Chapter 4: Dopamine Treatment in Brain-Dead Rats Mediates Anti-Inflammatory Effects: The Role of Hemodynamic Stabilization and D-Receptor Stimulation

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ABSTRACT

Background Brain death (BD) is associated with profound inflammation in end-organs. Dopamine (DA) treatment reduces this inflammatory response, but the underlying mechanisms remain thus far largely unknown. In the present study we investigated if the anti-inflammatory effect of DA was related to hemodynamic stabilization and by which receptors it was mediated.

Methods BD was induced in F344 donor rats. DA was given either before BD for 24 hrs or after brain death induction during a definite time. Adrenergic or D-receptor blockers were administered to inhibit the receptor stimulation mediated by DA. Hemodynamic changes were recorded and kidneys were harvested after 6 hrs of BD.

Results Mean arterial pressure (MAP) was completely normalized by DA treatment. DA pre-treatment before BD induction and treatment during BD both significantly inhibited monocyte infiltration. The anti-inflammatory as well as its blood-pressure stabilizing effect was abrogated by concomitant application of adrenergic receptor blockers. In contrast, concomitant application of D-receptor blockers only abrogated the anti-inflammatory effect but did not affect blood pressure stabilization. In contrast, pergolide and adrenergic receptor blockers completely normalized blood pressure but did not affect renal inflammation.

Conclusions Hence, DA might reduce BD induced monocyte infiltration possibly by hemodynamic stabilization, D-receptor activation or a combination of both.
INTRODUCTION
The majority of renal allografts are still retrieved from deceased donors. Brain death is considered to be an important cause of pre-transplantation injury. It is associated with hemodynamic instability, hypotension, hypothermia, coagulopathies and electrolyte abnormalities [1]. In addition a number of immunological alterations occur, e.g. cytokine release and upregulation of adhesion molecules that eventually lead to inflammation in end-organs [2-4]. It is believed, that the release of endogenous catecholamines plays a pivotal role in this process. During brain death serum catecholamine concentrations increase rapidly, resulting in profound vasoconstriction. As brain death ensues, serum catecholamine concentrations drop again. The process of vasoconstriction and subsequent vasodilatation dramatically influences blood pressure and defines the so-called “autonomic storm” [5, 6].
Catecholamines are frequently used in intensive care medicine to stabilize blood pressure in critically ill patients and in patients with irreversible brain injury [7-9]. There are conflicting data in the literature regarding the mode of action and effects of dopamine in clinical kidney transplantation [10-13]. In two retrospective clinical studies however, Schnuelle et al. [14, 15] demonstrated that dopamine treatment of brain-dead donors have a beneficial effect on delayed-graft function and long-term renal allograft survival. Although the precise mechanisms by which dopamine may improve transplantation outcome after renal transplantation are not known so far, several mechanisms have been suggested. Dopamine is able to induce the expression of the heme oxygenase 1 (HO-1) in endothelial cells [16], delays the expression of adhesion molecules after TNFα stimulation, inhibits the production of chemokines in renal tubular epithelial and endothelial cells [17], and protects endothelial cells against cold preservation injury [18]. Moreover, treatment of brain-dead rats with dopamine reduces monocyte infiltration and MHC class II expression in donor kidneys [19].
Since dopamine treatment also improves hemodynamic instability in BD donors it is unclear to what extent the beneficial effect of donor dopamine treatment on transplantation outcome is primarily mediated by blood pressure stabilisation. In the present study we therefore tested the hypothesis that the anti-inflammatory effect of dopamine is independent of blood pressure stabilisation. We investigated the involvement of α- and β-adrenergic- as well as dopaminergic receptors in this regard.
MENTHODS

Animals

Inbred male Fisher (F344, RT1¹vr) rats weighing 200 to 250 g were obtained from Harlan-Winkelmann (Borchen, Germany). Animals were kept under standard conditions and fed standard rodent chow and water ad libitum. All procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the National Academy of Sciences and were approved by the local authorities (RP Karlsruhe, AZ 35–9185.81/27/04).

