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麻醉劑의 心臟藥理學的 硏究

第1報 全身麻醉劑 Halothane의 心臟代謝 抑制作用에 關한 基礎的 考察

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Cardiac Pharmacology of Anesthetics

1. Preliminary Observation of Halothane's Inhibitory Action on Cardiac Metabolism

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ABSTRACT

Certain metabolic aspects of halothane's cardiac depressant action on the contractility of the myocardium were elucidated from a study of the effect of pyruvate on halothane-depressed rat atria. Approximately 6 mg% halothane was required to maintain a 50% depression of the contractility of rat atria suspended in a modified Krebs-Ringer bicarbonate glucose medium, pH 7.4, 30°C for a 2 hr. period. Pyruvate was found to restore partially the contractility of halothane-depressed atria. The maximally effective concentration of pyruvate was 2.5 mM. There was minimal pyruvate effect on the force of contraction of control atria. The effect of pyruvate on halothane-depressed atria was shown to be due to the pyruvate and not the sodium ion of the sodium pyruvate. Pyruvate was found to produce no increase in the contractility of atria depressed by hypertonic medium, but caused a further depression. Selected aspects regarding the action of halothane on glucose metabolism in myocardial cells are discussed. The results are consistent with the hypothesis that at least a part of the negative inotropic action of halothane is due to an inhibition of glucose uptake or utilization in the glycolytic pathway.

INTRODUCTION

Since the introduction of halothane as an anesthetic agent by Raventos in 1956[1], it is widely used in operating rooms. There is evidence, however, that halothane depresses the cardiac function[2,3], and its cardiac effect have attracted much attention. Although numerous reports concerning the direct depression of myocardium by inhalation anestetics appeared from the intact [2,3] and isolated hearts[4~8], the mechanism for this depression has not been completely clarified.

As an order of investigation to elucidate the mechanism of the myocardial depression by the anesthetic agent, it was of interest to as certain whether the myocardial depression due to halothane might be related to the metabolic state as well as the substrate utilization of the myocardium. Exogenous substrate pyruvate is commonly known as a useful tool for the study of cardiac

depressant and metabolism of the heart[9~16].

In the present investigation, it was found that the addition of pyruvate to the halothane-depressed isolated atria resulted in a marked increase in force of contraction. This paper advances the hypothesis that the cardiac depressant action of halothane is a manifestation of the inhibition of glucose uptake or glucose utilization of the myocardium.

METHODS

Male, Sprague Dawley rats weighing from 180 to 200 g having *ad libitum* access to food and water were employed. Atria were removed from decapitated rats and suspended in a modified Krebs-Ringer bicarbonate glucose medium of the following composition (mM): NaCl 120, KCl 4.8, $CaCl_2$, 1.22, $MgSO_4 \cdot 7H_2O$ 1.33, $NaHCO_3$ 25.3, glucose 5.55[16,17]. The medium was gassed with 95% O_2-5% CO_2 at pH 7.4 and 30°C. A constant resting tension of 750 mg was exerted on the atria with a micrometer head, the developed tension was recorded through a Statham strain gauge, and the atria were electrically stimulated at a rate of 200 pulses/min. An equilibration period of 60 min was allowed before readings were taken. The experimental values of contractility (peak tension) were compared with those of the control records obtained at zero-time (following equilibration) and expressed as per cent changes in developed tension. In the experiments with hypertonic medium the increase of osmotic pressure was made by the addition of sodium chloride at a concentration of 100 mM to the normal Krebs-Ringer bicarbonate glucose medium. Halothane was administered into the medium by means of the anesthetistat[5,6]. The halothane concentration in the medium was determined at 10 to 30 min intervals with a gas chromatograph throughout the experimental period.

RESULTS

Effect of halothane on atrial contractility

The behavior of atria in the presence of halothane was determined to provide data with which the responses of depressed atria to pyruvate could be compared. Halothane administration was begun at zero time (following a 1 hr equilibration period). Adjustments in the anesthetistat used to deliver the halothane were made as necessary during the first 30 min in order to achieve 50% depression of the contractility. Following this 30 min period no further adjustments were made. Relatively stable concentrations of halothane were observed in the bathing medium during the following 90 min (5.6 to 5.8 mg% mean values). The force of contraction declined slightly as approximately the same rate as the normal atria not exposed to halothane(Fig. 1).

