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ORIGINAL ARTICLE

Postmortem determination of concentrations of stress hormones in various body fluids—is there a dependency between adrenaline/noradrenaline quotient, cause of death and agony time?

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Abstract To find out whether a certain cause of death or a certain length of an agonal period shows specific adrenaline or noradrenaline profiles, heart blood, femoral vein blood, liquor, urine and vitreous humour were taken from corpses (n=98) at the Medical School Hannover, and noradrenaline and adrenaline were determined using high-performance liquid chromatography (HPLC). Corpses were classified according to the following five categories: short agony, long agony, state after hanging, state after asphyxiation and state after CPR with documented administration of epinephrine. Once results were collected the adrenaline/noradrenaline quotient was determined. It became clear that there were no significant differences regarding the concentration of adrenaline and noradrenaline in the various body fluids in relation to the above-mentioned categories. The means adrenaline/noradrenaline quotients in femoral vein blood were 0.21±0.29 for hanged persons, 0.38±0.47 for asphyxiated persons, 0.17±0.19 for those with short agony and 0.42±0.43 for those with long agony, significantly below 1 (p<0.001; p=0.001; p=0.003). For condition after CPR we found an adrenaline/noradrenaline quotient of 2.81±5.8. In liquor the adrenaline/noradrenaline quotients for short agony was 0.17±0.17, for hanged persons 0.18±0.19 and for asphyxiated ones 0.30±0.38, significantly lower than 1 (p<0.001). In urine the adrenaline/noradrenaline quotients for all categories are lower than 1 (p<0.001); short agony (0.13±0.09), long agony (0.21±0.16), hanged (0.15±0.16), asphyxiated (0.14±0.08) and CPR (0.14±0.06). In vitreous humour the quotients for short agony (0.14±0.28), long agony (0.13±0.12), hanged (0.07±0.09) and asphyxiated (0.09±0.11) are lower than 1 (p<0.001). The spread of data for the adrenaline/noradrenaline quotient did not allow for any conclusions about cause of death and length of agony in individual cases.

Keywords Postmortem · Adrenaline/noradrenaline quotient · Hanging · Asphyxiation

Introduction

A desirable postmortem tool for forensic diagnostic considerations would be a link between the level of adrenaline and noradrenaline in various body fluids in relation to a certain cause of death or a clear association to the length of the agonal period. Previous studies do not present uniform results.

Berg [1, 2] proved a significantly higher level of adrenaline and adrenaline metabolites in the blood of the vena cava inferior for various causes of death with a semi-quantitative UV-fluorescence method. In this paper an obvious relation between adrenaline concentration and cause of death such as pulmonary embolism, cardiac arrest attack and external asphyxiation became clear.

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Using a modified quantitative trihydroxyindol method, Berg and Bonte [3] determined adrenaline and noradrenaline in the blood of the vena cava inferior in 130 autopsies and furthermore in the liquor of 20 corpses. They proved that a storage time of a corpse of up to 60 h in a cool environment did not have a significant influence on the level of catecholamines. The authors related the autopsy cases with regard to cause of death to a certain period of agony: short (internal or external asphyxiation, bleeding and cardiac death), protracted (poisoning and serious head injury), or missing (very short) agony (reflex death, drowning, bolus death, sudden death). The study showed an increase of catecholamines in the blood corresponding to a prolonged agonal period. In protracted agony adrenaline values were twice as high as in short agony. Cases with short and protracted agony presented significantly higher adrenaline and noradrenaline values in comparison to the group with extremely short agony. Concerning liquor, they also proved an increased concentration of both catecholamines for prolonged agony.

Hausdörfer et al. [7] showed in 119 cases a correlation between length of agony and catecholamine concentration in serum without distinguishing between noradrenaline and adrenaline. The recorded values for extremely short agony could be compared with those of a living human at rest, those of protracted agony with a human under maximum strain. There is a clear tendency that length of agony was correlated to the level of catecholamines for the mean values.

Hirvonen and Huttunen [10] examined 11 corpses and 14 rabbits killed by injection of Mebumat and subsequent removal of adrenal gland and determined the adrenaline/noradrenaline quotient, which were determined using high-performance liquid chromatography (HPLC) . Samples were taken of heart blood and femoral vein blood from human corpses. The first samples were taken immediately after admission to the forensic institute; a second sample was taken in the course of the examination after a maximum period of 4 days. In femoral vein blood, they found a significant increase of the mean adrenaline/noradrenaline quotient from 0.34±0.38 to 0.81±1.33. As regards the heart blood, they detected a decrease from 0.78±0.71 to 0.62±0.60. Both were declared as a result of a postmortem increase of adrenaline and noradrenaline through diffusion and redistribution of blood. These results do not support the findings of Berg [1], who declared that a storage time of the corpse up to 60 h in a cool environment would have no significant influence on catecholamine levels.

The first sample of the rabbits was taken antemortem from a vein of the ear; three further samples were taken from the heart ventricles over the next 3 days. In rabbits a continuous increase of noradrenaline was found, which was linked to the discharge and diffusion from the sympathetic nerve ending. The removal of the adrenal gland was regarded as responsible for the fact that adrenaline concentrations could not be quantified postmortem.

