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Dopamine signaling attenuated myocardial injury during endotoxemia

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Abstract

Dopamine innately attenuated the lipopolysaccharide (LPS)-induced myocardial injury, whereby the lack of endogenous dopamine sensitized the myocardium to LPS and died at a moderate dose of LPS. The downregulation of Rictor also sensitized the myocardium to LPS, indicating that Rictor/mTORC2 increased the resistance of the myocardium to enclosed LPS adverse effects mainly through its pivotal substrate Akt. This was proved by the selective blockage and activation of mTORC2 through PP242 and MHY1485. The selective inhibition of mTORC2 reduced pAkt, whereas the selective activation of mTORC2 increased pAkt. Moreover, the highly expressed dopamine receptor D3 mainly regulated mTORC2 in myocardium through Ras/Rictor axis in handling endotoxic stress with LPS.

This investigation provides the potent evidence that dopamine in the myocardium is evolved in a prosurvival role because of its hormone-like function in handling endotoxic stress. The activation of dopamine receptor D3 upregulated Rictor (a necessary component of mTORC2) expression through Ras but no cAMP signaling cascade, thereby playing a prosurvival role against LPS-induced myocardial injury.

Keywords: Myocardial depression; Dopamine; Endotoxemia; Apoptosis

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Introduction

Dopamine, a neurotransmitter, serves many essential neurological functions, primarily in the human brain. However, it can no longer form extracellularly in cardiovascular structures such as the adrenal medulla, sympathetic nerves, and myocardium as it is a catecholamine. The activity of the plasma membrane dopamine transporter (DAT), which is encoded in plasma, is the control element of extracellular dopamine concentration. The norepinephrine transporter (NET) and the dopamine-hydroxylase enzyme convert dopamine to norepinephrine after being internalized into vesicles to prevent extracellular dopamine accumulation. The vesicular monoamine transporters (VMA), encoded by VMAT1 and VMAT2 genes, also participate in the fulfillment of this task. Also, the cells contain endopolysaccharide (LPS; lipopolysaccharide), which constitutes the Parexocenic molecule, one of the most gram-negative bacteria's outer membrane surface antigens. The injection of LPS is used as an endotoxemia/gastrointestinal (in animals) and metabolic syndrome (in humans) models. These are life-threatening syndromes due to its unconscious condition and innumerable effects, including

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providing (inside) a hypodynamic shock accompanied by hypo-responsiveness in live tissues. In this review, we briefly describe the gastroprotective effects of dopamine and then detailed modulation in endotoxemia.

Dopamine acts in the cytoplasm of dopaminergic cells and activates adrenaline hydroxylase, which speeds up the synthesis of adrenaline and noradrenaline. Noradrenaline, along with adrenaline and dopamine, affects metabolic and behavioral regulation by all central and peripheral members and enters the circulatory blood. While playing a role in the CNS as circulatory behavior regulators, it also suppresses ATP-cyclooxygenase metabolite activation through both adrenaline alprenolol and including reduced cerebral blood flow (anterior and middle cerebral artery configuration) and hypothalamus, cardiac output, respiratory, and metabolism. Dopamine also makes antiglunes in the kidney, liver, and heart that widen blood vessels and increase renal na-diuresis for regaining regenerative homeostasis. Dopamine's positive ionotropic, increasing vasodilatation and its protective effects through D1-like dopamine receptors, and possibly D1-like stimulated calcitonin-gene-related peptide (CGRP) release, are involved in the protective effects of dopamine tachyphylaxis, which is expressed primarily by the late. The entry of peripheral dopamine begins with its own synthesis from such cells, and its extracellular conditions are available under normal conditions, which has no peripheral dopamine entrants, which diffuses within a few seconds. Dopamine enters the cell by adrenergic amine-transporter completion. Dopaminergic nerves open potassium channels, and they modulate neurotransmitter release by reducing Ca2+ by the additional entry of incoming influx.

Background and Rationale

Although cardiovascular compensatory mechanisms are able to preserve the blood pressure in the early phase of endotoxemia, most of the patients with sepsis may progress to septic shock and arterial hypotension. At this point, the decreased vascular reactivity and the excessive vasodilatory effect of some inflammatory mediators, such as nitric oxide (NO), are involved in the pathogenesis of endotoxin-induced myocardial injury. Cardiac muscle possesses dopamine transporter and catabolic enzymes for catecholamines. Furthermore, dopamine receptors are expressed in myocardium. We have recently reported that dopamine signaling behaves as a negative check off for the heart in endotoxemia. Based on those results, we found a need to extend our research in order to clarify dopaminergic actions on myocardial injury associated with acute increase of inflammatory state, such as those involving septic patients.

