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Hormonal changes in brain death and immune activation in the donor

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Abstract Kidneys obtained from brain dead donors show inferior graft survival compared to living donation. The effects of brain death itself are thought to be partly responsible for these results. We, therefore, examined levels of catecholamines, the vasoconstricting hormones AT II, ET-1 and renin activity, pituitary hormones, and their correlation to pro-inflammatory cytokines and cytokine receptors. In 17 brain dead patients and 19 preoperative neurosurgical patients, these parameters were measured by HPLC, RIA and ELISA. Brain death resulted in massive increases in serum catecholamines, AT II and ET-1, as well as PRA, whereas thyroid and adrenal hormone levels remained unchanged. We found a significant correlation with rises in IL-6 and soluble TNF and IL-2 receptors as markers for the activation of immunological cascades. We concluded that these effects could be directly and indirectly responsible for the impaired organ perfusion and function observed in brain death.

Key words Brain death · Hormones · Catecholamines · Interleukins · Inflammation · Soluble receptors

Introduction

The difference in the outcome of transplanted organs retrieved from living donors compared to brain dead donors has lead to a growing interest in the pathophysiology of the latter situation. By analysis of the UNOS database, it has been demonstrated that kidneys explanted from living donors – independent of the genetic relation to the recipient – show superior survival and function [1]. Brain death itself causes massive disturbances in the haemodynamic, hormonal and immunological homeostasis, which could explain at least part of the inferior outcome of organs originating from this kind of donor (reviewed in [2]). It is known that the type of primary injury to the brain and donor demographic data correspond to the short- and long-term prognosis of the transplanted organ [3]. It is further known that, in particular, the initial changes in this situation – the herniation of the brain stem in the foramen magnum – cause a massive activation of the sympathetic nervous system called "autonomic storm" with consequences for the perfusion of all parenchymal organs [4]. In order to characterize the processes in the brain dead donors that may cause these effects, we compared hormone and cytokine levels between a group of patients declared brain dead and control patients before neurosurgical intervention.

Materials and methods

Two groups of patients were compared, 17 brain dead patients (BD; 9 female; 8 male; mean age 31.5 ± 3.6 years) versus 19 control patients (NS; 10 female; 9 male; mean age 46.1 ± 2.7 years) immediately before a neurosurgical operation. Brain death was testified according to the rules of the German Bundesärztekammer. Death in the BD group was due to trauma ($n = 9$), intracerebral haemorrhage ($n = 4$), intracerebral infarction ($n = 3$) and cerebral gunshot trauma ($n = 1$). Neurosurgical intervention in the NS group was due to meningioma ($n = 7$), brain tumour ($n = 6$), acoustic neuroma ($n = 4$) and aneurysm clipping ($n = 2$). Patients in the NS group

Table 1 Peripheral hormones, mean ± SEM

	Epinephrine pg/ml	Norepinephrine pg/ml	PRA ng/ml × h	AT II pg/ml	ET 1 pg/l
BD-group 1	105.3 ± 42*#	470 ± 135*#	6.3 ± 1.7*#	16.5 ± 3.6*#	10.7 ± 0.8*#
BD-group 2	29.3 ± 7#	211 ± 67#	1.1 ± 0.7	4.4 ± 1.9	25.6 ± 15.5
NS-group	6.9 ± 1	47 ± 10	2.2 ± 0.7	7.7 ± 2.1	18.3 ± 4.4

* $P \leq 0.05$ compared to BD group 2; # $P \leq 0.05$ compared to NS group

Table 2 Pituitary hormones, mean ± SEM

	ACTH pg/ml	Cortisol ng/ml	TSH mU/l	fT3 pmol/l	fT4 pmol/l
BD group 1	13.8 ± 2.5	10.5 ± 2.2	1.5 ± 0.5	4.0 ± 0.4	15.2 ± 1.3
BD group 2	13.8 ± 6.6	8.1 ± 3.6	1.1 ± 0.4	3.7 ± 0.2	16.5 ± 1.3
NS group	13.9 ± 2.5	7.7 ± 1.6	1.0 ± 0.2	4.7 ± 0.4	17.9 ± 1.1

