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Uncoupling Obesity-related Hypertension by Targeting Hypothalamic IKKβ/NF-κB

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Obesity-related hypertension has become an epidemic health problem and a major risk factor for the development of cardiovascular disease (CVD). Recent research on obesity pathophysiology has targeted the hypothalamus and illustrated that various hypothalamic dysregulations can mechanistically mediate obesity pathogenesis[1-3]. However, it remains unknown whether the coupling of obesity with hypertension can be directed through hypothalamic dysfunction, despite the emerging appreciation that many forms of hypertension can be neurogenic[4-13]. Here, our studies revealed that acute activation of pro-inflammatory nuclear factor κB (NF-κB) and its upstream activator IκB kinase-β (IKKβ) in the mediobasal hypothalamus rapidly elevated blood pressure in mice. This form of hypothalamic inflammation-induced hypertension involved the sympathetic upregulation of hemodynamics and was reversed by sympathetic suppression. Loss-of-function studies further demonstrated that NF-κB inhibition in the mediobasal hypothalamus counteracted obesity-related hypertension in a manner that was dissociable from body weight changes. In addition, we found that pro-opiomelanocortin (POMC) neurons critically accounted for the hypertensive effect of hypothalamic IKKβ/NF-κB which underlied obesity-related hypertension. In conclusion, IKKβ/NF-κB in the mediobasal hypothalamus—in particular in the hypothalamic POMC neurons—represents a primary pathogenic link between obesity and hypertension, and a potential target for uncoupling obesity-related hypertension and CVD.

Chronic inflammation is a common feature of both obesity[14-20] and hypertension[21-23]. The molecular pathways that mediate obesity-associated inflammation include IKKβ/NF-κB[14, 24, 25]. Various recent studies have demonstrated that IKKβ/NF-κB activation in peripheral metabolic tissues can cause diverse types of metabolic dysregulations[14, 24, 25]. Most recently, it was revealed that IKKβ/NF-κB in the hypothalamus can be activated under obesogenic condition to promote energy, body weight and glucose imbalance[26-28]. In this

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Author contributions DC conceived and designed the study. SP performed experiments involving Figures 1, 2 and 4 with assistance from GZ and DC. GZ also performed experiments involving Figure 3. All authors performed data analysis and interpretation. DC wrote the paper.

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work, we investigated whether obesity and hypertension can be mechanistically coupled by IKKβ/NF-κB in the hypothalamus, and if hypothalamic IKKβ/NF-κB inhibition can uncouple obesity-related hypertension. To do this, we first performed experiments using virus-mediated gene delivery of constitutively-active IKKβ ($IKK\beta^{CA}$) to activate IKKβ/NF-κB in the mediobasal hypothalamus. Normal chow-fed, adult C57BL/6 mice were pre-implanted with telemetric BP radio-transmitter, and following post-implantation recovery, they received mediobasal hypothalamic injections of $IKK\beta^{CA}$- vs. control GFP-expressing adenoviruses. Our telemetric monitoring revealed that BP values of $IKK\beta^{CA}$-expressed mice gradually increased since Day 5 post viral injection, and reached the magnitude of hypertension on Day 7–8 post injection (Fig. 1a–d and supplementary Fig. 1a–d). $IKK\beta^{CA}$ did not significantly affect the heart rates of the mice (supplementary Fig. 1e,f). The hypertensive effect of $IKK\beta^{CA}$ was independent of body weight or fat mass, because the induction of $IKK\beta^{CA}$ in this protocol was only short-term which was insufficient to significantly alter these parameters (supplementary Fig. 1g–i). Furthermore, using high-resolution telemetric recording, we calculated low-frequency BP variability (LF-BPV) and low-frequency to high-frequency ratio of heart rate variability (LF/HF-HRV), since both parameters can reflect the sympathetic modulation of hemodynamics[29]. LF-BPV (Fig. 1e) and LF/HF-HRV (Fig. 1f) in $IKK\beta^{CA}$-expressed mice were significantly higher than that in the control mice, suggesting that $IKK\beta^{CA}$ upregulated the sympathetic activity. Thus, activation of hypothalamic IKKβ/NF-κB can acutely cause hypertension which can be dissociated from body weight change.