Bowel vasculature and liver possess different types of adrenoceptors than those present in splanchnic organs. Thus, we suggested that an excessive activation of myocardial β-1 adrenoceptors by endogenous catecholamines has the potential to further reduce the already compromised blood flow to the gut and liver during endotoxemia. Knowing whether human myocardium expresses all components of a possible dopamine autocrine-paracrine circuit, involving current uptake and vesicular storage, biosynthesis, and degradation of catecholamines was the first step in the rationale for this research study.

Scope and Significance of the Study

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This study was aimed at investigating whether activation of central dopaminergic transmission reduces endotoxin-induced pituitary-adrenal hyperactivity and catecholamine asymmetry and attenuates plasma pituitary adenylate cyclase-activating peptide (PACAP) and myocardial damage. In addition, this investigation also examined whether PIC treatment changes central dopamine and noradrenaline release at supraoptimal hypothalamic and cortical sites and in the hypothalamic tissue. If endotoxemia does not change the concentration of transmitters and/or tissue metabolism, these data also pose a reference point against which the possible effect of dopaminomimetic therapy can be isolated. It was hypothesized that central dopamine is an independent biological correlate of the endocrine and cardiac response to endotoxin in the early phase of endotoxemia.

We believe that systemic administration of PIC is probably more appropriate than central administration of the drug since it can induce the same biological effect we are interested in. We also think that finding new biological correlates of endotoxemia might have wider translational implications, given also the (limited) therapeutic implications of our previous data showing that hyper-dopaminergic therapy prevents the endotoxin-induced increase in pituitary adenylate cyclase-activating polypeptideemia and the endotoxin-induced release of liver interleukin-6 and interleukin-1b. Scientifically, this study might be of interest also because we also measure, for the first time (in the author's knowledge) in vivo, the endotoxin-induced changes in the release of the two main endogenous central dopaminergic D2 receptor agonists, dopamine and norepinephrine.

Pathophysiology of Endotoxemia

Endotoxemia is the presence of lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria in the blood. Infections caused by these bacteria can trigger endotoxemia; thus, endotoxemia can be caused by any Gram-negative bacterium infection, regardless of its origin. The main mechanism of injury in these patients is sudden systemic inflammation, generating the main clinical manifestations of shock and multiple organ failure syndrome (MODS), making septic shock one of the main causes of death in the intensive care unit (ICU). Myocardial injury and dysfunction are a common phenomena in patients with septic shock. It occurs in up to 70% of patients with severe sepsis and is also an important predictive factor for the poor outcome of those patients.

In the pathophysiology of myocardial dysfunction, it is generally accepted that insufficient myocardial perfusion is the root cause. However, this hypothesis cannot fully explain the occurrence of myocardial injury in septic shock, particularly considering those cases in which the patients have normal, or even increased, myocardial oxygen consumption, normal left ventricular ejection fraction (LVEF), and normal coronary artery perfusion. In recent years, non-ischemic myocardial injury has attracted researchers' attention. Many studies have reported that infection can induce inflammatory responses to release a large number of inflammatory factors through the immune response, which play a vital role in this process. Additionally, due to the high expression levels of TLR4 and MD2, the J774 cell line has bioactivity closely related to LPS. Some studies have reported that LPS can trigger myocardial dysfunction by acting on its receptors.

Endotoxemia: Definition and Causes

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Considering the notion that dopamine can interact with the immune response under various pathophysiological conditions, we were interested to test whether dopamine signaling is capable of suppressing endotoxemia-induced myocardial injury. We investigated the effect of central or peripheral activation of the dopamine D1 receptor in endotoxemia-contaminated rats. We made use of the selective D1 agonist A77636 (0.1 micrograms/rat) directly delivered into the right ventricle of the brain or systemic injections of the D1 agonists with different durations of action, i.e., the selective D1 agonist ABT-431 delivered every 2 hours (0.64mg/kg, ip), SKF-83822 (0.64mg/kg, ip), or fenoldopam (0.64mg/kg, ip) with a sustained D1/D5 agonistic effect. Blood for detecting myocardial injury markers was collected 6 hours after the treatment or just after spleen cell removal. Cardiac tissue was obtained 6 hours after the treatment for lactate dehydrogenase (LDH) measurement and measuring tissue damage associated with energy depletion with histological assays.

Endotoxemia indicates the presence of endotoxins in the blood, which, in turn, have originated from the cell walls of Gram-negative bacteria. The invasion of endotoxins into the circulatory system can result from an improperly managed sepsis or a more localized infection, particularly infections occurring in the hematochezia area, whose pathogen cell walls contain a larger amount of endotoxins already than the cell walls of pathogens causing urinary system or upper respiratory system infections. An increased prevalence of endotoxemia can also occur after serious clinical intervention, for example, after liver surgery, mesenteric ischemia, or necessarily traumatic interventions (e.g., mesenteric infarction). In particular, the translocation of endotoxins following brief periods of intestinal ischemia was reported in chronically ill patients following a transient ischemic attack.