Table 3 Cytokines and soluble cytokine receptors, mean ± SEM

	IL 1-β pg/ml	IL 2 pg/ml	IL 6 pg/ml	sIL2-receptor U/ml	sTNF-receptor II ng/ml
BD-group 1	15.3 ± 2.8	0.7 ± 0.2	399 ± 145*#	600 ± 123*#	37.9 ± 9.7*#
BD-group 2	19.8 ± 3.1	0.7 ± 0.3	134 ± 88#	213 ± 6#	16.3 ± 7.5#
NS-group	17.3 ± 3.2	0.9 ± 0.4	3 ± 1	359 ± 50	9.8 ± 0.9

* $P \leq 0.05$ compared to BD group 2; # $P \leq 0.05$ compared to NS group

gave informed consent for study participation, as did the relatives of the deceased donors. The study was approved by the local ethics committee.

Blood was drawn in the BD group immediately (BD group 1), and in a mean of 6.7 h after the definitive diagnosis of brain death before organ retrieval (BD-group 2). Blood was drawn in the NS group before induction of aneasthesia.

Epinephrine (E) and norepinephrine (NE) in plasma were analysed by HPLC. The following hormones were measured by RIA: plasma renin activity (PRA; Nichols Institute, Bad Nauheim, Germany), angiotensin II (AT II; Biermann Diagnostika, Bad Nauheim, Germany), endothelin-1 (ET1; Amersham, Munich, Germany), TSH (Henning, Berlin, Germany), fT3 and fT4 (Kodak Diagnostik, Braunschweig, Germany), ACTH and cortisol (Biermann Diagnostika, Bad Nauheim, Germany). The cytokines IL1β (Quantikine Research and Diagnostic Systems, Minneapolis, USA), IL2 (Immuno Biological Laboratories, Hamburg, Germany), IL6 (Biermann Diagnostika, Bad Nauheim, Germany), as well as the soluble receptors sIL2-R (Biermann Diagnostika, Bad Nauheim, Germany) and sTNF-R II (Quantikine Research and Diagnostic Systems, Minneapolis, USA) were measured by ELISA according to the recommendations of the manufacturer.

Statistical analyses were computed by the Kruskal-Wallis H-test for intra-individual changes over time and the Mann Whitney *U*-test for detection of differences between two groups. Testing for correlations was done by using the product moment correlation and the Spearman rank correlation where appropiate. A *P*-value of 0.05 or less was considered significant.

Results

The detailed results are depicted in Tables 1–3. E and NE levels were significantly elevated at the first and second time points after brain death compared to the NS group. In parallel, brain death was also marked by a transient increase in PRA, as well as AT II followed by a drop to values comparable to the NS group. ET 1 increased over time, with significantly lower plasma levels immediately after the diagnosis of brain death compared to both BD group 2 and the NS group. The levels of PRA, AT II an ET1 are depicted in Fig. 1.

Hormone analysis showed no significant differences between plasma levels of ACTH and cortisol or TSH and fT3. Only fT4 showed a lower level at both time points in the BD groups compared to the NS group with a slight increase over time to the second blood collection (15.2 ± 1.3 vs 16.5 ± 1.3 vs 17.9 ± 1.1 pmol/l). However, we did not demonstrate significant differences between the three groups.

