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Central Monoamines and the Death Process Time (Antemortem Time) During Asphyxia

An Experimental Study in the Mouse Brain

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Summary. The changes of the brain monoamines, norepinephrine (NE), dopamine (DA), and serotonin (5-HT), during acute asphyxia, caused by strangulation, anoxia, and drowning, were studied in the mouse.

In several asphyxiated animal groups significant linear correlation was found between the level of monoamines, NE, DA, and 5-HT, and the death process times or antemortem times were $r = 0.50$, 0.98 ($P < 0.05$), and 0.57, respectively.

It is concluded that the level of brain NE and DA increased in the mouse that died of asphyxia, and the level of 5-HT showed only an apparent decrease in anoxia groups as compared with the control group and showed a twice as high increase in drowning groups. Especially, there was a tendency that the longer the death process times or antemortem times, the higher was the level of DA.

Key words: Monoamines, asphyxia – Antemortal alternation, catecholamine and serotonin in mouse brain

Zusammenfassung. Die Konzentrationsänderung der Monoamine Norepinephrin (NE), Dopamin (DA) und Serotonin (5-HT) im Gehirn wurde während der akuten Asphyxie (Strangulation, Anoxie und Ertrinken) experimentell an Mäusen untersucht. In verschiedenen Versuchsgruppen wurde eine signifikante, lineare Abhängigkeit der Monoamin-Werte NE, DA, und 5-HT zur Länge des agonalen Stadiums beobachtet, wobei folgende Korrelationskoeffizienten errechnet wurden: $r = 0{,}50$, 0,98, ($P < 0{,}05$) und 0,57.

Hieraus wird geschlossen, daß die Konzentration des NE und 5-HT im Gehirn bei den asphyktischen Tieren zunimmt, die das 5-HT bei den Tieren unter Anoxie abfällt, hingegen einen etwa zweifachen Anstieg bei den Tieren aufweist, die ertranken. Darüber hinaus ließ sich eine Tendenz zu

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höheren DA-Werten feststellen, die in Abhängigkeit zur Länge der agonalen Phase steht.

Schlüsselwörter: Ersticken, Konzentrationsänderung der Monoamine – Monoamine, Verhalten bei Asphyxie

Introduction

Explaining the cause of death at autopsy, as far as the determination of any cause of death is concerned, we occasionally require some considerable judgements and understandings based on experience in part of the practical forensic science because the recognition of asphyxia is very difficult and complex. The mechanism of asphyxia in physiologic and biochemical aspects has been examined in detail (Cohen 1973; Bryan and Jones 1980; Komura and Fujimura 1974).

Fatal asphyxia is a condition caused by lack of oxygen in the respiratory interchanges between the air in the lung alveoli and interruption of the blood circulation in the pulmonary capillaries, resulting in impending or actual cessation of apparent life. The term „asphyxia", as generally employed, refers to cessation of effective respiration. Furthermore, there is a condition due to lack of oxygen in the respiratory gas interchanges, oxygen insufficiency, between the blood in the capillaries and the tissue cells in all parts of the body. The term „tissue asphyxiation" is also used in this condition.

From the medicolegal standpoint general asphyxia comprises anoxia as oxygen deprivation due to a reduction of oxygen in the body tissue below physiologic levels, strangulation including hanging; strangulation by ligature, and manual strangulation, due to occulusion of the air passage of the upper airways; drowning, due to suffocation; and death resulting from filling of the lungs with water or other fluids. The tissue asphyxia comprises the interruption of blood circulation in the brain and cardiac failure.

The postmortem appearances in several cases of death by asphyxia have been described in detail, but the changes of some neurotransmitters associated with the cause of death, especially asphyxia, and the death process time or antemortem time have been studied minimally, to our knowledge.

This paper was mainly aimed at studying the effects of asphyxia, especially anoxia resulting from oxygen deprivation, strangulation, and drowning on catecholamines and serotonin levels in mouse brain.

Materials and Methods

C57Bl/6N male mice (22.4 ± 1.7 g body weight, aged 5–7 weeks) were used during the experimental works. Five mice each were kept in the department in separate cages and allowed free access to food and water. The animals were kept under regular dark-light condition (light period 8 a.m.–8 p.m.).

Induction of asphyxia, especially strangulation, anoxia resulting from oxygen deprivation, and drowning is shown in the following. Strangulation group I was produced by compressing and choking mouse neck of upper airways by two triple winding rubber bands as ligature. Strangulation group II was produced by one triple winding rubber band as described already.

