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Tissue surface pH monitoring during reduced blood flow: Metabolic implications and sources of error*

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1 Introduction

Low tissue surface pH in the absence of low blood pH has been observed in both adults and children suffering from occult blood loss [4, 5, 6, 9, 14, 15] or peripheral vascular disease [11, 12, 13]. For this reason, it has been suggested that continuous measurement of tissue surface pH may be useful for monitoring metabolic acidosis resulting from reduced blood flow [13]. However, clinical application of these observations is difficult since a direct relationship has neither been established between tissue surface acidosis and the degree of impaired aerobic metabolism nor between it and peripheral blood flow. Although preliminary observations relating improved oxygen delivery and increased tissue surface pH (independent of blood pH) have been reported following transfusion in neonates [10] and following the establishment of adequate blood flow in patients with peripheral vascular disease [13], the relationship of the acid-base status of the blood to aerobic metabolism and to tissue surface pH during periods of reduced blood flow (reduced oxygen delivery) is not well defined. Finally, although tissue surface pH has been monitored in neonates for up to 28 days [5], calibration and sources of error are often not discussed in great detail.

To evaluate the possible application of tissue surface pH monitoring as an indicator of the acid-base status of arterial blood in infants at risk for acidosis and underperfusion, we examined the relationship between tissue surface pH and the acid-base status of the blood during periods of reduced blood flow in cats and also identified the sources of error in its measurement.

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Curriculum vitae

EDMUND FRANCIS LA GAMMA was born in New York City in 1952. He completed his pediatric training and neonatal fellowship at The New York Hospital-Cornell Medical Center in 1980 and was a Postdoctoral Scholar at the Cardiovascular Research Institute, Moffit Hospital, University of California, San Francisco in 1981. He is currently investigating the role of endogenous opiates (e.g. endorphin and enkephalin) in neural regulation of the perinatal circulation at the Division of Developmental Neurology and at the Perinatology Center of the New York Hospital-Cornell Medical Center.

2 Methods

2.1 Electrode calibration and maintenance:

Two micro-pH electrodes (Microelectrodes, Inc., Londonderry, New Hampshire) were used for all experiments in this study. Reference electrodes included a standard silver-silver chloride wire, bathing in a saturated solution of potassium chloride or ordinary, pregelled electrocardiogram skin leads (Sentry Medical Products, Santa Clara, California).

A sterile pair of electrodes was calibrated in vitro at room temperature (25 °C) with 6.864 or 7.413 phosphate buffers (Instrumentation Laboratories, Lexington, Massachusetts; 6.840 and 7.385 ± 0.005 pH units at 37 °C) and in vivo as shown in Fig. 1. In vivo and in vitro recalibration (see below) was performed after in vivo use. After being used, the electrodes were cleaned with sulfa-

Fig. 1. Arrangement of electrodes, buffer, and pH meter for in vivo calibration of tissue surface pH electrodes.

chromic acid or a 70 millimolar solution of sodium hypochlorite. They were then sterilized by either 4½ hours exposure to ethylene oxide gas at 60 °C or by soaking for 24 hours in 2% glutaraldehyde (Cidex, Arbrook Incorporated, Texas). They were then stored dry or in a bacteria free buffer (Millipore filter 0.22 micron, Millipore Corporation, Bedford, Massachusetts).

2.2 Sources of error:

Recalibration drift of the electrodes was further evaluated by comparing in vivo and in vitro calibration before and after an experiment. Furthermore, since recalibration drift could either be due to electronic drift of the pH meter (Corning Model 130, Medfield, Massachusetts) or to a change in electrode function during in vivo use, we measured electronic drift of a sealed micro-pH and calomel electrode buffer cell over a 5 day period and electrode drift by comparing pH values at recalibration to those values first obtained at insertion.

Sensitivity of the electrodes (response time) was evaluated by determining the time necessary to achieve greater than 95% of the final pH value in a buffer solution. Response times in excess of one minute were considered unsuitable for in vivo use. Electrode calibration drift and response times were also evaluated after contaminating the pH or reference electrodes with blood for 24 hours. The effects of cleaning and sterilization procedures on these electrode factors were also examined.