Analysis of IL1β, as well as IL2, showed no differences between the three groups, whereas IL6 measured at the time of brain death diagnosis was significantly higher compared to the second time point in the BD group and the NS group (Fig. 2a; 399 ● 145 vs 134 ± 88 vs 3 ± 1 pg/ml). Levels of IL6 at both time points correlated significantly with E and NE levels ($P = 0.001$ and

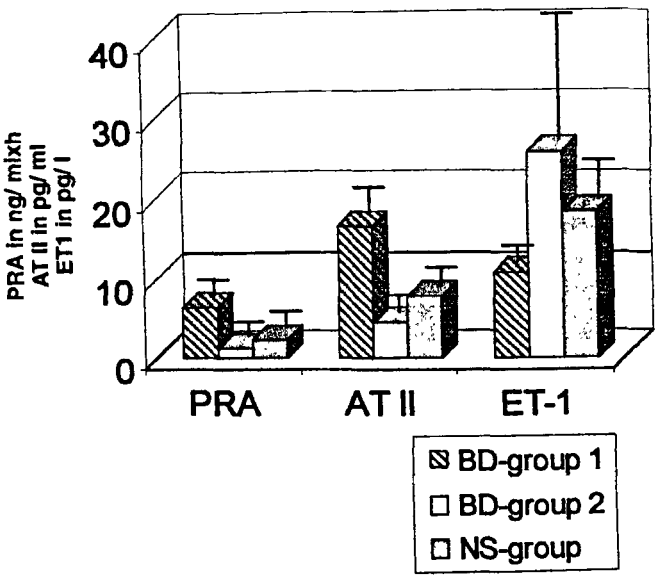

Fig. 1 Vasoconstricting hormones, mean ± SEM

$P = 0.0003$, respectively), as well as with AT II ($P = 0.003$) and PRA ($P = 0.0002$).

Plasma levels of sIL2-R (Fig. 2b) analysed immediately after BD were significantly increased compared to both other groups, with lower levels in BD group 2 compared to the NS group (600 ± 123 vs 213 ± 6 vs 359 ± 50 U/ml). Also, for sTNF-R II, a distinct peak at BD time point 1 was shown (Fig. 2c), with a decrease over time, but still higher levels compared to the NS group. Cytokine receptor levels were both significantly correlated with IL6 levels (sIL2-R: $P = 0.02$; sTNF-R II: $P = 0.002$).