In parallel, we examined whether NF-κB inhibition in the mediobasal hypothalamus could reverse obesity-related hypertension. To do this, we employed viral delivery of dominant-negative IκBα ($I\kappa B\alpha^{DN}$) to suppress hypothalamic NF-κB in C57BL/6 mice with chronic, severe obesity induced through 6-month high-fat diet (HFD) feeding. Using the procedure described above, the acute effects of NF-κB loss-of-function on the BP of HFD-fed mice and matched chow-fed mice were analyzed. Concomitant to HFD-induced obesity (supplementary Fig. 1g–i), the control mice under HFD feeding showed higher BP levels than control mice under chow feeding (Fig. 1c,d and supplementary Fig. 1a–d). In contrast, hypothalamic delivery of $I\kappa B\alpha^{DN}$ significantly reduced obesity-related hypertension (Fig. 1c,d and supplementary Fig. 1a–d). Also, while chronic HFD feeding elevated both LF-BPV and LF/HF-HRV, both changes were blocked by $I\kappa B\alpha^{DN}$ (Fig. 1e,f). Notably, body weight and adiposity of HFD-fed mice were not significantly affected by the short-term $I\kappa B\alpha^{DN}$ expression (supplementary Fig. 1g–i), therefore, the acute hypotensive effect of NF-κB did not involve the long-term action of hypothalamic NF-κB inhibition in changing body weight[26]. On the other hand, $I\kappa B\alpha^{DN}$ did not affect the normal BP values in chow-fed mice (Fig. 1c,d and supplementary Fig. 1a–d), consistent with the previous finding that hypothalamic NF-κB is mostly inactive under normal chow feeding[26]. Finally, we examined if hypothalamic delivery of $IKK\beta^{CA}$ could escalate hypertension in mice with HFD-induced obesity. Data showed that the BP-raising effect of $IKK\beta^{CA}$ was minor in HFD-fed mice which had already developed hypertension (supplementary Fig. 2a–d). The lack of an additive hypertensive effect from $IKK\beta^{CA}$ in HFD-fed mice suggests that the hypertensive mechanism of obesity is mediated significantly through hypothalamic IKKβ/NF-κB.

In addition to viral injection, we adopted a pharmacologic approach by injecting TNF-α into the third ventricle of mice to instantly activate hypothalamic IKKβ/NF-κB. Normal chow-fed C57BL/6 mice were implanted with a BP radio-transmitter and simultaneously with an injection cannula into the third ventricle. Following post-operative recovery, mice received an injection of TNF-α at various doses. Telemetric recording revealed that TNF-α significantly elevated systolic BP (Fig. 2a), diastolic BP (Fig. 2b) and mean BP (Fig. 2c), and the hypertensive effect of TNF-α was manifested in a dose-dependent manner (Fig. 2d). TNF-α significantly increased LF-BPV and LF/HF-HRV (Fig. 2e,f), indicating that the sympathetic up-regulation underlied the hypertensive action of TNF-α. Consistently, TNF-α injection increased norepinephrine release into the blood (Fig. 2g). To test if sympathetic up-regulation could account for the hypertensive role of TNF-α, we intraperitoneally injected the α-adrenergic antagonist prazosin 45 min prior to a brain injection of TNF-α. Indeed, prazosin completely prevented TNF-α from causing hypertension (Fig. 2h–j). Thus, TNF-α, an obesity-associated cytokine and a pivotal IKKβ/NF-κB activator, leads to the neural induction of hypertension without involving body weight or feeding changes.

Subsequently, we asked which neuronal subpopulations in the mediobasal hypothalamus might mediate the hypertensive action of IKKβ/NF-κB. We focused on two neuronal subtypes that are critically involved in obesity development, i.e., POMC neurons and NPY/AGRP neurons which co-express neuropeptide Y (NPY) and agouti-related peptide (AGRP). We first examined whether IKKβ/NF-κB in POMC neurons and NPY/AGRP neurons responded to TNF-α similarly or differentially. POMC neurons and NPY/AGRP neurons were detected using two lines of reporter mice, *Pomc/ROSA* mice and *NPY-GFP* mice, in which POMC neurons and NPY/AGRP neurons are fluorescently labeled, respectively. We profiled IKKβ activation in these neurons in response to TNF-α using immunostaining of phosphorylated IKKβ (at Tyr 188). Basal levels of IKKβ phosphorylation were barely detectable in the hypothalamus except for a few cells in the median eminence (supplementary Fig. 3). Upon TNF-α stimulation, IKKβ phosphorylation was evidently induced in the arcuate nucleus, median eminence, ventromedial nucleus, and tanycyte ependyma (supplementary Fig. 3). TNF-α-induced IKKβ phosphorylation was either modest or negligible in other hypothalamic regions. We examined POMC neurons in the arcuate nucleus, and found that induction of IKKβ phosphorylation by TNF-α occurred in the majority of POMC neurons (Fig. 3). In contrast, induction of IKKβ phosphorylation by TNF-α was relatively minor in NPY/AGRP neurons (supplementary Fig. 4). Hence, IKKβ/NF-κB in POMC neurons vs. NPY/AGRP neurons is differentially activated by TNF-α.