2.3 Reduced blood flow experiments:

Adult laboratory cats were anesthetized with 30 mg/kg of intraperitoneally administered pento-

barbital and then restrained, supine on an animal board inclined 10 degrees from the horizontal. The animals were divided into a control group, and two experimental groups in which blood flow was reduced. In the first experimental group, six spontaneously breathing animals were bled from 16 to 64% of their total blood volume [1] in 8% aliquots until their blood pressure dropped to 50% of their control levels [19]. In three of these animals, lost blood was reinfused to examine whether a relationship to the metabolic effects of reduced blood flow was reversible and detectable by tissue surface pH monitoring. In the second experimental group, six additional animals received 10 mcg/kg/min of norepinephrine in 5% glucose water for approximately one hour to reduce peripheral blood flow without lowering blood pressure (perfusion pressure). Three other animals were not manipulated after anesthesia was produced and served as controls.

Tissue surface pH was measured by electrodes that were inserted on the tissue surface through a 2 mm incision in the skin and positioned subfascially along the upper medial thigh of the animals. In addition, all cats were monitored continuously for heart rate, respiratory rate (Hewlett-Packard Cassette Recorder, model 78901A, Paramus, New Jersey), aortic blood pressure (Ailtech Model MSD20-E, Physiological Transducer, Industry, California), and temperature (Telethermometer, model 43TA, Yellow Springs Instrument Company, Yellow Springs, Ohio). Arterial blood was drawn every fifteen minutes to 1 hour to measure pH, pCO₂, pO₂, as well as to calculate the base deficit. Serial arterial lactates, drawn every hour, were determined using a commercial spectrophotometric method (Boehringer-Mannheim Company, Indianapolis, Indiana). Animals were sacrificed at the conclusion of each experiment to confirm catheter position in the descending aorta and inferior vena cava.

3 Statistical methods

Tissue surface pH was compared to simultaneously drawn blood pH, base deficit, and arterial lactic acid concentrations by linear correlation analysis. The correlation coefficients were tested for significance using appropriate tables [20]. When a rela-

tionship existed for independently related points ($N = 15$), data from all the experiments were plotted and analyzed as a group ($N = 63$). The unpaired t-test was used for comparing variables from before and after a transfusion [20].

4 Results

4.1 Sources of error

Recalibration drift was always less than 0.1 pH units after in vivo use. When examined after contamination with blood for 24 hours, the pH electrode drifted less than 0.03 pH units in 7 experiments, while drift in the reference electrode ranged from 0 to 0.67 pH units. Electronic drift of the pH meter over 5 days was small and averaged ± 0.08 (SD) pH units.

Response times (sensitivity) of the electrodes after ethylene oxide sterilization followed by dry storage were in excess of 1 minute in two of the three situations where it was used. These response times returned to normal after rehydration in buffer solution. In contrast, response times were all less than one minute when the pH electrode was cleaned using either sulfachromic acid or the sodium hypochlorite solution followed by sterilization in 2% glutaraldehyde and storage in bacteria free buffer.

4.2 Reduced blood flow experiments

After anesthetic induction, baseline recordings of heart rate and blood pressure were made for at least thirty minutes prior to experimental manipulation. Each of the 15 animals studied received a total dose of 40 mg/kg of intraperitoneally administered pentobarbital over the period in which they were studied (2½ to 8 hours). During baseline recordings tissue surface pH averaged 7.25 ± 0.10 pH units ($\bar{x} \pm SD$) and lactic acid concentrations were not elevated in arterial blood (1.53 ± 0.60 meq/L, $\bar{x} \pm SD$).

In each of the animals studied tissue surface pH was noted to be lower than arterial blood pH ranging from 1 to 1.2 pH units below the blood value. The greatest difference between tissue surface pH and blood pH occurred just before reinfusion and at the end of the hour of norepinephrine infusion (Figs. 2 and 3).

