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Effect of Lidocaine on Utilization of Endogenous Substrates for Contractile Process of Isolated Rat Atria

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ABSTRACT

The experiments were performed to determine whether the cardiac depressant action of lidocaine is directly associated with the utilization of endogenous substrates in isolated rat atria, by using citrate and bicarbonate-free medium known as potent inhibitors of phosphofructokinases (PFK) enzyme step. Citrate and bicarbonate-free medium produced negative inotropic action of isolated rat atria incubated in normal Krebs-Ringer bicarbonate glucose medium. Pyruvate and acetate increased the force of contraction of atria depressed by citrate or bicarbonate-free medium, whereas fructose was without effect indicating the inhibitory effect of citrate and bicarbonate-free medium at some point in the glycolytic pathway such as the PFK step in atria. In the absence of exogenous substrate, citrate and bicarbonate-free medium produced a marked depression of the force of substrate-depleted atria indicating that utilization of endogenous substrate above the PFK step, probably cardiac glycogen, is also impaired by citrate or bicarbonate-free medium.

Lidocaine produced further depression of the contractile force of atria depressed by citrate. These results argue strongly for an additional mechanism of cardiac depression caused by lidocaine involving the sites below the PFK.

Key Words: Lidocaine, Heart, Citrate, Bicarbonate-free medium, Phosphofructokinase

INTRODUCTION

Since the cardiac depressant action of anesthetic agents attracted attention, the negative inotropic action of anesthetics has been well documented by means of many studies dealing with the mechanism of the cardiac depressant action of inhalation anesthetics (Ko and paradise, 1969a, 1970a, 1970b, 1971a, 1971b, 1972a, 1972b, 1973a, 1975; Blanck *et al.*, 1992) and barbiturates (Ko and Yoon, 1980; Ko, 1981; Ko and Paradise, 1983; Ko. 1989) on isolated rat atria and human hearts. We focused our attention on glycolysis. That pyruvate in rat and human atria, and acetate, lactate, and frouctose 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; 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 confirmend 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). Local anesthetics, lidocaine, procaine, and bupivacaine are known to depress cardiac contractility in a dose-dependent fashion (Ahn, 1994; Austen WG and Moran JM, 1965; Dontion *et al.*, 1969; Ko *et al.*, 1986, 1990; Park *et al.*, 1992; Lim and Kim, 1984; Liu *et al.*, 1982; Sage *et al.*, 1983; Ko, 1994), but the mechanism by which they do this is not fully clar-

ified.

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). It is also reported that lidocaine has little or no effect on the utilization of endogenous lipid for contractility by rat atria (Ko, 1994). The purpose of this study is to determine whether lidocaine interferes with the utilization of endogenous substrates as source of energy fuel for the contractile process by the isolated heart, by using citrate and bicarbonate-free medium known to inhibit PFK enzyme step of glyotytic pathway of the heart (Ko and Yoon, 1980).

MATERALS AND METHODS

Male rats weighing 180 to 200 g were decapitated, and the atria were removed and suspended in modified Krebs-Ringer bicarbonate glucose medium (Gimeno *et al.*, 1965, 1966, 1969, 1972c).

The medium was gassed with 95% O_2~5% CO_2 at pH 7.4 and 30°C. 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.*, 1969; Ko and Paradise, 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 period. In the experiments with bicarbonate-free medium, the experimental procedures were conducted by means of techniques previously described by Ko *et al.* (Ko and Paradise, 1971b; Paradise and Ko; 1970). The bicarbonate-free medium was prepared by replacing the sodium bicarbonate from the Krebs-Ringer bicarbonate glucose medium with an equivalent concentration of sodium chloride and bubbling with 100% oxygen. The pH of the bicarbonate-free medium was initially adjusted with dilute sodium hydroxide to 7. 4 just prior to experimental procedure. The electrode, placed in the tissue bath to monitor pH, demonatrated no significant change from 7.4 throughout the course of the bicarbonate-free medium experiments.