IκBα is the NF-κB-specific inhibitor which undergoes protein degradation in response to IKKβ activation[30-33]. Under the basal condition, IκBα was abundant throughout the hypothalamic neurons including POMC neurons (Fig. 3). The strong baseline expression of IκBα indicates that IKKβ/NF-κB is normally inactive in hypothalamic neurons, as observed previously[26]. However, TNF-α injection via the third ventricle clearly incurred IκBα protein degradation in the majority of POMC neurons (Fig. 3). Induction of IκBα degradation by TNF-α was also evident in the ventral medial nucleus and the dorsal medial nucleus, but not evident in the paraventricular nucleus or the lateral hypothalamic region (supplementary Fig.

5). All these data corroborated the spatial pattern of IKKβ phosphorylation induced by TNF-α (Fig. 3 and supplementary Fig. 3). Altogether, TNF-α can sensitively activate IKKβ/NF-κB in POMC neurons, implicating that hypothalamic POMC neurons may be mechanistically relevant for the development of obesity-related hypertension directed by hypothalamic inflammation.

We next explored the molecular basis which could underlie the differential activation of IKKβ/NF-κB in POMC neurons and NPY/AGRP neurons by TNF-α. Since both TNF-α receptor 1 (TNF-α R1)[30-33] and TNF-α receptor 2 (TNF-α R2)[34] convey TNF-α stimulation to IKKβ/NF-κB activation, we examined the distribution patterns of these two receptors in the hypothalamus. TNF-α R1 was barely detected in hypothalamic neurons, while it was mostly expressed in non-neuronal cells including tanycytes localized in the median eminence and the third-ventricle wall (supplementary Fig. 6). In contrast, TNF-α R2 was abundantly expressed in neurons of the hypothalamic arcuate nucleus (supplementary Fig. 7). Immunostaining using the fluorescent reporter mice revealed that TNF-α R2 was expressed in POMC neurons but only modestly in NPY/AGRP neurons (supplementary Fig. 7). TNF-α R2 was detected in the membrane as well as the cytoplasm of the neurons, indicating that TNF-α R2 in the hypothalamus underwent constant protein synthesis with a fast protein turnover rate. Overall, TNF-α R2 distribution and TNF-α-induced IKKβ phosphorylation in the hypothalamus spatially correlated with each other (Fig. 3). Taken together, TNF-α R2 expression pattern may represent a basis for the differential IKKβ/NF-κB activation in POMC neurons vs. NPY/AGRP neurons by TNF-α.

Supported by the IKKβ/NF-κB signaling profile in POMC neurons, we tested whether IKKβ/NF-κB inhibition in POMC neurons could prevent the acute induction of hypertension by TNF-α. To do so, we bred *IKKβ*$^{lox/lox}$ mice[26] with *Pomc-Cre* mice[35] to generate the mouse line with IKKβ ablated in POMC neurons, termed *Pomc/IKKβ*$^{lox/lox}$ mice. For comparison, we also included the mouse line with IKKβ ablated in AGRP neurons (termed *Agrp/IKKβ*$^{lox/lox}$ mice), which were generated through crossing *IKKβ*$^{lox/lox}$ mice with *Agrp-Cre* mice as described previously[26]. All mice were pre-implanted with cannula into the third ventricle at ~2 weeks prior to the pharmacological injection. These mice were maintained on normal chow feeding, and food intake and body weight of age-matched *Pomc/IKKβ*$^{lox/lox}$ mice, *Agrp/IKKβ*$^{lox/lox}$ mice and control mice were comparable (supplementary Fig. 8a,b). In response to TNF-α injection, systolic, diastolic and mean BP values in *Agrp/IKKβ*$^{lox/lox}$ mice and control mice increased similarly (Fig. 4a–c). However, BP levels of *Pomc/IKKβ*$^{lox/lox}$ mice failed to increase following TNF-α stimulation (Fig. 4a–c). Further, while TNF-α upregulated the sympathetic modulation of hemodynamic response in *Agrp/IKKβ*$^{lox/lox}$ mice and the control *IKKβ*$^{lox/lox}$ mice, it did not do so in *Pomc/IKKβ*$^{lox/lox}$ mice (Fig. 4d,e). Since the protocol of this experiment required only a few hours, the anti-hypertensive effect displayed in *Pomc/IKKβ*$^{lox/lox}$ mice was unrelated to feeding or body weight. Thus, IKKβ/NF-κB inhibition in POMC neurons significantly prevents TNF-α from causing hypertension in a manner which is independent of feeding or body weight.

