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Changes in Adenosine Nucleotides in the Heart Tissue of Rats Following Different Types of Death

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Abstract: Forty-eight Sprague-Dawley rats were killed by cervical dislocation, electric shock and drowning to investigate the correlations between types of death and levels of adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) in heart tissue after death. The hearts were taken out after death and ATP, ADP and AMP levels in the heart muscle were measured.

The ATP and ADP levels were lower in rats killed by electric shock and with drowning. This may be due to ventricular fibrillation caused by electric shock in the electric shock group, and debilitation of rats in the drowning group before death, which both may diminish energy sources and cause the ATP level decline.

We conclude that measurement of ATP, ADP, and AMP levels in the heart tissue after death may give us an idea about the type and time of death.

Key Words: Adenosine nucleotides, heart tissue, cervical dislocation, electric shock, drowning

Değişik Şekillerde Öldürülen Sıçanların Kalp Dokusunda Adenozin Nükleotidlerinde Oluşan Değişiklikler

Özet: Bu çalışmada servikal dislokasyon, elektrik akımı ve suda boğularak öldürülen 48 adet Sprague-Dawley tipi sıçanda ölüm zamanının ve sebebinin belirlenmesi amacıyla, ölüm tipi ile kalp dokusu adenozin trifosfat (ATP), adenozin difosfat (ADP) ve adenozin monofosfat (AMP) düzeyleri arasındaki ilişki araştırıldı. Her grupta sıçanlar öldürüldükten sonra kalp dokuları alınarak modifiye Jaworek metodu ile kalp dokusu ATP, ADP, AMP düzeyleri ölçüldü.

Elektrik şoku ve suda boğularak öldürülen sıçanların kalp dokusu ATP ve ADP düzeyleri servikal dislokasyon ile öldürülenlere göre daha düşük bulundu. Bu durum elektrik şoku ile öldürülen sıçanlarda şokun oluşturduğu ventriküler fibrilasyon sonucu, suda boğularak öldürülen sıçanlarda ise fazla fiziksel aktivite sonucu enerji azalmasına bağlı olabilir.

Ölüm sonrasında kalp dokusunda ATP, ADP ve AMP düzeylerinin ölçülmesinin ölüm zamanı ve şekli konusunda fikir verebileceği kanısındayız.

Anahtar Sözcükler: Adenozin nükleotidleri, kalp dokusu, servikal dislokasyon, elektrik şoku, suda boğulma

Introduction

Myocardial contraction occurs by the sliding of actin and myosin on each other. Myocardium contracts slower than skeletal muscle. Cardiomyocyte contains more

mitochondria and is richer in collagen tissue than is skeletal muscle (1).

After death, ATP synthesis by oxidative phosphorylation declines because of hypoxia, and ATP is

synthesised only by anaerobic glycolysis from glucose and glycogen. The end product of anaerobic glycolysis, lactic acid, accumulates. In living tissue there is a dynamic balance between ATP synthesis and its utilisation. This balance is disturbed after death, ATP utilisation becomes more than its production and ATP level decreases. When ATP decreases below a critical level, actin and myosin combine irreversibly (2,3).

An electric shock to a living body results in death causing ventricular fibrillation, respiratory muscle spasm, stroke and burns. The reason for death after an electric shock is mostly cardiac arrhythmias. In a 110-220 V alternating flow electric shock, death is usually due to ventricular fibrillation. The electrical flow affects the syncytium of the myocardium and the conduction system is disturbed. At the end, cardiac dysrhythmia and following it fibrillation is seen, and cardiac arrest occurs. In the case of the loss of resistance of the skin, a much lower ampere electrical flow may cause ventricular fibrillation (4,5).

Cell membrane destruction is an important feature of electric shock; an 800-1000 mV electric flow disturbs most of the membranes of the cells of humans. Cytoplasmic ATP concentrations decrease as a result of the rupture of the membranes of muscle cells (6).

Drowning is a kind of death based on anoxia, and occurs as a result of aspiration of water instead of air into upper and lower airways, after the reflex apnea period. The features of death change according to the water temperature and effort of the victim during drowning. All organ system may be affected in terms of pathophysiology. The cause of pathology in other systems is hypoxia, due to disturbance in the lungs. The prior status of the organism, the salinity and chemical contents of the water, the amount of water aspirated, and the physiological and anatomical properties of the victim affect the outcome. Although the beginning is the same, drowning in salt water and normal water differs in terms of pathophysiology. Alveolar damage is seen both in salt and normal water (surfactant disturbance and noncardiogenic pulmonary oedema). Metabolic acidosis appears due to anoxia in both cases. Death occurs because of the filling of the lungs with water (7,8).

