Mechanism of Action of Tris (Hydroxymethyl) Aminomethane on the Negative Inotropic Effect of Carbon Dioxide

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ABSTRACT

Administration of isotonic THAM to the blood of the heart-lung preparation induced a decrease in the negative inotropic effect of CO₂, suggested by the fact that, at constant stroke work, an increase in Pco₂ of 26 ± 1.9 mm Hg was followed by an increase in left ventricular end-diastolic pressure (LVEDP) of 3.8 ± 0.2 mm Hg before the infusion of THAM; after THAM was given, a similar increase in Pco₂ induced an increase in LVEDP of 1.8 ± 0.2 mm Hg (P<0.01). When pH was increased by 0.15M NaHCO₃, no changes in myocardial contractility or in the tolerance of the heart to CO₂ were found. On the other hand, hemodilution with isotonic sucrose reproduced the effect of THAM. Either sucrose or THAM produced a decrease in plasma Na. Myocardial contractility, previously increased by THAM or sucrose, returned to control after plasma Na was restored. When cat papillary muscles were immersed in Ringer’s solution with normal (142 mM) and low (112 mM) Na concentration, it was observed that a similar increase in Pco₂ originated a smaller decrease in contractility when the muscles were in the low Na solution. It is concluded that the effect of THAM in the present experiments is due, at least to an important extent, to changes in extracellular Na.

KEY WORDS myocardial contractility cat papillary muscle dog heart-lung preparation low extracellular [Na] extracellular pH hemodilution osmolarity

Tris (hydroxymethyl) aminomethane (THAM) is an amine buffer that was introduced in 1960 (1) in the clinical treatment of acid base disorders. Its use has been stimulated by the observation that it apparently buffers the intracellular as well as the extracellular space (2, 3).

The cardiovascular actions of THAM have been extensively studied (4-12). It has been shown that the infusion of THAM in acidotic dogs increases myocardial contractile force (4-9) and that the replacement of NaHCO₃ by THAM is associated with an increase in the developed tension of the isolated atrium (11). However, when THAM titrated to pH 7.4 is injected into the coronary circulation, no changes in myocardial contractile force are produced (9). In preliminary experiments from this laboratory (13) we observed that the administration of 0.3M THAM solution increased myocardial contractility in the isolated heart-lung preparation. On the other hand, the in vitro replacement of NaHCO₃ by THAM was followed by an increase in contractility of cat papillary muscles only when a significant decrease in Na concentration of the medium was achieved (12).

To supply further information about the effect of THAM upon myocardial contractility and the mechanism of its action and to study the influence of extracellular Na upon the effect of THAM, experiments were under-
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This study was performed using two different preparations—the heart-lung preparation and the isolated cat papillary muscle.

HEART-LUNG PREPARATION

The conventional heart-lung preparation, in which some modifications were introduced, was used. Mongrel dogs weighing 14 to 18 kg were anesthetized with Na pentobarbital, 30 mg/kg iv. The venous blood reservoir was filled with approximately 1.5 liters of heparinized blood obtained from donor dogs under light pentobarbital anesthesia. An electromagnetic flowmeter was connected to the inflow line, the cardiac inflow being then monitored. The inflow, which was controlled by a screw clamp in the inflow line, was maintained constant throughout the experiment. A metal cannula was introduced in the thoracic aorta, and the aortic flow was returned to the blood reservoir. The resistance to arterial outflow was controlled by a screw clamp in the outflow line, and was regulated in such a way as to maintain mean aortic pressure constant throughout the experiment. A heat exchanger connected to the inflow line maintained the blood temperature at 38°C. Heart rate was kept constant by pacing the right atrium. Aortic pressure was measured with a pressure transducer connected to a cannula inserted in a carotid artery. To measure left ventricular diastolic pressure a 15-gauge blunt needle was passed through the apex into the left ventricular chamber providing a constant flow of Ringer's solution. The mural end of the muscle was fixed with a metal clamp and was connected to a muscle holder by a small loop of silk thread.