Finally, we employed *Pomc/IKKβ*$^{lox/lox}$ mice to test if IKKβ ablation in POMC neurons could prevent the induction of hypertension by obesity. Mice were maintained under long-term HFD feeding. Unlike *Agrp/IKKβ*$^{lox/lox}$ mice which displayed an obesity-resistant

phenotype due to the counteraction against neuroendocrine dysfunctions[26], *Pomc/IKKβ*$^{lox/lox}$ mice were however prone to dietary obesity (supplementary Fig. 8c), an outcome which was predictedly related to the lack of counteraction against other obesogenic mechanisms. Notably, *Pomc/IKKβ*$^{lox/lox}$ mice were significantly protected from the induction of hypertension under HFD-induced obesity (Fig. 4f). Altogether, the pathological impacts of obesity-associated inflammation through different types of neuronal subpopulations in the hypothalamus can be divergent. While IKKβ/NF-κB in AGRP neurons is critical for metabolic disorders through neuroendocrine alterations[26], the disease relevance of IKKβ/NF-κB in POMC neurons involve sympathetic alterations which can lead to obesity-related hypertension and CVD (Fig. 4g).

The so-called “metabolic syndrome” refers to a spectrum of metabolic disorders, such as obesity, dyslipidemia, insulin resistance and hypertension that all increase the risk for developing cardiovascular disease. A common feature of these health problems is a sustained but low-grade level of inflammation in the circulation and many peripheral tissues[14, 24, 25]. Using a combination of viral, pharmacologic and genetic approaches, the current study demonstrated that the pro-inflammatory IKKβ/NF-κB pathway in the hypothalamic arcuate nucleus or POMC neurons is significant for obesity-related hypertension. In conjunction with our recent research[26], the current research unveiled that IKKβ/NF-κB-mediated hypothalamic inflammation can convey obesity to the development of not only metabolic problems (e.g., feeding and glucose disorders) but also CVD. These two important categories of obesity co-morbidities are often intertwined, making it difficult to dissect which process is the pivotal pitfall. The findings in the present study indicate that these two pathological conditions can be disentangled to certain extent by targeting IKKβ/NF-κB in POMC neurons. A potential point in diverting the metabolic and cardiovascular consequences of obesity can be determined by the impact of the hypothalamus on endocrine vs. neural pathway. Although the connection between POMC neurons and the sympathetic nervous system might not be prominent in normal physiology, our data suggest that this connection can be reinforced under obesogenic conditions by disease factors like IKKβ/NF-κB. Indeed, a few recent studies have begun to recognize a potential role of the hypothalamic POMC-melanocortin system in modulating the sympathetic pathway to affect BP balance[36, 37]. We also realize that, although hypertension is often a ramification of dietary obesity, there are certain obesity models, such as melanocortin-4 receptor knockout[38], which are not clearly accompanied by hypertension. In light of this study, it might be interesting to examine whether POMC neurons are under the protection of counter-inflammation property in normotensive obesity models.

METHODS

Animal Models and Phenotyping

IKKβ$^{lox/lox}$ mice, *AGRP-Cre*[26] and *POMC-Cre* mice[35] were previously described. We obtained C57BL/6 mice, *ROSA-STOPFlox-YFP* mice, and *NPY-GFP* mice from Jackson Laboratory, and generated *Pomc/ROSA* mice by crossing *Pomc-Cre* mice with *ROSA-STOPFlox-YFP* mice. The Institutional Animal Care and Use Committee at Albert Einstein College of Medicine approved all the procedures. We measured plasma norepinephrine

using ELISA kit (Rocky Mountain Diagnostics). HFD was purchased from Research Diets, Inc. We used a laboratory scale to regularly measure food intake and body weight of mice. Magnetic resonance imaging was performed at the metabolic core of Albert Einstein College of Medicine.

Third Ventricle Cannulation and Chemical Injection

As previously described[26], under an ultra-precise small animal stereotactic apparatus, we implanted guide cannula into the third ventricle of anesthetized mice at the midline coordinates of 1.82 mm posterior to the bregma and 5.0 mm below the bregma. Following post-implantation recovery, we injected TNF-α (Sigma) dissolved in 2 μl artificial cerebrospinal fluid into the third ventricle of mice through the pre-implanted cannula over 5 min. In some experiments, mice received an intra-peritoneal injection of prazosin (Sigma) to reduce the sympathetic nervous system activity.