In the experiments with citrate, sodium citrate produced dose-dependent decrease in the force of contraction of isolated rat atria. Thus, 1.5 mM of sodium citrate was chosen for this experiment because it produced about the smae degree of depression as that seen with other cardiac depres-

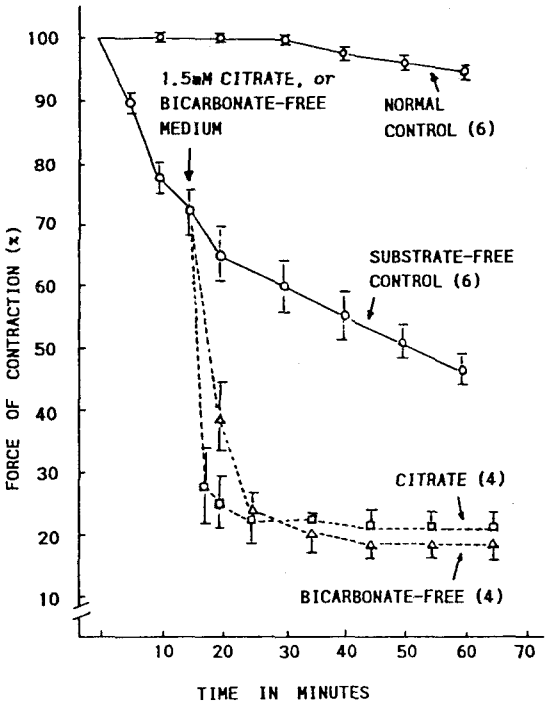

Fig. 1. Effect of citrate and bicarbonate-free medium on contractility of substrate-depleted atria.
Zero time represents a one hour equilibration period in the normal Kredbs-Ringer bicarbonate medium containing 5.55 mM glucose.
At zero time, medium was changed to one free of lucose (substrate-free).
Fifteen minutes later, 1.5 mM citrate was added or medium was changed to bicarbonate-and substrate-free.

sants (Ko and Paradise, 1970c; Ko, 1981; Ko and and Paik, 1983). After sixty minutes equilibration period in the normal Krebs-Ringer bicarbonate glucose medium, 15 mM sodium citrate was added to the bathing medium.

RESULTS

Effects of citrate and bicarbonate-free medium on contractility of substrate-depleted atria

Following a one hour equilibration period in the normal Krebs-Ringer bicarbonate glucose medium (zero time in Fig. 1) the medium was changed to one free of glucose (substrate free). After 15 minutes incubation of atria in this substrate-free medium, 15 mM citrate was added to atria, or the substrate-free medium was again changed to bicarbonate-free medium.

The results are shown in Fig. 1. It is evident from the Fig. 1 that the force of contraction of atria declined due to prolonged activity in substrate-free medium, in comparison with the normal control level. However, it is also evident from the Fig. 1 that substrate-free treated atria were markedly depressed by citrate, or by bicarbonate-free medium.

Effect of pyruvate and fructose on contractility of atria depressed by citrate or bicarbonate-free medium

Having established the ability of fructose as an energy source for contraction, it is important to determine the pathway by which fructose is utilized. It has been previously reported that car-

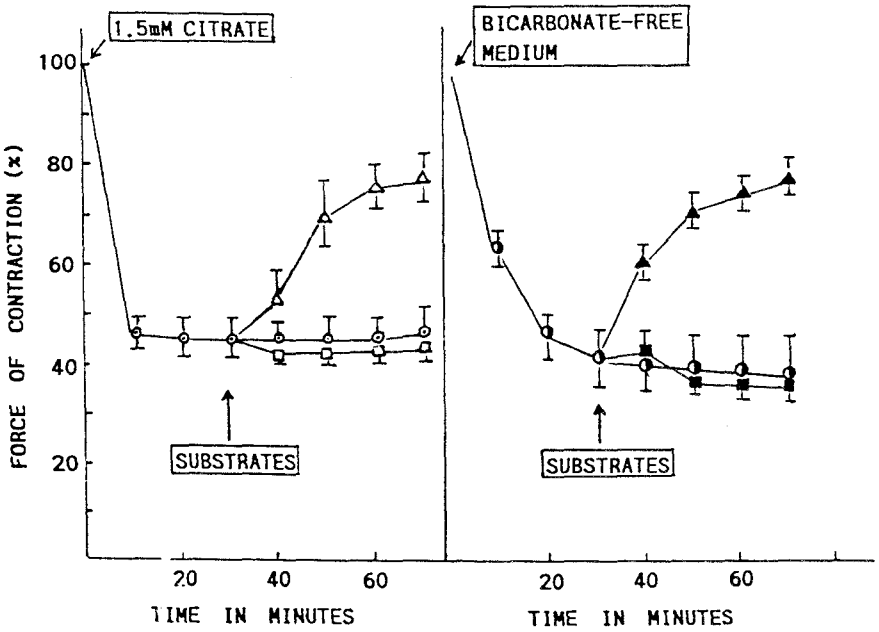

Fig. 2. Effect of pyruvate or fructose on contractility of isolated rat atria depressed by citrate or bicarbonate-free medium.