Once the preparation was completed the cardiac inflow and aortic resistance were set to obtain a cardiac output of 80 ml/kg body weight and a mean aortic pressure of 80 to 85 mm Hg; both cardiac output and mean aortic pressure, as well as heart rate, were maintained constant during the entire experiment. Before starting with the experimental procedure, 4 to 5 mg of propranolol was added to the blood reservoir. This dose proved to block the chronotropic and inotropic effect of 50 μg of norepinephrine.

The lungs were ventilated for 15 to 20 minutes with gas mixtures of different CO₂ concentrations (1 to 3%, 5 to 7%, and occasionally 10 to 15%). Blood samples and fast recordings were obtained in each period when the hemodynamic parameters were in a steady state. Each preparation was successively exposed to at least two gas mixtures with different CO₂ concentrations. After this procedure was completed, 12 experiments, 250 ml of 0.3M THAM was added to the reservoir, and the preparation was exposed to gas mixtures of the same CO₂ concentration as those employed in the control period.

In nine experiments, the same sequence was followed, but the inotropic effect of CO₂ was studied before and after the addition of 250 ml of 0.15M NaHCO₃.

In seven experiments, the same procedure was followed before and after the addition of 250 ml of 0.3M sucrose.

In six additional experiments, Pco₂ was maintained constant at approximately 35 mm Hg for the entire experiment. After a control recording and blood samples were obtained, 250 ml of 0.3M THAM was added to the blood in three of the experiments. In the remaining 3 experiments, 250 ml of 0.3M sucrose was added. Blood samples and recordings were obtained 15 to 20 minutes after infusion of the solutions, and then 250 ml of 0.3M NaCl was added to the blood. Fifteen to 20 minutes later, blood samples and fast recordings were again obtained. In all the samples, pH, Pco₂, and also plasma Na concentration was measured.

Myocardial contractility was evaluated by changes in left ventricular end-diastolic pressure (LVEDP), left-ventricular circumference segment length, and maximal dp/dt at constant heart rate, cardiac output, and mean aortic pressure.

ISOLATED CAT PAPILLARY MUSCLE

The principal features of this preparation have been described previously (15).

Papillary muscles were isolated from the right ventricle of cats using a specially designed chamber providing a constant flow of Ringer's solution. The mural end of the muscle was fixed to a muscle holder by a small loop of silk thread. Another small loop of silk thread was tied to the tendinous end of the muscle and was connected with an extending wire to a Statham transducer (model B78-O 75-350).

Isometric mechanograms were recorded on a Sanborn model 7100 oscillographic recording system equipped with a model 350-1100C Carrier
The first derivative of the developed tension (dT/dt) was recorded by electronic differentiation, and calibration was accomplished by a linear sawtooth voltage. The dV/dt of the sawtooth is a square wave with an amplitude which corresponds to a known rate of change. The time required to reach peak tension, as measured from the first development of tension, was estimated from records obtained at paper speed of 50 or 100 mm/sec. Tension values were expressed as g/mm² cross-sectional area (15).

The resting tension was usually 100 to 300 mg and remained constant throughout the experiment.

The average cross sectional area of the muscles used was 1.21 ± 0.11 mm². The electrical stimuli were delivered by two platinum electrodes that covered the entire length of each muscle. Rectangular pulses of 10 msec and an amplitude 20 to 30% higher than the threshold voltage of each preparation (16) were produced by an electronic stimulator. The frequency of contraction was 10/min in all of the experiments. The chamber was connected with a four-way tap that permitted the rapid replacement of the solution.

The composition of the control Ringer solution in mM was: NaCl, 112.8; KCl, 4.74; CaCl₂, 2.54; KH₂PO₄, 1.18; MgSO₄, 1.18; NaHCO₃, 29.3; glucose, 3.6.

A heat exchanger connected to the inflow line maintained a constant temperature of 30°C within the chamber. Before the start of each experiment, the muscle was kept immersed in the control Ringer solution, through which a mixture of 5% CO₂-95% O₂ was continuously bubbled; electrical stimulation was begun at the beginning of the second hour.

