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Endogenous Opioids Do Not Mediate HCl-Induced Myocardial Dysfunction

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ABSTRACT. We evaluated the hypothesis that increased endogenous opioid activity mediates part or all of the left ventricular contractile and pump dysfunction previously demonstrated in HCl-induced metabolic acidemia. Eighteen Western newborn lambs were catheterized and instrumented; pacing wires were sutured to the right atrial appendage; a catheter mounted micromanometer pressure transducer was inserted into the left ventricle; and a 2.5 F thermistor was inserted into the distal abdominal aorta. The lambs were studied 3 days after surgery. Metabolic acidemia was produced with an infusion of 0.5 N HCl into the inferior vena cava. Inhibition of endogenous opioids was achieved with a bolus of 2 mg/kg of intravenous naloxone, which was demonstrated to inhibit morphine sulfate-induced myocardial dysfunction. The effects of opioid inhibition were contrasted with our previously published results after restoration of a normal arterial pH with intravenous sodium bicarbonate. In agreement with our previous study, we found that reducing the arterial pH from 7.41 ± 0.01 to 6.97 ± 0.04 was associated with a 45% reduction in cardiac output which resulted from a 50% reduction in stroke volume. These changes in turn were mediated by a 35% reduction in the maximal first derivative of left ventricular pressure and/or a 63% increase in systemic vascular resistance which we used to estimate contractility and afterload, respectively. Left ventricular end diastolic pressure increased during acidemia. Although opioid inhibition produced a consistent increase in the maximal first derivative of left ventricular pressure, this increase was relatively small and was not associated with a significant change in cardiac output, stroke volume, or systemic vascular resistance. In contrast, restoration of a normal arterial pH was associated with increases in cardiac output, heart rate, stroke volume, and dP/dt to greater than control values, as well as a normalization of systemic vascular resistance. These hemodynamic changes were not attributable to direct effects of naloxone. Herein we suggest that although opioids may exert a small role in mediating the contractile dysfunction that occurs during HC- induced metabolic acidemia, there is no apparent influence of opioids on pump performance. Opioids participate very little in the myocardial dysfunction of this model of metabolic acidemia in lambs. (*Pediatr Res* 23: 643–646, 1988)

Abbreviations

IV, intravenous
LVEDP, left ventricular end diastolic pressure
dP/dt, first derivative of left ventricular maximal pressure

Myocardial dysfunction has occurred in conjunction with the metabolic acidemia of birth asphyxia in human newborns (1–5). To examine the effects of metabolic acidemia on neonatal cardiovascular function, we recently used an IV HCl infusion to produce a metabolic acidemia in unanesthetized lambs (6). In this model, metabolic acidemia was associated with a significant reduction in cardiac output, which resulted from a simultaneous reduction in left ventricular contractility and/or from the increase in cardiac afterload. These studies did not distinguish among several possible mechanisms that may have been responsible for these hemodynamic changes during acidemia.

During the past decade, endogenous opioids have been demonstrated to exert significant regulation over the circulation, especially during periods of severe hemodynamic stress. Endogenous opioids such as β-endorphin appear to be important mediators of the depression of cardiac output and left ventricular contractility that occurs in endotoxic or hemorrhagic shock in mature cats and dogs (7–9). To the best of our knowledge, endogenous opioids have not been examined as possible regulators of the hemodynamic depression that occurs during metabolic acidemia (10, 11). On considering that metabolic acidemia occurs frequently in newborn humans with asphyxial cardiomyopathy, that umbilical cord arterial blood pH and β-endorphin concentrations are inversely correlated in asphyxiated human newborns, that metabolic acidemia depresses cardiovascular function in lambs, and that β-endorphins are present and may be increased by certain conditions in lambs (12, 13), we formulated the hypothesis that increased endogenous opioids mediate part or all of the hemodynamic depression that occurs in HCl-induced metabolic acidemia in lambs. We tested this hypothesis in unanesthetized lambs by examining the hemodynamic effects of IV naloxone, an opioid inhibitor, when administered before and after the metabolic acidemia was produced.