Effect of pyruvate on atria depressed with halothane

Sodium pyruvate(5 mM) was added to the bathing medium 30 min after the start of the halothane administration. Despite the continued administration of halothane and maintenance of halothane levels in the medium similar to the control atria, the addition of pyruvate resulted in a gradual increase in the force of contraction (Fig. 2). The maximally effective concentration of pyruvate was 2.5 mM(Fig. 3). Addition of 2.5 or 5 mM pyruvate to normal atria resulted in only slight alterations in contractile activity in contrast to the marked positive inotropic effects seen when administered to halothane-depressed atria(Fig. 4).

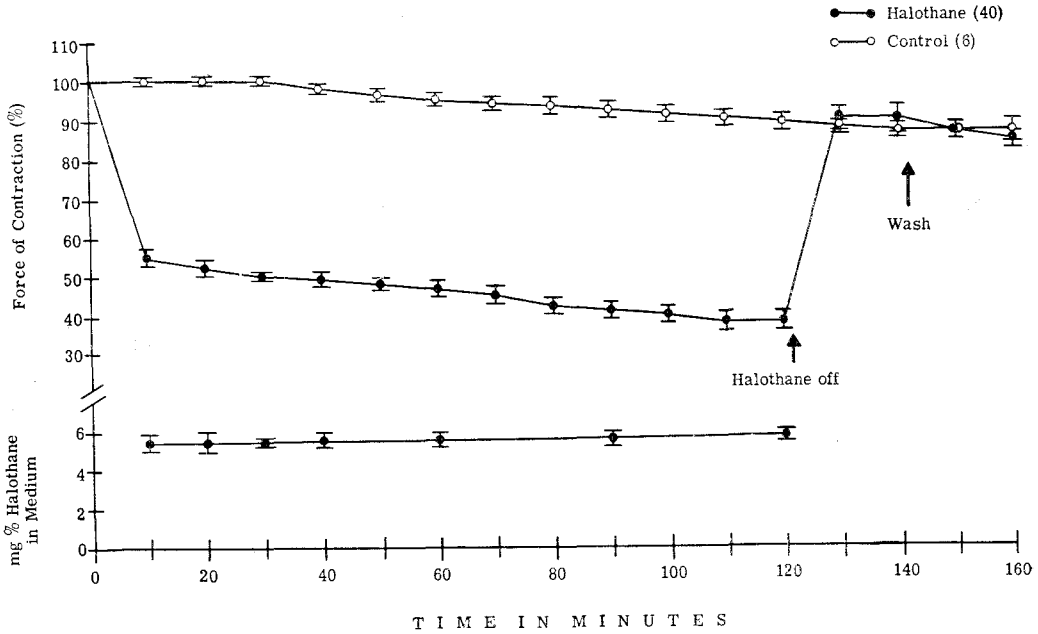

Fig. 1. Effect of halothane on isolated rat atria. Halothane was added at zero time(i.e. following a 60 min equilibration period in the normal Krebs-Ringer bicarbonate glucose medium).

