Anaerobic Metabolism in Dogs after Organismal Death

Jpn J Legal Med
37 (1), 75-78, 1983

Anaerobic Metabolism in Dogs after Organismal Death

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(Received Jul. 22, 1982; accepted Sept. 16, 1982)

Abstract. Energy metabolism of tissues after organismal death was investigated in dogs. Cardiac blood was taken from dogs killed by ligation of coronary artery at 1, 2, 4, 7 and 24h after cardiac arrest. For comparison, venous blood was removed from living dogs. Plasma concentrations of lactate, pyruvate, hypoxanthine plus xanthine, uric acid and allantoin were determined. Both lactate and the sum of the three oxypurines (hypoxanthine, xanthine and uric acid), recognized as good indicators for anoxia increased rapidly till 4h after death, then increased gradually till 24h. These changes appear to reflect anaerobic metabolism in cardiac muscle after death. Although further study is needed, the present results may find application in the estimation of postmortem intervals in human.

Key words: Postmortem changes, Anaerobic metabolism, Lactate, Oxypurines

Introduction

Cells fight a battle against the tendency to greater entropy even after organismal death. Since aerobic respiratory ATP formation is put out of commission, shift of aerobic to anaerobic metabolism (Pasteur effect) should occur at organismal death similar to the case of acute cyanide poisoning[1]. These metabolic changes may be useful for the estimation of postmortem intervals.

In the present study, plasma metabolites used for indicators of anoxia such as lactate[1)~3)], lactate/pyruvate ratio (L/P)[4)], hypoxanthine[5)] and uric acid[6)] were determined in dogs at various postmortem intervals to investigate the postmortem energy supply in cells after organismal death.

Materials and Methods

Adult mongrel dogs of both sexes weighing 10 to 15kg were anesthetized with pentbarbital (50mg/kg i.p.). The animal was loosely tied on its back and the trachea was intubated. The heart was exposed, and cardiac arrest was introduced by ligating left coronary artery. Approximately 3ml of postmortem blood was obtained from the right ventricle of the dogs held at 20-25℃ with a 19-gauge needle attached to a 5ml syringe at 1, 2, 4, 7 and 24h after cardiac arrest. For comparison, venous blood was removed from four living dogs using a catheter placed in cervical vein under light anesthetics (0 time control). Blood was centrifuged to obtain plasma. Plasma concentrations of lactate and pyruvate were determined using commercial kits (Lactate-UV-Test and Pyruvate-UV-Test, Boehringer Mannheim GmBH, Mannheim, W-Germany). Plasma concentrations of hypoxanthine plus xanthine and uric acid were determined as described by Katsumata *et al*[1)]. *using a commercial kit for uric acid (Uricolor-400, Ono Yakuhin Co., LTD, Osaka, Japan) and xanthine oxidase (110434, Boehringer Mannheim). Plasma concentrations of allantoin were determined by the method of Young and Conway*[7)].

Results

Changes in plasma lactate concentrations with postmortem time were shown in Fig. 1. The mean value of plasma lactate in antemortem blood was 27.8mg/dl. The value increased rapidly till 4h after death and then increased gradually till 24h. Plas-

ma L/P values increased rapidly till 4h after death from 27.3 to 357, and then remained fairly constant till 24h (Fig. 2). Although plasma concentrations of both hypoxanthine plus xanthine and uric acid increased with postmortem time, the ratio of uric acid to total oxypurines (hypoxanthine, xanthine and uric acid) varied from dog to dog possibly due to organismal variation in xanthine oxidase activity; dogs in which plasma concentrations of uric acid were high showed low levels of hypoxanthine plus xanthine in plasma and *vice versa*. Therefore, plasma concentrations of total oxypurines were plotted against postmortem time in the present study (Fig. 3). The pattern was strikingly similar to that of plasma lactate; the value increased rapidly till 4h after death and then increased gradually till 24h. Since uric acid is degraded to allantoin by uricase in living dogs, plasma concentrations of allantoin were also determined (Fig. 4). The mean value of allantoin in antemortem plasma was 1.51 mg/dl, increased slightly at 1h after death and then increased very slowly till 24h to the value of 4.21 mg/dl.

Discussion

It is reasonably assumed that permeable metabolites such as lactate, pyruvate, oxypurines and allantoin in cardiac blood mainly reflect the metabolic changes in cardiac muscle after death because systemic circulation ceases at cardiac arrest. Rapid accumulation of lactate after organismal death (Fig. 1) indicates that the shift from aerobic to anaerobic metabolism occurred in cardiac muscle. Since glycogen content in dog heart muscle is shown to be 400-495mg/100g wet wt[8]. the mean value of plasma lactate at 24h after death (414mg/dl) shows that most glycogen in the muscle converted to lactate through glycolysis within 24h. It has been pointed out that L/P represents the cytoplasmic $NADH_2/NAD$ ratio and becomes a good indicator for tissue anoxia[4]. In the present study, L/P in cardiac plasma rapidly increased till 4h, then remained fairly constant till 24h after death (Fig. 2). Although the present conditions were extremely abnormal, these results indicate that $NADH_2/NAD$ ratio in the cytoplasm of cardiac muscle increased at least till 4h after death.