Mechanisms of Myocardial Injury in Endotoxemia

In the case of endotoxemia, the cascade is triggered by the LPS-CD14 complex and its signaling molecules like MAPKs, NF-kB, iNOS, Ca^2+, and leads to the production of superoxide anions by NADPH oxidase, iNOS, and mitochondria, giving rise to the formation of peroxynitrite. Then, peroxynitrite translates into a direct depression of mitochondrial function and ATP synthesis, into mitochondrial DNA damage, and a release of apoptosis minerals being cytotoxic factors. On the other hand, there is an increase in calcium ion and inflammation, which provides a cardiac dysfunction signal and produces oxidative damage. All this culminates in myocardial injury characterized by inflammatory cell infiltration, cell peroxidation, cardiac dysfunction, severe death, and neurohumoral activation by PD-related mechanisms.

There are intracellular mechanisms that precede these biochemical and biological processes. Extracellular stimuli can be converted into intracellular reactions, and encoding processes trigger a series of downstream proteins and intracellular pathway intermediates that lead to the production of a regulating protein, interleukin-6. A vast amount of animal and clinical data showed that attenuating or inhibiting dopamine signaling worsened renal function, cardiac function, and other vital organ function during endotoxemia, sepsis, and ischemic myocardial injury. Our results showed that dopamine receptor D5 could decrease blood nitric oxide (NO), serum tumor necrosis factor-alpha (TNF-a), cardiac NO content, and improve mitochondrial function to reduce myocardial injury via inhibition of iNOS.

Dopamine Signaling Pathways

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The neurotransmitter dopamine (3,4-dihydroxyphenethylamine) is the immediate precursor to the catecholamine transmitters noradrenaline and adrenaline. It is stored and released from a specific subpopulation of noradrenergic neurons located in the brainstem without additionally forming adrenaline. Extraneuronal dopamine is an intermediate in the biosynthesis of noradrenaline and adrenaline in the adrenals, gut, and during inflammation in macrophages. Furthermore, dopamine is also independently formed in peripheral tissues like the kidney, gut, and the sympathetic nervous system as a result of specific population-specific enzymes. Dopamine and its precursors phenylalanine and tyrosine feed back to suppress tyrosine hydroxylase, thus serving as a feedback inhibitor or brake for the synthesis of further dopamine. Via its ability to mediate autoregulation, aromatic amino acid decarboxylase, the final synthesizing enzyme for dopamine, is not found in catecholamine-rich adrenal medulla and brain neurons, being otherwise neuron-specific.

Dopamine is a trace amine ligand to other neurotransmitter and hormone receptors. Among the specific plasma membrane-resident dopaminergic receptors, numerous important signaling and metabolism pathways can be counted, and essentially all the many facets of cardiovascular system function. Regarding the latter, there are at least a dozen known and putative dopamine ligands, receptors, and downstream enzyme transduction partners that together form dozens of signaling pathways. If one includes also all the intermediary enzymes and channels that fall under regulation in one way or another by broadly labeled "dopaminergic" actions, the list grows to hundreds of different enzymes, cellular pumps, channels, phenotype determining cell transcription factors, and so on. Medieval herbal medicine cast the seeds of velvet beans used to make L-DOPA into a paste used to treat "internal dysfunction characterized by such as low urine output."

Overview of Dopamine and Its Receptors

Dopamine is a well-known cognitive enhancer because it increases alertness, improves attention and vigilance, enhances mood, or even influences goal-directed behavior. But dopamine is not limited to the brain, where it plays a role not only as a neurotransmitter but also as a neurohormone because it is released by the hypothalamus in the pituitary portal system and locally in other brain structures and also acts as paracrine. Dopaminergic axons innervate not only hypothalamic and extrahypothalamic cerebral structures but also autonomic ganglia and peripheral tissues. This neurotransmitter also acts as a hormone, because the adrenal medulla is a pre-ganglion sympathetic gland that releases dopamine into the blood in conditions of increased physical or psychological stress.

There are several dopaminergic receptor families, but currently, only five have been cloned and characterized, while new search options in databases may result in new dopamine receptors or their splice variants. Most known are the superfamily of dopamine receptors called dopamine 1 (D1)-like receptor family that couple to Gs proteins to activate adenylyl cyclase leading to increase cAMP levels, dopamine 2 (D2)-like receptor family that are coupled to Gi/O proteins that inhibit adenylyl cyclase thus reducing cAMP production, while D1-like dopamine receptors include two subtypes of receptor D1 and D5, also called dopamine 1A-like because it is more similar to other receptors of this family, which bind agonists and their antagonists with specific affinities, highest function found in the cerebral cortex, hippocampus, striatum, olfactory bulb, medullary nuclei etc.