Anoxia was caused by replacing air with pure nitrogen gas (99.99%). Anoxia group I showed that the concentration of air was reduced by 5% (v/v) and anoxia group II showed the concentration of air being reduced by 15% (v/v). Five animals each were transported to these experimental surroundings in plastic cages. Drowning group I animals were drowned in the fresh water completely and the other five animals of drowning group II were drowned without a physical restriction in the fresh water. The fresh water temperature was 16–18°C.

The death process time (antemortem time) was measured by stopwatch still the stop of respiratory in the final stage after the stage of apnea as shown asphyxia symptoms.

After confirmation of death, the whole mouse brain (without the olfactory lobes) was quickly removed, and the whole brain was stored in liquid nitrogen to measure its weight. Then it was used for the quantitative analysis of several monoamines. The stored whole brain was homogenized by ultrasonic homogenizer in 5 ml heptane containing 250 ng DHBA (dihydroxybenzylamine) as internal standard, 100 µl 0.1 *M* EDTA (ethylenediaminetetra-acetic acid disodium salt) and 750 µl 0.1 N HCl.

Then samples were prepared according to published procedures (Sasa and Blank 1977) and modifications. All experiments were performed using a model 2000 L High Performance Liquid Chromatography (Yanagimoto MGF, Co., Ltd.) with a electrochemical detector (Model VMD). The electrode potential was set at 0.8 V for the catecholamines (vs Ag-AgCl reference electrode). The column used was a type of ODS-T C18 reversed-phase column (10 µm particle size range, 250 × 4 mm i.d. Yanaco, Yanagimoto MGF, Co., Ltd.). Control animals were killed immediately by cervical dislocation. Eventually, the death process time (antemortem time) showed as zero second.

The mobile phase was prepared with K-phosphate buffer, 0.1 *M* (potassium dihydrogen phosphate, KH2P04), adjusted to the fine pH 3.1 with phosphoric acid, containing 1% acetnitrile and 500 mg/l phosphate buffer EDTA.

The monoamines, norepinephrine (NE), dopamine (DA), and serotonin (5-HT), in brain tissue were analyzed simultaneously. The concentration of each of the compounds in brain tissue was established from the chromatographic peak heights, by calculating the calibration factor for each component.

Means and their standard deviations were determined, and the effects of the various treatments on monoamine concentrations were compared statistically by Student's *t*-test.

Results

The death process time or antemortem time and monoamine concentrations in seven groups including the control group are shown in Table 1.

Comparison Between Group I and Group II in Three Asphyxial Types

There were significant increases in the death process times or antemortem times in three asphyxial types of group II. The longest death process time was about 47 min in drowning group II. Except for the level of dopamine in all three asphyxial types and 5-HT of drowning groups I, II we observed that there was a tendency for a significant increase in the concentration of norepinephrine (NE) and 5-HT in the longer death process time of group II than the shorter death process time of group I.

In the drowning groups, there was a significant decrease in the levels of DA and 5-HT, as compared to the short death process time group (drowning group I), from about 1,743 ng/g to 1,689 ng/g and from about 1,159 ng/g to 895 ng/g, respectively. Nevertheless, the level of brain 5-HT in animals that died of drowning was higher than in the other asphyxial groups (strangulation and

Table 1. The death process time or antemortem time and the changes of the level of brain monoamines in mice that died of asphyxia

Method	Death process time	NE (ng/g)	DA (ng/g)	5-HT (ng/g)
Control group	0 s	433.25 ± 120.14	1,222.47 ± 273.16	517.16 ± 133.93
Strangulation group I	25.0 ± 1.87 s	470.21 ± 34.26	1,450.58 ± 36.70*	485.89 ± 73.81
Strangulation group II	47.7 ± 0.48 s[b]	610.58 ± 74.69[b]***	1,611.56 ± 191.63*	796.48 ± 201.1[b]*
Anoxia group I	15.2 ± 3.27 s	472.45 ± 38.88	1,421.87 ± 126.81	394.76 ± 59.35*
Anoxia group II	66.6 ± 9.29 s[a]	622.17 ± 32.37[a]***	1,632.15 ± 337.17*	432.27 ± 104.25
Drowning group I	78.2 ± 18.27 s	496.63 ± 31.61	1,743.54 ± 71.25***	1,158.59 ± 76.85****
Drowning group II	46.7 ± 10.9 min[a]	464.78 ± 39.34	1,689.24 ± 227.89**	894.94 ± 175.97[c]**

Each value is the mean of five animals ± SD
**** $P<0.001$, *** $P<0.005$, ** $P<0.01$, and * $P<0.05$ as compared to control in all groups
[a] $P<0.005$, [b] $P<0.01$, and [c] $P<0.05$ as compared to group II in three asphyxial types