In tissue anoxia, ATP rapidly degraded to AMP, followed by formation of IMP, hypoxanthine, xanthine and uric acid[9]. Rapid accumulation of total oxypurines in cardiac plasma of dogs (Fig. 3) shows that rapid degradation of ATP occurred in cardiac muscle after death. Although uric acid is degraded to allantoin by uricase in most animals[9], allantoin increased only slightly in cardiac plasma (Fig. 4). Therefore, products of ATP degradation after death seemed to accumulate mainly as oxypurines in cardiac muscle. Since cardiac muscle of dogs contains around 5mM of ATP[10] and the mean concentration of oxypurines in plasma was calculated to be around 2mM at 24h after death, 40% of ATP in cardiac muscle degraded to oxypurines within 24h.

The present results that the increasing pattern of

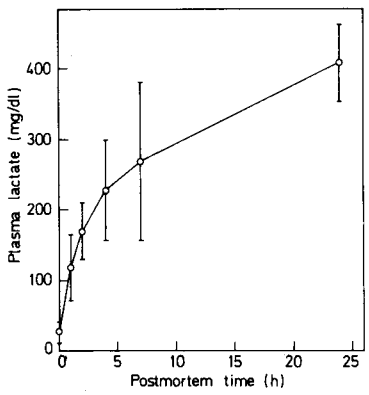

Fig. 1. Postmortem changes in plasma lactate in dog cardiac blood. Each point represents the mean ± standard deviation with 4 dogs.

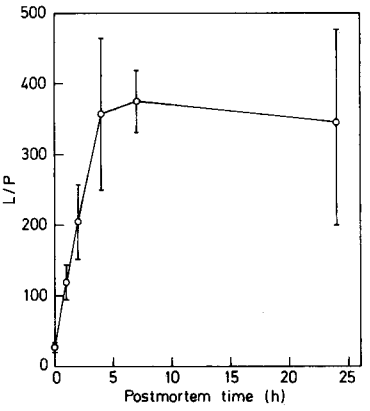

Fig. 2. Postmortem changes in plasma L/P in dog cardiac blood. Each point represents the mean ± standard deviation with 4 dogs.

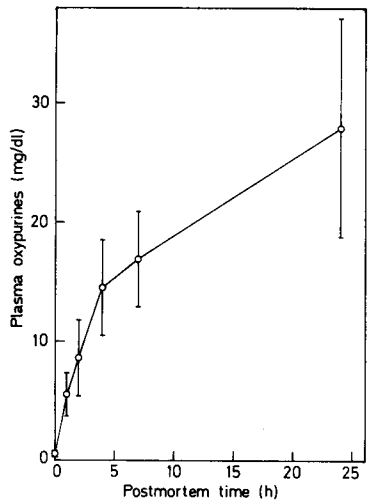

Fig. 3. Postmortem changes in plasma oxypurines in dog cardiac blood. Each point represents the mean ± standard deviation with 4 dogs.

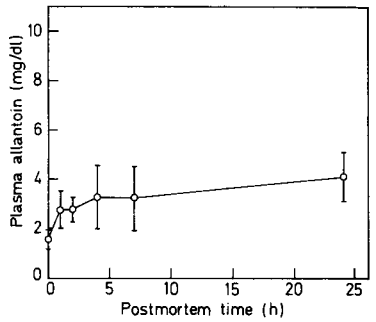

Fig. 4. Postmortem changes in plasma allantoin in dog cardiac blood. Each point represents the mean ±standard deviation with 4 dogs.

lactate was greately similar to that of oxypurines (Figs. 1 and 3) indicate that both lactate and oxypurines in plasma are good indicators of anoxic metabolism in tissue, and that anaerobic formation of ATP by glycolysis (accumulation of lactate) and degradation of ATP (accumulation of oxypurines) occur simultaneously after death. Anoxic metabolism seemed to become slow at 4h after death probably due to decrease in temperature and pH, deterioration of relating enzymes by proteolysis and so on.

Although further study is needed with human cadavers, indicators for anaerobic metabolism such as plasma lactate or plasma oxypurines may find applications for the estimation of postmortem intervals because these metabolites specifically change with postmortem time.

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犬の死後における嫌気的代謝

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摘要 個体の死後における組織のエネルギー代謝について犬を用いて検討した．冠動脈を結さつすることにより死に至らしめた犬より，心停止したのち1，2，4，7，24時間経過後心臓血を採取した．比較のため生犬の静脈血を採取した．血しょうの乳酸，ピルビン酸，ヒポキサンチン，キサンチン，尿酸およびアラントインを測定した．低酸素症のよい指標とされる乳酸および3種のオキシプリン類（ヒポキサンチン，キサンチンおよび尿酸）の総和は死後4時間まで急激に上昇し，その後24時間までゆるやかに上昇した．これらの変化は死後における心筋の嫌気的代謝を反映しているものと思われた．さらに検討が必要であるが，これらの結果はヒトにおける死後経過時間の推定に役立つ可能性があると思われる．