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Time-dependent effect of leptin on renal Na^+,K^+-ATPase activity

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Leptin, secreted by adipose tissue, is involved in the pathogenesis of arterial hypertension, however, the mechanisms through which leptin increases blood pressure are incompletely elucidated. We investigated the effect of leptin, administered for different time periods, on renal Na^+,K^+-ATPase activity in the rat. Leptin was infused under anesthesia into the abdominal aorta proximally to the renal arteries for 0.5–3 h. Leptin administered at doses of 1 and 10 μg/min per kg for 30 min decreased the Na^+,K^+-ATPase activity in the renal medulla. This effect disappeared when the hormone was infused for ≥1 h. Leptin infused for 3 h increased the Na^+,K^+-ATPase activity in the renal cortex and medulla. The stimulatory effect was abolished by a specific inhibitor of Janus kinases (JAKs), tyrphostin AG490, as well as by an NAD(P)H oxidase inhibitor, apocynin. Leptin increased urinary excretion of hydrogen peroxide (H_2O_2) between 2 and 3 h of infusion. The effect of leptin on renal Na^+,K^+-ATPase and urinary H_2O_2 was augmented by a superoxide dismutase mimetic, tempol, and was abolished by catalase. In addition, infusion of H_2O_2 for 30 min increased the Na^+,K^+-ATPase activity. Inhibitors of extracellular signal regulated kinases (ERKs), PD98059 or U0126, prevented Na^+,K^+-ATPase stimulation by leptin and H_2O_2. These data indicate that leptin, by acting directly within the kidney, has a delayed stimulatory effect on Na^+,K^+-ATPase, mediated by JAKs, H_2O_2 and ERKs. This mechanism may contribute to the abnormal renal Na^+ handling in diseases associated with chronic hyperleptinemia such as diabetes and obesity.

Keywords: leptin, Na^+,K^+-ATPase, obesity, arterial hypertension, mitogen-activated protein kinases, hydrogen peroxide

Leptin, the peptide hormone secreted by white adipose tissue, acts on hypothalamic centres to decrease food intake and stimulate energy expenditure. Leptin has also multiple peripheral effects on carbohydrate and lipid metabolism, hormone secretion, immune and inflammatory response, reproductive and cardiovascular function (Fruhbeck, 2004). Plasma leptin concentration is proportional to the amount of adipose tissue and is markedly increased in obese humans and in animals with dietary-induced obesity. Recent studies indicate that hyperleptinemia is involved in the pathogenesis of obesity-associated cardiovascular complications, including arterial hypertension (Correia & Haynes, 2004).

Renal sodium handling is the main long-term regulator of arterial pressure. The amount of sodium excreted in urine is primarily determined by the rate of its tubular reabsorption, the process driven by Na^+,K^+-ATPase contained in the basolateral membrane of tubular cells (Féraille & Doucet, 2001). Abnormal regulation of the renal Na^+ pump leading to increased sodium reabsorption plays an important role in the pathogenesis of various animal models of hypertension, including obesity-induced hypertension (Hussain *et al.*, 1999). However, the role of leptin in the regulation of renal Na^+,K^+-ATPase is poorly understood. Acutely administered leptin increases natriuresis, in part by reducing renal medullary Na^+,K^+-ATPase activity (Jackson & Li, 1997; Bełtowski *et al.*, 2004a). The inhibitory effect of leptin on the sodium pump is mediated by phosphatidylinositol-3-kinase (PI3K) (Sweeney *et al.*, 2000; Bełtowski *et al.*, 2004b). In contrast, chronic hyperleptinemia induced by 7-day administration of exogenous hormone increases the Na^+,K^+-AT-

Abbreviations: CV, coefficient of variation; ERK, extracellular signal regulated kinase; JAK, Janus kinase; MAPK, mitogen- activated protein kinase; PI3K, phosphatidylinositol 3-kinase; ROS, reactive oxygen species; SOD, superoxide dismutase.

Pase activity; an effect also observed in dietary-induced obesity (Bełtowski *et al.,* 2004a). Systemic hyperleptinemia could modulate renal Na^+,K^+-ATPase through multiple indirect mechanisms such as changes in insulin sensitivity, renin-angiotensin system, nitric oxide production etc. On the other hand, in acute studies leptin was administered either as a bolus or was infused for a short time not exceeding 30 min, so it is unclear whether leptin has any direct Na^+,K^+-ATPase-stimulatory effect when administered locally in a more prolonged manner. Therefore, in the present study we examined the effect of leptin administered for different time periods on renal Na^+, K^+-ATPase activity.