METHODS

Experimental preparation. We performed a left thoracotomy under 1% halothane anesthesia on 18 Western lambs at 2–6 days after birth when they weighed 4.6 ± 0.3 (mean ± SEM) kg. Using techniques described previously, pacing wires (no. 5633 hookup wire, Cooner Wire Co., Chatsworth, CA) were sutured to the right atrial appendage, and fluid filled catheters (inside diameter = 0.10 cm; outside diameter = 0.15 cm) were placed in the ascending aorta and the left atrium (6). Through a separate incision, a catheter mounted pressure transducer (no. PC-350, Millar Instruments, Houston, TX) was inserted into the left carotid artery and manipulated into the left ventricle. A separate catheter was placed into a small leg vein and advanced so that its tip was in the distal inferior vena cava. A 2.5 F thermistor was inserted into a small leg artery and advanced so that it was in the distal descending aorta. All catheters were tunneled subcutaneously to the left posterior chest and protected by a nylon

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mesh vest. The wounds were sutured, the anesthesia was discontinued, and the lambs recovered with their ewes.

Experimental protocols. Three days after surgery, the lambs were placed in a nylon-mesh animal support sling and we began a continuous recording of the electrocardiogram, heart rate, LVEDP, aortic blood pressure, and dP/dt. Intermittent arterial blood withdrawals were made for the measurement of blood gasses, oxygen saturation, and Hb concentration. Cardiac outputs were measured intermittently by the thermodilution technique, using a 4-ml iced saline left atrial injection and the distal aortic thermistor (14). These intermittent measurements were performed as the final part of each condition described in the protocols that follow.

In eight of the lambs, we made sequential repetitions of the above measurements during a control period, during HCl-induced metabolic acidemia, and 5 and 15 min after an IV bolus injection of 2 mg/kg of naloxone (Sigma Chemical Co., St. Louis, MO). In another group of eight lambs, we made the measurements during a control period, 5 min after 2 mg/kg of naloxone, and during metabolic acidemia. Metabolic acidemia was produced by infusing 0.5 N HCl into the distal inferior vena cava, using a protocol described previously (6). Our goal of reducing the arterial pH below 7.10 was achieved after 31 ± 3 min of HCl infusion. The measurements that were made during acidemia were made 15 min after the desired arterial pH was reached.

Four of the above lambs developed sinus bradycardia (60–75 bpm) during metabolic acidemia. Each acidemia protocol contained two of these lambs. These lambs were paced at their control heart rates to avoid the hemodynamic instability that may develop when bradycardia occurs during acidemia with this model (6). These lambs were not distinguished by their age or body weight from the lambs that did not develop bradycardia during acidemia.

Two additional lambs were used to determine the bioactivity of the naloxone concerning opioid inhibition. On one day hemodynamic variables were measured after administration of 0.5 mg/kg of IV morphine. Two days later hemodynamic variables were measured when 2 mg/kg of naloxone was given before the same dose of morphine.

There was no evidence of pain or discomfort when the HCl or naloxone were infused through the central inferior vena caval catheter, as evidenced by the absence of agitation or anxiety. The lambs rested quietly in the animal support sling during all parts of the study. A single study was performed in each lamb using one of the above protocols. After each study, the lambs were killed with an IV euthanasia solution and the correct position of each catheter was verified at necropsy.

Measurements and calculations. PO_2, PCO_2, and pH were measured with a blood gas analyzer (no. 158, Corning Instruments, Medfield, MA). Measurements of PO_2 were made at 37° C, and the results were corrected to the body temperature that was measured with the indwelling thermistor. Blood oxygen contents were calculated by multiplying the measured oxygen saturations and Hb concentrations (15).

Aortic and left atrial blood pressures were measured with Statham P23Db transducers referenced to atmospheric pressure with zero obtained at the midchest position. The left ventricular pressure transducer was calibrated by matching the LVEDP with the left atrial a wave, and the peak systolic pressures in the left ventricle and ascending aorta. Electronic differentiation was performed with a Gould no. 13-4615-71 differentiator. This allowed us to measure the maximal positive dP/dt, which we used to estimate left ventricular contractility (see "Discussion"). LVEDP was used to estimate preload. All signals were recorded on a Gould no. 2800S physiologic recorder.