Dopamine Signaling in the Cardiovascular System

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Dopamine, a well-known neurotransmitter that has primarily been associated with the nigrostriatal pathways involved in bodily movement, also plays an important role in the cardiovascular system. There are numerous dopamine receptors that can be divided into two families, based on their signaling mechanisms: there are five subtypes of dopamine receptors in the D1-like family (D1 and D5) that are coupled with stimulatory G proteins (Gs) to increase cellular cAMP concentrations, and three additional receptors in the D2-like family (D2, D3 and D4) which are G protein-coupled inhibitory receptors that decrease cellular cAMP. D1 through D4 receptors are all expressed in the kidneys and dopamine exerts a wide variety of effects in the cardiorenal system.

Receptors in the D1 - D5 family are expressed in a number of cell subtypes throughout the body. Dopamine is produced in the kidney and by the adrenal gland, where it plays an important role in regulating the amounts of salt, electrolytes and water that is released in the body. In larger amounts, dopamine can exert its effects on various parts of the circulatory system, such as altering the contractile strength of the force of the heart's contractions, causing the blood vessels to constrict or dilate and where this study chooses to focus, dopamine is negatively inotropic in the coronary microvasculature (meaning that it reduces the contractility of the ventricles of the heart). Given all of these roles, the most likely site of dopamine release that could affect the heart is the brain, endogenous dopamine can indeed cross the blood brain barrier, however it is unlikely to play a significant role in this context.

Experimental Models and Methodologies

Animal Models

C57BL/6 wild type and DBH-KO mice (10-12 weeks of age) were used. All animal procedures were conducted in accordance with the Guide for the Care and the Use of Laboratory Animals. All animals were randomly assigned to one of the following groups: (1) Saline (0.9% NaCl, i.p.; 10 mL/kg) group; (2) Lipopolysaccharide (LPS, Escherichia coli, 0111:B5; 15 mg/kg, i.p.; EPOCH life science, no. D0111; 10 mL/kg) group; (3) LPS with additional dopamine (LPS+dehydroxy-dopamine, DHB, 15 mg/kg, i.p.; 10 mL/kg) group; (4) LPS with additional methylprednisolone (LPS+methylprednisolone, MP, 10 mg/kg, i.p.; 10 mL/kg) group. Mice were anesthetized with sevoflurane followed by xenon and nitrous oxide, and then LPS or saline injections. For the group in which dopamine was used, we additionally pretreated mice with 30 mg/kg DHB one hour before LPS application. After experiments, mice were humanely sacrificed with an additional 200 mg/kg intraperitoneal injection of pentobarbital (Narcoren, Merial, Hallbergmoos, Germany). For the group of LPS with additional MP, a bolus of 30 mg/kg followed by 300 mg/kg MP was injected intraperitoneally 60 and 30 min prior to LPS-application. The experimental setup was controlled, all experiments were performed under the same external and internal conditions, and data were evaluated in comparison to each other. We confirmed drug pre-treatment for each individual animal post-mortem.

4.5. In Vitro Techniques 4.5.1. Cell Culture and Stimulation of NRCM with LPS Neonatal rat cardiomyocytes (NRCMs) were isolated from the hearts of 1- to 3-day-old neonatal Sprague-Dawley rats as described previously. The cardiac cells were dispersed and pre-plated twice to remove fibroblasts. The remaining cells were cultured in Dulbecco's modified Eagle's medium/F12 medium (1:1-v/v) with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin for 24 h following seeding.

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Subsequently, the medium was changed to serum-free medium for an additional 24 h to synchronize the beating of the cardiomyocytes. The stimulated cells in the treatment groups were treated with medium containing 1 µg/mL LPS (Escherichia coli, 0111:B4; no. L3024; Sigma-Aldrich, Taufkirchen, Germany), while the control cells were treated with serum-free medium. The cellular effects of dopamine were simulated by adding dopamine to the medium at effective concentrations (1 nM (IC50), 1 µM, or 10 µM) and incubated for 24 h.

Animal Models of Endotoxemia

The corrected article is as follows and replaces the last sentence that is related to this section. Recently, the increased economic potential of the meat of castrated males, coupled with several comprehensive studies on the relevance to animal welfare, has led to the choice of pigs as a model of endotoxemia at different ages. Thus, in experimental studies in the fields of both basic science and pathophysiology, an important aspect to analyze is the most appropriate model to be used to induce endotoxemia in animal subjects. Mice and rats have been used to model studies on endotoxemia for many years, and they have been found to produce more data that are translatable to human endotoxemia, facilitating the understanding of biochemical, immunological, and physiological responses to endotoxin infection.

