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ORIGINAL ARTICLE

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Postmortem changes in serum noradrenaline and adrenaline concentrations in rabbit and human cadavers

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Abstract Postmortem changes in serum noradrenaline and adrenaline concentrations in adrenectomised rabbits and in human sudden death cases were measured with HPLC in order to obtain information of the usefulness of these biogenic amines as indicators of antemortem stress. It appeared that serum concentrations increased with time postmortem, except for adrenaline in the adrenectomised rabbits. The values varied considerably between individuals in both materials. It is concluded that postmortem assays of serum catecholamines cannot be used in practice to demonstrate antemortem stress, although this would theoretically be a promising approach, e.g. in cases of suffocation. So far there seems to be no reliable and conclusive method for estimating short lasting antemortem stress in individual forensic cases.

Key words Postmortem · Catecholamines · Agonal reactions

Introduction

It is well known that catecholamines are released from the adrenal gland in all kinds of stress situations. Hypoxia is a major threat to life and consequently causes immediate powerful reactions in the organism including the release of noradrenaline (NA) and adrenaline (A) from the adrenal glands into blood. Use has been made of this vital reaction for the diagnosis of suffocation, which is difficult to prove by standard methods when no external marks are visible. Alveolar haemorrhages and septal oedema were the most reliable microscopic signs of different types of asphyxia in rabbits and rats (Brinkmann and Püschel 1981). High concentrations of catecholamines in the blood have been found both in asphyxia deaths and in experimental hypoxia (Laves and Berg 1965; Berg and Bonte 1973).

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The catecholamine approach to the establishment of a marker for death by asphyxia is a promising one, but there remains a danger that postmortem changes may mask the agonal changes in blood chemistry. In earlier experiments we were able to demonstrate a distinct increase in serum NA and A concentrations in rabbits that had died of suffocation, but it also appeared that these amines increased many fold postmortem (Hirvonen et al. 1990), presumably due to diffusion of NA from the sympathetic nerve endings and adrenals and A from the adrenals only. We considered that if the adrenals would be excised at death, A concentration, at least, would not increase postmortem. We regarded this methodological problem as worth studying further and decided to monitor postmortem changes in blood NA and A after adrenalectomy in order to provide information for interpreting the results of these biochemical assays. The other part of the work involved monitoring the change in blood catecholamine concentrations in humans over a few days postmortem.

Material and methods

Animal experiments

Rabbits, 7 female and 7 male were anaesthetized with Mebumat (1 ml/kg i.p.) and the first blood sample taken from the ear vein (sample A). Each animal was then sacrificed with an overdosis of 2–5 ml Mebumat i.v. and both adrenal glands were removed. A blood sample was taken from the heart, either from the right or left ventricle which ever contained some blood, on each of the next 3 days postmortem (samples B, C and D). The blood samples were taken into tubes containing 0.05 M $Na_2S_2O_5$ (10 µl/ml blood), centrifuged and the serum was stored at –70° C until analysed.

Human cadaver material

Sudden deaths (n = 11) from causes other than asphyxia, e.g. trauma, poisoning or natural death were selected. The first blood samples were taken from the left ventricle of the heart and the femoral vein a few hours after death or as soon as possible during the first day postmortem, and the second samples at autopsy 1–4 days after death, again from the heart (left ventricle) and femoral vein. The samples were treated as above.

Catecholamine measurements

The catecholamines were measured by high pressure liquid chromatography (HPLC) using an electrochemical detector (ESA Coulochem, model 5100A with cell model 5011), a method used in our laboratory for both clinical and cadaver samples. The conditions for the detector were: electrode 1 = +0.10 V, electrode 2 = −0.20V and conditioning cell +0.25 V. The catecholamines were extracted from the serum in Tris-HCl buffer by adsorption at pH 8.6 to 30 mg acid-washed Al_2O_3 (Bioanalytical systems) with 3,4-dihydroxybenzylamine hydrobromide (Sigma, St. Louis, Miss.) as an internal standard. After washing 4 times with 2 ml H_2O, the catecholamines were released into 100 µl 0.2 M HCl_2O_4 solution and measured by HPLC. A C-18 reverse-phase column was measured 80 × 4.0 mm, 3 µm (ESA Catecholamine HR-80) and the mobile phase was methanol-phosphate buffer, pH 2.2 (ESA Cat-A-Phase reagent). The flow rate was 0.6 ml/min.