Fig. 2. Data illustrating the effects of norepinephrine infusion (10 mcg/kg/min) on tissue surface pH and monitored variables in an anesthetized 2200 g cat that was allowed to breath spontaneously. Note the lack of a relationship between tissue surface pH and arterial blood pH.

During periods of reduced blood flow there was a statistically significant inverse correlation between tissue surface pH and lactic acid concentration ($r = -0.822$, $p < 0.001$, $N = 15$) and between tissue surface pH and base deficit ($r = -0.665$, $p < 0.02$, $N = 13$; Figs. 4a and 4c). There was no statistical relationship between tissue surface pH and arterial blood pH ($r = 0.517$, $p < 0.1$, $p < 0.1$, $N = 13$). These relationships remained when data from the entire period of study were included ($N = 63$) for lactic acid concentration ($r = -0.558$, $p < 0.001$) and for base deficit ($r = -0.666$, $p < 0.001$). Tissue surface pH again did not correlate well with blood pH ($r = 0.336$) by this analysis.

Fig. 3. Data illustrating the effects of hemorrhage and reinfusion of lost blood on tissue surface pH and monitored variables in an anesthetized 1800 g cat. Note that blood pH is 0.9 pH units above tissue surface pH just prior to reinfusion at the peak blood concentration of lactic acid.

In the three cats where lost blood was reinfused after hemorrhage, tissue surface pH rose by 0.16, 0.44, and 0.04 pH units ($p < 0.06$, $p < 0.001$, and $p < 0.001$, respectively). Corresponding arterial blood pH increased less than tissue surface pH after reinfusion of the lost blood (0.05, 0.10, and 0.02, respectively, $p > 0.1$). In the three cats not reinfused with their lost blood after hemorrhage, tissue surface pH continued to fall until death. The rise in tissue surface pH after transfusion was associated with increased blood pressure and decreased heart and respiratory rates towards baseline values (Fig. 3).

TISSUE pH vs ARTERIAL LACTATE

TISSUE pH vs ARTERIAL pH

5 Discussion

The terminology concerning arterial blood pH is well standardized and accepted in clinical as well as research laboratories. This cannot be said for pH terminology in regard to other organs since it has received various labels from several authors. For example, FILLER, et al. [5, 6] have implanted electrodes subfascially and originally coined the

Fig. 4. Linear correlation analysis of tissue surface pH with (A) lactic acid concentrations in arterial blood, (B) blood pH, and (C) base deficit. Data are from 15 spontaneously breathing, anesthetized cats subjected to reduced blood flow as indicated in the figure.

phrase "muscle surface pH" for their measurement. More recently, this pH measurement has been referred to as tissue pH [11] or similarly tissue surface pH. The more precise and descriptive convention would be to state exactly where the measurement was made and therefore to refer to it as "subfascial pH". In an effort that is not breaking with recent tradition, we have applied the current and conventional terminology in this paper by using the phrase "tissue surface pH".

5.1 Sources of error

In vitro and in vivo calibration of tissue surface pH electrodes demonstrated that electrical drift at the reference electrode is the major limiting factor in producing reliable long term tissue surface pH values in vivo. The amount of drift is variable, unpredictable, and probably results from protein contamination and leakage of potassium ion at the liquid junction of the reference electrode [2].

Since this junction is an integral feature of conventional electrode systems, it cannot be avoided and so all current implantable pH and reference electrode systems are subject to its problems. Unless this drift can be measured in vivo or the liquid junction be eliminated from the design, reliable long term (greater than 8 hours) tissue surface pH data can presently only be confirmed upon removal of the probes for recalibration or by periodically calibrating the electrodes and reinserting them again. The latter method is probably unacceptable in humans and would be particularly unsuitable in very low birth weight premature infants, since it would involve reinsertion of the probe through the same non-sterile wound or require moving it to multiple other locations after each recalibration.

