BLOOD PRESSURE LOWERING EFFECTS OF CENTRAL TNF-α BLOCKADE DEPENDS ON FUNCTIONAL LEPTIN SIGNALING

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BLOOD PRESSURE LOWERING EFFECTS OF CENTRAL TNF-α BLOCKADE DEPENDS ON FUNCTIONAL LEPTIN SIGNALING (Abstract) Background: Obesity is a major independent risk factor for the development and progression of arterial hypertension. Leptin-mediated sympathoexcitation is a common phenomenon in obesity. Since leptin leads to the synthesis of Tumor Necrosis Factor (TNF)-α in the central nervous system, we hypothesized that the pathological activation of the sympathetic nervous system in obesity-associated hypertension may be mediated by central leptin-related TNF-α mechanisms. Material and methods: We compared the long-term effects of TNF-α inhibition on mean arterial blood pressure, heart rate, baroreflex sensitivity and sympathetic tone in animals with a functional leptin signaling (i.e. lean Zucker rats - LZR) and in animals insensitive to leptin (i.e. obese Zucker rats - OZR). Results: central inhibition of TNF-α in normotensive LZR significantly lowered mean arterial blood pressure, decreased sympathetic activity and improved baroreflex sensitivity but not in the OZR group. Conclusions: These findings suggest that a functionally central leptin-TNF-α signaling plays a key role in mediating the central sympathetic outflow and may represent a promising approach to ameliorate the pathophysiology of obesity related-hypertension. Keywords: OBESITY, LEPTIN, TNF-α, HYPERTENSION, BAROREFLEX SENSITIVITY.

Arterial hypertension is a significant contributor to cardiovascular morbidity and mortality worldwide, and the activation of the sympathetic nervous system has been shown to play a key role in its pathogenesis (1-4). Obesity is a major independent risk factor for the development and progression of arterial hypertension, as obese individuals often have excessively high levels of renal and muscle sympathetic nerve activity due to pathological sympathoexcitation (2, 3, 5). The excessive activation of the sympathetic nervous system has been demonstrated as a crucial mechanism in the development of obesity-induced hypertension. By counteracting the activation of the sympathetic nervous system, pharmacological and non-pharmacological sympatholytic therapies have been shown to lower blood pressure in obesity-associated hyper-
tension (3-7). Leptin-mediated sympathoexcitation is a common phenomenon in obesity, with various central mechanisms such as the melanocortin system or phosphoinositide 3-kinase (PI3K) signaling pathways playing a key role (6, 8). Additionally, TNF-α-leptin mediated mechanisms may also contribute to the pathological activation of the sympathetic nervous system, as leptin leads to the synthesis of TNF-α in the central nervous system, which subsequently causes sympathoexcitation and high levels of arterial blood pressure (9-11). Peripheral TNF-α may also contribute to an increased sympathetic activity in obesity-associated hypertension, as shown in both clinical and experimental studies (10). Furthermore, sympathetic activation and hypertension in such physiopathological settings are associated with impaired baroreflex control of heart rate. At the level of the nucleus tractus solitarii, leptin impairs baroreflex control of heart rate in response to increase in arterial pressure (12). Moreover, central TNF-α impairs baroreflex sensitivity and increases arterial blood pressure and heart rate (13). Thus, our aim was to test the hypothesis that the pathological activation of the sympathetic nervous system in obesity-associated hypertension is mediated by central leptin and TNF-alpha. Therefore, we compared the cardiovascular responses to TNF-α blockade with etanercept in animals with a functional leptin signaling and in animals insensitive to leptin. To investigate whether leptin-TNF-α induced sympathoexcitation is driven by central or peripheral mechanisms, we administered etanercept both peripherally and centrally.

MATERIAL AND METHODS

All procedures were performed considering the reduction of animal distress and minimizing as much as possible the number of the animals for statistical significance. Six adult male obese Zucker (fa/fa) rats and six littermate lean Zucker rats (28-32 weeks of age) were used. Rats were housed in a temperature (21-23°C) and humidity-controlled environment with a 12 h light/dark cycle, with ad libitum access to food and water. All procedures involving surgical intervention were conducted under isoflurane anesthesia (2-3%). All experiments were performed in accordance to the European Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes, the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Council of Europe No. 123, Strasbourg, 1985) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Research Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy, Iași.

Continuous Blood Pressure Recording

For continuous measurement of blood pressure a telemetric system was used as previously described (14). Briefly, the body of the telemetry transmitters (TRM54PB, Millar, Inc., Houston, TX, United States) was placed intraabdominally and secured to the abdominal wall. The tip of the pressor sensor was inserted into the femoral artery and advanced into the abdominal aorta below the emergence of the renal arteries. At the end of the implantation procedure all the incisions were closed with non-absorbable sutures (3-0 Prolene; Ethicon, NJ, United States) and antibiotic prophylaxis administered intraperitoneally was continued for 72 hours.