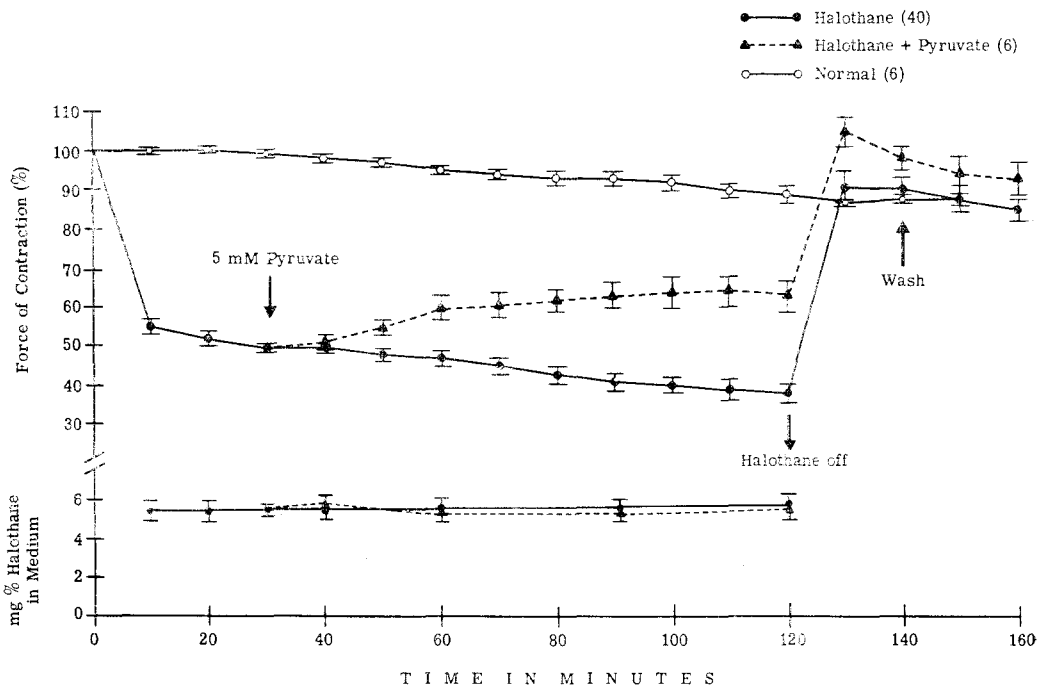

Fig. 2. Effect of 5 mM pyruvate on halothane-depressed isolated rat atria. 5 mM pyruvate was added 30 min after start of halothane administration. Vertical bars represent one standard error of the mean.

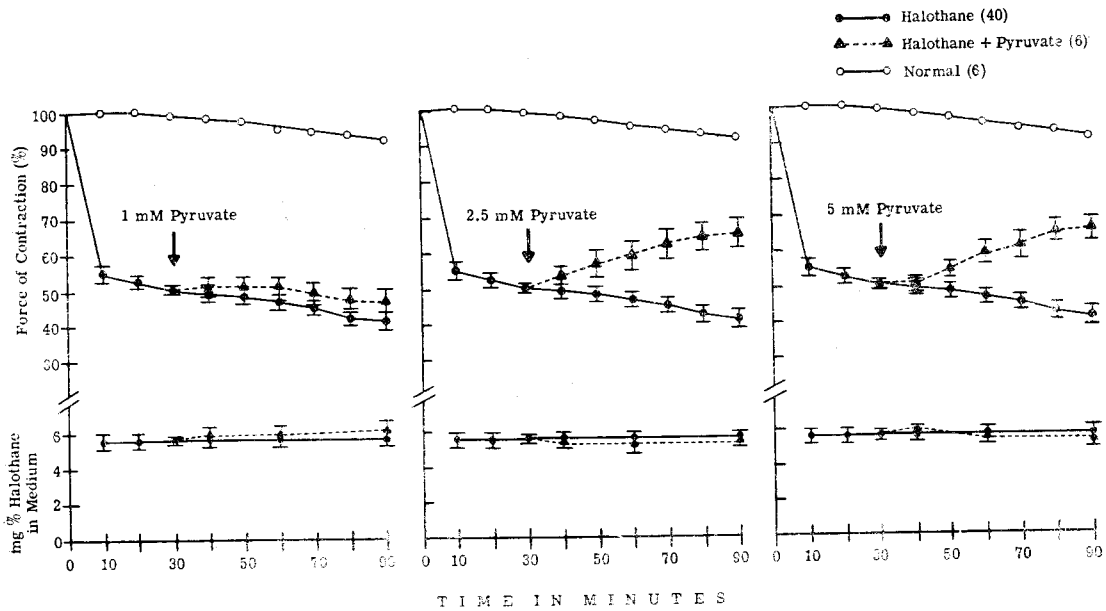

Fig. 3. Effect of 1, 2.5 and 5 mM pyruvate on halothane-depressed isolated rat atria.