In this series of experiments, the muscle was immersed in the control Ringer solution, equilibrated with a Pco₂ of approximately 40 mm Hg. Once a period of stabilization of two hours had elapsed, the muscle was exposed successively, for periods of 30 minutes, to control Ringer's solutions equilibrated with a Pco₂ of approximately 85 and 40 mm Hg. Once this was done the muscle was exposed to another Ringer's solution with a Na concentration of 112 mM; osmolarity was maintained by the addition of appropriate amounts of sucrose. The procedure of exposing the muscle to Pco₂ of approximately 40 and 85 mm Hg was repeated. The solutions with low and high Pco₂ were switched in a random sequence. At the end of the exposure to a given solution, fast recordings and samples for pH and Pco₂ were obtained.

Pco₂ and pH were measured with a Radiometer PHM 27 pH meter with anaerobic electrodes maintained at 37.5°C for the heart-lung experiments and at 30°C for the papillary muscle experiments. Plasma Na concentration was measured by flame photometry.

To quantify the effect of the various treatments employed in this experiment upon the tolerance of the heart and the isolated papillary muscle to carbon dioxide, the following procedure was employed. In the heart-lung preparation experiments, a ΔPco₂ and its corresponding ΔLVEDP were obtained for every individual experiment before and after addition of THAM, NaHCO₃, or sucrose. A theoretical change in LVEDP that would correspond to an increase in Pco₂ of 30 mm Hg was then calculated. The difference between the calculated ΔLVEDP before and after addition of any of the substances employed was obtained for every individual experiment. The individual differences were averaged and the standard error was calculated. In the cat papillary muscle experiments a ΔT and a Δ maximal dT/dt corresponding to a ΔPco₂ of 45 mm Hg were calculated for every individual experiment. The results were then expressed as percent difference with respect of the values obtained at Pco₂ of 40 mm Hg.
It can be seen that addition of THAM is followed by an increase in contractility evidenced by the decrease in LVEDP and left ventricular circumference segment length (LVCS) and the increase in maximal dp/dt at constant stroke work. When Pco2 was higher (Fig. 2), addition of THAM exerted a more profound effect upon myocardial contractility.

Table 1 shows the changes in LVEDP originated by changes in Pco2 before and after adding THAM. It can be seen that an increase in Pco2 is followed by an increase in LVEDP at constant stroke work both before and after adding THAM. However, as indicated in the last column of Table 1 for a theoretical change in Pco2 of 30 mm Hg, the increase in LVEDP is significantly higher before than after adding THAM, suggesting a different heart response to hypercapnic acidosis after THAM addition.

Table 1 shows the changes in LVEDP (mm Hg) at constant stroke work, during CO2 changes before and after THAM in 12 experiments.

<table>
<thead>
<tr>
<th>Pco2</th>
<th>ΔLVEDP</th>
<th>ΔLVEDPcalc</th>
<th>ΔLVEDPcalc - ΔLVEDP</th>
<th>ΔLVEDPcalc - ΔLVEDPcalc - ΔLVEDP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>26.0 ± 1.9</td>
<td>3.8 ± 0.2</td>
<td>4.8 ± 0.3</td>
<td>33.0 ± 6.3</td>
<td>1.8 ± 0.2</td>
</tr>
</tbody>
</table>

ΔLVEDP = Actual mean increase in left ventricular end-diastolic pressure. ΔLVEDPcalc = calculated mean increase in left ventricular end-diastolic pressure, for a theoretical ΔPco2 of 30 mm Hg. Last column = mean difference obtained by paired samples between the calculated increase in left ventricular end-diastolic pressure after THAM and during the control period for each experiment.

Values are means ± SE.
An increase in myocardial contractility can also be observed when plasma Na concentration is decreased by adding 0.3M sucrose (Fig. 6); upon restoration of plasma sodium by administration of 0.28M NaCl, contractility returned to control levels.

A record of an experiment using the isolated cat papillary muscle is shown in Figure 7. An increase in PCO₂ is followed by a decrease in developed tension and maximal dT/dt when Na concentration is either 142 or 112 mM. However, when Na concentration is lower, contractility is higher, and an increase in PCO₂ is followed by a smaller decrease in developed tension and maximal dT/dt at the lower Na concentration. The summary of the results in Tables 4 and 5 show that when PCO₂ is increased to the same extent, the decrease in contractility is smaller at the lower Na concentration, suggesting that low extracellular Na concentration diminishes the effect of CO₂ upon myocardial contractility.