Adenoviruses and Mediobasal Hypothalamic Injection

Ad5-CMV-driven IKKβ^{CA} adenovirus, Ad5-CMV-driven IκBα^{DN} and Ad5-CMV-driven GFP were described previously[26]. Using the stereotaxic apparatus described above, we performed bilateral injections to the mediobasal hypothalamus (predominantly the arcuate nucleus) at the coordinate of 1.5 mm posterior to the bregma, 5.8 mm below the skull surface, and 0.4 mm lateral to midline. Purified adenoviruses in 0.2 μl aCSF were injected over 10 min through a 26-gauge guide cannula and a 33-gauge injector connected to a Hamilton Syringe and an infusion pump.

Telemetric Measurement of Mouse Blood Pressure

We performed invasive measurement of artery BP on conscious freely moving mice using a radiotelemetry monitoring system (Data Sciences International). Surgical procedure for radiotelemetric probe (model TA11PA-C10, DSI) implantation was performed according to the standard module[39]. Briefly, mice were anesthetized, and a midline skin incision was made from the tip of the mandible to the sternal notch. The left common carotid artery was isolated; while the proximal end of the vessel was ligated, the distal end was occluded with a microclip. Through an incision made near the proximal end, pressure transmission catheter was guided into the artery, and radiotransmitter body was placed into subcutaneous pocket. Following the probe implantation, the neck incisions were closed using 4-0 sutures, and animals were allowed to fully recover from the surgery. Pharmacologic experiments: we performed implantation of guide cannula into the ventral third ventricle at the same time with intra-artery implantation of telemetric BP probe. Virus injection experiments: we implanted mice with telemetric probe into the artery, and after 1-week post-implantation recovery, we performed intra-hypothalamic injection of indicated viruses. In all experiments, we monitored BP levels of mice on daily basis following telemetric implantation. Continuous 24-hour BP signals were recorded at a sampling rate of 2,000 Hz over segment duration of 300 seconds.

Power spectral analysis of heart rate and blood pressure variabilities

We analyzed heart rate variability (HRV) and blood pressure variability (BPV) in frequency domain[29]. Multiple short-term (5 min) HRV on frequency domain were assessed, analyzed and averaged using the ART 4.0 software (DSI). Data was extracted from waveform pressure and after inter-beat-interval (IBI) interpolation, detrending and resampling at 50 Hz (20 ms). Power spectral density was computed by averaged periodogram method using maximum periodogram setting and an overlapping subseries of 5. Cut-off frequencies for power in the low frequency (LF) and high-frequency (HF) ranges were 0.4 – 1.5 Hz and 1.5 – 4 Hz, respectively. We analyzed BPV using Hemolab Software Suite 7.5. For spectral analysis of BP, artifact free beat-by beat systolic BP were spline interpolated at a sampling rate of 20 Hz and converted from non-equidistant to equidistant time series. Power spectra were computed as area under curve from equidistant data by Fast Fourier Transformation (FFT) with 50% overlapping segments of 512 values. The cut-off frequency boundary for LF was 0.2 – 0.6 Hz and a fixed HF boundary of 2.8 ± 0.5 was maintained for all the analyses.

Cardiac Perfusion and Immunostaining

We perfused mice transcardially under anesthesia with 4% PFA, and the brains were removed, post-fixed in 4% PFA, and infiltrated with 20% – 30% sucrose. Brain sections (15 μm thickness) were blocked with serum of appropriate species, penetrated with 0.2% Triton X-100, treated with rabbit antibody against IκBα (Santa Cruz), pIKKβ (Abcam), TNF-α R1 (Santa Cruz), TNF-α R2 (Santa Cruz), or mouse antibody against HuCD (Invitrogen), and then subjected to reaction with fluorescence-conjugated secondary antibodies (Invitrogen). We obtained images under a con-focal microscope.