Kauert [16] examined heart blood, urine and adrenal glands of corpses. As regards heart blood, he pointed out that due to the spread of the data, a correlation between a certain cause of death and the level of adrenaline and noradrenaline could not be proven.

Hirvonen et al. [12] investigated adrenaline and noradrenaline values in the venous blood of 26 anesthetised rats, which were afterwards strangled, and compared them to 13 rats killed with a Mebumat injection. For the strangled rats, the mean value of noradrenaline concentration was two times higher, for adrenaline a third higher compared to the group of rats killed with an injection of Mebumat.

Kernbach-Wighton et al. [15] examined heart blood in two subgroups with short and medium agony length, and a third subgroup with hypothermia. They found in short agony an adrenaline/noradrenaline quotient of 16.99, in medium agony 8.47, and in hypothermia 0.10.

Earlier studies showed inconsistent results in liquor and most of all in urine. Hirvonen and Lapinalampi [11] investigated noradrenaline and adrenaline in urine and liquor in guinea pigs after controlled hypothermia and rewarming. They found a strong increase of both catecholamines in urine, as well as their metabolites in liquor. By excluding other stress factors they suggested that the level of noradrenaline and adrenaline and their metabolites in the liquor could be seen as a forensic indicator of recent hypothermia.

Biliakov [4] compared liquor adrenaline and noradrenaline concentrations between corpses after hanging and corpses after heart disease. He found a quantitative dominance of both catecholamines for the hanged group. He therefore suggested using increased concentrations of adrenaline and noradrenaline in liquor as an indicator of death by asphyxiation.

As mentioned above, Kernbach-Wighton et al. [15] also investigated liquor in corpses and determined adrenaline and noradrenaline as well as adrenaline/noradrenaline quotient. In liquor, they found a quotient of 3.81 for short agony, 0.17 for medium agony and 0.08 for hypothermia.

Hirvonen and Huttunen [8], Hirvonen and Lapinalampi [11], and Hirvonen and Huttunen [9] conducted different trials about postmortem noradrenaline and adrenaline determination in guinea pigs, rats and human corpses. They were primarily looking for chemical laboratory indicators to show that hypothermia had taken place. In 24 cases of humans that died from hypothermia, they found higher noradrenaline and adrenaline concentrations in urine compared to the control groups (natural death and sudden violent death). The group of victims of hypothermia

showed catecholamine concentrations in urine of 0.20±0.16 μg/ml vs 0.07±0.07 μg/ml for natural death and 0.02±0.02 μg/ml for violent death.

In this study, Hirvonen et al. presented total catecholamine concentrations, without distinguishing between adrenaline and noradrenaline. Examining guinea pigs, they showed a 24- to 40-times increase of the adrenaline/noradrenaline quotient in urine in controlled hypothermia and subsequent rewarming of the animals. Further examination of urine of hypothermic rats by Hirvonen et al. showed a strong increase of noradrenaline compared to adrenaline. The authors suggested determination of the quotient as an indicator for hypothermia.

Sadler and Pounder [18] examined urine catecholamines in three cases who had died from hypothermia and were not able to show the suggested hypothermia adrenaline/noradrenaline quotient of >1. They found values of 0.92, 0.16 and 0.11.

Mancini and Brown [17] presented a significant increase of noradrenaline within 24 h after admission to hospital in the urine of patients after attempting suicide.

Tormey et al. [21] examined noradrenaline, adrenaline and dopamine in the urine from 30 specimens from unselected autopsies. They found a general increase in adrenaline and noradrenaline in comparison to ordinary cases, but could not prove a correlation between length of agony and level of catecholamine concentration in individual cases.

There are a few postmortem examinations of aqueous and vitreous humour in the literature. Lapinlampi and Hirvonen [16] examined vitreous fluid and urine in guinea pigs in relation to hypothermia. They found a 20-times-higher noradrenaline concentration in hypothermic rats in the vitreous fluid compared to the control group. The adrenaline values showed a fourfold increase. The authors suggested significantly increased catecholamine values in vitreous fluid as an indicator for hypothermia. In the above-mentioned investigation, Kernbach-Wighton et al. [15] examined fluid of the corpus vitreum and detected an adrenaline/noradrenaline quotient of 5.99 for short agony, 0.16 for medium agony and 0.03 for hypothermia.

In this context we wanted to examine if a particular cause of death presents a typical catecholamine profile, at this juncture death by hanging and death by asphyxiation, both as a separate group. Within this study the authors pointed out the special position of acute asphyxia, which is seen as a sufficient stimulus for a clear premortem and agonal catecholamine discharge.

Furthermore, we wanted to examine if the length of the agonal period has a significant influence on the level of catecholamines noradrenaline and adrenaline. Finally, we wanted to assess whether the adrenaline/noradrenaline quotient allows us any conclusions with regard to the above-mentioned categories.

Materials and methods

In relation to the above-mentioned studies, some of which presented significant differences in adrenaline and noradrenaline concentrations, we distinguished between short agony, a period of a few seconds up to a few minutes, e.g. after a fall from a window, or a shot into the heart, and long agony, of several minutes up to hours, e.g. general paralysis after alcohol intoxication, hypovolemic shock after gastric perforation and serious head injury.