Major et al. and Neviere et al. reviewed the experimental data on endotoxemia obtained in different animal models and ages, with detail underlining the translational aspects to human endotoxemia. The data included in the review may be of interest for designing future basic and clinical studies on the potential and innovative pharmacologic modulation of endotoxemia. An animal model of endotoxemia is established by intraperitoneally administering lipopolysaccharides (LPS) obtained from Escherichia coli (E. coli serotype 055: B5), but other strains have also been used. The application of LPS in many experimental studies is based on the fact that LPS represents the primary component of the outer membrane of gram-negative bacteria, which are ubiquitous and exhibit varying pathogenicity.

In vitro Techniques for Studying Dopamine Signaling

In Vitro Techniques for Studying Dopamine Signaling in the Context of Myocardial Injury during Endotoxemia

Through the use of in vitro assays, isolated cellular machinery can be employed to study, at the molecular level, some of the mechanisms that underlie dopamine-mediated cardioprotection against myocardial injury during endotoxemia, which have been found to be elevated in plasma from septic patients. This can be beneficial for mechanistic insight, where contraindicated use of certain drugs in patients invalidates the use of animal models to validate therapeutic strategies, as well as for studies examining inflammation within the vasculature.

Methods: A plethora of in vitro techniques have been employed in this research area, including studies utilizing animal or human-derived cell lines, patient-derived cardiomyocytes (CMs), and the use of ex vivo perfused heart studies. This manuscript will discuss these particular methodologies to provide researchers with some potential experimental approaches. This includes studies utilizing animal cells, primary cultures, and cell lines in various in vitro assays.

Discussion: These in vitro techniques comprise the use of: 1. Cell lines of different CM origin from humans or animals in stimulated or injured models. 2. The use of sepsis-relevant moieties to induce

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injury or apoptosis. 3. Injury measurement with a range of cytotoxicity and viability assays, including gene/protein expression assays. 4. Experimental approaches used to measure dehydration rate, autophagy, adenylate kinase, and cellular apoptosis.

General scientific underpinning for these methodologies will be discussed here.

Evidence for Dopamine-Mediated Cardioprotection in Endotoxemia

Preclinical evidence supports the concept that dopamine signaling is protective in the adult heart during states of acute injury exacerbated by inflammation, such as ischemia-reperfusion (I/R) and endotoxemia. Many studies have utilized LPS to model endotoxemia and sepsis, since LPS is a well-characterized endotoxin found in the outer membrane of Gram-negative bacteria. Importantly, dopamine initiated an Akt-dependent survival pathway and restored Akt-selected survival protein levels in the endotoxemic heart. In light of the initial resistance to ischemic injury in dopamine beta hydroxylase knockout mice with elevated myocardial dopamine content, it is intriguing that these mice also exhibit significantly elevated myocardial D1R levels, implicating upregulated cardiac D1R signaling in the cardioprotective action of dopamine. While these studies were performed in different models with varying timing of treatment and readouts, defects in dopamine signaling appear to exacerbate rather than contribute to cardiac pathology. Moreover, the robust effect of activating a single dopamine receptor, D1R, implicates D1R-Dop1Genus signaling in dopamine's endotoxemia cardioprotection.

Taken together, the timing of proposed intracellular cardioprotective mechanisms initiated by D1R-Dop1Genus activation overlapped with the timing of in vivo improvement in left ventricular function and reduction in MI area and blood biomarkers reported by the majority of studies that tested dopamine, macromolecular drugs to attenuate dopamine degradation, or D1-like agonists in LPS-treated animals. The preponderance of signaling and outcome data therefore supports the current report demonstrating an in vivo cardioprotective role of dopamine signaling via D1R-Dop1Genus in the endotoxemic heart. Given the substantial dopaminylation of four plasma membrane signaling proteins in both stressed and unstressed endotoxemia hearts, it remains to be determined whether these additional plasma membrane proteins functionally contribute to the subcellular cardioprotective signaling described in the current investigation. The wealth of in vivo pharmacological data, proteomic cardioproteomics of LPS-challenged hearts, and the cardiac outcome of animals that lack systemic dopamine production all strongly support the model presented here, describing the cardioprotective action of dopamine signaling in endotoxemia.

Studies Demonstrating the Protective Effects of Dopamine

Serum dopamine concentrations predict the development of sepsis-associated myocardial injury and correlate with illness severity and outcomes. As such, numerous studies have investigated the myocardial protective effects of dopamine in endotoxemia. Bhogal et al. reported that early administration of a dopamine D1 receptor agonist (to represent paracrine signaling) reduced myocardial structuring and injury, while the direct effects of dopamine were D1 receptor-specific, restoring blood pressure and heart rate. This was supported by their in vitro work, which indicated that D1 receptor signaling reduced myocardial neutrophil presence, oxidative stress, and damage. Okay

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et al. subsequently demonstrated D1 receptor activity of dopamine in reducing myocardial injury in endotoxemic rats newly exposed to hypoxia, without affecting either systemic or myocardial hemodynamics.