Experimental Protocol

Before induction of brain death, donor animals were anesthetized with ketamine (Ketanest, Pfizer, Karlsruhe, Germany, 100 mg/kg intraperitoneally) and xylazine (Rompun, BayerVital, Leverkusen, Germany, 6 mg/kg intraperitoneally) and placed on a heating table to keep their body temperature constant. A 3F Fogarty catheter was inserted epidural in an occipital burr hole and gradually inflated during 1 minute with 200 µL of saline. The state of brain death was verified by the occurrence of autonomic storm, the absence of corneal reflexes and by an apnoea test. All animals were mechanically ventilated by a tracheostoma with a rodent ventilator (Ugo Basile, Comerio, Italy). Systemic blood pressure (mean arterial pressure [MAP], mm HG) was continuously measured during the whole experiment (6 hrs) by a femoral arterial catheter (Statham pressure transducer P23Db and a Gould pressure processor, FMI, Ober-Beerbach, Germany). Anesthetized, non brain-dead ventilated donor animals without inserted Fogarty catheter served as controls. The animals were divided into 10 groups. Donor animals were treated intravenously by microinjection pumps (CMA/100, CMA/Microdialysis, Sweden) according to the following scheme (Fig. 1):

**Group 1**: Vehicle not brain death (VEH-NBD), Fisher rats non brain death ventilated plus NaCl 0.9%.

**Group 2**: Vehicle brain death (VEH-BD), Fisher rats brain death plus NaCl 0.9%.

**Group 3**: Dopamine brain death (DA), Fisher rats brain death plus dopamine 10 µg/min/kg [19, 20] during the first three hours of brain death.

Duration of DA infusion was based on a time-response-analysis, in which 3 hours of DA infusion from the onset of BD on appeared to be the shortest period for effectively inhibiting monocyte infiltration in renal tissue.

**Group 4**: Dopamine + adrenergic Receptor-antagonists (DA+ADR.BLOCK), Fisher rats brain death plus dopamine 10 µg/min/kg and propranolol/doxazosin (Sigma-Aldrich Deisenhofen, Germany/Pfizer GmbH, Karlsruhe, Germany; 10/5mg/kg body weight/d p.o.
day -1.0 prae op) during the first 3 hours of brain death [21].

**Group 5**: Dopamine + dopaminergic Receptor-antagonists (DA+D-BLOCK), Fisher rats brain death plus dopamine 10 µg/min/kg and SCH-23390 (10µg/kg body weight/min, Sigma-Aldrich Deisenhofen, Germany) and domperidone (10mg/kg body weight p.o. day -1.0 prae op, Sigma-Aldrich Deisenhofen, Germany) during the first 3 hours of brain death [22, 23].

**Group 6**: Dopaminergic Receptor-agonist pergolide (D-Agonist), Fisher rats brain death plus pergolide (0.5 mg/kg body weight i.p., Sigma-Aldrich Deisenhofen, Germany) during the whole duration of the experiment [24].

**Group 7**: Pergolide + adrenergic Receptor-antagonists (D-Agonist+ADR.BLOCK), Fisher rats brain death plus pergolide (0.5 mg/kg body weight i.p., Sigma-Aldrich Deisenhofen, Germany) and propranolol/doxazosin during the whole duration of the experiment.

To investigate if dopamine could already evolve its protective effects before brain death occur, separate groups of animals were pre-treated with dopamine for 24h with osmotic minipumps (Alzet 2ML1) implanted subcutaneously in the animal’s back. A catheter attached to the pump was placed in the left femoral vein and the pre-treatment solution was administered with a mean pumping rate of 10 µL/hr. After implantation of the osmotic minipump, animals were kept in single cages and had free access to water and chow. After 24-hours pre-treatment, dopamine infusion was discontinued and brain death was induced. Animals were divided into 3 groups according to the following scheme:

**Group 8**: Vehicle not brain death 24 hours pre-treatment with saline (VEH-NBD 24), Fisher rats non brain death ventilated pre-treated intravenously for 24 hrs before anaesthesia with saline.


**Group 10**: Dopamine pre-treatment for 24 hours (DA24), Fisher rats plus dopamine 10 µg/min/kg [20] pre-treated intravenously for 24 hrs before brain death induction with dopamine.