Death by hanging and asphyxiation each constituted a separate group in relation to absolute concentration of noradrenaline and adrenaline as well as with regard to the adrenaline/noradrenaline quotient. Asphyxiation is defined here as a condition of severely deficient supply of oxygen, e.g. carbon monoxide inhalation and physical obstruction of the flow of air to the lungs.

Another group consisted of various ways of dying, but all of them leading to CPR with documented administration of catecholamines during emergency medical treatment.

During autopsy at the Institute of Forensic Medicine at the Medical School Hannover heart blood was taken by opening the vena cava inferior inside the pericardial sac close to the atrium and was put into ethylenediaminetetraacetic acid (EDTA) tubes.

Femoral vein blood was taken by opening the femoral vein during postmortem investigation or autopsy and was also placed into EDTA tubes.

Liquor was extracted through suboccipital puncture of the cisterna cerebellomedullaris with injection between squama occipitalis and spinal crest of Rauber of the second cervical vertebra during the postmortem examination.

Urine was extracted through catheterization of the urethra during postmortem investigation or through direct opening of the bladder and collecting the urine during autopsy.

Vitreous humour was extracted through direct puncture of the vitreous body. In a separate trial to clarify whether there are significant differences to be expected in catecholamine concentration between fluid from the vitreous body and fluid from the anterior eye chamber, liquid was extracted from one corpse's anterior eye chamber and another corpse's eye's vitreous body. Immediate storage of samples took place for liquor and vitreous fluid in ordinary plastic tubes, for urine in urine tubes.

Furthermore, gender, age, size and weight, time of death and way of dying according to the death certificate, resting time of the corpse between detection of corpse and admission to our department and the storage period of the corpse in the refrigerator at 6±2°C until taking of samples was recorded. Right after the extraction heart blood, femoral vein blood, liquor, urine and vitreous humour were deep-frozen at −18°C until further analysis took place.

To get blood plasma from heart blood and femoral vein, blood was centrifuged for 10 min at 3,000 rpm. Liquor samples also were centrifuged for further sample preparation at 3,000 rpm for 10 min. Catecholamines from the plasma, liquor and vitreous humour were measured using HPLC with electromechanical detection analogue to determine plasma catecholamines of patient blood in hospital.

In this procedure catecholamines were—before being chromatographically separated—isolated from plasma, liquor and vitreous humour, through selective adsorption by aluminium oxide [6]. To prepare the samples, 0.5 ml of extraction buffer was used for the samples, calibration standard and the control measurements. Half (0.5) or 1 ml of plasma was extracted from the samples and, with 50 µl internal standard put into cartridges, these were closed, shaken and centrifuged at 2,500 rpm for 1 min. In three steps 1 ml washing buffer was added, the sample again centrifuged, and the eluate discarded. To elute the samples cartridges were dried and again centrifuged. Small vials were connected to the cartridges and 120 µl elution buffer was added and left for 5 min. Then the eluates were shaken for 30 s and centrifuged for 1 min.

The HPLC pump was adjusted to a flow speed of 1 ml/min, an equilibrated column for plasma catecholamines was connected to the system and the performance potential of the electrochemical detector was set at 500 mV. After calibration with 50 ml internal standard analysis of samples and control group took place (intra-assay -precision 1.7–11.4%, inter-assay precision 3.7–12.7%) [13].

As all data categories, except age, presented a positive skewed distribution although not universally in all partial populations, the *U* test by Mann–Whitney was used for all comparisons of groups, and the "Wilcoxon signed rank test" for comparisons within groups. All tests were conducted on two-sides with a level of significance of 0.05. As parameters for the descriptive statistic mean values and standard deviation were used.

To answer the question how catecholamines respond postmortem in relation to the resting time of corpse and temperature, femoral vein blood was taken and a part of the sample was cooled to 4°C and used at 24-h intervals for 4 days to determine catecholamines. The second part of the sample was left at room temperature. The first determination was carried out immediately, the second after 4 days. To determine the declining curve blood was taken from the brachial vein from four healthy volunteers. Samples were left at room temperature, and catecholamines were determined first at hourly intervals, and then after 2 h, and finally after 8 and 24 h, respectively.

Determination of catecholamines in the urine samples was also made using HPLC and electrochemical detection. Through pre-separation with ion exchange material catecholamines were separated from the urine matrix. To stabilise and predilute a 3-ml urine sample was mixed with 100 µl internal standard and 6 ml dilution buffer. The pH value was checked using ordinary pH sticks and, when necessary, adjusted with 1 N NaOH to a value of 3–7. Sample extraction took place with cation exchange pillars and a subsequent washing with 20 ml distilled water. For elution the pillars were put on prepared test tubes, 6 ml elution buffer was added and the eluate was collected. One milliliter of this was put into autosampler vials and mixed with 20 µl 5 M HCl. For injection of the eluate into the HPLC system the flow rate was adjusted to 0.8 ml/m and the detection range of the electrochemical detector was set at 610 mV (Intra-assay -precision <5%, inter-assay-precision <6%) [19, 20].