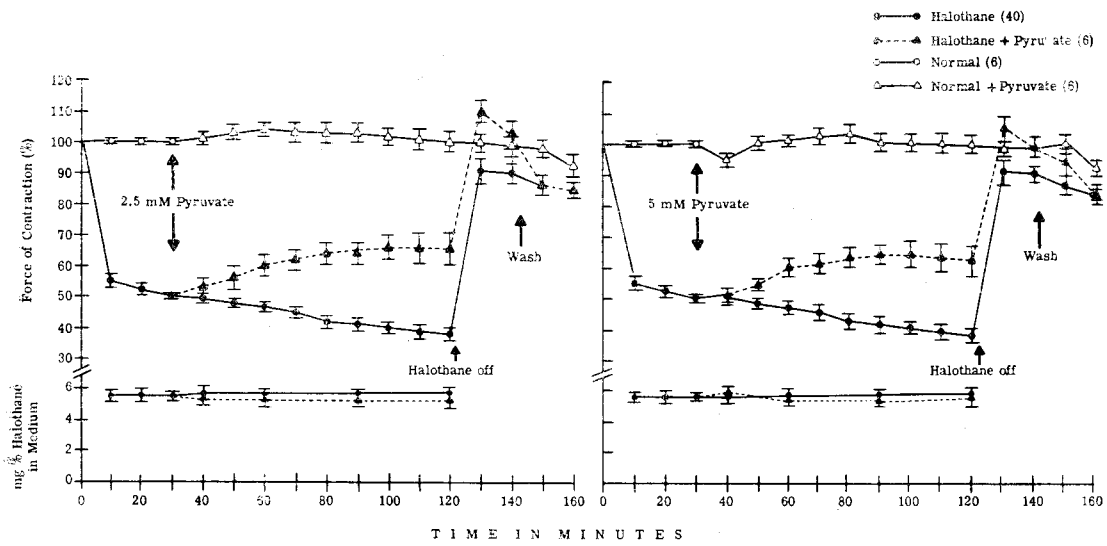

Fig. 4. Effect of 2.5 and 5 mM pyruvate on normal and on halothane-depressed isolated rat atria.

Effect of NaCl on atria depressed with halothane

In order to elucidate the nature of the positive inotropic effect of pyruvate on halothane-depressed atria, experiments were designed in which sodium chloride was employed under the same conditions as pyruvate. It has been reported that

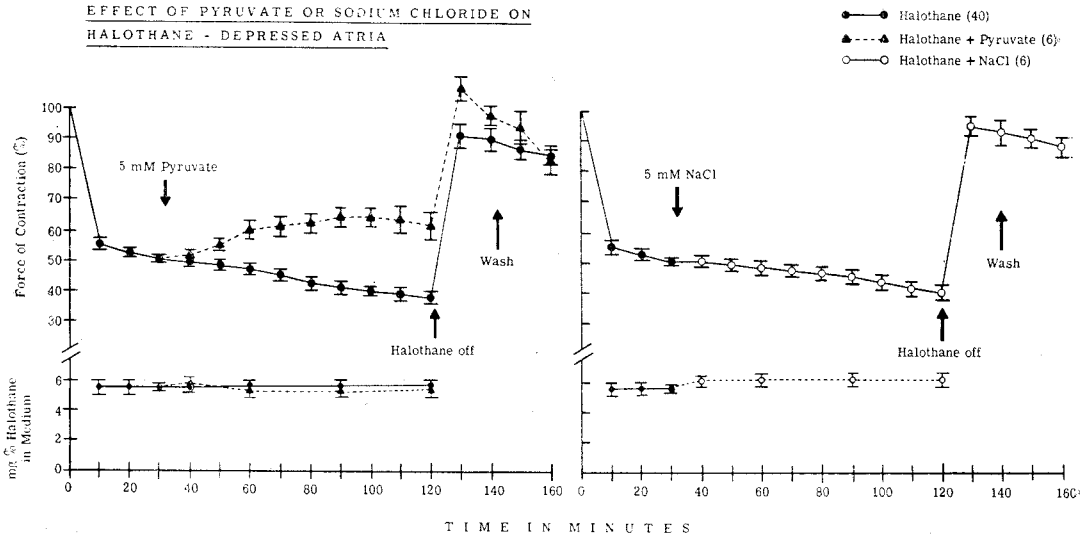

Fig. 5. Effect of 5 mM pyruvate and 5 mM NaCl on halothane-depressed isolated rat atria.

no demonstrable effect of the sodium ion can be detected at concentrations below 5 mM[18]. Hence, the administration of 5 mM sodium chloride may eliminate the possibility of any demonstrable factor in these comparative studies. Figure 5 demonstrates that 5 mM sodium chloride is ineffective in restoring the force of contraction of halothane-depressed atria when administered under the same conditions where sodium pyruvate is effective. This indicates that the effect of sodium pyruvate is due to pyruvate itself and not to the presence of the sodium ion.