In this study, adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) levels were investigated in homogeneous heart muscle after death in rats.

Materials and Methods

Forty-eight Sprague–Dawley rats (24 female, 24 male) were allocated for the study. The rats were weighed carefully. These 185-270 g rats were fed a similar diet in equal amounts. Light anaesthesia with diethyl-ether was applied to the rats before killing by cervical dislocation (control group) (8 male, 8 female), by electric shock (8 male, 8 female), or by drowning (8 male 8 female). Cervical dislocation was performed by pulling the tail after squeezing the neck of the rat with a hard material, and the rats died immediately. In the first group the electric shock (220 V) was applied to the tail and left anterior extremity and the rats died in 3-4 min. For drowning, rats were put into a water filled container, and pushed down to prevent them coming up. The rats died in 4-6 min in this second group. Immediately after death, the thorax was opened and the heart was removed. The heart was weighed carefully, and put into saline with ice. The heart tissue was cut with a blade and each sample was homogenised in 6 ml (0.6 N) of perchloric acid in a homogeniser. The resultant homogenates were centrifuged at 3000X g for 15 min. Later the resultant supernatant was neutralised with 5 N KOH from pH 4 to pH 6. The neutralised supernatants were put into tubes, and placed in a deep freeze until the day of analysis. The spectrophotometric analysis of ATP, ADP and AMP was performed in these supernatants with the modified Jaworek method (9,10).

Statistical analyses were performed with SPSS for Windows version 10 (SPSS Inc.). ANOVA analysis was used for comparison of groups, and Pearson's correlation coefficient test was used for the correlation analysis. A value of $p < 0.05$ was considered statistically significant. Variables are reported as mean \pm SD.

Results

The measured analytes are presented as mean \pm SD in the Table. The ATP level of the electric shock and drowning group was significantly lower than that of the control group. The ATP level in the electric shock group was lower than that in the drowning group (Figure 1). Similar results were obtained as far as the ADP and AMP levels are concerned; the ADP and AMP levels of the electric shock group were lower than those of the drowning and control groups. Furthermore, the ADP and AMP levels of the drowning group were lower than those

Table 1. ATP, ADP, and AMP levels of all groups.

	Cervical Dislocation (Control) (n = 16)	Electric Shock (n = 16)	Drowning (n = 16)
ATP (mg/g wet muscle tissue)	43.98 ± 7.89	32.48 ± 5.44*	35.81 ± 2.95*
ADP (mg/g wet muscle tissue)	10.80 ± 1.95	8.34 ± 1.32*	8.95 ± 0.85*
AMP (mg/g wet muscle tissue)	4.41 ± 0.51	2.95 ± 0.97*	3.21 ± 0.87*

*p < 0.05, compared to control group

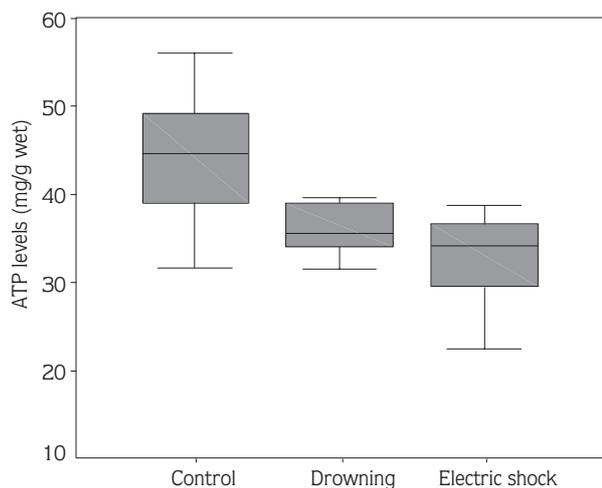


Figure 1. ATP level of groups.

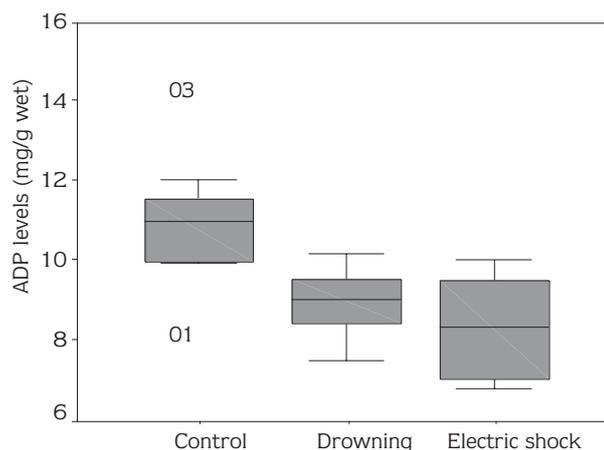


Figure 2. ADP level of groups.