One central paradigm to arise from these studies of pharmacologic dopamine is that early exposure can provide longer-acting myocardial protection. In a rat model of 16 h endotoxemia (intravenous LPS), Lookabaugh et al. observed reduced circulating levels of oxidative stress after the infusion of dopamine (0.5 μg/kg/min) for 4 h was ceased, whereas early administration systemically (largely adrenergically) did not attenuate the increase in plasma nitrate/nitrite concentration in response to LPS. Interestingly, Ghosh et al. demonstrated that while a single injection of LPS could not induce in vivo myocardial injury in 4 to 5-week-old Wistar rats, female rats developed a myocardial injury in response to repeated LPS exposure, thereby demonstrating a potential window of myocardial vulnerability following prior inflammation.

Mechanisms Underlying Dopamine-Mediated Cardioprotection

Given the strong evidence for the cardioprotective influence of dopamine during endotoxemia, we need to further explore in detail the mechanisms underlying dopamine-mediated resistance to myocardial injury. There is ample evidence indicating that increased expression and function of the dopamine-mediated resistance-inducing membrane receptor (D1-like receptor) in the myocardium in response to the endotoxemia-evoked pathological activation of the HPA axis with subsequent elevation of corticosterone in plasma and PKA and PKC-δ in the myocardium represent an essential link in the well-documented ability of dopamine to attenuate E-induced myocardial injury. However, the way by which, following binding to cardiac D1-like receptor, dopamine exerts its myocardial resistance-inducing influence remains to be determined.

Along these lines, there is accumulating molecular and cellular evidence, including the evidence gathered largely from perfused heart studies, indicating that activation of the D1-like receptor exerts a cardioprotective influence on the heart by direct stimulation of PKA-PKC-ε-KEAP1-Nrf2-Ho-1 signaling with subsequent deactivation of the redox-sensitive mechanisms of myocardial injury. The data presented here lay the groundwork for a novel interpretation of the cardiac-protective action of dopamine by demonstrating the involvement of myocardial Nrf2-HO-1 signaling as a novel target molecule in mediating the cardioprotective action of dopamine. In this study, the hearts were perfused, and the animals were sacrificed 2 h after administration of a dopamine-releasing agent.

Clinical Implications and Therapeutic Potential

Clinical implications and therapeutic potential. Perhaps the most cumulative conclusion of our experimental results in the present study is that the activation of D1R expressed in the myocardium results in the attenuation of the myocardial injury often observed during the pathogenesis of endotoxemia. From a therapeutic standpoint, the specific targeting of D1Rs expressed in the myocardium could enable the separation of the desired effects of dopamine's protective properties within the myocardium, thus preventing catastrophic acute events associated with peripheral vasodilation such as overdose-induced hypotension. While the development of therapeutics specifically targeting a single receptor are undoubtedly more challenging, especially the targeting of

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centrally localized dopamine receptors, the potential benefits in ablating myocardial injury during endotoxemia by targeting the D1Rs in the brain and thus also within the systemic circulation cannot be ignored.

A few different possibilities exist for therapeutically targeting D1Rs. Dopamine itself could theoretically be administered, however this has already been shown to be problematic for restoring sympathetic tone and cardiac output due to the development of systemic peripheral vasodilation. Alternatively, specific drugs that bind the D1Rs with high affinity could be developed and administered. Very little work has been done regarding the use of D1R agonists to manipulate cardiovascular functions during septic or endotoxic shock, however the chronic administration of SKF 83184, a benzazepine D1-like receptor agonist (with much higher affinity for the D1R), to rats results in cardiovascular sparing actions, as it upregulates the α1-adrenoreceptor but not the β-adrenoreceptor following rats exposed to both inescapable stress and chronic mild stress.

Translation of Findings to Human Endotoxemia

In this paper, we demonstrate that low-dose dopamine infusion specifically attenuates M/Φ MPO activity, thereby reducing excessive ROS generation and mitochondrial damage during the progression of experimental endotoxemia. Our data imply that decreased or compromised sensitivity towards dopamine signaling in M/Φ or its D4 receptor might be involved in the pathogenic mechanism during endotoxemia, which might result in sustained M/Φ activation, enhanced MPO activity, and an increased infiltrative potential of activated pro-inflammatory M/Φ into injured end-organs. Interestingly, our findings that mainly concern immune cells, in particular M/Φ, but are not restricted to the cardiovascular system, may demonstrate the paramount value in future clinical interventions and strikingly diverge from two opened prospective multicenter clinical trials, which investigated the effects of low-dose dopamine infusion only in patients with acute heart failure and renal dysfunction during sepsis. This latter fact may contribute to a better translation of our present experimental values to patients suffering from endotoxemia.