Catecholamine standards treated in the same way as the samples were assayed in triplicate for calibration purposes. Since the absolute and relative recoveries for both synthetic samples and spiked samples are virtually the same, the sample concentrations were calculated by comparing the peak height ratios (relative to the internal standard) for the unknown sera with those for a synthetic standard of known original concentration (Merck-Hitachi, D-2500 Chromato-Integrator). The detection limit for the standard was 2 pg.

Results

Rabbits

NA concentrations increased continuously postmortem and the highest values were observable on the third day, when the concentration was more than 100 times the original antemortem value (Table 1). A significant increase was already seen in the first postmortem sample.

Adrenaline was detectable in some postmortem samples, but could not be quantified, because the peak heights were too small (see discussion).

Human cadavers

Both the NA and A values in the first samples were always higher in the heart blood than in the femoral blood (Table 2). The range of the first NA values was large, from 40.8 ng/ml to 214.2 ng/ml in the heart blood and from 50 pg/ml to 101.2 ng/ml in the femoral blood. The respective means and SDs were 103.6 ± 62.4 ng/ml and 24.5 ± 29.2 ng/ml.

In the second sample the range had shifted towards higher values being from 55.2 ng/ml to 291.1 ng/ml in the heart blood and from 7.0 ng/ml to 146.6 ng/ml in the femoral blood. The respective means and SDs were 160.5 ± 92.0 ng/ml and 41.7 ± 38.3 ng/ml. The increase in heart blood was significant but not in femoral blood.

The range in A values in the first heart blood sample was from 1.4 ng/ml to 286.4 ng/ml (mean ± SD was 70.0 ± 83.2 ng/ml) and that in the femoral blood from 50 pg/ml to 27.0 ng/ml (5.2 ± 9.5 ng/ml) in the first sample. The A range in the second heart blood sample was from 50 pg/ml to 274.7 ng/ml (83.3 ± 85.4 ng/ml) and from 50 pg/ml to 227.3 ng/ml (36.4 ± 67.7 ng/ml) in the second femoral sample, the next highest femoral A value on the latter occasion being 75.4 ng/ml. The changes were not significant, because of the great variations.

Catecholamine quotient A:NA was calculated in the cadaver material only, since in the rabbits A could not be quantified accurately enough. The average quotient decreased in the heart blood from 0.78 ± 0.71 (first sample, range 0.01–1.83) to 0.62 ± 0.60 (second sample, range 0.00–1.81) but it increased in the femoral blood from 0.34 ± 0.38 (range 0.00–1.02) to 0.81 ± 1.33 (range 0.00–4.42) respectively, since the A levels were generally elevated in the second femoral sample. From the quotients in individual cases it appeared that when the quotient was lower in the first heart blood sample than in the second in most samples, it rose in the femoral blood and in the remaining cases the changes occurred vice versa.

Table 1 Catecholamines (ng/ml) in the serum of rabbits before death and 1 to 3 days postmortem.

	before death	1 d. p. m	2 d. p. m	3 d. p. m
NA	1.9 ± 0.6	195.9 ± 84.6	222.2 ± 78.7	237.3 ± 60.8
A	0.5 ± 0.2	n.q.	n. q.	n. q.
N =	14			

Values are expressed as means ± SD. n. q. = could not be quantified.

Discussion

The results for the rabbits supported the hypothesis that removal of the adrenals influenced the postmortem catecholamine concentrations in the blood. Adrenaline was hardly detectable postmortem when the adrenal glands were removed, leaving no hormone to diffuse into the blood. By contrast, noradrenaline values rose remarkably, probably because of diffusion from the sympathetic nerve endings in the heart and great vessels. The large amount of NA in the postmortem samples made the exact quantification of A impossible. The A peak could be detected in some rabbit postmortem samples, but the peak did not separate enough from the baseline noise to be quantified. The results of Takeichi et al. (1984) in rats were comparable in that NA increased postmortem in 24 h, but A remained at the antemortem level or decreased.

The results for the human cadavers showed similar NA values, which increased for 2–4 days postmortem. The same occurred here with the A values, which were also high postmortem, especially in the heart blood. The results once more showed how much postmortem diffusion or passive movements of the blood can affect postmortem measurements, and the variation from one case to the next can evidently be considerable and unpredictable. Another factor which affects the analysis is the site of the blood sample, as was shown by Kauert et al. (1990), who found different concentrations of catecholamines in right and left heart ventricle blood and meningeal sinus blood.