Cardiac output, which we used to estimate cardiac pump performance, was measured using left atrial injection of iced saline (0–3° C) and distal abdominal aortic sampling. Measurements were performed in duplicate. If the individual measurements were more than 5% from their mean, the measurements were repeated. Injectate temperatures were measured directly. Cardiac output measurements were calculated with an American Edwards Laboratories no. COM1 cardiac output computer. Afterload was estimated from systemic vascular resistance, which was calculated by dividing aortic mean blood pressure by cardiac output. Central venous pressure was not measured and is not included in the estimate of afterload. This omission may have induced a small overestimation of systemic vascular resistance during acidemia (Tables 3 and 4). However, this omission could not have accounted for the entire change in systemic vascular resistance during acidemia.

The mean ± SEM was calculated for each variable. Significant effects of treatments were determined with analysis of variance for repeated measurements (16). When the computed F ratio exceeded the critical F ratio at $p = 0.05$, the modified t test was used to identify significant within group differences (16).

RESULTS

Consistent with our previous results (6), a 30-min IV infusion of 0.5 N HCl produced a significant metabolic acidemia with a partially compensatory respiratory alkalemia (Tables 1 and 2). The HCl-induced acidemia also was associated with a significant increase in arterial PO_2, and with significant decreases in arterial PCO_2 and in oxygen saturation. These data suggest that a Bohr shift had occurred during acidemia. There were no significant effects of naloxone administration either before or during acidemia on the blood gasses, oxygen saturations, or Hb concentrations (Tables 1 and 2).

In agreement with our previously published results (6), HCl-induced metabolic acidemia was associated with a 35–45% reduction in cardiac output, which resulted from a reduction in stroke volume (Tables 3 and 4). The mean heart rate of each group increased during acidemia. These heart rate data include the two lambs in each study protocol that were paced at their control heart rate as soon as they developed sinus bradycardia during acidemia. The mean heart rate during each acidemia period (Tables 3 and 4) would have been unchanged if the spontaneous heart rate had been included in the measurements (149 ± 15 and 156 ± 13 for the data in Tables 3 and 4, respectively). Nonetheless, it is apparent that stroke volume decreased during acidemia. Of the determinants of stroke volume, LVEDP and systemic vascular resistance increased during acidemia whereas the maximal positive dP/dt decreased.

Table 1. *Effect of acidemia and naloxone on arterial blood gasses Hb concentration and oxygen saturation (mean ± SEM, n = 8)*

	Control	Acidemia	Naloxone-5	Naloxone-15
pH	7.41 ± 0.01	6.97 ± 0.04*	6.98 ± 0.04	6.98 ± 0.04
PCO_2 (torr)	38 ± 1	22 ± 3*	24 ± 2	24 ± 1
PO_2 (torr)	81 ± 2	100 ± 7*	97 ± 7	98 ± 7
Hb (g/dl)	8.7 ± 0.03	9.2 ± 0.4	9.2 ± 0.4	9.5 ± 0.5
O_2 saturation (%)	97 ± 2	88 ± 2*	88 ± 2	88 ± 2
O_2 content	5.1 ± 0.3	4.9 ± 0.2	4.9 ± 0.2	5.1 ± 0.2

* Significantly different from the preceding measurements at $p < 0.05$. O_2 content is in mmol/liter. Naloxone 5 and 15 represent observations made 5 and 15 min after 2 mg/kg of IV naloxone.

Naloxone did not alter the spontaneous heart rate of the four lambs that developed sinus bradycardia during acidemia. There was a trend toward an increase in dP/dt after naloxone administration during acidemia. This reached statistical significance 15 min after naloxone administration, but naloxone was not associated with any change in cardiac output or in its other determinants.