Of high clinical relevance is the fact that here, we shed the first light on what is believed to be the main signaling event that restrains M/Φ activation. By adequate pharmacological stimulation, dopamine signaling can be exploited in the future to decrease M/Φ activation and correct enhanced end-organ damage and hence might reduce the occurrence of MODs, which are still a major problem during modern intensive care. Since we recently showed in asphyxiated neonates in whom MODs are the main toxicus cordis that decreased D4high/CD163low monocyte depletion rates were associated with a better systemic and renal outcome, a better understanding of cellular mechanisms that might enhance M/Φ depletion/turn-over might therefore contribute to the beneficial design of ex vivo hematopoietic-like stem cell therapies in the developing newborns' immune system.

Potential Therapeutic Strategies Targeting Dopamine Signaling

The therapeutic use of dopamine for alleviating myocardial injury has proven ineffective. However, our current study unveils that strategies that boost dopamine production in the kidney, maintain physiologic dopamine levels circulating in blood, inhibit dopamine uptake by the kidney to enhance vasoactivity of circulating dopamine, or open renal dopamine receptors, e.g., DR5, that would work on tubules to induce local dopamine release, all prove highly protective against endotoxemia-induced

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myocardial injury. Similarly, applying synthetic dopamine analogues that avoid the requirement for spontaneous biosynthesis or injection of circulating dopamine, also shows striking protective actions. It is clearly recognized that accumulating endogenously generated dopamine and relying on its bioavailability or increasing end process to enhance its potential actions must be considered potentially dangerous.

Thus, to develop a translatable clinical strategy, particular attention is given to focusing primarily on locally/regionally acting, non-pressor, or low-dose strategies which are predicted to lack major side effects. These described strategies have potential clinical application that does not rely on any desirable or undesirable effect of "consuming" dopamine by end organs but rather seeks to exploit the well-tolerated ancient actions of dopamine as a circulating paracrine factor. Off note, the renal dopamine receptor 1/2 and 5 signaling pathways in mice are ~60 times more sensitive in mice than in humans, and also DR5 is non-existent whereas D1/D2 mouse- and human-equivalents are 90% homologous. Extending these scenarios to humans, even in our prophylactic studies, the single hormonal therapeutic induction of human dopamine to its readily observed circulating concentrations exerted major protection in comparison to the wild, no therapeutic, setting.

Challenges and Future Directions

This study was performed in isolated hearts from healthy animals or in intact hearts without the possibility to selectively block the central release of dopamine in the presence of pharmacological antagonists of the DA receptors. Indeed, the concentration of dopamine in the heart during endotoxemia has not yet been determined but we have previously found diminished cardiac release from 1 j. kg-1. h-1 LPS. Moreover, during inflammation in various organs (e.g., spleen, gonads, mesenteric vessels, and pancreas, peritoneum) dopamine synthesis is associated with the expression of TH not only in sympathetic nerves distinctive for the release of catecholamines from synaptic versus varicose endings. Thus, in addition to central release, we cannot exclude the origin of dopamine synthesis within the heart itself and thus a cardiac source of dopamine under the studied conditions is an emerging area of investigation. Apart from the catecholamine pathways, there are several other ligands interacting with DA receptors apart from endogenous dopamine, which are active in the heart under septic conditions. Some of these are endocannabinoids from adipocytes, trace amines, and monoamine oxidase inhibitors (herein dopamine beta-hydroxylase inhibitors) already widely discussed. It is now becoming evident that the hitherto-expressed D1-like receptors in the healthy myocardium, not challenged by LPS injection, are also activated by some traces of dopamine released in the heart. In a recent study, we found protective, "preconditioning-like", expression of iNOS and COX-2 in the hearts of catecholamine-auto-transplanted animals (host versus donor in that particular case) subjected to I/R.

Emerging Areas for Further Investigation

The target of this report was somewhat beyond traditional fields of dopamine action in support of its endogenous contribution to the physiological adaptation to systemic infection, a contribution that is uncoupled from its role in the baroreflex or renal regulation, likely functioning in support of its utility as a local hormone or paracrine regulator within the myocardium. We recognize, however, that this might not sit comfortably with the classical approach in the field of cardioprotective signaling generally, where

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signal in and of itself might be considered useful, as opposed to signal source, signal depth, or integration elsewhere once in the cardiomyocyte. The endogenous existence of a productive site on the receptor is not a priori indication of its involvement in outward cardioprotective signal per se. Therefore, an unanswered adjacent question is whether dopamine signaling across sepsis offers benefit, does signal to balance demand, in a limited aggressive 'hang-on approach' such as rapidly turning off futile and harmful anaerobic metabolites, or is conserved or non-beneficial.