In all groups the kidneys were collected after 6 hours for immunohistochemical stainings and PCR analysis. Each group consisted of a minimum of six animals.
### Fig 1: Schematic representation of all intervention studies. BD was induced in groups (II-VII, IX+X) at timepoint $t = 0$, and routine data monitoring was performed for 6h thereafter (kidney explantation $t = 6$). Drugs were applied to the BD rats at various time frames as indicated by the lines.

### Immunohistochemistry

Serial paraffin sections (4 µm) were fixed in 10% neutral buffered formalin for immunohistochemical staining. After phosphate-buffered saline rinsing and blockade of endogenous peroxidase (3% hydrogen peroxide) and endogenous biotin (Avidin blocking kit, Vector, Burlingame, CA), sections were incubated for 1 hr with primary antibody for monocytes and macrophages ED1 (monoclonal mouse anti-rat, Linaris Biologische Produkte GmbH, Germany), major histocompatibility complex (MHC II) (F-17–23–2, monoclonal mouse anti-rat, Linaris), T-cells (CD3) (monoclonal mouse anti-rat, Linaris) followed by incubation with species-specific secondary antibodies for 1 hr. Irrelevant murine or rabbit control antibody or omission of the primary antibody served as controls. Standard avidin-biotin complex staining was performed according to the manufacturer’s instructions (ABC kit, Vector); 3,3′ diaminobenzidine substrate was applied, and the sections were counterstained with hematoxylin. After dehydration in graded ethanol and xylene, slides were mounted in

<table>
<thead>
<tr>
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<th>Renal Explantation ($t = 6$)</th>
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<td>I</td>
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Permount for evaluation. ED1 and MHC II positive cells were counted in a blinded fashion under the microscope at 400× enlargement. At least six animals per group and 20 fields or more per view per sample were evaluated in a blinded fashion. Analysis was performed using a magnification of 400×.

**Light cycler polymerase chain reaction**

Kidneys from naive-, ventilated non BD-, NaCl treated and dopamine treated BD rats were investigated. Snap-frozen tissue samples were homogenized using a Polytron homogenizer (IKA Labortechnik/Fischer Scientific). 500 ng of total RNA was reversed transcribed into cDNA according to the manufacturer’s instructions, using the 1st Strand cDNA Synthesis Kit. cDNA was diluted in 20 µl DEPC-treated water and stored at -80°C until use. Specific DNA standards were generated by PCR amplification of cDNA, purification of the amplified products, and quantification by spectrophotometry. Light cycler PCR of cDNA specimen and DNA standards were conducted in a total volume of 25 µl, containing 2 µl FastStart DNA Master SYBR GreenI, 10 pMol of HO-1 forward and reverse primers and 2mMgCl₂. The amplification profile for each sample was as follows: 2 minutes at 50°C, 5 minutes at 95°C followed by 45 cycles of amplification each cycle consisting of denaturation at 95°C for 15 seconds, annealing for 20 seconds at 55°C and extension for 30 seconds at 72°C. Standard curves were generated in all experiments. PCR efficiency was assessed from the slopes of the standard curves and was found to be between 90% and 100%. Linearity of the assay could be demonstrated by serial dilution of all standards and cDNA. Primer sequences were as follows: HO-1, forward: ATG TCC CAG GAT TTG TCC GA; reverse: TAA AGC CTT CCC TGG ACA CCT). S16, forward: CAC CTA ACC ATA CGC CTT GCT T; reverse: TGG ATC ATA GGA GCC CAA CTG). All samples were normalized for an equal expression of S16. Each experiment was repeated 3 times with similar results.
**FACS-Analysis**

Peripheral blood was drawn through a femoral arterial catheter before and 6 hours after brain death induction. The animals were either treated with NaCl or dopamine (N=3 in both groups) as described earlier. Peripheral Blood Mononuclear Cells (PBMC) were isolated by Ficoll density gradient and stained with directly conjugated monoclonal antibodies (ED1 FITC; MHCII RPE; CD 11a FITC; CD18, FITC; CD62L FITC, all from BD Biosciences, Heidelberg, Germany). After incubation with monoclonal antibodies (30 minutes at 4°C) the samples were extensively washed and analysed on a FACScalibur flow cytometer (BD Biosciences, Heidelberg, Germany).