The opioid inhibitory activity of the naloxone was demonstrated in two additional lambs. In these studies, 0.5 mg/kg of IV morphine sulfate alone was associated with 32, 13, and 16% (mean) reductions in cardiac output, heart rate, and stroke volume, respectively, whereas these variables changed by no more than ± 3% when 2 mg/kg of IV naloxone was given before the morphine.

DISCUSSION

Methodological considerations. As an index of left ventricular contractility, the maximal positive dP/dt is known to be sensitive to changes in inotropic function as well as to changes in loading conditions. Although dP/dt is relatively unaffected by changes in afterload, dP/dt changes directly with preload (17, 18). In our study, LVEDP either was unchanged or changed in the opposite direction compared to maximal dP/dt (Tables 3 and 4). Thus, the changes in dP/dt do not appear to have resulted from changes in loading conditions and should represent changes in left ventricular contractility.

In contrast to the lactic acidemia of birth asphyxia, our model used an HCl infusion to produce metabolic acidemia. This method was used because it mimicks many of the hemodynamic effects of birth asphyxia in humans by reducing cardiac output and contractility (6). However, it must be noted that we did not directly measure endogenous opioid activity. The inverse relationship between pH and circulating β-endorphin activity has been demonstrated only for the lactic acidemia of birth asphyxia (13). We do not know if the increased endogenous opioid activity of birth asphyxia is specific to the lactic acidemia or if it is related to other factors. Thus, the apparent lack of effect of opioid blockade in reversing the hemodynamic effects of HCl-induced acidemia could suggest either the absence of a regulatory role for opioids during metabolic acidemia, or that this particular model of acidemia does not stimulate endogenous opioid activity. We favor the former possibility because of the similar depression of function in the two models. It seems unlikely that one would involve a cardiac depressive mechanism that would not be present in the other.

Effect of opioid inhibition on hemodynamic effects of HCl-induced metabolic acidemia. The results herein do not support our hypothesis. Endogenous opioid activity contributes remarkably little to left ventricular contractile or pump performance in the normal newborn lamb or in lambs with HCl-induced metabolic acidemia (Tables 3 and 4). Opioid inhibition did result in a statistically significant, small increase in left ventricular contractility during acidemia, but there was no measurable effect on cardiac output (Table 3). This is in contrast to much larger effects of pH normalization on acidemic dysfunction, and of opioid inhibition on morphine-induced cardiac dysfunction (6).

There are several possible ways to explain the apparent minimal effects of naloxone on the hemodynamic effects of HCl-induced metabolic acidemia. First, it was possible that the naloxone that we used might have no bioactivity. In the two lambs in which it was tested, however, naloxone prevented morphine sulfate-induced depression of cardiac output, heart rate, and stroke volume. Thus, the naloxone appears to have had adequate bioactivity. Alternately, it is known that there are several different opioid receptor subtypes, and that naloxone and morphine sulfate have the highest affinity for the μ receptor subtype (19). We specifically chose naloxone as our opioid inhibitor because several previous studies have suggested that the μ opioid receptor was particularly involved in the cardiovascular regulation by opioids (7–9, 20). However, agonists and receptors of other subtypes have recently been identified directly in the heart and elsewhere (21). Although the affinity of naloxone is low for these other receptor subtypes, the high dose of naloxone that was used in this study should have blocked all of the opioid receptors (22). Thus, the results herein suggest that endogenous opioids are

Table 2. *Effect of naloxone and acidemia on arterial blood gasses, Hb concentration, and oxygen saturation (mean ± SEM, n = 8)*

	Control	Naloxone	Acidemia
pH	7.39 ± 0.02	7.41 ± 0.02	7.04 ± 0.03*
PCO_2 (torr)	37 ± 2	39 ± 2	24 ± 2*
PO_2 (torr)	79 ± 2	78 ± 2	95 ± 5*
Hb (g/dl)	9.0 ± 0.2	9.1 ± 0.2	9.3 ± 0.3
O_2 saturation (%)	96 ± 2	96 ± 2	88 ± 3*
O_2 content	5.2 ± 0.3	5.1 ± 0.3	4.8 ± 0.3

* Significantly different from the preceding measurement at $p < 0.05$. O_2 content is in mmol/liter.