5.2 Reduced blood flow experiments

Tissue surface acidosis was present in cats after hemorrhage or norepinephrine infusion. These causes of reduced blood flow result in acidosis because too little oxygen is provided to meet metabolic needs [7, 19] or too little of the acid products of metabolism are removed. This was demonstrated in the present study since lactic acid concentrations and base deficit were increased in arterial blood during norepinephrine infusion and during hemorrhage. These variables fell sharply following transfusion and unlike blood pH, tissue surface pH varied inversely with these values (Fig. 4). Thus, tissue surface pH may bear a specific relationship to peripheral oxygen requirements under the conditions of this study (but not to blood pH) as previously suggested in similar work [6, 10]. On the other hand, tissue surface pH has also been shown to vary directly with arterial blood pH when peripheral perfusion is presumed normal [18]. Consequently, tissue surface pH alone does not appear to be an accurate reflection of arterial blood pH unless normal peripheral blood flow can also be demonstrated. Furthermore, the lack of a clear relationship to blood pH is indicative of the enormous buffering capacity of the bicarbonate, phosphate, respiratory and renal buffering mechanisms. This contrasts sharply with the tissue's dependence on adequate perfusion for continued pH homeostasis.

Although a clinically reliable, continuous indicator of peripheral oxygen requirements would be useful to monitor neonates at risk for tissue underperfusion hypoxia and acidosis [3, 8, 16, 17], the usefulness of long term tissue surface pH monitoring as an indication of this not only requires further proof but will depend primarily on stable

reference electrodes or on developing methods for sterile in vivo recalibration to avoid removal of the probes. Continued use of tissue surface pH electrodes should take these limitations into consideration when they are used for extended periods of time or when blood contamination of the reference electrode is inevitable.

Summary

To evaluate tissue surface pH as an indicator of the acid base status of arterial blood during periods of reduced blood flow, we examined the relationship between tissue surface pH, arterial pH, arterial lactic acid levels, and base deficit in fifteen spontaneously breathing anesthetized cats. Blood flow was reduced by hemorrhage to 50% of control blood pressure values (Fig. 3) or by infusing norepinephrine (10 mcg/kg/min intravenously) for one hour (Fig. 2). During these procedures, tissue surface pH was inversely related to arterial base deficit ($r = -0.665$, $p < 0.02$) and lactic acid ($r = 0.822$, $p < 0.001$) but not related to blood pH (Fig. 4). The errors resulting from measuring tissue surface pH continuously for 2½ to 8 hours were examined by comparing the in vivo recalibration drift of the electrodes. The pH and reference electrodes drifted less than 0.1 pH units during this time.

However, when examined in separate experiments for recalibration drift in vitro, we observed up to 0.67 pH units drift after 24 hours of reference electrode contamination with blood, but only 0.02 pH units drift in the pH electrode after this period. Since tissue surface pH is linearly correlated with the blood concentration of lactic acid or the base deficit during periods of acute blood flow reduction, tissue surface pH monitoring may be helpful for following the severity of the resulting anaerobic metabolism in neonates at risk for reduced peripheral perfusion or hypoxia. However, improvements in the reference electrode will be necessary to make long term tissue surface pH monitoring reliable if there is a reasonable risk of contamination of the reference electrode with blood.

Keywords: Neonates, reduced blood flow, shock, tissue surface pH.

Zusammenfassung

Gewebs-pH-Messung bei reduziertem Blutfluß unter Einbeziehung metabolischer Parameter und möglicher Fehlerquellen

Unsere Fragestellung war, ob der pH im Oberflächengewebe auch bei reduzierter Durchblutung als Indikator für den Säure-Basen-Status im arteriellen Blut dienen kann. Wir untersuchten den Zusammenhang zwischen dem pH im Oberflächengewebe, dem arteriellen pH, den arteriellen Lactatspiegeln sowie dem Basenmangel an 15 anästhesierten spontan atmenden Katzen. Der Blutfluß wurde zum einen durch Aderlaß reduziert, und zwar auf 50% der RR-Werte der Kontrollgruppe (Abb. 3); zum anderen wurde 60 Minuten lang Noradrenalin infundiert (10 mcg/kg/min i.v.) und so die Durchblutung gedrosselt (Abb. 2). Während dieser Phasen korrelierte der Gewebe-pH umgekehrt mit dem arteriellen Basenmangel ($r = -0.665$, $p < 0.02$) und dem Lactatspiegel ($r = -0.822$, $p < 0.001$); bezogen auf den arteriellen Blut-pH zeigte sich jedoch keine Korrelation (Abb. 4). Eine mögliche Fehlerquelle