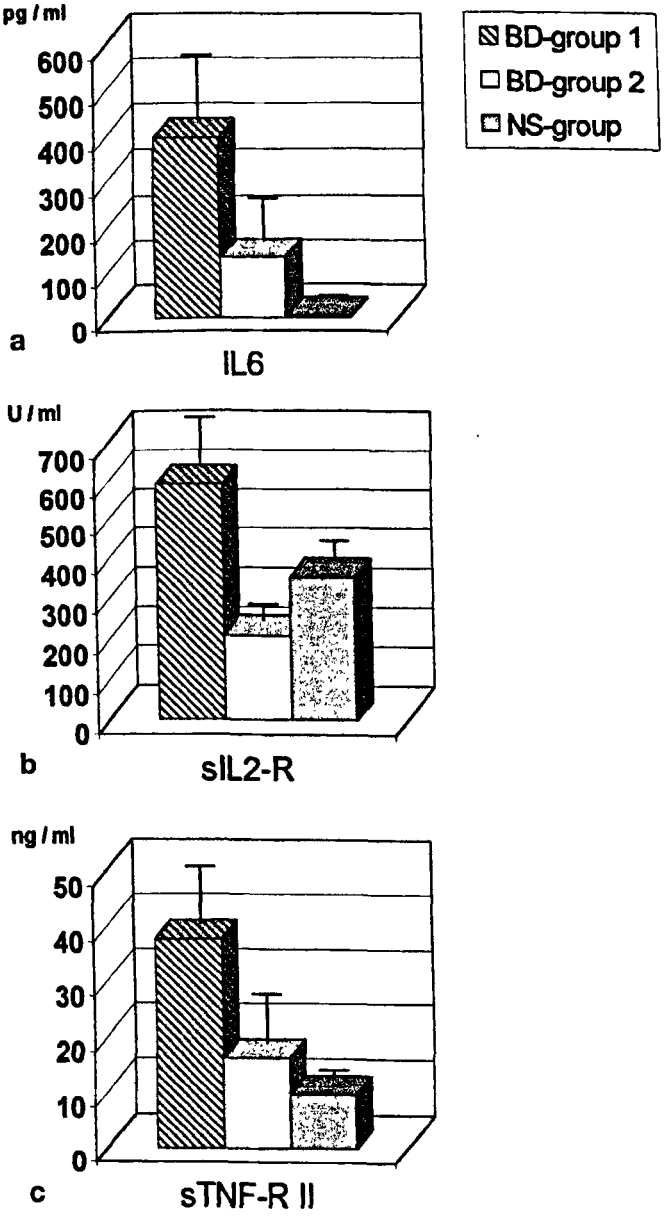

Fig. 2 Mean ± SEM of **a** IL6 serum levels, **b** soluble IL-2 receptor levels and **c** soluble TNF receptor II levels

Discussion

As one reason to account for the poorer results obtained by transplantation of kidneys from brain dead donors, compared to living – related or unrelated – donors [1], brain death itself and the changes resulting from massive cerebral tissue damage have to be taken into account. In this study, we looked for the hormonal changes implied by brain death in a longitudinal way as well as for markers of immune activation in the donor before any intervention for organ retrieval took place.

As has been observed by Cushing in 1902, we found increased levels of catecholamines immediately after the diagnosis of brain death was made. The serum levels declined again 7 h later but were still significantly higher compared to the neurosurgical patients taken as a control group. This is in accordance with observations of several other groups, as for example Powner et al. [5]. A similar pattern with a positive statistical correlation was also observed for the vasoconstrictory hormones AT II and PRA as an indirect parameter for the plasma renin content. A high PRA has also been seen by Amado et al. who analysed plasma only at one time point directly after the establishment of brain death [6]. A similar peak in plasma AT II has been described by a French group in domestic pigs over a 4-h observation protocol [7]. This group also looked for ET1 levels in plasma in these animals and only found an insignificant increase directly after experimental induction of brain death with rapid normalization of ET1 after 90 min, studies in brain dead humans have, to our knowledge, not been undertaken. These results are in contrast to

ours; we showed a steep increase in plasma ET1 at the second blood collection. In the rat, it has already been shown that these massive increases in catecholamine levels (together with the parallel activation of the RAS and the endothelin system) can cause massive disturbances in the perfusion of parenchymal organs, especially the kidneys and lungs, in brain dead organisms [8].

Hormones synthesized by the pituitary gland, as well as their peripheral effector hormones, showed no significant differences compared to the NS group levels in discordance to the results of Amado et al. [6], in particular we did not find any correlation to the levels of IL-6. The reason for this remains unclear in view of the use of similar assays and protocols. However, in the case of a total cessation of cerebral perfusion, pituitary blood flow could be spared due to different vascularization.

In the second part of the study we investigated several parameters of stimulation of the immune system. We found significantly elevated levels of IL6, together with IL1β and and IL2 levels in the normal range, confirming results obtained by Amado [6]. This points to an unspecific activation of the cytokine network; the synthesis of IL6 as a potential product of nearly every mesenchymal cell could not be attributed to any special cell line. The question remains if IL6 is released directly from necrotic cerebral tissue or from other cells activated by some systemic trigger, as for example the massive doses of catecholamines in the initial phases of brain death, which has previously been shown in rats [9].

We also found increased levels of soluble IL2 and TNF receptors directly after the diagnosis of brain death. Both markers are thought to be released from the surface of activated cells upon contact with their substrate [10, 11] and are responsible for inactivation and clearance of IL2 and TNF-α. The latter is known to be a pleitrophic and multifunctional cytokine with immune-activating and pro-inflammatory properties in a multitude of tissues [12], whereas IL2 is the strongest and most important cytokine promoting T-cell activation [13]. The rise in both soluble receptors pointed to a further down-stream activation of parts of the immune cascade, which could result in endothelial activation and microcirculatory deterioration in numerous organs eventually procured for transplantation.

In conclusion we demonstrated massive increases in hormones with vasoconstricting effects correlated to profound increases in pro-inflammatory cytokines and cytokine receptors. This could be responsible for at least part of the detrimental effects of brain death on the function and structure of peripheral organs.

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