**Statistical Analysis**

Numerical data are expressed as mean ± standard deviation. For immunohistological parameters and PCR-analysis statistical analysis was performed using the Kruskal-Wallis test with option for multiple comparisons (StatsDirect 2.2.8, Aswell, UK). For analysis of the blood pressure data two-way ANOVA was applied. A P-value of less than 0.05 was considered as significant.
RESULTS

**MAP and inflammatory response in brain dead rats after dopamine intervention**

In untreated Fisher rats mean arterial pressure (MAP) was approximately 110±13 mm Hg. Two minutes after inflation of the fogarty-catheter MAP decreased slightly (82±5 mm Hg), followed by a sharp increase (161±5 mmHg) in the next two minutes. Within 20 min after induction of brain death, MAP decreased to 70±5 mmHg and remained unchanged thereafter in untreated animals. In ventilated, non brain-dead animals, MAP was stable during the first 3 hours but gradually declined during the last 3 hrs (Fig. 2 A). When dopamine was applied during the first three hours after brain death, the hypotensive period was completely abrogated when compared to untreated brain-dead controls. Hypotension reoccurred upon cessation of dopamine infusion (Fig. 2 B).

![Fig 2A](image1)

![Fig 2B](image2)

*Fig. 2: Hemodynamic changes in BD rats. MAP was recorded as described in the method section in VEH-BD (closed circles) and VEH-NBD (open circles) rats (Fig. 2 A). Significant*
differences in MAP between both groups were observed from the onset of BD on until 3 hrs after BD (P<0.05 VEH-BD vs VEH-NBD). No differences in MAP were found before induction of BD. Dopamine treatment during BD (grey squares) significantly improved MAP compared to NaCl treated BD (closed circles) rats (P<0.05, DA vs VEH-BD) (Fig. 2 B). After cessation of dopamine treatment MAP was not different from the NaCl treated BD (closed circles) rats. The results are expressed as MAP (mmHg) of at least 4 animals in each group.

In kidneys of untreated brain-dead rats infiltration of ED1+ and MHC class II+ cells was significantly higher compared to kidneys from ventilated non-brain-dead rats (30±6 vs 22±3 for ED1+ cells; P<0.005 and 101±15 vs 68±20 for MHC class II+ cells; P<0.005). Three hours of dopamine treatment from the onset of BD on significantly diminished the infiltration of these cells (22±5 vs 30±6; P<0.005, for ED1+ cells Fig. 3 A, B and 56±23 vs 101±15; P<0.005, for MHC class II+ cells Fig. 3 C). BD also significantly increased the number of CD3 positive T-cells in renal tissue compared to ventilated non-brain-dead animals (13±5 vs 8±2, P<0.05). Although dopamine treatment showed a tendency to reduce CD3 infiltration, this did not reach statistical significance (13±5 vs 11±2; P=NS; 8±2 vs 11±2; P=NS; Data not shown).

![Graph](image-url)

*Fig 3A: Analysis of ED-1 positive cells in the kidneys of ventilated non-brain-dead rats, brain-dead rats and brain-dead rats treated with dopamine (Fig. 3 A, B). BD rats were...*
treated with dopamine for 3 hrs from the onset of BD on (DA). NaCl treated BD (VEH-BD) and non BD (VEH-NBD) rats served as controls (VEH-NBD vs VEH-BD, VEH-BD vs DA, #: P<0.01). The results are expressed as mean number of positive cells per 10 fields of view ± standard deviation. At least 120 fields of view were analysed comprising 6 to 8 animals per group. Analysis was performed using a magnification of 400x.

Fig 3B Immunohistological staining of ED-1 positive cells
Fig 3C: Analysis of MHC class II positive cells was assessed in Fig. 3 C. The animals were
treated similar as in A (VEH-NBD vs VEH-BD, *: P<0.05; VEH-BD vs DA #: P<0.01). The
results are expressed as mean number of positive cells per 10 fields of view ± standard
deviation. At least 120 fields of view were analysed comprising 6 to 8 animals per group.
Analysis was performed using a magnification of 400x.