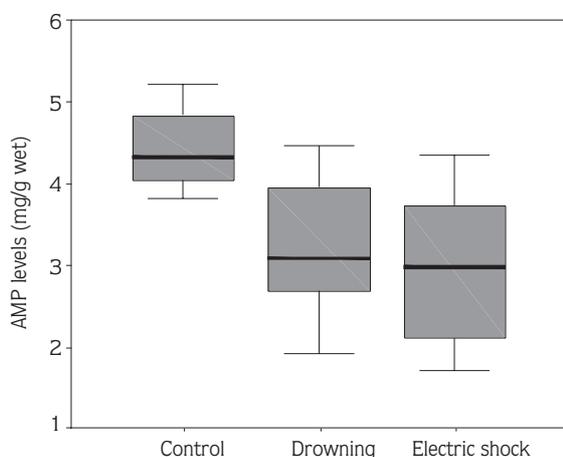


Figure 3. AMP level of groups.

of the control group (Figures 2,3). All differences were statistically significant ($p < 0.05$).

There was a positive linear correlation between ATP, ADP and AMP levels in all groups ($p < 0.05$).

Discussion

The ATP level in this study was lowest in the electric shocked rats and highest in the cervically dislocated animals (control). This difference was substantial and indicates that ATP does not decrease in the same manner in all types of death.

Reduced ATP is naturally associated with increased ADP and AMP levels, since the latter are the product of ATP breakdown. As ATP decreases, ADP increases. However, the accumulated ADP is converted into ATP as in the reaction of $2 \text{ ADP} \rightarrow \text{ATP} + \text{AMP}$. In other words, ATP and ADP are sources of each other and the positive correlation between the two analytes is accepted (11).

Stapleton and Allshire (12) investigated the relationship between myosin ATPase in rat cardiomyocytes in the postmortem period and rigor mortis, and found that the increased myosin ATPase caused ATP levels to decline. Since muscle contraction in rigor mortis increases ATPase activity, ATP decreases with time after death. In our study we took samples as

soon as the rats died, before rigor mortis occurred, in order to obtain correct results.

It is reported that various mammalian cells have the ability to downregulate their energy expenditure when the oxygen supply becomes limited, so that cellular ATP remains unchanged (13). However, Knull and Bose (14) reported that oxygen deficiency in tissues causes mechanical and biochemical changes. Oxygen deficiency causes ATP to decline even if glucose is present. In our study, the difference in the decline of the ATP level was thought to be related to the work of muscles, as the work changes the oxygen demand and energy utility of the tissues.

Since electric shocks cause ventricular fibrillation, before death, the energy sources diminish and the ATP level decreases rapidly after that shock. In drowning, rats were very debilitated, and this caused the ATP level to decline. During cervical dislocation, the rats died immediately and ATP did not decrease as much as in the other groups. This explains why the ATP level of the electric shock and drowning groups was lower than that of the cervically dislocated animals, and also explains why the ATP level of electric shocked rats was lower than that of drowned rats.

The changes in cardiovascular function that occur in victims of near-drowning are predominantly secondary to changes in the partial pressure of arterial oxygen tension and acid base balance (15). Complete recovery is possible

even after a prolonged period of asphyxia in near-drowning patients (16). Siebke et al. (17) reported that some persons regained normal cerebral function after submersion for as long as 40 min in extremely cold water. This may be due to the energy (ATP) preservation of the heart in such cases.

Doering et al. (18) measured the ATP, lactic acid and glycogen levels and extension of the muscles in rabbit gastrocnemius muscle. Their results indicate that ATP formation continued for some time after death. Kobayashi et al. (19) showed that lactic acid and ADP were independent of the ATP level in different muscles. These results indicate that the production and consumption of ATP progressed differently in each muscle. They also showed that the rate of decrease of ATP differed in different muscles, and it did not decrease in quantity in each muscle per unit mass. This may be because of the work of muscles before death. In our study, there was a correlation between ATP, ADP and AMP levels. This difference may be because of the type of muscle that was investigated.

In conclusion, measuring ATP and ADP levels in the heart after death, may give us a clue about the effort of the heart before death, and it can be used in the determination of the time and the type of death. Complete recovery of near-drowning patients is possible even after a prolonged period of asphyxia, possibly due to energy (ATP) preservation in the heart tissue.

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