Statistical Analyses

Data are presented as mean ± SEM. We analyzed data using Student's t-tests, ANOVA and appropriate *post hoc* analyses. Telemetric BP data were analyzed using Repeated Measures ANOVA. $P<0.05$ was considered significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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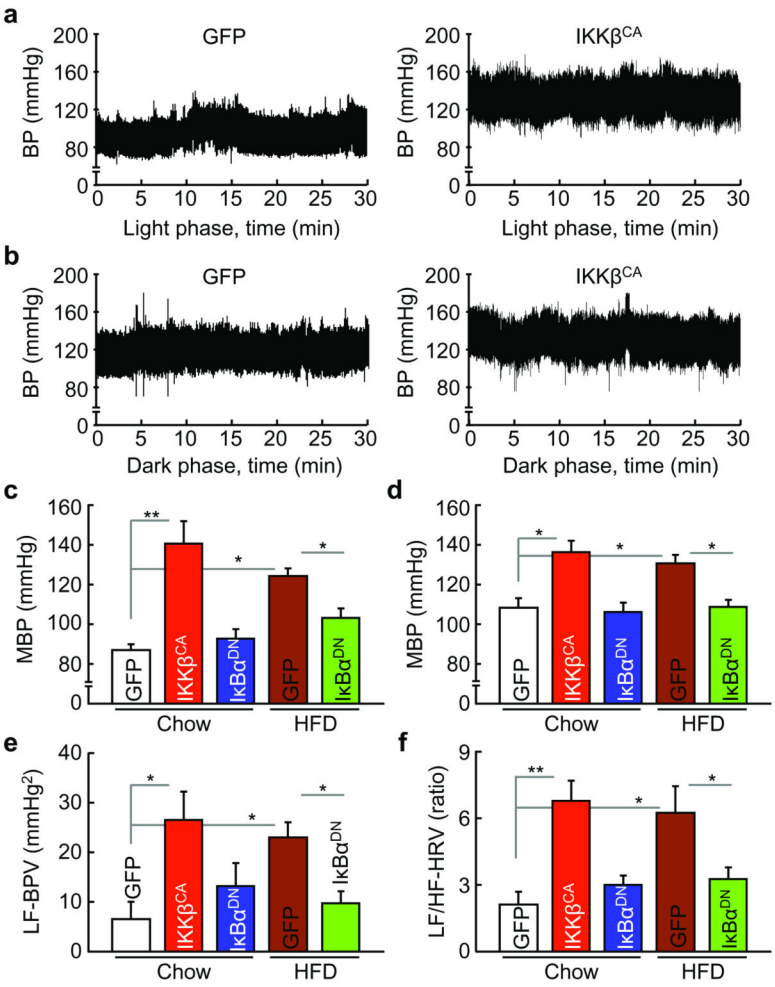

Figure 1. Effects of manipulating hypothalamic IKKβ/NF-κB on BP in C57BL/6 mice

Representative 30-min telemetric BP tracing during the light phase (**a**) vs. the dark phase (**b**), average values of mean BP (MBP) in the indicated mice during the light phase (**c**) vs. the dark phase (**d**), and LF-BPV (**e**) and LF/HF-HRV (**f**) in chow-fed or HFD-fed mice at 1 week post hypothalamic injection of IKKβ^{CA}-, IκBα^{DN}-, or GFP-expressing adenoviruses. *P < 0.05, **P < 0.01; data represent 3 – 4 mice per group. Error bars reflect mean ± SEM.

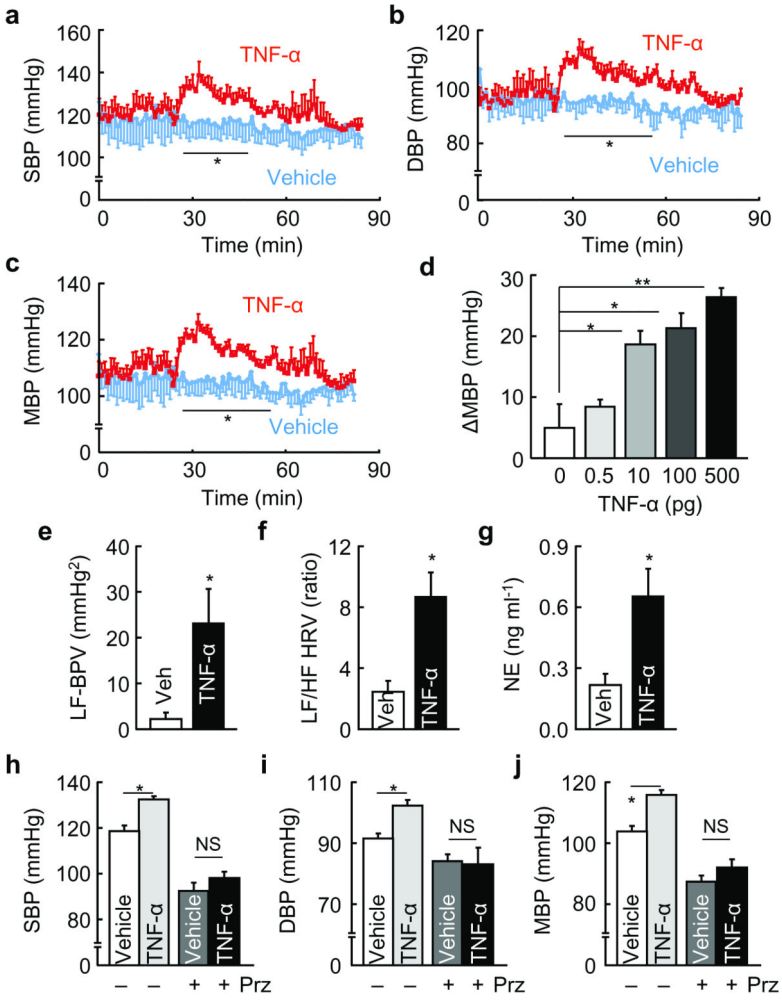

Figure 2. Effects of TNF-α injection via the third ventricle on BP in C57BL/6 mice