Continuous Recording of BP Waveform

Using a PowerLab 16/35 acquisition system (ADInstruments, Bella Vista, NSW,
Blood pressure lowering effects of central TNF-α blockade depends on functional leptin signaling

Australia), 24-h blood pressure waveforms from implanted telemeters were acquired continuously at a sampling frequency of 2000 Hz. TR181 smartpads (Millar, Inc., Houston, TX, United States) were used for both blood pressure signal acquisition and wireless charging of the implanted telemeters. Daily mean arterial pressure (MAP) and heart rate (HR) were calculated from all 24-hrs individual cardiac cycles identified using LabChart® built-in BP module (ADInstruments, Bella Vista, NSW, Australia) as previously described (14).

Peripheral and central inhibition of TNF-α

Peripheral inhibition of TNF-α was attained by daily intraperitoneal administration of Enbrel® in doses of 5 mg/kg as reported by others to have a proper TNF-α inhibition (15). For central TNF-α inhibition we have used osmotic minipumps (Alzet model 2002, pumping rate ± 0.5 μL/hr) connected to a stainless steel 23 G canula which was inserted into right lateral cerebroventricle using a stereotactic system (Stoelting Co, IL, USA). Continuous intracerebroventricular administration of Enbrel® was made in doses of 10 μg/kg/day which were reported in other studies to have a proper central TNF-α inhibition (16). Briefly, after isoflurane anesthesia, the head of the rat was gently fixed into stereotactic apparatus and a longitudinal incision was made on the middle line of the skull. A small hole was drilled at the following coordinates: bregma -0.84 mm; lateral + 1.8 mm; depth + 4.3 mm and the 23 G stainless steel canula was inserted into the right lateral cerebroventricle. The osmotic minipump was inserted subcutaneously and connected to the infusion cannula via the catheter tube to deliver etanercept into the right lateral cerebroventricular. After anchoring the canula to the cranium with dental cement, the incision was closed with stainless steel clips (Stoelting Co., Wood Dale, IL, United States).

Estimation of Sympathetic Activity

As we previously described (17), the original daily BP signal sampled at 2000 Hz was analyzed in the frequency domain using the implementation of the LabChart® software based on the Fast Fourier Transform algorithm. Power spectra were calculated for all artifact-free segments ~1 min in duration, overlapping by 50% and windowed using a Hamming function and finally averaged to generate daily BP spectra. Analysis of the direct BP signal was preferred over extraction of cardiac cycle-related variables such as the systolic BP as it avoided the inherent issues related to resampling for unequally spaced time-series (17). Since the frequency band between 0.25 and 0.75 Hz (low frequency, LF) contains BP oscillations originating from sympathetically driven variations in arterial vascular tone (18), the power in this band was integrated and expressed as percentage of the total power below the HR (0.01-3 Hz).

Time-domain analyses

The sequence technique was used to calculate spontaneous baroreflex sensitivity from the slope of the linear regression functions between SBP and the subsequent PI of the following 3rd, 4th and 5th heartbeat, as we have previously described (17). Up and down sequences of at least three intervals with changes in SBP of >1 mmHg and in PI of >0.5 msec were analyzed only if the correlation coefficients were > 0.85.

Statistical Analysis

Results are expressed as mean ± SE. One-way repeated measures ANOVA followed by Dunnett’s multiple comparison test were used (Prism 6.01, GraphPad Software) to indicate the presence or ab-
Results

Responses to peripheral inhibition of TNF-α

As shown in Fig. 1, obese Zucker rats (OZR) are hypertensive while lean Zucker rats (LZR) are normotensive. Although there is a significant difference between mean arterial pressure (MAP) in LZR vs. OZR (105 ± 3 mmHg vs. 116 ± 3 mmHg), peripheral TNF-α inhibition did not induce any changes in either group. Heart rate (HR) significantly differs, LZR showing an increased heart rate when compared to OZR as shown in figure 2. No difference of baroreflex sensitivity (BRS) between LZR and OZR was observed during the control period. Baroreflex sensitivity in LZR increased after intraperitoneal administration of etanercept, but this effect did not reach statistical significance. No changes in baroreflex sensitivity were observed in the OZR during peripheral blockade of TNF-α as shown in figure 3. As shown in figure 4, the degree of the estimated sympathetic activity is higher in OZR compared to LZR during control period and no other significant changes were observed during peripheral inhibition of TNF-α.

Responses to central inhibition of TNF-α

The MAP significantly decreased in LZR during central inhibition of TNF-α achieved by continuously intracerebroventricular administration of etanercept. No significant changes were observed in the obese group, the values of the MAP being comparable to those from control period (fig. 1). HR was maintained approximately at the same levels during the entire period of continuously intracerebroventricular administration of etanercept for both groups (fig. 2). In the LZR group, the BRS was significantly higher during central TNF-α inhibition. Conversely, in the OZR the BRS was slightly lower at the end of the intracerebroventricular administration of anti-TNF-α (fig. 3). The activity of the sympathetic nervous system significantly decreased during central TNF-α inhibition but only in the LZR group (fig. 4).