At zero time, 1.5 mM sodium citrate was added to normal medium or the normal medium was changed to bicarbonate-free medium.

Thirteen minutes later, sodium pyruvate(5 mM) or fructose (30 mM) were added to the atria.

●–●: Citrate control(6)
△–△: citrate + pyruvate(6)
□–□: Citrate + fructose
◑–◑: Bicarbonate-free control (6)
▲–▲: Bicarbonate-free+pyruvate(6)
■–■: Bicarbonate-free+fructose(6)

diac contractility is depressed by citrate (Ko and Paradies, 1970c; Webb, 1950) or bicarbonate-free medium (Berman and Saunders, 1955; Ko *et al.*, 1969b). Biochemical studies using homogenates of various tissues indicate that citrate is a potent inhibitor of phosphofructokinase (PFK) (Ko and Paradise, 1970c). Bicarbonate-free medium also inhibits the PFK activity of diaphragm muscle in the rat (Show and Statie, 1959). In the absence of citrate or bicarbonate, fructose could be converted to fructose diphosphate. Fig. 2 shows that after 30 minutes incubation of atria in the citrate (1.5 mM)-contained medium or bicarbonate-free medium, substrates were added to the atria depressed by citrate (left in Fig. 2) or bicarbonate-free medium (right in Fig. 2). It is evident from the Fig. 2 that pyruvate partially restored the contractility of atria depressed by citrate or bicarbonate-free medium, but fructose had no effect. Since fructose can serve as a source of fuel for the contractile process in atria (Paradise and Ko, 1970), these studies indicate a defect in the utilization of this substrate prior to its conversion to pyruvate, and are consistent with the previous data indicationg that the PFK enzyme is inhibited by citrate and bicarbonate-free medium (Show and Stadie, 1959). Thus, it appears that fructose is metabolized via the PFK step.

Effect of acetate and glucose on contractility of atria depressed by bicarbonate-free medium

By the use of other substrates acetate and glucose, experiments were designed to examine the effects of substrates on contractile activity of atria depressed in bicarbonate-free medium.

The atria were exposed to bicarbonate-free medium immediately after the 60 minutes equilibration period in Krebs-Ringer bicarbonate glucose medium. The contractility of rat atria was mark-