a–g. Longitudinal profiles of systolic BP (**a**), diastolic BP (**b**) and mean BP (**c**), dose-dependent increases (Δ) in mean BP averaged over a 15-minute peak change period (**d**), LF-BPV (**e**) and LF/HF-HRV (**f**) over the 15-minute period, and blood norepinephrine (NE) concentrations (**g**) in C57BL/6 mice following TNF-α vs. vehicle injection. **h–j.** Systolic (**h**), diastolic (**i**) and mean (**j**) BP levels over a 15-min peak change period in adult C57BL/6 mice received TNF-α or vehicle injection in the presence (+) or absence (−) of prior IP injection of adrenergic blocker, prazosin (Prz). The dose of injected TNF-α in **a–c** and **e–j** was 0.5 ng. **a–j:** **P* < 0.05, ***P* < 0.01, NS, non-significant; *n* = 4 – 6 per group. Error bars reflect mean ± SEM. SBP: systolic BP; DBP: diastolic BP; MBP: mean BP.

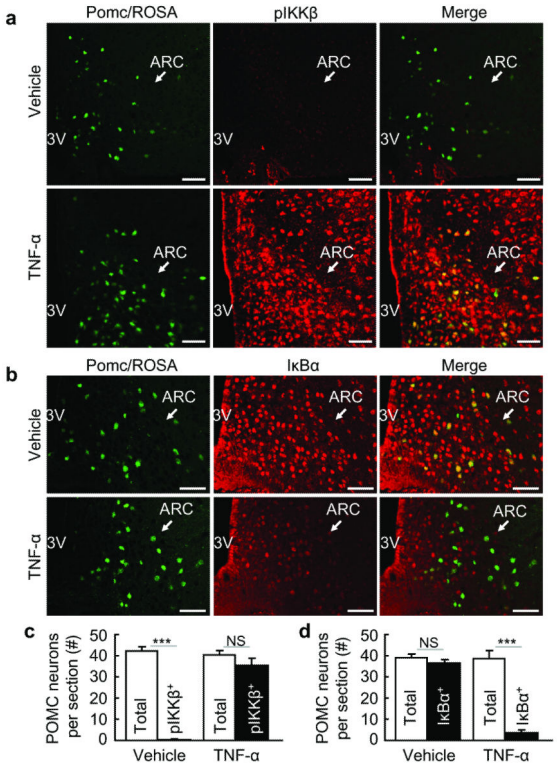

Figure 3. Activation of IKKβ/NF-κB by TNF-α in POMC neurons

Immunostaining of phosphorylated IKKβ (red) (**a**) or IκBα (red) (**b**) in POMC neurons (green) (**a, b**) of *Pomc/ROSA* mice treated with TNF-α vs. vehicle via the third ventricle. pIKKβ: phosphorylated IKKβ; scale bar = 50 μm. The cell numbers of pIKKβ-positive (pIKKβ^{+}) POMC neurons (**c**) or IκBα-positive (IκBα^{+}) POMC neurons (**d**) as well as the total numbers (Total) of POMC neurons (**c, d**) in the brain sections across the ARC were counted. Data represent average cell numbers unilaterally in the median ARC. ***$P <$ 0.001; NS: non-significant; $n = 3 – 4$ per group, error bars reflect mean ± SEM.