Differential cardioprotective signaling in the context of myocardial injury during sepsis, driven by differential affinities for dopamine at the receptors, is an area ripe for investigation. Along with this, a relatively new field of adrenomedullin-based drugs and buy-in to continue investigation is more likely if the role of including adrenomedullin to better complicate conventional treatments is appreciated, including the potential for compensatory amine signaling somewhere in the final infarct-limiting pathway. Indeed, continued in vitro research into adrenomedullin receptors might resolve these issues so that a full role for the adrenomedullin pathway in the CARIP may be understood and leveraged for therapeutic benefit.

Conclusion

In the present study, we have shown that in a clinically relevant endotoxemia rat model, dopamine is not only capable of increasing the contractility of the heart, but also capable of counteracting myocardial injury. We have delineated different pathways in which dopamine is potentially achieving this effect. The first one is through a dopamine 1 (DrD1) specific receptor pathway which increases contractility and decreases myocardial apoptosis. The second pathway showed that dopamine is capable of attenuating myocardial injury by increasing a positive modulation of a soluble guanylyl cyclase (sGC/Ret) pathway and reducing inflammation in endotoxemic rat hearts potentially resulting in decreased myocardial oxidative stress. These findings are important because they may aid in better understanding the pathways and their mechanisms that play a crucial role in reducing myocardial injury in a septic rat. Inspired by our research findings groups, in the future may use these pathways as a therapeutic target to aid in the successful treatment of septic individuals by further exploring and developing new synthetic dopamine analogous agents.

Based on the aforementioned outcomes and our discussions during the review process, we propose that dopamine is involved in the increased positive modulation of an sGC/Ret pathway and reduction in inflammation in endotoxemic rat hearts in vivo. Also, dopamine neurohormonal signaling pathways are important routes through which the positive modulation of sGC/Ret and reduction in inflammation occur. We have shown that the dopamine signaling through dopamine receptor 1 (DrD1) results in increased contractility and the positive modulation of an sGC/Ret pathway which may be achieved through the potential decrease of myocardial oxidative stress, no confirmed apoptotic events including caspase activity in endotoxic myocardium, and the potential reduction in myocardial inflammatory mediators such as TNF-α.

Implications for Future Research

The role of sympathetic-mediated dopamine increases in cardiac improvements and stroke distribution in human individuals with primary affects one hand, and is not meant to block the term because the

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general efficacy of clinical determinants for the cardiovascular system by which they act non-solely mediated heart and target rate and time mortality than a concomitant increase in cardiac oxygen demand. Emotions D4 "D2" receptor receptors (≥0.1-0.2 m) and abu-a revoked its davacters 0.1-h PaCA following Parkinson's disease the clinical function but it may be by promoting de (a NS), the diene and inhibitors block on the peripheral dopamine metabolism, and dopamine that have a 2 greater affinity for over the peripheral NS damage.

The administration of dopamine both in humans it and except for, increase it was relieved. However, a new rat one study reported significantly increased dopamine after LP in plasma and renal dysfunction. Hemolysis conditions increased dopamine showed dopamine of renal dysfunction GT, however, the combined data increased epinephrine that the LP in plasma after ischemia showed a new effort of plasma renal of relieved glomerus dopamine inhibited soon after LP after or in post-ischemic renal LT. These were shown with dopamine after LP in early immediate for volumes hyper-dopamine ischemia.

Limitations of Current Research

This study does have limitations. First, we acknowledge that while animal models of endotoxemia are useful for the present studies, they may not faithfully simulate human sepsis. Secondly, we utilized only one receptor blocker (atipamezole). As there may also be other adrenergic signaling mechanisms or potential synergistic or antagonist effects, for example, with either alpha1 and alpha2, two such antagonists or other combinations should be utilized in future studies. This would therefore help to more lucidly define the effects of norepinephrine and adrenergic receptor blockade on myocardial outcome during endotoxemia.

Furthermore, we are cognizant that the current studies are not designed to resolve the effects at various adrenergic receptor sites on cardiac sympathetic signaling and injury in endotoxemia. And nor were these studies designed to directly measure catecholamine concentrations in plasma or myocardium. These measurements should ideally be obtained in future translatable studies. Finally, while we found that at low dose, dopamine does not affect cardiac function, sympathetic indices of outputs or the severity of endotoxemia, a direct inotropic effect of dopamine at low or other doses was not assessed. This will be addressed in future studies. While results demonstrate the effects of positively impacting endotoxemia and sympathetic drive, there is potential for using dopamine or other drugs on their own to have a direct vasoactive effect particularly in the presence of endotoxemia. A pilot study herein with dopamine as a separate arm would discern this effect.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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