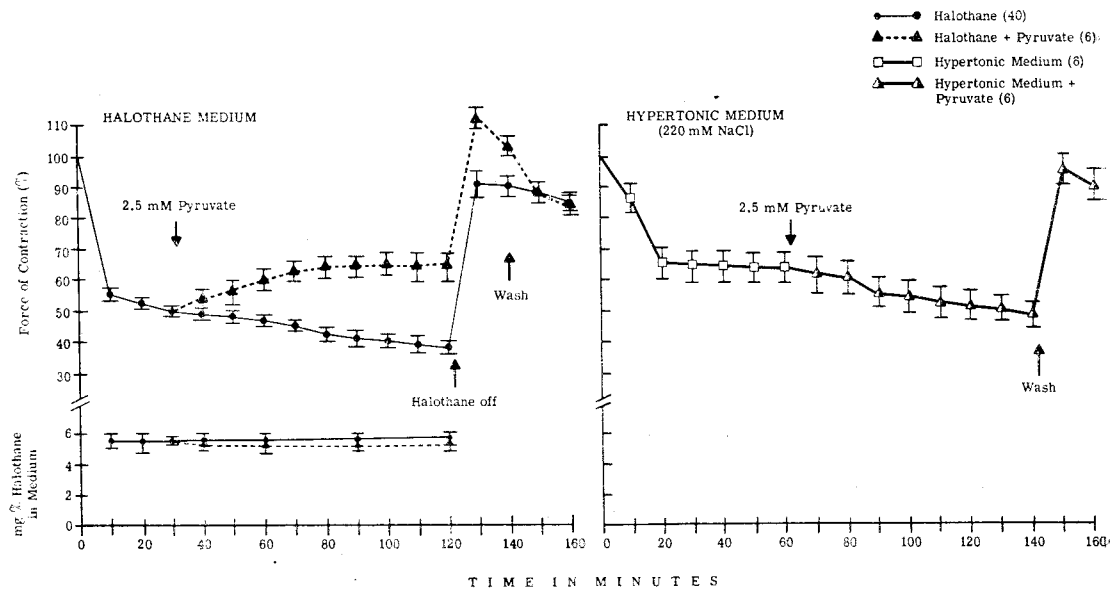

Fig. 6. Effect of 2.5 mM pyruvate on atria depressed with halothane or hypertonic medium.

Effect of pyruvate on atria in hypertonic medium

Experiments to further clarify the positive inotropic activity of pyruvate on the halothane depressed atria were designed employing different experimental conditions. Hypertonic medium, prepared by the addition of 100 mM sodium chloride to the normal medium, was used rather than halothane to depress the atrial contractility. After 60 min of exposure to this medium, the force of contraction stabilized at approximately 65% of the control value(Fig. 6). The addition of 2.5 mM pyruvate at this time resulted in no increase in contractility. The force of contraction appeared to undergo further depression. These results indicate that depression of the force *per se* is not a sufficient condition to permit pyruvate to effect a recovery in contractility.

DISCUSSION

Although the effects of halothane on functional properties of the heart have been studied with isolated heart preparations[4,7,8,19], few investigations of halothane on metabolism have been made.

Halothane has been shown, in hepatic studies in man, to result in diminished perfusion pressure, hepatic blood flow and venous oxygen tension without effect on the overall oxygen consumption and with no excess lactate liberation[20]. Investigations of the effects of halothane on cerebral metabolism have demonstrated that there were no changes or minimal variation in the rate of cerebral oxygen consumption[21]. Using various substrates, halothane was found to uncouple oxidative phosphorylation in rat liver mitochondria[22]. However, the concentration of halothane used were extremely large (7-14 mM). This is in the order of 30 times the amount required to depress the force of contraction of the beating rat heart by 50%.