Determination and interpretation of catecholamines in urine presented some difficulties. Normal hospital routine takes in urine catecholamine determination of urine collected for 24 h as its basis. Postmortem urine collection presents a time window of unknown length. In addition, catecholamine production is subject to daytime fluctuation and is stress-dependant. The immediate premortem urine secretion into the bladder is a further unknown variable. Sadler and Pounder [18] suggested a determination in urine based on a calculation of catecholamines in urine collected 24 h, which takes into account the relation between catecholamines and urine creatinine. This procedure was not applied in this investigation.

Results

Heart blood (n=46), femoral vein blood (n=84), liquor (n=88), urine (n=47) and vitreous humour samples (n=93) were taken from a total of 98 corpses (30 women, 68 men).

Mean age was at 53.05±19.3 years. Mean time between death and admission to the forensic institute was 9.1± 12.7 h, resting time of corpse from detection till admission was 7±9.2 h. The time between placement of corpse in the refrigerator and extraction of sample was at 39.6±34.2 h. Mean time between taking samples and laboratory examination was 39.5±63.6 h.

Absolute values for adrenaline and noradrenaline in heart blood displayed no significant differences in relation to type of death and length of agony, contrary to the view that concentration rises with length of agony. Mean values of noradrenaline are higher in the group with short agony, 211.62±158.14 µg/l, than those in the group with long agony, 135.15±89.76 µg/l. The following mean values were found for the following groups: hanging, 157.23±98.84 µg/l, asphyxiation, 161.15±73.39 µg/l and for state after CPR, 202.11±172.47 µg/l.

For adrenaline, no increase in concentration could be found when comparing short agony, 195.27±160.99 µg/l,

with long agony, 194.49±307.26 μg/l, hanging, 249.55±263.81 μg/l and asphyxiation, 194.67±177.85 μg/l. The mean values for the group of corpses having undergone CPR reflect the amount of epinephrine administered during CPR with 371.47±246.29 μg/l. When calculating the adrenaline/noradrenaline quotient the following results were found for heart blood: short agony 0.84±0.5, long agony 1.25±1.06, hanging 1.19±0.81, asphyxiation 1.02±0.77 and state after CPR 2.80±2.3.

The analysis of femoral vein blood showed no significant differences for noradrenaline concentrations: short agony 36.36±27.70 μg/l; long agony 34.44±43.46 μg/l; hanging 43.55±38.96 μg/l, asphyxiation 51.2±56.78 μg/l and CPR 28.80±21.32 μg/l. For adrenaline, a relation to length of agony can be suspected based on the mean values for short agony with 3.96±3.71 μg/l and long agony with 16.61±29.1 μg/l; however, because of the wide dispersion these are not definite values and cannot be distinguished significantly from each other. For hanging, a mean value of 12.5±40.04 μg/l was found, for asphyxiation 28.76±55.89 μg/l and for condition after CPR a mean value of 45.04±48.74 μg/l. Mean values of the adrenaline/noradrenaline quotients, as shown in Fig. 1, for hanging with 0.21±0.29 and for asphyxiation 0.38±0.47 are significantly below 1. This is also true for short agony with 0.17±0.19 and long agony with 0.42±0.43 ($p<0.001$; $p=0.001$ and $p=0.003$; Wilcoxon signed-rank test).

The differences between short and long agony are significant (U test according to Mann–Whitney: $p=0.022$).

Table 1 shows the results of the declining trial from femoral vein blood of a corpse. Noradrenaline showed a significantly slower progress of catecholamine processing for the refrigerated sample when analysing the values after 96 h with 37.42 μg/l in relation to the sample at room temperature with 15.18 μg/l. After 96 h the refrigerated adrenaline sample is with 3.5 μg/l lower than the sample at room temperature with 4.16 μg/l. When analysing the refrigerated samples separately, we found that for up to 72 h catecholamine processing in noradrenaline and in adrenaline showed a similar kinetics and examination produced valid data.

Table 2 shows the result of the declining trial after blood sample taking from a brachial vein from healthy candidates. Compared to postmortem values in general, the absolute values show concentrations several times lower. At the moment of sample taking noradrenaline concentrations moved from 0.13 to 0.45 μg/l; adrenaline concentrations —as far as they could be determined—moved from 0.04 and 0.09 μg/l. On average, concentration went back to about 50% of the initial value after 24 h, at the next measurement after another 24 h a significantly slower speed of decomposition was observed.

Contrary to the expectation that prolonged agony presents significantly higher catecholamine concentrations in liquor, the recorded absolute values of these groups showed no significant differences. For noradrenaline, the group of long agony showed a slightly higher concentration, with mean values of 52.46±43.45 μg/l, than the group of short agony,