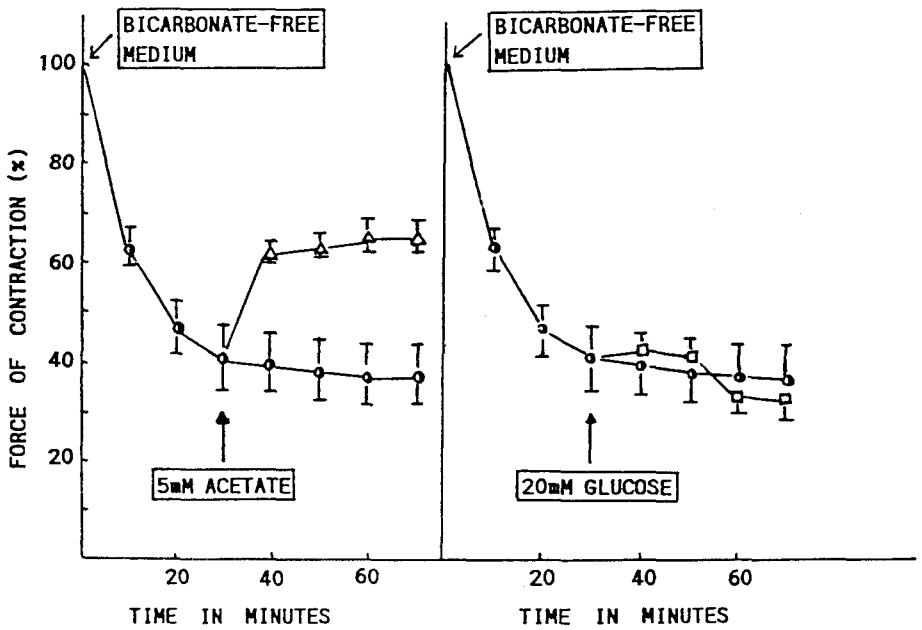

Fig. 3. Effect of acetate or glucose on contractility of isolated rat atria depressed by bicarbonate-free medium.
Normal medium was changed to bicarbonate-free medium at zero time.
Sodium acetate(5 mM) or glucose (20 mM) were added at 30 minutes.
Vertical lines represent standard error.
◑-◑: Bicarbonate-free control (6) △-△: Bicarbonate-free + acetate(6)
□-□: Bicarbonate-free + glucose(6)

edly depressed in the bicarbonate-free medium, despite the fact that the medium contained 5.5 mM glucose (Fig. 3). After 30 minutes of incubation in the bicarbonate-free medium, sodium acetate(5 mM) and glucose (20 mM) were added to the depressed atria. It is evident from the Fig. 3 that acetate partially restored the contractility of atria depressed with bicarbonate-free medium, but additional glucose had no effect. It is evident from the Fig. 3 that acetate was utilized for the contractile activity of the depressed atria by bicarbonate-free medium, but glucose could not be utilized by the depressed atria in the bicarbonate-free medium, despite the fact that same concentration of glucose produced marked increase in the depressed atria by substrate-free medium (Fig. 4). These studies indicate a defect in the utilization of glucose prior to its conversion to pyruvate, which is consistent with biochemical data obtained by Show and Stadie in rat diaphragm muscle in which PFK enzyme in the Embden-Meyerhof pathway was inhibited by bicarbonate-free medium (Show and Stadie, 1959).

Effect of substrates on contractility of substrate-depleted atria

By the use of various substrates employed in this study, experiments were performed to observe the nature of their ability to support the conractile force of isolated atria depressed by omission of exogenous substrates. Then, the behavior of isolated atria in the absence of exogenous glucose as an energy fuel for the contractile activity was determined to provide the control data with which the responses to atria depressed by lidocaine, citrate, or bicarbonate-free medium might be compared.

Atria were suspended in Krebs-Ringer bicarbonate medium containing 5.5 mM glucose, and allowed for a one-hour equilibration period before the experiments were begun. Immdiately after the equilibration period, the normal nedium was changed to substrate-free medium. The results are summarized in Fig. 4. It is evident from the Fig. 4 that the developed tension of atria decreased progressively, and was depressed approximately 45% by 30 minutes in the substrate-free medium. The addition of pyruvate (5 mM), acetate (5 mM), or fructose (30 mM) at 30 minutes after the glucose removal partially restored the contractile activity of depressed atria. However, glucose at concentration with 20 mM, added to the atria exposed to stubstrate-free medium for 30 minutes, produced a marked increase in the force of contraction to higher than the control level like previous demonstration (Ko and Paradise, 1973b), whereas this concentration of glucose did not produce significant effect on the depressed by bicar-

