical vars indicate standard error of the mean.

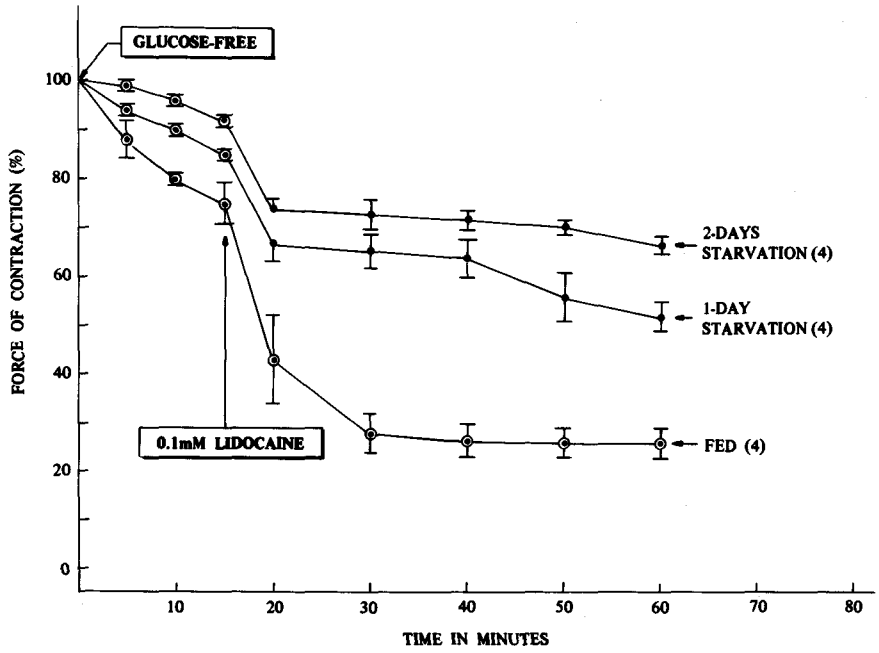

Fig. 4. Effects of lidocaine on contractility of substrate-depleted atria from fed and starved rats. At zere time (following one hour eqilibration period), the normal glucose medium was changed to substrate-free medium. Fifteen minutes later 0.1 mM of lidocaine was administered to this substrate-free medium. Vertical bars represent standard error of the mean. Parentheses represent number of experiments.

medium (free of glucose). The results are summarized in Fig. 3. The Fig. 3 shows the effects of omission of exogenous substrate glucose from the medium on the tension developed by atria from fed and starved rats following a one-hour equilibration period in the normal medium. The Fig. 3 also shows that when the atria from starved rats are suspended in substrate-free medium, they have a significantly smaller reduction in contractility than those of fed atria. It is also evident from the Fig. 3 that the maximal depresseion of contractile force of atria from two days starved rats was even less than those of atria from one or three days starved rats. The results are in agreement with the previous report that two days of starvation is the optimum time period for accumulation of readily utilizable endogenous substrates by the atria (Ko, 1977). Thus, the atria from one and two days starved rats were chosen for the experiments

Effects of lidocaine on contractility of atria from starved rats in the absence of glucose

Experiments were performed to determine the effect of lidocaine (0.1mM) to produce a maximal decrease in the force of contraction of substrate-depleted atria from starved rats in comparison to those on substrate-depleted atria from fed rats. In these series of experiments, the atria were obtained from one and two days starved rats. The atria from starved rats were exposed to substrate-free medium immediately after the one hour equilibration period in Krebs-Ringer bicarbonate glucose medium. After 15 minutes incubation of atria from the starved rats in this substrate-free medium, lidocaine, at concentration of 0.1mM as a same amount in the experiments with the atria from fed rats, was added to the bathing medium in which the atria from starved rats were beating. The results are shown in Fig. 4. It is evident from Fig 4 that lidocaine produced less depression in

the force of contraction of substrate-depleted atria from two days starved rats than those from one day starved rats.

DISCUSSION

In an effort to determine the mechanism of the depressant action of local anesthetics on cardiac contractility, we have carried out investigations in isolated rat heart preparation (Ko *et al.*, 1986, 1990). Depression of atrial contractility by lidocaine was overcome by the metabolizable substrates pyruvate, acetate, and fructose, but not by additional glucose in rat atria (Fig. 1). However, it has been demonstrated in the literatures (Ko and Paradise, 1973b) that additional glucose produced the dose-dependent increase in the force of contraction of normal atria, whereas the addition of pyruvate, acetate, and fructose produced no significant effect in the contractile activity of the normal atria. The results are similar to those from the previous experiment with inhalation anesthetics halothane (Ko and Paradise, 1969a; Paradise and Ko, 1970) and methoxyflurane (Ko and Paradise, 1973a) or intravenous anesthetics of pentobarbital and thiopental (Ko and Yoon, 1980; Ko and Paik, 1983).