Association of inflammatory response with MAP
To investigate in more detail if the anti-inflammatory effect of dopamine was related to an
improved MAP, we performed additional experiments using dopamine together with
alpha/beta-receptor-antagonists (i.e. doxazosin/propranolol). Application of alpha/beta-
receptor-antagonists together with dopamine resulted in a significantly reduced MAP
comparable to untreated brain-dead controls (Fig. 4 A). Propranolol/doxazosin completely
abrogated the beneficial effect of dopamine on monocyte infiltration (35±8 vs 22±5 for ED1+
cells and 87±10 vs 56±23 for MHC class II+ cells; DA+ADR.BLOCK vs DA, P<0.001 for
both ED1 and MHC class II, Fig. 4 B).
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Fig. 4A: Influence of adrenergic and dopaminergic receptor antagonists on hemodynamic changes and monocyte infiltration in dopamine treated BD rats. Adrenergic receptor antagonists (grey triangle) completely abrogated blood pressure stabilisation by dopamine during BD (Fig. 4A). Additional MAP curves represent NaCl treated VEH-BD (closed circles) and dopamine treated (closed squares) rats.

Fig. 4B: Adrenergic receptor antagonists also abrogated the effect of dopamine on monocyte infiltration (VEH-NBD and DA vs DA+ADR.BLOCK, #: P<0.01, Fig. 4B).
An additional group received dopamine simultaneous with D1/D2-receptor-antagonists (SCH23390/ domperidone). In this group (DA+D-BLOCK) MAP was not significantly different from the group receiving DA only (Fig. 4 C).

*Fig. 4C: D1/D2 receptor antagonists (white squares) did not affect blood pressure stabilisation by dopamine (Fig. 4 C). Additional MAP curve represent NaCl treated BD (closed circles) rats. For reasons of clarity the MAP curve of dopamine treated rats is not depicted, but was similar as in Fig. 2 B.*

However, monocyte infiltration was significantly stronger when D1/D2-receptor-antagonists were applied (42±8 vs 22±5 for ED1+ cells and 97±15 vs 56±23 for MHC class II+ cells; DA+D-BLOCK vs DA; *P*<0.0001 for both ED1 and MHC class II, Fig. 4 D).
Fig. 4 D Although D1/2-receptor-antagonists did not influence dopamine mediated blood pressure stabilisation during BD, they completely abrogated the anti-inflammatory effect of dopamine as assessed by immunohistochemistry for ED-1 positive cells (VEH-NBD and DA vs DA + D-BLOCK, #: P<0.01, Fig. 4 D).

In addition, the D-receptor Agonist pergolide reduced monocyte infiltration to a similar extent as dopamine (23±3 vs 30±6 for ED1 positive cells, D-Agonist vs VEH-BD) but did not stabilise MAP (Fig. 4 E, F).
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Fig. 4 E

Fig. 4 E+F: The D-receptor agonist pergolide had no blood pressure stabilizing properties (grey circles) (Fig. 4 E). Nevertheless it inhibited monocyte infiltration (VEH-BD vs D-Agonist, *: P<0.05 and DA vs D-Agonist, P=NS, Fig. 4 F).

Fig. 4 F
Pergolide did not reduce monocyte infiltration when used in combination with propranolol/doxazosin although hemodynamic destabilisation during BD did not occur under this condition (Fig. 4 G, H).

Fig 4 G

Fig. 4 H+G: The influence of pergolide together with adrenergic receptor blockers on hemodynamic changes and monocyte infiltration is depicted in Fig 4 G and H respectively. In Fig 4 H, the group of dopamine plus adrenergic blockers (DA+ADR.BLOCK) is also depicted for comparison (D-Agonist vs D-Agonist+ADR.BLOCK, *: P<0.05 and DA+ADR.BLOCK vs D-Agonist+ADR.BLOCK, P=NS). For hemodynamic changes the results are expressed as MAP (mmHg) of at least 4 animals in each group. For immunohistochemical analysis at least 120 fields of view were analysed comprising 6 to 8 animals per group. Analysis was performed using a magnification of 400x.
Influence of dopamine pretreatment on MAP and renal inflammation

To study if dopamine was also effective before induction of brain death we pretreated animals for 24 hrs with dopamine. The animals did not receive dopamine during the 6 hours brain-death period. Hemodynamic changes during brain death were not affected by dopamine pre-treatment (Fig. 5 A). In contrast, dopamine pre-treatment markedly reduced monocyte infiltration in the kidneys (44±7 vs 37±5 for ED1+ cells, untreated vs DA pre-treatment brain dead rats, P<0.05; 63±17 vs 43±16 for MHC class II + cells, untreated vs DA pre-treatment brain dead rats, P<0.01, Fig. 5 B), indicating that inhibition of monocyte infiltration per se is not mediated by restoring MAP.
Fig. 5A