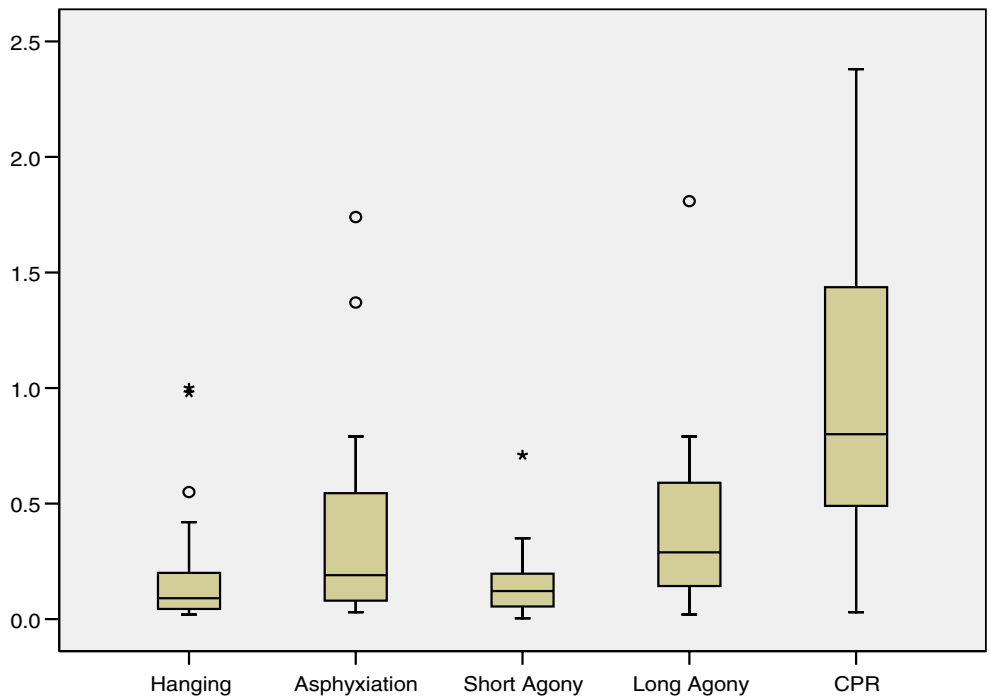

Fig. 1 Box-and-whisker plot of the adrenaline/noradrenaline quotient in the femoral vein blood according to cause of death and agony time displayed. The *circles* show outliers, the *stars* extreme values. One extreme value was cut off (CPR 18.16)

Table 1 Declining trial from femoral vein blood of a corpse in comparison at storage temperature 4°C and room temperature, concentration in μg/l

Variable	Time (h)				
	0	24	48	72	96
NA at 4°C	48.58	85.02	84.6	76.24	37.42
NA at room temperature	38.56				15.18
A at 4°C	4.02	19.1	19.0	16.14	3.5
A at room temperature	5.8				4.16

NA Noradrenaline, *A* adrenaline

with 36.19±24.67 μg/l. The group of hanging had the highest noradrenaline concentration, with 78.1±170.73 μg/l, asphyxiation 50.47±24.98 μg/l. For state after CPR, a noradrenaline concentration with 41.86±37.65 μg/l was found. See Table 3.

The values for liquor adrenaline, as displayed in Fig. 2, showed in the mean in comparison of short with 4.68±4.28 μg/l to long agony with 30.87±53.15 μg/l, an increase in concentration; however, due to the variability this increase is statistically not significant. The groups of hanging, with 10.62±16.19 μg/l, and asphyxiation, with 14.15±18.66 μg/l were among these values. The concentration for state after CPR, with 142.27±203.85 μg/l, can be clearly distinguished from the group of short agony ($p<0.05$; Mann–Whitney test).

The calculated adrenaline/noradrenaline quotients were significantly smaller than 1 for short agony (0.17±0.17), for hanging (0.18±0.19) and for asphyxiation (0.30±0.38) ($p<0.001$, Wilcoxon signed ranks test). Due to the variability of the data, the quotients for long agony (0.48±0.76) and CPR (2.84±4.19) were neither significantly higher nor lower than 1.

In urine, the noradrenaline concentrations were in the mean 93.9±82.51 μg/l for short agony, 99.64±79.25 μg/l for long agony, 94.0±56.84 μg/l for hanging, 63.0±36.51 μg/l for asphyxiation and 109.2±77.39 μg/l for CPR.

For adrenaline, we found the following values: short agony 12.40±15.78 μg/l, long agony 21.36±22.63 μg/l, hanging 18.0±26.02 μg/l, asphyxiation 7.72±3.85 μg/l and CPR 16.10±17.14 μg/l.

The adrenaline/noradrenaline quotients are significantly lower than 1 for all groups ($p<0.001$, Wilcoxon signed ranks test); short agony 0.13±0.09, long agony 0.21±0.16, hanging 0.15±0.16, asphyxiation 0.14±0.08 and CPR 0.14±0.06.

Concerning vitreous humour, we found the following noradrenaline concentrations: short agony 10.36±11.97 μg/l, long agony 12.25±12.97 μg/l, hanging 10.89±11.56 μg/l, asphyxiation 18.09±11.79 μg/l and CPR 17.63±18.15 μg/l.

Adrenaline values were detected with 0.87±1.25 μg/l for short agony, 1.28±2.04 μg/l for long agony, 0.81±2.13 μg/l for hanging and 1.39±2.31 μg/l for asphyxiation. Adrenaline values for state after CPR with 36.55±69.29 μg/l can be clearly distinguished form short agony ($p<0.001$) and long agony ($p<0.005$; *U* test according to Mann–Whitney).