Since fructose is apparently metabolizable via phosphofructokinase (PFK) step, this implicated glucose uptake, phosphrylation of glucose to glucose-6-phosphate, or isomerization of glucose-6-phosphate to fructose-6-phosphate as the site of lidocaine blockade, similar to those of halothane (Ko and Paradise, 1971b; Paradese and Ko, 1970).

In this study, rats were starved for one or two days in order to increase the lipid content of the heart, since it has been demonstrated that starvation increases endogenous lipids in the heart (Evans, 1964). Atria from starved rats suspended in substrate-free medium showed a significantly smaller reduction in contractility than those from fed rats (Fig. 3).

This implies a greater availability of endogenous substrate for the contractile process in atria from starved rats. It is in agreement with the previous report by Ko and Paradise (Ko, 1977), that the starved rats effectively accumulate the endogenous substrates during the starvation period and actively utilize the endogenous substrates which are important for the contractile process (Fig. 3). However, the body weight of starved rats was markedly decreased with time (Fig. 2). It is further demonstrated that when the atria from starved rats are suspended in substrate-free medium, the atria from two days starved rats have a significantly smaller reduction in contractility than do those from one day or three days starved rats (Fig. 3). The results are consistent with the previous report by Ko and Paradise that the starved rats effectively accumulate the endogenous substrates during the starvation period and actively utilize the endogenous substrates which are important for the contractile process (Ko and Paradise, 1972c).

Lidocaine (0.1 mM) produced less depression of the force of contraction of atria from starved rats than those from fed rats in substrate-free medium (Fig. 4), and it was further observed that the same concentration of lidocaine produced much less depression of contractility of atria from two days starved rats than those from one day starved rats (Fig. 4). The results indicating that lidocaine less depress the contractility of atria from starved rats than those from fed rats may be explained by the following possibilities. Endogenous substrates, less sensitive or insensitive to anesthetic lidocaine, accumulated in the atria during the starvation process and served as part of the energy supply during lidocaine's action. It is suggested that endogenous substrate accumulate during starvation and can be used as source of fuel for the contractile process. Lipid, as well as glycogen, accumulates in the heart during starvation and is utilized in virto (Evans, 1964; Ko and Paradise, 1972c).

Thus, the most likely explanation for the smaller lidocaine's depressant action on contractility of atria from starved rats than those from fed rats is the gearter accumlation of endogenous lipid during the starvation process. This lipid is utilized for maintenance of the force of contraction of atria in the presence of lidocaine. Sites between fatty acids to acetyl CoA in the myocardial cells is not affected by lidocaine or at least is less sensitive than an early site in glycolysis (above the PFK step). These experiments provided evidence that lidocaine produced apparently less depression of

contractility of atria from starved rats than those from fed rats, indicating that lidocaine has little or almost no effect on the utilization of endogenous lipid for contractile activity of rat atria. Furthermore, it has been recently reported that inhalation anesthetics do not alter the affinity of cardiac troponin C for calcium binding site for the negative inotropic action of inhalation anesthetic agents (Blank *et al.*, 1992).

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=국문초록=

Lidocaine 억제 심장의 수축성에 대한 내인성 지질의 영향

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고 계 창

기아(starvation)기간중 흰쥐의 심근세포내에 심장의 수축 기능에 필요한 내인성 대사 기질인 지질의 축적이 증가된다는 사실이 알려져 있다. 본 연구에서는 lidocaine의 심근내 지방대사에 대한 영향을 기능적 측면에서 관찰하기 위하여, 굶긴 쥐 적출 심장의 수축성에 대한 lidocaine의 영향을 검토한바 다음과 같은 실험결과를 얻었다.

1. 굶긴 쥐 체중은 정상쥐에 비하여 현저히 감소되었으며, 기아시작 4일에서 약 30%의 체중감소를 나타냈다. 그러나 정상쥐의 체중은 증가되었다.

2. 정상 쥐의 적출 심방은 기질제거 용액에서 30분에 약 40%의 현저한 수축력의 감소를 보였다. 그러나 2일간 굶긴 쥐의 적출 심장은 기질제거용액에서 30분에 약 13%의 수축력의 감소를 보여 정상 쥐에서의 수축력 저하보다 현저히 낮은 감소율을 나타냈다.

3. Lidocaine(0.1mM)에 의한 흰쥐 적출 심장의 수축력 감소는 정상쥐에 비해 굶긴 쥐 적출 심장의 수축력이 작게 감소되었다. 또한, lidocaine에 의한 굶긴 쥐 적출심장의 감소율은, 1일간 굶긴 쥐 보다 2일간 굶긴 쥐의 적출심장이 현저히 더 작게 감소되었다.

이상의 결과로 미루어 보아, 굶기는 동안 쥐 심근 내에 축적된 내인성 대사 기질인 지질이 lidocaine에 의해 해당과정이 억제된 심장의 수축과정에 energy원으로 쓰여지고 있을 가능성을 시사하고 있다.