Fig. 5: Influence of dopamine treatment before BD induction on hemodynamics and monocyte infiltration. Rats were treated for 24 hrs with dopamine using an osmotic mini-pump (DA 24). Control groups (VEH-NBD 24, VEH-BD 24) were treated with saline for the same time. Dopamine was not applied during the period of BD. MAP was not affected by dopamine pre-treatment (grey triangle, Fig. 5 A). ED-1 (shaded bars) and MHC class II (black bars) positive cells were assessed as described (VEH-BD 24 vs DA 24, *: P<0.05, Fig. 5 B). For hemodynamic changes the results are expressed as MAP (mmHg) of at least 4 animals in each group. For immunohistochemical analysis at least 120 fields of view were analysed comprising 6 to 8 animals per group. Analysis was performed using a magnification of 400x.
Influence of dopamine on HO-1 gene expression

In renal tissue of ventilated non brain-dead rats HO-1 gene expression was slightly but significantly increased compared to naive controls (P<0.05). Brain death led to a profound upregulation of HO-1 gene expression (naive vs. brain death: P<0.0001), which was completely abrogated by dopamine treatment (VEH-BD vs DA: P<0.05). Similarly, HO-1 expression was significantly lower when pergolide was applied to the BD donors (VEH-BD vs D-agonist: P<0.05). Application of pergolide in the presence of propranolol/doxazosin restored HO-1 expression to that observed in untreated BD animals (Fig. 6)
Fig. 6: Quantitative PCR-Analysis for HO-1 gene expression in kidneys of naive (NAIVE), ventilated non BD (VEH-NBD), NaCl treated brain death (VEH-BD) dopamine treated brain death (DA), pergolide treated brain death (D-Agonist) and pergolide + propranolol/doxazosin treated brain death rats (D-Agonist+ADR.Block). The results are expressed as mean HO-1/S16 ratio ± SD. In each group kidneys from 4 animals were analysed (NAIVE vs VEH-BD, #: P<0.01; VEH-NBD vs VEH-BD, *: P<0.05; VEH-BD vs DA, §: P<0.05; VEH-BD vs D-Agonist, $: P<0.05 and VEH-BD vs D-Agonist+ADR.Block: P=NS).

FACS-Analysis
To investigate if BD influences the expression of adhesion molecules on circulating peripheral blood leucocytes we performed FACS-Analysis using anti-CD11a, CD18, and CD62L monoclonal antibody. No significant changes in the expression of these molecules were observed during BD. The expression of adhesion molecules on circulating leucocytes was also not affected in animals receiving dopamine during the BD period (data not shown).
DISCUSSION
The present study demonstrates that dopamine in a clinically relevant dosage reduces monocyte infiltration in renal tissue of brain death donors irrespective if the treatment started before or after brain death induction. This protective effect is partly mediated by improving MAP during BD. In addition dopamine seems to have a direct anti-inflammatory effect mediated via D-receptor stimulation.

Brain death is considered to be an important alloantigen-independent risk factor in transplantation [25-27], as it is associated with a rapid up-regulation of inflammatory mediators and consequently with mononuclear cell infiltration in end-organs [28, 29] [30, 31]. It thus seems that allografts obtained from brain-dead donors are more immunogenic compared to grafts obtained from living donors [32].

A major problem during brain death is the occurrence of hypotension. This in turn leads to hypoperfusion of organs, tissue ischemia and consequently to generation of reactive oxygen species (ROS) [27, 33]. Blood pressure stabilization to physiological values seems therefore to be auspicious in preventing tissue injury in BD donors before organ procurement. We have recently demonstrated in an animal model, that dopamine treatment during brain death improves renal perfusion [19]. A correlation between hemodynamic stabilization and reduced renal inflammation has also been demonstrated by other groups [2, 27, 30, 34]. The finding that the beneficial effects of dopamine on MAP and monocyte infiltration were abrogated when blood pressure lowering substances were applied to brain death rats, are in line with these studies. It is therefore tempting to postulate that the anti-inflammatory effect of dopamine is largely mediated by improving hemodynamics. There are however, several arguments against this assumption. Firstly, dopamine pretreatment reduced monocyte infiltration although MAP was not influenced during brain death. Secondly, D-receptor antagonists abrogate the anti-inflammatory effect of dopamine without changing its blood pressure stabilising properties. Thirdly, application of a D-receptor agonist reduced monocyte infiltration, but it did not improve MAP during BD.