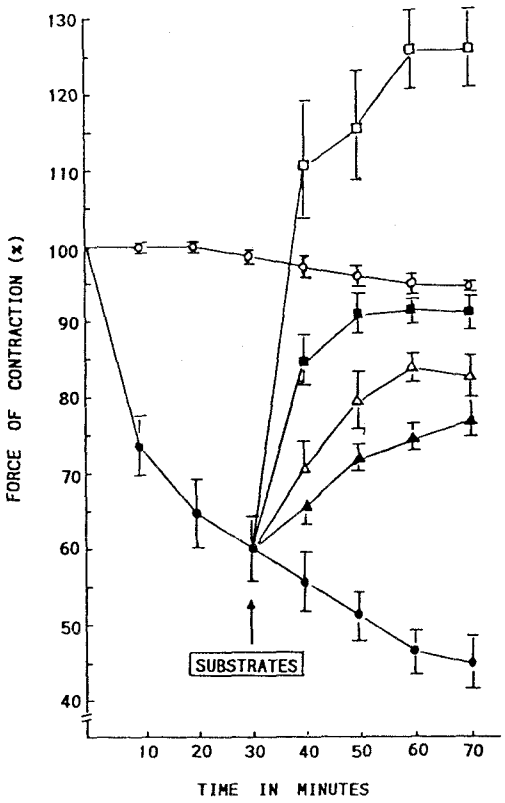

Fig. 4. Effect of substrates on contractility of substrate-depleted atria.
5 mM of pyruvate, acetate (5 mM), 20 mM of glucose and 30 mM of fructose were added 30 minutes after the normal Krebs-Ringer bicarbonate glucose medium was changed to glucose-free medium at zero time (following a one hour equilibration period).
○-○: Normal control(6)
●-●: Substrate-free control(7)
△-△: Substrate-free + 5 mM acetate(4)
▲-▲: Substrate-free + 5 mM acetae(4)
□-□: Substrate-free + 20 mM glucose(4)
■-■: Substrate-free+30 mM fructose(6)

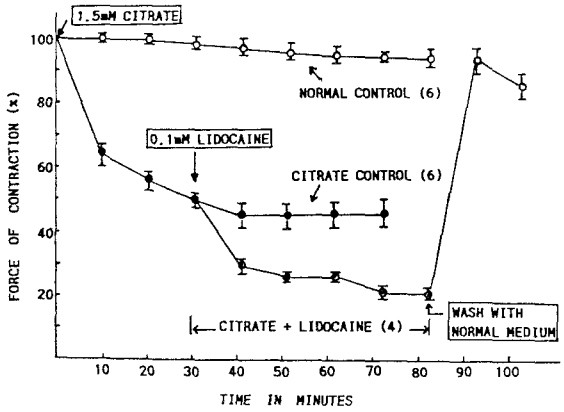

Fig. 5. Effect of lidocaine on contractility of isolated rat atria depressed by citrate.
Sodium citrate (1.5 mM) was added to normal medium at zero time (after a one-hour equilibration period). Lidocaine (0.1 mM) was added at 30 minutes after incubation of atria in the citrate-contained medium.
Vertical bars represent standard error of the mean.
○-○: Normal control(6)
●-●: Citrate control(6)
◉-◉: Citrate + Lidocaine(4)

bonate-free medium (Fig. 3).

Effect of lidocaine on citrate-depressed atria

Experiments were designed to determine the effect of lidocaine on the utilization of endogenous substrates in rat atria. Following the equilibration period of 60 minutes in normal Krebs-Ringer bicarbonate glucose medium, 1.5 mM sodium citrate was added. After 30 minutes incubation of atria in this citrate-contained medium, the contractility of atria declined about 50%. At this time, 0.1 mM of lidocaine was added to the bathing medium. It is evident from Fig. 5 that the addition of lidocaine further depressed the contractility of citrate-treated atria.

DISCUSSION

The local anesthetic lidocaine is known to depress the cardiac function (De Jong and Heavner, 1973; Li *et al.*, 1982; Ko *et al.*, 1986; 1990), and it has been previously reported that pyruvate, acetate, and fructose partially restored the contractility of isolated rat atria depressed by lidocaine, but glucose was without effect on the lidocaine-depressed atria (Lim and Kim, 1984; Ko *et al.*, 1986; 1990). Thus, it was postulated that the negative inotropic action of lidocaine is at least in part linked to a block at an early step in the glycolytic pathway of rat atria (Ko *et al.*, 1986). This investigation was undertaken to ascertain whether the cardiac depressant action of lidocaine is directly associated with the utilization of endogenous substrates in rat atria, by using citrate and bicarbonate-free medium known as potent inhibitors of phosphoructokinase (PFK) enzyme step (Webb, 1950; Show and Stadie, 1959; Ko and Paradise, 1970c) and (Fig. 2, 3). It has been also established that cardiac contractility is depressed by citrate and bicarbonate-free medium (Ko *et al.*, 1969; Ko and Paradise, 1970c). The fact that substrate-free treated atria were markedly depressed by citrate or bicarbonate-free medium suggests that a substrate above the PFK step, probably glycogen, is a main endogenous substrate to maintain the force of contraction in the absence of exogenous substrate.