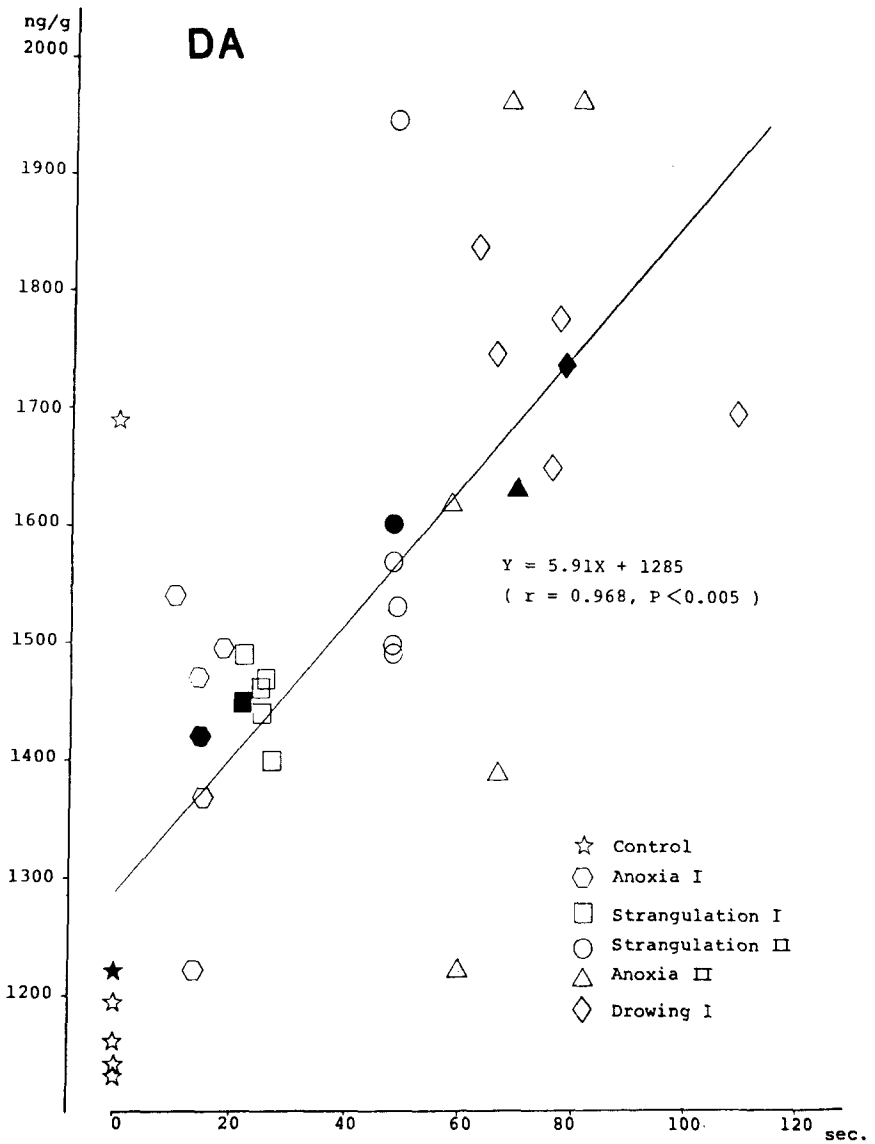

Fig. 1. The significant linear correlation between the level of dopamine and the death process time or antemortem time in several asphyxial types. Six *filled symbols* show the means of all groups, respectively

anoxia). With regard to the concentration of DA, there was no significant decrease in the three types of asphyxia.

Comparison Between Control Group and Three Asphyxial Types

In asphyxiated animals, the level of DA was significantly different from the control in almost all types of asphyxia except for anoxia I, and there was a tendency for an increase in the concentration of NE. However, the level of 5-HT was

significantly lower in anoxia groups and higher in drowning groups as compared with the control.

In six groups, except for drowning groups II, significant linear correlation values found between the level of monoamines, NE, DA, and 5-HT, and the death process time or antemortem time were $r = 0.50$, 0.98, and 0.57, respectively. Especially, the significant linear correlation found between the level of DA and the death process time was shown in Fig. 1 ($Y = 5.91X + 1285$, $P < 0.005$).

Based on the results of the present study, as a consequence, the levels of brain NE and DA increased in mice that died of asphyxia. There was a tendency that the longer the death process time or antemortem times was, the more increased the level of DA.