Our results indicate that dopaminergic/adrenergic receptor-antagonists inhibit the anti-inflammatory response mediated by dopamine in kidneys of brain-dead donors. While adrenergic receptors most likely exert a beneficial effect on hemodynamic stabilization, D1/D2 receptors inhibit monocyte infiltration independent of hemodynamic effects. D1/D2-antagonists do not influence renal perfusion as demonstrated by Asico et al. [22]. It must be
stressed however that D1/D2 receptor stimulation itself not necessarily results in an anti-inflammatory effect, since application of pergolide in the face of adrenergic receptor blockade did not affect BD induced monocyte infiltration. Thus, pergolide and dopamine do not display completely similar effects in this regard. Although it is unclear from our data what the underlying mechanism for this discrepancy might be, additional anti-inflammatory effects mediated by dopamine and not by pergolide must be considered. Inhibition of IL-8 production by dopamine [17] is a plausible explanation and needs to be investigated in future studies.

It also needs to be discussed if the anti-inflammatory effect of dopamine is due to changes in renal tissue or if it is due to changes in monocyte behaviour. Previously, we already have shown that the expression of adhesion molecules in kidneys of BD rats is inhibited by dopamine treatment [19]. In the present study we show that neither BD nor dopamine treatment during BD changes the expression of CD11a/CD18 and CD62L on circulating mononuclear cells. Nevertheless, we can not rule out that dopamine treatment also influences the inflammatory properties of monocytes. Several studies indeed indicate that catecholamines have the propensity to inhibit TNF\(\alpha\) and NO production in monocytes [35-40] [41] [42].

An increasing number of studies have suggested the involvement of heme oxygenase 1 (HO-1) in the regulation of inflammation [43-46]. This is largely mediated by carbon monoxide (CO), a by-product of heme catabolism [44]. In BD animals renal HO-1 was significantly upregulated. These findings were unexpected since in these animals monocyte infiltration was much higher than in ventilated non BD rats. Similar to dopamine, pergolide also abrogated the upregulation of HO-1. HO-1 expression was comparable in pergolide and adrenergic receptor blocker treated rats as in untreated BD animals.

The findings on HO-1 seem to be in conflict with previous data reported by our group, showing that HO-1 expression was upregulated by dopamine [16]. It must be stressed however that in vitro induction of HO-1 was strictly dependent on the pro-oxidative effects of dopamine. Moreover, dopamine mediated HO-1 mRNA and protein expression required a longer period of time than the 3 hrs of dopamine infusion used in the present study. Since HO-1 expression is upregulated during cellular stress, e.g. ischemia, it is conceivable, that upregulation of HO-1 during BD was related to the hemodynamic instability of the donor rats. This is in line with the finding that upregulation of HO-1 was abrogated in dopamine treated BD rats. However, also in pergolide treated rats hemodynamic instability occurred during BD and this was not reflected by an increased HO-1 mRNA expression. In addition, pergolide
treatment together with adrenergic receptor blockers, increased HO-1 expression to the extent of that observed in untreated BD rats. Thus, neither the involvement of D-receptor stimulation nor the effects of hemodynamic destabilisation on HO-1 expression can unambiguously be demonstrated in this study. Nevertheless, our results imply that the anti-inflammatory effect of dopamine is not mediated by HO-1.

In conclusion, we demonstrate that although hemodynamic stabilisation is beneficial to limit BD induced inflammation, the anti-inflammatory effect of dopamine involves more than just blood pressure stabilisation. Activation of D1/D2 receptors and even non-receptor mediated effects of dopamine might collectively contribute to the anti-inflammatory effect of dopamine in BD donors.
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REFERENCES

Chapter 4 – Dopamine Treatment in Brain-Dead Rats Mediates Anti-Inflammatory Effects

[References]