**Discussion**

The results in the present experiments are in agreement with the common notion that CO₂ exerts a deleterious effect upon the isolated heart (15, 17-22).

It seems apparent that addition of THAM to the blood of the heart-lung preparation is followed by an increase in myocardial contractility, evidenced by a decrease in LVEDP and LVCS and an increase in maximal dp/dt, while mean aortic pressure, cardiac output and heart rate are maintained constant. The effect of the administration of THAM is more important at higher PCO₂ levels; so the decrease in myocardial contractility that follows an increase in PCO₂ is smaller after THAM has been added to the blood. This is
This would suggest that the change in myocardial contractility that followed THAM administration would not depend upon extracellular pH changes.

Up to this point, we were tempted to conclude that THAM increases myocardial contractility and that its effect is more important at higher Pco2 levels, increasing the tolerance of the isolated heart to CO2. This effect would not depend upon the extracellular pH changes originated by THAM, since similar pH variations produced by NaHCO3 did not mimic the observed effect of THAM; the fact that THAM would influence intracellular hydrogen ion activity could possibly account for the difference between the effect of THAM and that of NaHCO3.

However, when THAM is added to the blood, as it was in our experiments, some associated changes can occur. The dilution of blood with a 0.3M THAM solution will obviously lead to a decrease, among other effects. Better illustrated in Table 1, where it can be seen that for a given increase in Pco2, the increase in LVEDP is smaller after THAM administration. In the absence of changes in myocardial distensibility, a change in LVEDP reflects a change in fiber length (23) and, although variations in myocardial distensibility could theoretically induce a change in fiber length with a concomitant change in filling pressure, or vice versa, the changes in LVEDP observed in the present experiments cannot be explained solely on the basis of changes in distensibility, for parallel changes in LVCS were associated with variations in LVEDP.

Administration of NaHCO3 was not followed by significant changes in myocardial contractility. This gives additional support to previous experiments from this laboratory using the isolated heart (24, 25) and the cat papillary muscle (15). The effect of CO2 upon myocardial contractility is not influenced by administration of NaHCO3, as shown in Table 2.
Effect of changes in plasma Na concentration produced by sucrose and NaCl on myocardial contractility. Left: Control period. Middle: 15 minutes after adding 0.3M sucrose. Right: 15 minutes after plasma Na was restored by adding 0.28M NaCl.

Effect of CO₂ on contractility of an isolated cat papillary muscle at two Na concentrations in Ringer's solution. T = developed tension; dT/dt = rate of rise of tension; [Na]₀ = Na concentration in the Ringer solution.
TABLE 3
Changes in LVEDP at Constant Stroke Work during CO₂ Changes before and after Sucrose in Seven Experiments

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sucrose</th>
<th>Sucrose - Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔPCO₂</td>
<td>LVEDP</td>
<td>LVEDP</td>
<td>LVEDP</td>
</tr>
<tr>
<td>27.0 ± 1.6</td>
<td>4.3 ± 0.8</td>
<td>4.6 ± 0.6</td>
<td>27.0 ± 2.9</td>
</tr>
</tbody>
</table>

See footnotes to Table 1.

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TABLE 4
Changes in Developed Tension during Changes in CO₂ (mm Hg) at Normal and Low Extracellular Na Concentration in 21 Experiments

<table>
<thead>
<tr>
<th>[Na] (mM)</th>
<th>ΔT (mm Hg)</th>
<th>ΔTcalc (mm Hg)</th>
<th>ΔT/dt (mm Hg)</th>
<th>ΔT/dtcalc (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 mM</td>
<td>47 ± 2.4</td>
<td>44 ± 2.3</td>
<td>27.6 ± 2.7</td>
<td>29.4 ± 4.0</td>
</tr>
<tr>
<td>112 mM</td>
<td>44 ± 2.4</td>
<td>33.2 ± 2.7</td>
<td>35.7 ± 2.3</td>
<td>35.7 ± 2.3</td>
</tr>
</tbody>
</table>