The acquired adrenaline/noradrenaline quotients for short agony with 0.14±0.28, for long agony with 0.13±0.12, for hanging with 0.07±0.09 and for asphyxiation with 0.09±0.11 are lower than 1 ($p<0.001$, *U* test according to Mann–Whitney; see Table 3). The value for state after CPR is 4.33±7.7.

Figure 3 shows the results of the separate trials of five corpses from whom vitreous body fluid was taken from one eye and fluid from the anterior eye chamber was taken from the other eye. No significant differences in noradrenaline concentration with regard to place of extraction could be proven. Due to technical measurement reasons only two samples could be analysed within adrenaline (sample 1 vitreous body fluid 0.133 μg/l vs 0.162 μg/l in the eye

Table 2 Declining trial with four healthy volunteers, blood taken from brachial vein

Sample	Variable	Time (h)						
		0	1	2	3	4	24	48
1	Noradrenaline	0.13	0.14	0.13		0.09	0.07	0.07
2	Noradrenaline	0.30	0.25	0.24		0.23	0.16	0.14
	Adrenaline	0.07	0.07				0.04	0.05
3	Noradrenaline	0.41	0.35	0.30		0.27	0.16	0.14
	Adrenaline		0.09	0.08		0.07	0.05	
4	Noradrenaline	0.45	0.37	0.30	0.33	0.30	0.17	
	Adrenaline	0.06	0.06	0.05	0.04	0.05	0.04	

Samples were left at room temperature; concentration in μg/l.

Table 3 Mean values (μg/l) ±standard deviation (SD) for heart blood, femoral vein blood, liquor, urine and vitreous humour in the following groups: hanging, asphyxiation, short agony, long agony and condition after CPR

Variable	Specimen	Cause of death, agony time				
		Hanging	Asphyxiation	Short agony	Long agony	CPR
Noradrenaline mean±SD	Heart blood	157.23±98.84	161.15±73.39	211.62±158.14	135.15±89.76	202.11±172.47
	Femoral vein blood	43.55±38.96	51.2±56.78	36.36±27.70	34.44±43.46	28.80±21.32
	Liquor	78.1±170.73	50.47±24.98	36.19±24.67	52.46±43.45	41.86±37.65
	Urine	94.0±56.84	63.0±36.51	93.90±82.51	99.64±79.25	109.20±77.39
	Vitreous humour	10.89±11.56	18.09±11.79	10.36±11.97	12.25±12.97	17.63±18.15
Adrenaline mean±SD	Heart blood	249.55±263.81	194.67±177.85	195.27±160.99	194.49±307.26	371.47±246.29
	Femoral vein blood	12.5±40.04	28.76±55.89	3.96±3.71	16.61±29.10	45.04±48.74
	Liquor	10.62±16.19	14.15±18.66	4.68±4.28[a]	30.87±53.15	142.27±203.85[a]
	Urine	18.0±26.02	7.72±3.85	12.40±15.78	21.36±22.63	16.10±17.14
	Vitreous humour	0.81±2.13	1.39±2.31	0.87±1.25[g]	1.28±2.04[h]	36.55±69.29[g, h]
Adrenaline/noradrenaline quotient mean±SD	Heart blood	1.19±0.81	1.02±0.77	0.84±0.50	1.25±1.06	2.80±2.32
	Femoral vein blood	0.21±0.29[b]	0.38±0.47[b]	0.17±0.19[c, e]	0.42±0.43[d, e]	2.81±5.80
	Liquor	0.18±0.19[f]	0.30±0.38[f]	0.17±0.17[f]	0.48±0.76	2.84±4.19
	Urine	0.15±0.16[f]	0.14±0.08[f]	0.13±0.09[f]	0.21±0.16[f]	0.14±0.06[f]
	Vitreous humour	0.07±0.09[f]	0.09±0.11[f]	0.14±0.28[f]	0.13±0.12[f]	4.33±7.7
n	Heart blood	6	11	10	13	6
	Femoral vein blood	24	19	14	18	9
	Liquor	30	20	14	16	9
	Urine	10	9	10	14	5
	Vitreous humour	27	20	10	18	10

[a] $p<0.05$ short agony vs CPR
[b] $p<0.001$ or [c] $p=0.001$ or [d] $p=0.003$ vs <1
[e] $p=0.022$ short vs long agony
[f] $p<0.001$ vs <1
[g] $p<0.001$ short agony vs CPR
[h] $p<0.005$ long agony vs CPR