Hoch et al.[23] have reported on the effect of halothane with respect to the oxygen consumption of rat brain, liver and heart and anaerobic glycolysis of rat brain. They have concluded that clinical concentrations of halothane(1%) caused a significant decrease in oxygen consumption of unstimulated rat brain slices and that two percent halothane decreased the oxygen consumption of both heart and liver slices. They also found 5% halothane to be without effect on anaerobic glycolysis of rat brain. However, these studies were done on homogenates where the actions of halothane may be very different from its action on intact tissue. It has been recently reported that halothane reduces the ATPase activity of both myocardial and skeletal muscle myofibrillar preparations. The hypothesis has been presented that[1] there is a close association between the myocardial tension developed and the enzymatic hydrolysis of ATP and[2] halothane exerts its cardiac effects by inhibiting this enzymatic hydrolytic process[24]. However, the concentrations of halothane in the medium in contact with the myofibrillar preparations were exceedingly high (the lowest concentration reported was 20mM). Depression of the isolated perfused rat heart by 50% and 29°C requires about 0.33 mM[6].

The direct action of halothane on isometric and isotonic contractions of cat papillary muscles has recently been studied[19]. The negative inotropism induced by halothane was suggested to be the result of a dosedependent depression of the intensity of energy conversion by the contractile element of the heart. Evidence presented by Goldberg and Ullrick[8] and Goldberg and Phear[25], concerning the influence of halothane on the mechanical properties of the rat trabeculae carnae preparation, points to an indirect action of halothane on the contractile machinery. Such an action could be elicited by an inhibition of subs-

trate uptake or utilization.

Several investigators have been able to demonstrate the relative abilities of various substrates to maintain the contractile activity of myocardial function[9~17]. It is commonly emphasized that efficient operation of energy mechanisms is essential for cardiac function; thus, cardiac depression and failure must be explained ultimately on a biochemical and/or biophysical basis. It has not been established whether uptake or utilization of certain substrates to support the mechanical function of the myocardium is directly associated with the action of halothane in depressing the isolated rat heart.

The observation in the present study that pyruvate was partially effective in restoring the contractile activity of the isolated rat heart in the hypodynamic state induced by halothane, has interesting implications of a metabolic role for the action of halothane on cardiac function. It has been demonstrated with rat atria[13] and rabbit atria[26] that either the uptake of glucose or operation of the glycolytic pathway are important for a fraction of contractile activity since pyruvate is only partially effective in restoring the developed tension in the absence of glucose or during block with 2-deoxy-d-glucose. Similar investigations have been made by Ko and Berman which indicate that pyruvate partially restored the decreased contractility of isolated rat atria[15] in phosphate medium either with or wihtout glucose. Pyruvate was relatively effective as an energy source for contraction of isolated rat ventricles in phosphate medium[11] and in glucose impaired metabolism in rat heart[27]. Shaw and Stadie[28] proposed that in diaphragm muscle the Embden-Meyerh of pathway was inhibited at the phosphofructokinase step when the tissue was incubated in phosphate medium.

If it could be assumed that the effect of pyruvate on the atria depressed with halothane may be similar to that described above, our investigations might be concerned with the manner in which halothane influences the myocardial metabolism. Either the uptake or utilization of glucose may be impaired by halothane. No direct evidence with respect to the action of halothane on cardiac metabolism has been reported in the literature.

Using partition coefficient data of Larson et al.[29], a saline concentration of 6 mg% halothane would be equivalent to a blood concentration of 19.8 mg%. Blood levels of 17.9~20.3 mg% were found necessary to anesthetize a dog to produce a loss in the pain reflex of the foot pad[6]. Thus, the concentration of halothane employed in this study was similar to that assumed to produce anesthesia in the dog. The rat atria, however, were at 30°C and the dogs at 37°C. It has been shown that for haothane a 10 degree increase in tempelrature requires approximately a doubling of the partial pressure required to produce anesthesia in goldfish[30], and in dogs[31]. The concentration of halothane used in this study would be almost twice the concentration necessary to produce a minimal degree of anesthesia in the dog at 37°. The details of studies relating the cardiac depressant activity to anesthetizing partial pressures will be described in a later publication.

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