stellen Eichpunktabweichungen von Meß- und Bezugselektrode bei der Langzeitmessung über 2,5 bis 8 Stunden dar. In vivo betrugen diese Abweichungen aber weniger als 0.1 pH-Einheiten. In einer Versuchsanordnung in vitro, wo über 24 Stunden gemessen wurde, zeigte sich jedoch ein anderes Bild: hier betrug die Eichpunktabweichung der Bezugselektrode bis zu 0.67 pH-Einheiten, wenn die Elektrode mit Blut kontaminiert war, während die pH-Elektrode nach der Meßperiode nur eine Abweichung von 0.03 pH-Einheiten aufwies. Da der pH im Oberflächengewebe eine lineare Korrelation mit dem Lactatspiegel und dem Basenmangel bei akuter Minderdurchblutung aufweist, ist die Überwachung schon sinnvoll, um das Ausmaß des anaeroben Stoffwechsels bei Neugeborenen mit peripherer Minderdurchblutung oder Hypoxie abzuschätzen. In jedem Fall muß aber bei der Langzeitüberwachung die Bezugselektrode überprüft werden, besonders wenn das Risiko einer Blutkontamination besteht.

Schlüsselwörter: Minderdurchblutung, Neugeborenes, pH im Oberflächengewebe, Schock.

Résumé

Enregistrement du pH tissulaire de surface pendant la diminution du débit sanguin. Implications métaboliques et sources d'erreur

Afin d'étudier le pH tissulaire de surface en tant qu'indicateur de l'état acido-basique du sang artériel, pendant des phases de diminution du débit sanguin, les auteurs

ont examiné la relation entre le pH tissulaire de surface, le pH artériel, le taux artériel d'acide lactique, et le déficit basique chez 15 chats anesthésiés en respiration spontanée. Le débit sanguin a été diminué par hémorragie jusqu'à 50% des valeurs tensionnelles du groupe témoin (Fig. 3) ou par perfusion de morepinephrine (10 mcg/kg/min en intraveineux) pendant une heure (Fig. 2) Pendant ces procédés, le pH tissulaire de surface est en relation inverse avec le déficit basique artériel ($r = 0,822$, $p < 0,001$) mais il n'y a pas de relation avec le pH sanguin (Fig. 4).

Les erreurs provenant de la mesure du pH tissulaire de surface en continue pendant 2 heures ½ à 8 heures ont été examinées en comparaison avec la dérive de recalibration in vivo des électrodes. Le pH et les électrodes de référence dévièrent de moins de 0,1 unité pH pendant cette période.

Toutefois, après examen lors d'essais séparés de recalibrage in vitro, les auteures ont observé jusqu'à 0,67 unités pH de variation après contamination de l'électrode de référence par du sang pendant 24 heures, mais seulement 0,03 unités pH de variation au niveau de l'électrode à pH après ce délai. Du fait que le pH tissulaire de surface est en corrélation linéaire avec la concentration sanguine d'acide lactique ou avec le déficit basique pendant les phases de diminution aigüe du débit sanguin, la surveillance du pH tissulaire de surface peut être utile pour suivre la gravité du métabolisme, anaérobie qui en résulte chez les nouveau-nés qui risquent d'avoir une circulation périphérique diminuée ou une Hypoxie. Cependant, des progrès au niveau de l'électrode de référence seront nécessaires pour que l'on puisse réaliser la surveillance au long cours du pH tissulaire de surface de façon fiable même s'il existe un risque raisonnable de contamination de l'électrode de référence avec du sang.

Mots-clés: Choc, diminution du débit sanguin, nouveau-nés, pH tissulaire de surface.

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