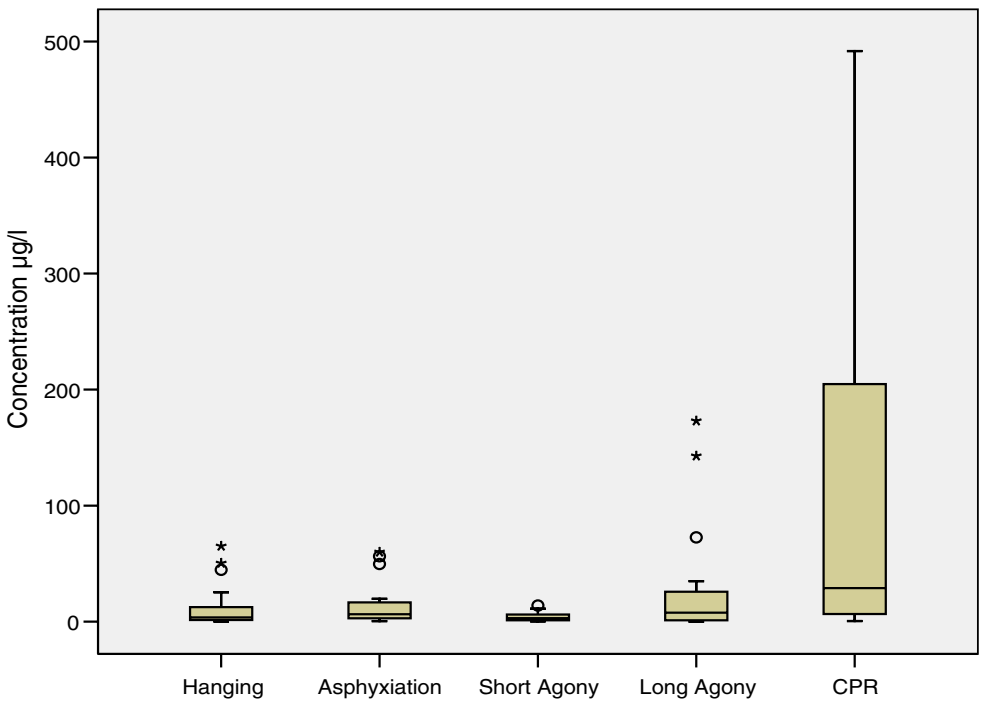

Fig. 2 Box-and-whisker plot of the adrenaline concentration in the liquor according to cause of death and agony time. The *circles* show outliers, the *stars* extreme values

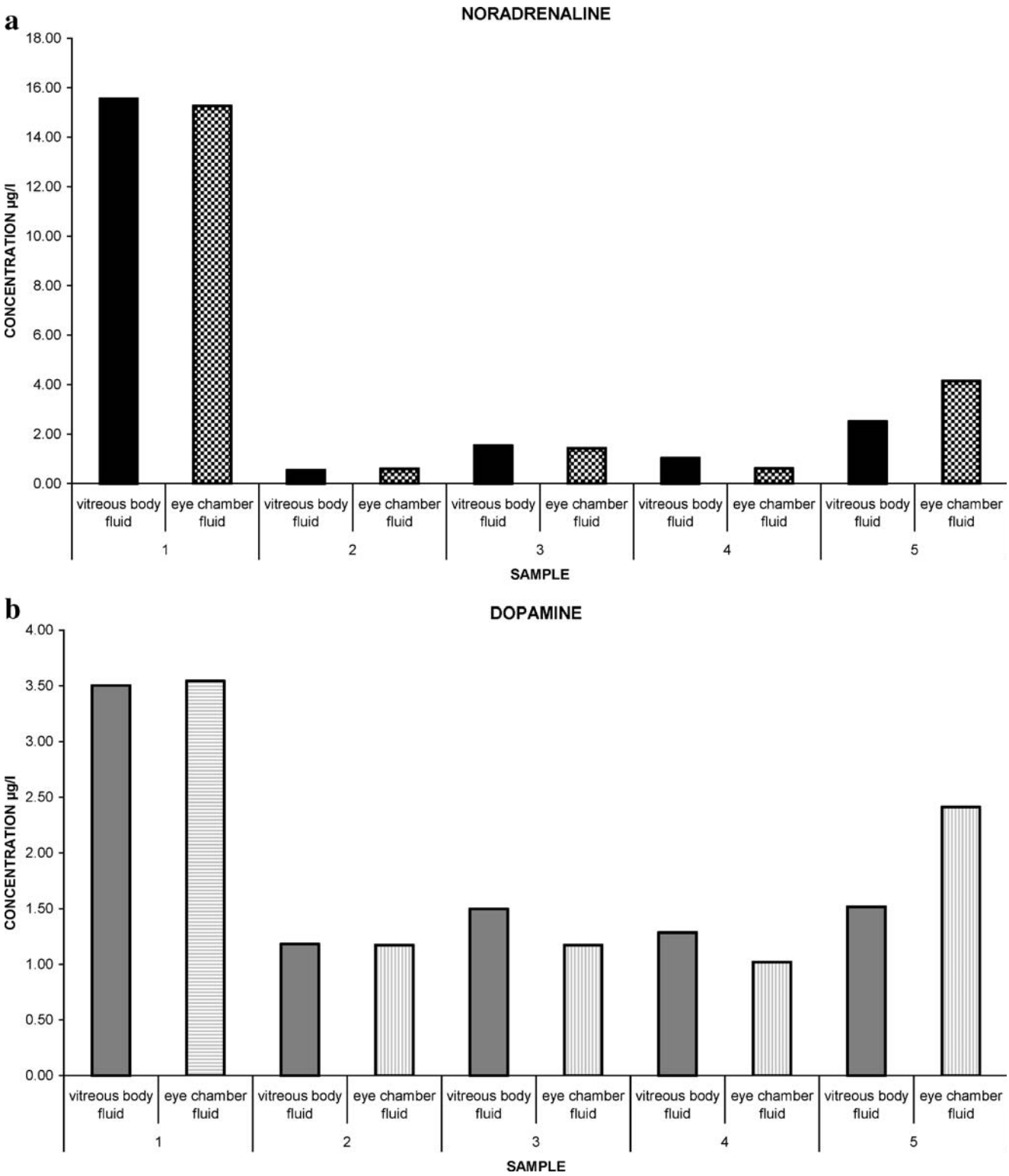

Fig. 3 Results of the separate trial of five corpses, in which vitreous body fluid was taken from one eye and fluid from the other eye's anterior chamber, concentrations in µg/l. **a** noradrenaline, **b** dopamine

chamber fluid, sample 5 vitreous body fluid 0.428 μg/l vs 0.358 μg/l in the eye chamber fluid). In addition, the dopamine concentration was determined.