Discussion

Several studies on the well-defined symptoms of asphyxia have been published (Spitz 1980; Sherman et al. 1977) but only a few are available on the determination of the cause of death due to the various types of asphyxia.

To our knowledge only a few investigations of the relationships between the death process time or antemortem time and the levels of the brain monoamines in asphyxia have been published. Supposing the practical murder case and the violent death, we investigated only the changes of the monoamine levels in the brain of mice that died of asphyxia caused by strangulation, anoxia, and drowning.

Asphyxia refers to a condition due to lack of oxygen in respired air, resulting in impending or actual cessation of apparent life. The term „asphyxia" is used in the broader meaning of anoxia, the term „total asphyxia" includes the oxygen deprivation (anoxia), suffocation by external pressure on the upper airways (hanging and strangulation) and drowning, the term „tissue asphyxia" includes the interferences of blood circulation in the brain, cardiac failure as well as arsenic, carbon dioxide, carbon monoxide, and cyanide poisoning (Spitz 1980).

At the autopsy, there was a great number of causes of death due to asphyxia. The recognition of asphyxia as a cause of death is occasionally difficult and requires considerable attention.

The mechanism of asphyxia has been studied in detail, physiologically and biochemically (Gregory et al. 1982). As a consequence, the essential causes of death due to asphyxia are referred to as brain anoxia, may contribute to the restriction of cerebral blood flow, and there are many physical and biochemical changes in total asphyxia still processing to death.

Especially, a condition of asphyxia, such as oxygen deprivation, seems to result in the increase of adrenaline secretion by the stimulation of adrenargic sympathetic nerves. The changes of the monoamine levels, such as the increase of adrenaline secretion, are very interesting. In this paper, the relationship between the death process time or antemortem time and the quantitative changes of the monoamine (norepinephrine, dopamine, and serotonin) levels in several asphyxial types was published. The strangulation group used a rubber band as a

ligature-like object. The groups of anoxia and oxygen deprivation applied the method of replacing air with pure nitrogen gas, then decreased the volume of oxygen in the environment. Normal air contents, shown as percent by volume, were: nitrogen 78.084, oxygen 20.946, carbon dioxide 0.0033, and argon 0.934. In this experiment, consequently, the air contents of 5% (v/v) and 15% (v/v) fell to approximately 1.0% (v/v) and 3.0% (v/v), respectively, by replacing with nitrogen gas. Our investigations are different from the case published by Di Maio and Di Maio (1973) in so far as there was a striking deficiency of oxygen accompanied by a marked increase in carbon monoxide and methane.

As suggested, the mechanism of death by drowning in water may be based on sudden large shifts of fluids and electrolytes rather than acute asphyxia, if more significance exists between drowning in fresh and salt water. The death process time is much longer in drowning group II without a physical restriction rather than in drowning group I. One explanation for this finding may be a more active locomation and a lighter mouse body weight.

Herein, in all experimental groups, the confirmation of death refers to cessation of effective respiration after a stage of apnea. On the other hand, it is suggested that in the human being the heart may continue to beat for a brief period after respiration has caesed.

The principal aim of the experimental work in this thesis was to study the effects of asphyxia on the monoamine levels in the experimental animal brain. Consequently, as shown in Table 1, the differences in the death process times or antemortem times are proportional to the levels of monoamines (NE, DA and 5-HT) in asphyxial death, such as strangulation, anoxia, and drowning. There was a common tendency for acute asphyxia to find the significant linear correlation between the death process time and the monoamine levels.

The significant correlation factors between antemortem time, death process time as well as NE and 5-HT levels in asphyxia were $r = 0.50$, and $r = 0.57$, respectively, although there were no statistically significant correlation linears. On the other hand, the significant correlation factor between antemortem time and DA level was $r = 0.968$, and it was statistically highly significant ($P < 0.05$). Therefore, there were more increasing levels of NE and DA in the asphyxia groups than in the control group.

The level of 5-HT showed only an apparent decrease in the anoxia groups as compared to the control groups; in contrast, it was approximately twice as high in the drowning groups. Furthermore, the change of the 5-HT level may has been useful for classifying the death causes and estimating the time of death.

The postmortem changes of the monoamine levels (Sloviter and Connor 1977; Van Wijk and Kopf 1981) and the activity of various transmitter enzymes in the brain (Oehmichen 1980) have been published. It should be possible to estimate the time after death caused by several factors monoamine level rate, the monoamine levels at the time of death, the enzyme activity, monoamine synthesis, and metabolism rate of several brain enzymes during the postmortem intervals.

Furthermore, we should be able to classify the cause of death due to asphyxia by the available value indication. This idea should be valuable for the practical forensic science.

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