Values are means ± SE

TABLE 5
Changes in Rate of Rise of Tension during Changes in CO₂ (mm Hg) at Normal and Low Extracellular Na Concentration in 21 Experiments

<table>
<thead>
<tr>
<th>[Na] (mM)</th>
<th>ΔT/dt (mm Hg)</th>
<th>ΔT/dtcalc (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 mM</td>
<td>47 ± 2.4</td>
<td>38.6 ± 3.7</td>
</tr>
<tr>
<td>112 mM</td>
<td>44 ± 2.4</td>
<td>39.2 ± 3.9</td>
</tr>
</tbody>
</table>

ΔT/dt = difference in maximal rate of rise of tension between lower and the higher CO₂; ΔT/dtcalc = calculated difference in maximal rate of rise of tension between lower and the higher CO₂, using a theoretical ΔCO₂ of 45 mm Hg. Last column = mean difference obtained by paired sampling between ΔT/dtcalc at an extracellular Na concentration of 142 mM, and at one of 112 mM.

Values are means ± SE

On the other hand, addition of 0.15M NaHCO₃ will not decrease plasma Na concentration, although the remaining plasma constituents will decrease. The expected decrease in plasma Na concentration associated with THAM addition can account for an increase in contractility. When 0.3M sucrose was administered to the heart-lung preparation, an increase in myocardial contractility was observed. As with THAM, for a given ΔCO₂, LVEDP increased less after sucrose, indicating that the increase in contractility after sucrose was more marked at CO₂ was higher. Moreover, the fact that contractility, previously increased by THAM or sucrose, was restored to control values when plasma Na concentration was returned to normal values, induced us to consider that the effect of THAM was mediated, at least to an important
extent, through a secondary decrease in plasma Na concentration.

These results, together with the fact that NaHCO₃ did not change the effect of CO₂ upon the heart and a decrease in extracellular Na concentration exerts a positive inotropic effect (26-28), led us to study the effect of a change in Na concentration upon the inotropic action of CO₂ in a system in which the composition of the medium could be accurately controlled. It was observed that when the papillary muscle was exposed to a solution with low Na concentration, other factors being constant, its contractility increased as was expected on the basis of previous reports (26-28). Furthermore, in that case an increase in Pco₂ was followed by a decrease in contractility that was smaller than the one observed when the muscle was immersed in a Ringer solution with normal Na concentration. The low Na concentration is of the same order of magnitude of the Na concentration present in plasma after administration of sucrose or THAM.

The fact that propranolol was administered to the heart-lung preparation seems to exclude a mediation of endogenous catecholamine release in this phenomenon.

The results obtained in the present experiments seem to indicate that the effect of THAM can be reproduced by hemodilution with an isotonic solution of sucrose, which leads to the associated changes in plasma Na concentration. The question arises whether the change in Na concentration following THAM is the only mechanism playing a role in the effect reported in these experiments or if THAM exerts an inotropic action. We think that the results obtained in the present experiments do not help to elucidate that question. However, in in vitro experiments performed in our laboratory (12), we observed that the replacement of NaHCO₃ by 0.3M THAM at constant pH and Pco₂ was not followed by changes in developed tension or dT/dt of isolated cat papillary muscles. The association of the replacement of NaHCO₃ by THAM with a decrease in sodium concentration and a slight increase in osmolarity of the Ringer solution, both factors exerting a positive inotropic effect (26-30), would suggest that THAM actually exerts a negative inotropic influence.

Previous experiments have demonstrated that an increase in myocardial contractility originated by a decrease in sodium concentration can be reproduced by the increase in calcium concentration (26, 27). It has also been suggested that a decrease in sodium concentration enhances the release of Ca²⁺ by the sarcoplasmic reticulum (31, 32). In this way, low sodium or high calcium concentration could share a common mechanism in producing an increase in myocardial contractility. Perhaps one interesting contribution of this study is the finding that the contractile response to Pco₂ diminished in low sodium concentration, suggesting that either sodium or calcium ions may be involved in the mechanism by which CO₂ affects myocardial contractility. Experiments are in progress to supply information about the different contractile response to CO₂ at different calcium concentrations. A competition between calcium and hydrogen ions has recently been postulated (33).
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