Discussion

The results of Berg and Bonte [3], whereby adrenaline values in long agony were twice as high as in short agony, could not be confirmed from the results of the analysis of heart blood and femoral vein blood. However, it is interesting that the adrenaline/noradrenaline quotient in femoral vein blood was significantly different ($p<0.05$) when short agony (0.17±0.19) is compared to long agony (0.42±0.43). Standard deviation, however, shows that a clear and definite separation is not possible in individual cases.

With an altogether multifold quantitative dominance of catecholamines in heart blood shows in contrast to femoral vein blood a much higher adrenaline concentration. With the exception of short agony adrenaline concentration in heart blood in the mean surpasses noradrenaline concentration and therefore quotients from adrenaline and noradrenaline are >1.

For femoral vein blood the adrenaline/noradrenaline quotients in the groups asphyxiation and hanging and in short and long agony are significantly <1 ($p<0.001$; $p=0.001$ and $p=0.003$).

The adrenaline/noradrenaline quotients of 16.99 in short agony mentioned by Kernbach-Wighton et al. [15] could not be reproduced in this study, even when taking into account our maximum value (heart blood, max. 1.76). The value is closer to the maximum value in the group of corpses after CPR (heart blood, max. 7.12; femoral vein blood, max. 18.16).

The declining trial using femoral vein blood of a corpse has shown that the processing of catecholamines allows a determination until at least 72 h after sample taking when using appropriate refrigeration. The declining kinetic in the trial with healthy candidates showed that even at room temperature the catecholamines noradrenaline and adrenaline in vitro 24 or 48 h, respectively, after extraction display a similar decomposition process, which allows determination.

Assignment of catecholamine profiles to certain types of death or agony intervals does not seem to be possible according to the results of this study. Contrary to the view that length of agony is reflected in the level of adrenaline and noradrenaline concentration, heart blood showed a different result. Mean values of concentrations were higher in short agony than in long agony. As mentioned above a "special position" of death by asphyxiation or hanging cannot be confirmed from the data we found in heart blood [5]. Values for state after CPR are in line with the amount of epinephrine administered by physicians.

Only in femoral vein blood could a significant difference between short and long agony be found when analysing the quotient of adrenaline and noradrenaline. However, due to the wide distribution individual values could not be used for a clear distinction.

Conclusions about a certain cause of death or length of agony based on a catecholamine profile could not be shown for liquor. The increase of catecholamines in long agony shown by Hirvonen and Huttunen [8], Hirvonen and Lapinalampi [11], and Hirvonen and Huttunen [9] could not be confirmed with the results of this study. In addition, quantitative dominance of catecholamines in asphyxia or hanging could not be reproduced.

In this study the adrenaline/noradrenaline quotient in short agony (0.17±0.17) was significantly lower than 1 ($p<0.001$). This result is in contrast to the quotients of 3.81 found by Kernbach-Wighton et al. [15] in liquor for short agony.

Our results show that urine catecholamine concentrations do not allow for any conclusions on cause of death or length of agony. The quotient higher than 1 as an indicator of hypothermia suggested by some authors [11, 18], which in the present study could only be applied to the group of long agony, could not be confirmed. In reality, quotients of nearly all groups were significantly smaller than 1 ($p<0.001$). The already mentioned technical difficulties (24 h collected urine, urine creatinine) did not allow a solid interpretation. The combination of catecholamine values in urine with the creatinine value in urine seems to us a sensible approach, which we will apply in further investigations and methodical reassessment of the results.

The results for vitreous humour allowed significant conclusions, at least for the group of state after CPR, and could be clearly distinguished from the groups with short ($p<0.001$) and long ($p<0.005$) agony.

The adrenaline/noradrenaline quotients in vitreous humour found by Kernbach-Wighton et al. [15] of 5.99 for short agony and 0.16 for medium agony could not be reproduced in this study. The quotients from our groups were, with the exception of CPR, always smaller than 1 ($p<0.001$) [22].

In addition, different points of extraction of aqueous humour, vitreous body or eye chamber, yielded reliable and comparable results, so that the point of extraction in the eye did not matter and accidental incorrect aspiration could be neglected.

Therefore, analysis of adrenaline and noradrenaline concentrations—based on the results of this study and contrary to hitherto published data—cannot be used for further investigation in detailed examination of the type of death or clarification of agony interval.

Although some mean values, in particular, of some adrenaline/noradrenaline quotients, showed significant results, a biostatistical assessment of individual cases due

to the great widespread variability of individual values is impossible.

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