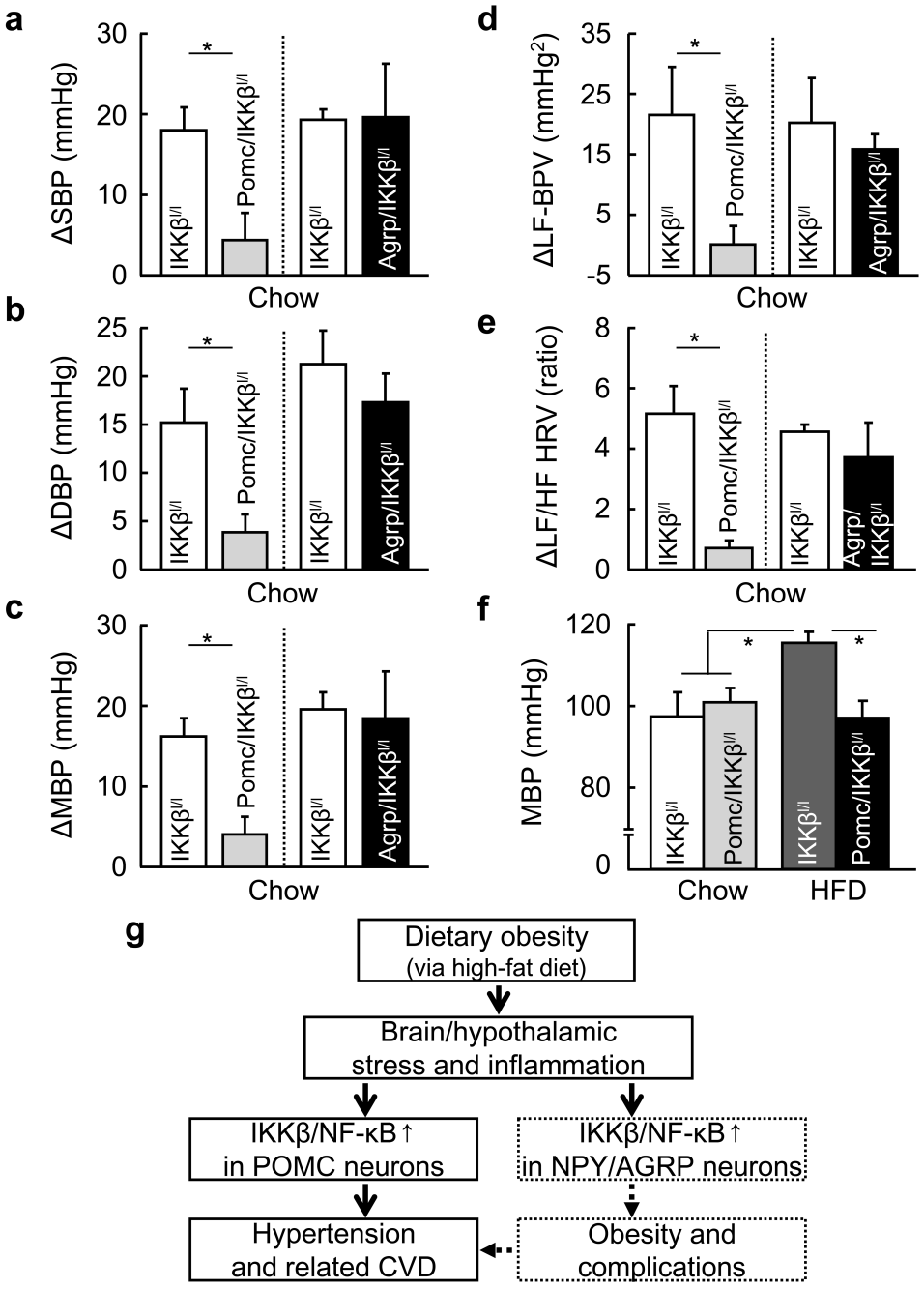

Figure 4. Hypotensive effect of POMC neuron-specific IKKβ ablation

a–e. Average increases (Δ) in systolic BP (**a**), diastolic BP (**b**), mean BP (**c**), LF-BPV (**d**), and LF/HF-HRV (**e**) of chow-fed *Pomc/IKKβ*$^{lox/lox}$ mice, *Agrp/IKKβ*$^{lox/lox}$ mice, and the genotype-matched control *IKKβ*$^{lox/lox}$ mice in response to a third-ventricle injection of TNF-α vs. vehicle during a 15-min peak change period. **f**. Mean BP values in chow- vs. HFD-fed *Pomc/IKKβ*$^{lox/lox}$ mice and control *IKKβ*$^{lox/lox}$ mice. **a–f:** *Pomc/IKKβ*$^{l/l}$: *Pomc/IKKβ*$^{lox/lox}$ mice; *Agrp/IKKβ*$^{l/l}$: *Agrp/IKKβ*$^{lox/lox}$ mice; *IKKβ*$^{l/l}$: *IKKβ*$^{lox/lox}$ mice. SBP: systolic BP;

DBP: diastolic BP; MBP: mean BP. *$P < 0.05$, $n = 3 – 4$ per group. Error bars reflect mean ± SEM. **g.** Proposed role of hypothalamic IKKβ/NF-κB in obesity-related hypertension.