It is further evident from this investigation that the contractile force of substrate-depleted atria supported by endogenous substrates above the PFK step is much greater than that supported by substances below the PFK step, indicating that probably cardiac glycogen is mainly utilized for the contractile process of the myocardium in the absence of exogenous substrates (Fig. 1). The fact that citrate and bicarbonate-free medium do not competely depress the contractile activity of substrate-depleted atria indicates that some endogenous substrates below the PFK step are used for the contractile porcess. The most likely substrate would be lipid (Evans, 1964) and perhaps lactate (Fig. 1). Fructose also partially restored the contractility of atria depressed by substrate-free medium (Fig. 4), but fructose had no effect on atria depressed by citrate and bicarbonate-free medium (Fig. 2). The results are again in agreement with the previous demonstration by using bicarbonate-free medium that fructose apparently is metabolized via the PFK step (Paradise and Ko, 1970).

The contractile activity of isolated rat atria was progressively decreased when substrate-free medium was substituted for the Krebs-Ringer bicarbonate glucose medium. Exogenous substrates glucose, acetate, pyruvate, and fructose resulted in marked increase in the force of contraction of depressed atria induced by substrate-free medium (Fig. 4). These results indicate that the contractile depression caused by substrate-free medium was not due to the blockade of glycolytic pathwasy of the atria. These results are consistent with the functional demonstration that acetate partially restored the contractility of atria depressed by bicarbonate-free medium, but glucose was without effect on the atria depressed by bicarbonate-free medium, but glucose was without effect on the atria depressed by bicarbonate-free medium (Fig. 3). However, glucose produced marked increase in the force of contraction of atria depressed by substrate-free medium (Fig. 4). It can be interpreted that the depression of atrial contractility by citrate and bicarbonate-free medium is due to the blockade of PFK enzyme activity in the Krebs-Ringer bicarbonate medium containing glucose. Thus, the exogenous substrates pyruvate and acetate, not metabolized via PFK step. It was found that the lidocaine further depressed the force of contraction of citrate-depressed atria (Fig. 5), indicating that some endogenous substrates below the PFK step of the myocardial glycolysis, utilized for contractile process, may also be partially impaired by the action of lidocaine. These results may be interpreted as indicating that lidocaine depress the force of contraction of isolated atria by acting probably at multiple cellular sites: one involving a block in glycolysis as previously described; another involving another mechanism (s).

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=국문초록=

심근 수축에 쓰여지는 내인성 기질 대사에 대한 Lidocaine의 영향

경희대학교 의과대학 약리학교실

고 계 창

Lidocaine의 심근 수축력 억제 기전이 적출 심장에서 내인성 기질의 사용과 관련이 있는가를 규명하기 위하여, 심장의 phosphofructokinase (PFK)에 대한 강력한 억제 작용을 나타내는 citracte와 bicarbonate-free medium을 이용하여 쥐의 적출 심방 수축성에 대한 lidocaine의 영향을 연구하여 다음과 같은 실험 결과를 얻었다. Citrate와 bicarbonate-free medium은 쥐의 적출 심방의 수축력을 현저히 저하시켰다. Pyruvate나 acetate는 citrate나 bicarbonate-free medium에서 저하된 심방 수축력을 현저히 증가시키는 반면, fructose는 수축력을 증가시키지 못했다. 이 결과는 citrate나 bicarbonate-free medium이 Embden-Meyerhof pathway의 일부, 즉 PFK step을 억제함을 시사한다. 외인성 기질이 없을때 citrate나 bicarbonate-free medium은 기질 제거 용액에서 심방 수축력을 현저히 감소시키며, acetate에 의해 수축력이 회복되었다. 이는 PFK step 이전 단계의 내인성 기질 (glycogen)이 citrate에 의해 억제됨을 시사한다. Lidocaine은 citrate에 의해 억제된 수축력을 더욱 감소시켰다. 이 결과는 lidocaine에 의한 심방 수축력 억제가 PFK step 이후 단계의 내인성 기질 억제에 의한 것임을 시사한다.

이상의 결과로 보아 lidocaine의 적출 심방에 대한 수축력 억제 작용은 두가지 (또는 그 이상)의 기전에 의한 것으로 사료된다: 하나는 PFK step 전단계의 해당과정의 억제기전이고 또 다른 기전은 PFK step 이후의 내인성 기질(들)의 억제인 것으로 사료된다.