Table 4. *Effect of naloxone and acidemia on cardiac output and its determinants (mean ± SEM)**

	Control	Naloxone	Acidemia
Cardiac output	382 ± 37	399 ± 34	248 ± 29†
Heart rate	161 ± 9	163 ± 9	177 ± 8†
Stroke volume	2.35 ± 0.29	2.39 ± 0.27	1.41 ± 0.21†
LVEDP	2.7 ± 0.4	3.2 ± 0.4	6.7 ± 0.6†
Aortic mean pressure	75 ± 2	77 ± 2	72 ± 3
dP/dt_{max}	3770 ± 274	3820 ± 252	2492 ± 217†
SVR	0.19 ± 0.02	0.20 ± 0.03	0.30 ± 0.03†

* SVR is systemic vascular resistance. Units are the same as in Table 3. Naloxone indicates results obtained 5 min after naloxone administration.

† Significantly different from the preceding measurement at $p < 0.05$.

Table 3. *Effect of acidemia and naloxone on cardiac output and its determinants (mean ± SEM)**

	Control	Acidemia	Naloxone-5	Naloxone-15
Cardiac output	406 ± 48	222 ± 28†	199 ± 20	230 ± 24
Heart rate	159 ± 8	179 ± 8†	174 ± 6	181 ± 6
Stroke volume	2.63 ± 0.34	1.31 ± 0.20†	1.21 ± 0.22	1.25 ± 0.22
LVEDP	3.0 ± 0.4	6.7 ± 0.6†	6.9 ± 0.7	6.8 ± 0.6
Aortic mean pressure	77 ± 2	72 ± 3	70 ± 3	71 ± 3
dP/dt_{max}	3900 ± 293	2550 ± 253†	2710 ± 343	2990 ± 277†
SVR	0.19 ± 0.03	0.31 ± 0.04†	0.33 ± 0.04	0.29 ± 0.04

* Cardiac output and stroke volume are in ml/min/kg. Aortic mean pressure and left ventricular end diastolic pressure are in mm Hg. dP/dt_{max} is the maximal positive first derivative of left ventricular pressure, in mm Hg/s. SVR is systemic vascular resistance in mm Hg/ml/min/kg.

† Significantly different from previous measurement at $p < 0.05$. Naloxone-5 and 15 indicate results obtained 5 and 15 min after 2 mg/kg of IV naloxone.

unlikely as mediators of the myocardial dysfunction that occurs during HCl-induced metabolic acidemia.

As previous studies had demonstrated that administration of several different exogenous opioids may result in sinus bradycardia, we were particularly interested to determine if opioid inhibition would prevent the sinus bradycardia that we noted previously in some of the lambs during acidemia (6, 23, 24). The irregular occurrence of sinus bradycardia also had been noted previously during HCl-induced acidemia in mature dogs (10). For this reason, we temporarily discontinued the atrial pacing and measured the spontaneous heart rate of the lambs that had developed sinus bradycardia during HCl-induced metabolic acidemia. Our study demonstrates that the sinus bradycardia that occurs in some lambs during HCl-induced acidemia does not appear to be mediated by increased endogenous opioids.

We previously demonstrated that HCl-induced metabolic acidemia results in a reversible contractile dysfunction. However, the mechanism(s) regulating the depression of contractility remains unknown. The results of our previous work suggest that the contractile dysfunction did not result from the aortic blood desaturation, or from the reductions in transmural myocardial blood flow or myocardial oxygen consumption (6). Similarly, acidemia did not produce myocardial dysfunction through selective subendocardial ischemia (6). Our study argues against a specific opioid regulatory mechanism. Although the mechanism remains unknown, it is possible that acidemia-induced contractile dysfunction may be related to previously described cellular mechanisms such as an inhibition by excess hydrogen ion of the interaction between calcium and troponin, and/or an interference with adrenergic receptor binding (25, 26).

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