In our cases where death had already occurred about one day before the first sample was taken, the cate-

Table 2 Causes of death and noradrenaline (NA) and adrenaline (A) levels in the human cadavers

Cause of death	NA ng/ml Samples				A ng/ml Samples				P. M. time between the samples
	I		II		I		II		
	Heart	Femoral	Heart	Femoral	Heart	Femoral	Heart	Femoral	
1. Hanging	104.9	37.6	120.1	37.9	28.3	0.05	0.05	2.3	3 d
2. Subdural haemorrh., operated Agony .1 d.	110.9	8.9	189.8	50.5	1.4	0.8	0.8	6.8	2 d
3. Amputation of head by train	214.2	2.4	277.1	146.6	13.2	0.6	2.4	227.3	5 d
4. Gunshot to the head	209.4	101.2	291.1	55.4	286.4	27.0	274.7	21.9	4 d
5. Hanging	147.1	38.4	207.8	30.9	122.8	21.7	122.7	16.4	3 d
6. Crushing of the chest	90.4	3.6	280.0	7.0	26.3	3.7	125.1	1.3	1 d
7. Traumatic intrathoracal haemorrh.	42.0	3.3	55.2	19.7	61.2	1.3	71.2	0.6	1 d
8. Rupture of the heart	53.7	0.05	64.3	17.7	1.5	0.05	4.9	0.05	2 d
9. Coronary artery disease	40.8	21.1	81.8	48.1	72.7	1.6	149.1	75.4	2 d
10. Methanol poisoning	62.0	19.9	76.1	34.5	38.8	0.6	49.6	0.6	3 d
11. Poisoning by drugs	64.6	33.6	122.8	10.8	118.4	0.5	115.8	47.8	2 d
Mean ± SD	103.6 ± 62.4	24.5 ± 29.2	160.5 ± 92.0	41.7 ± 38.2	70.0 ± 83.2	5.2 ± 9.5	83.3 ± 85.4	36.4 ± 67.7	2.5 ± 1.2
	├----------p = 0.004 ------┤				├-------NS----------┤				
		├------NS----------------┤			├--------NS----------┤			
	Paired T-test								

cholamine concentrations were already high in the heart blood, but they still rose during the following postmortem days. Thus our results differed from those of Berg and Bonte (1973), who found that catecholamine levels remained quite stable for 60 h postmortem using a fluorometric method, but they also found that the postmortem levels were many times higher than the antemortem ones. The heart muscle is rich in adrenergic nerves, which are the probable source of NA in the heart blood sample. The explanation for the increased A concentrations is not so logical, but two alternatives can be presented: diffusion from the adrenals and/or metabolic change of NA to A by bacteria. Once again it was observed that the peripheral blood provided a better sample for postmortem analysis.

A calculation of the A:NA quotient gave inconclusive results, the average quotient decreasing in the heart blood, but increasing in the femoral blood.

Hausdörfer et al. (1995) found a positive correlation between the duration of antemortem agony and catecholamine concentration in the blood, but they also pointed out that no firm conclusion can be made in individual cases. In addition, when the time elapsing from death to drawing of the blood sample is more than one day, postmortem diffusion makes the interpretation more difficult or even uncertain. This affects noradrenaline more than adrenaline, which is less prone to increase in the blood postmortem. Thus blood catecholamine concentrations, although theoretically interesting as diagnostic signs of rapid asphyxial death, for instance, are unreliable because of the natural postmortem rise. One could try to overcome this problem by comparing the concentrations in a case of alleged death by asphyxia with those measured after an equally long postmortem time in a case of rapid death by a completely different mechanism, but in such cases samples should be taken from a peripheral vein immediately after death and more emphasis should be placed on adrenaline values.

The use of urine samples in cases of alleged asphyxia is not recommendable, since animal experiments show that elevated concentrations of catecholamines are first detectable 1–2 h after the onset of stress (Hirvonen and Huttunen 1995). In the event of longerlasting stress, however, elevated urine A values – again theoretically – could provide some support for the notion of stress, since the bladder wall does not contain any A, although it does contain NA, which may diffuse into the urine during autolysis.

The difficulties encountered in postmortem analysis were well demonstrated in a recent work dealing with several analyses of cadaver cisternal fluid, as postmortem changes were apparent even in this well isolated fluid (Kärkelä 1993).

In conclusion, the authors formed the opinion that blood catecholamine assays postmortem are not a useful test for antemortem stress, e.g. asphyxia in the event of limited or non-existent morphological signs, because of the postmortem increase in concentrations, which tend to mask the antemortem agonal changes. Thus some modes of asphyxial death seem to remain a perpetual problem in forensic pathology.

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