Fig. 1. Mean arterial blood pressure responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; *P <0.05 vs. Recovery (days 20-21).

OZR – Obese Zucker Rats; LZR – Lean Zucker Rats
Blood pressure lowering effects of central TNF-α blockade depends on functional leptin signaling

**Fig. 2.** Heart rate responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; OZR – Obese Zucker Rats; LZR–Lean Zucker Rats

**Fig. 3.** Baroreflex sensitivity responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; In LZR (o) *P <0.05 vs. Recovery (days 20-21); in OZR (•) P < 0.05 vs. Recovery (days 20-21). OZR – Obese Zucker Rats; LZR–Lean Zucker Rats

**Fig. 4.** Sympathetic nervous system activity responses to peripheral and central TNF-α blockade; Values are mean ± SEM and n=6; In LZR (o) *P <0.05 vs. Recovery (days 20-21); OZR – Obese Zucker Rats; LZR–Lean Zucker Rats
DISCUSSION

The major findings of the present study are: 1) central inhibition of TNF-α in normotensive LZR, with functional leptin signaling, significantly improved BRS, decreased sympathetic activity and lowered MAP and 2) in the hypertensive OZR which are insensitive to leptin, TNF inhibition fails to lower sympathetic tone, did not improved BRS and did not lower MAP.

A state of low-grade chronic inflammation is considered a feature of obesity and is characterized by secretion from adipose tissue of various inflammatory adipokines such as resistin, monocyte chemoattractant protein-1, leptin, interleukin 6 (IL-6) and TNF-α (19-21). Leptin was shown to be involved in mediating inflammatory cytokines through the activation of dendritic cells, monocytes and macrophages (22-25). Experimental studies have demonstrated a close relationship between leptin and TNF-α production through various pathways such as phospholipase D1 (26), p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK) (27) or activation of human B cells via Janus activation kinase 2 (JAK2)/ signal transducer and activator of transcription 3 (STAT 3) signaling pathways (28). In the OZR, the TNF-α adipose expression starts around 3 to 4 weeks of age when usually the animals are already obese and insulin resistant (29, 30). The level of TNF-α expression is correlated with the degree of adiposity and the associated metabolic disturbances (30). Since systemic inflammation is known to impair cardiovascular homeostasis, we hypothesized that peripheral TNF-α may represent the linking mechanism between inflammation and cardiovascular dysregulation in obesity related-hypertension. Although OZR have high levels of circulating and tissue TNF-α (31,32), in our study its peripheral inhibition did not improved cardiovascular parameters since BRS, MAP or vascular myogenic sympathetic tone did not change. These results suggest that peripheral TNF may be involved in hypertensive organ damage, systemic inflammation, endothelial dysfunction, rather than in the physiopathology of sympathetic obesity-related hypertension.

When centrally administrated in normal rats, leptin was shown to increase sympathetic outflow through pathways within ventromedial hypothalamus which result in high levels of plasma norepinephrine and epinephrine concentrations (33). Other experimental studies have shown that intracerebroventricular infusion or microinjection of leptin into the arcuate nucleus induces sympathetic activation to various territories such kidneys or lumbar nerves and subsequent increase of arterial BP and alteration of BRS (34-36). Additionally, Bin Yu et al. have shown that increased concentrations of central leptin and TNF-α play a key role in the development of obesity-related hypertension (37). Moreover, in obese mice, Han et coworkers have shown that central leptin-induced TNF-α production was associated with high levels of diurnal blood pressure values, suggesting that this mechanism may contribute to obesity-related hypertension (9). Also, central TNF-α inhibition over the short-term reduced arterial blood pressure and heart rate suggesting that leptin-TNF-α interplay may play a critical role in the physiopathology of obesity hypertension (9). Also, in a model of renovascular hypertension, central TNF-α inhibition reduces blood pressure, restored baroreflex sensitivity and sympathetic tone (38). Taken together, these results strongly support the hypothesis that central leptin-TNF-α may play a key role in obesity-related hypertension.
The results from our study suggest that over the long term, leptin-TNF-α mediated central sympathetic activation is functional under normal conditions and is responsible for maintaining a basal physiologic sympathetic tone, as shown in LZR. This mechanism might become hyperactive in obesity when leptin increase and lead to subsequent sympathoexcitation and hypertension. This hypothesis is supported by the results from OZR with an altered leptin signaling mechanisms in which central TNF inhibition did not lower arterial blood pressure or sympathetic tone. On the contrary, in LZR with a functional leptin signaling, TNF-α inhibition significantly improved BRS, decreased sympathetic tone and lowered MAP. Although it is still a matter for further investigations, our results suggest that central leptin-TNF-α signaling may represent a promising approach to ameliorate the physiopathology of obesity related-hypertension.

CONCLUSIONS

These findings suggest that a functionally central leptin-TNF-α signaling plays a key role in mediating the central sympathetic outflow and may represent a promising approach to improve the pathophysiology of obesity related-hypertension.

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CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES


Blood pressure lowering effects of central TNF-α blockade depends on functional leptin signaling