MATERIALS AND METHODS

Reagents. The specific inhibitor of H^+,K^+-ATPases, Sch 28080, was provided by the Schering-Plough Research Institute (Kenilworth, NJ, USA). Recombinant human leptin was purchased from Calbiochem-Novabiochem (San Diego, CA, USA). Catalase (from bovine liver, specific activity 2000–5000 units/mg protein) and all other reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Experimental protocol. All studies were performed on adult male Wistar rats weighing 250–300 g. The study protocol was approved by the Bioethics Committee of the Medical University of Lublin. The animals were anaesthetized with pentobarbital (50 mg/kg i.p.) and a thin catheter was inserted through the femoral artery into the abdominal aorta proximally (<0.5 cm) to the renal arteries for the infusion of the investigated substances. In some animals a second catheter was inserted into the urinary bladder for urine collection. After the surgery, 0.9% NaCl was infused at a rate of 66 μl/min (4 ml/h) for 30 min (stabilization period). Then, leptin was infused for different time periods (0.5, 1, 2 or 3 h). Leptin was dissolved in a 15 mM HCl/7.5 mM NaOH mixture (5:3, v/v) and infused at a rate of 2 ml/h. Control animals received leptin vehicle solution starting from 31st min of the experiment for the corresponding time periods. When infusion was performed for >1 h, anesthesia was supplemented at hourly intervals by i.p. injections of 2.5 mg pentobarbital/kg. Rats were placed on a heating pad and the temperature was maintained at 37°C. Until otherwise stated, compounds expected to block leptin's effect were administered for 60 min, i.e. during the 30-min stabilization period and during leptin infusion in animals treated with leptin for 30 min and between 2 and 3 h of leptin infusion in rats receiving this hormone for 3 h. After the infusion, blood sample (0.5 ml) was withdrawn to an EDTA-containing tube for the measurement of plasma leptin. Then, the kidneys were excised and the animals sacrificed by a lethal dose of pentobarbital.

Na^+,K^+-ATPase assay. Microsomal fraction was isolated from the renal cortex and medulla as previously described (Bełtowski *et al.,* 2004a). Na^+,K^+-ATPase was assayed by measuring the amount of inorganic phosphate (P_i) liberated from ATP in a buffer containing 100 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA, 40 mM Tris/HCl (pH 7.4) and 3 mM Na_2ATP, and was calculated as the difference between the activities assayed in the absence and in the presence of 2 mM ouabain. Both samples contained 0.2 mM Sch 28080 to block ouabain-sensitive H^+,K^+-ATPase which otherwise interferes with the Na^+,K^+-ATPase assay. Enzyme activity was expressed in μmole of P_i liberated by 1 mg of microsomal protein during 1 h (μmol/h per mg protein). P_i and protein concentrations were assayed by the method of Hurst (1964) and Lowry *et al.* (1951), respectively.

Other assays. Hydrogen peroxide in urine was measured by the FOX method based on the oxidation of Fe^{2+} to Fe^{3+} which subsequently forms a blue-violet chromogen with xylenol orange (Yuen & Benzie, 2003). In brief, 200 μl of urine was mixed with 340 μl of FOX reagent (125 μM xylenol orange, 100 mM sorbitol and 250 μM ferrous ammonium sulphate in 25 mM H_2SO_4), incubated at room temperature for 60 min and the absorbance was read at 560 nm. To a separate set of urine samples 0.1 mg catalase was added 5 min before mixing with the FOX reagent. Standards of H_2O_2 (1–10 μM) were prepared from 30% stock solution and H_2O_2 concentration in urine was calculated from the difference between the absorbance of the samples assayed without and with catalase. The sensitivity of this assay (mean 0+S.D. of 10 zero standards) was 0.5 μM and the intra- and interassay CVs were <3% and <6%, respectively. Plasma leptin was measured using Leptin Enzyme Immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA).

Statistics. Data are presented as mean ±S.E.M. from 8 animals in each group. Statistical significance was evaluated by Student's *t*-test or ANOVA followed by Duncan's multiple range test for comparison of 2 or >2 groups, respectively. $P<0.05$ was considered significant.

RESULTS

Time-dependent effect of leptin on renal Na^+,K^+-ATPase: role of PI3K and JAKs

Consistently with our previous study (Bełtowski *et al.,* 2004b), leptin infused for 30 min at 0.1–10 μg/min per kg had no effect on the Na^+,K^+-

ATPase activity in the renal cortex but reduced its activity in the renal medulla in a dose-dependent manner (Fig. 1). However, this inhibitory effect was transient and disappeared if leptin infusion was continued for a longer time period. After 1 h of infusion, a significant decrease in medullary Na^+,K^+-ATPase activity was observed only in animals receiving the highest dose of leptin. Infusion of leptin for 3 h resulted in stimulation of Na^+,K^+-ATPase in both regions of the kidney in animals receiving either 1 or 10 μg leptin/min per kg (Fig. 1).

Plasma leptin in control animals was 3.96 ± 0.32 ng/ml. Infusion of leptin at 10 μg/min per kg for 30 min, 1, 2 and 3 h increased plasma leptin to 6.76 ± 0.74, 6.21 ± 0.53, 5.95 ± 0.57 and 6.42 ± 0.58 ng/ml, respectively. Lower doses of leptin had no effect on plasma leptin concentration. To exclude extrarenal effects, further experiments were performed with leptin at the dose 1 μg/min per kg, which did not elevate the systemic hormone level but exerted significant effects on renal Na^+,K^+-ATPase.

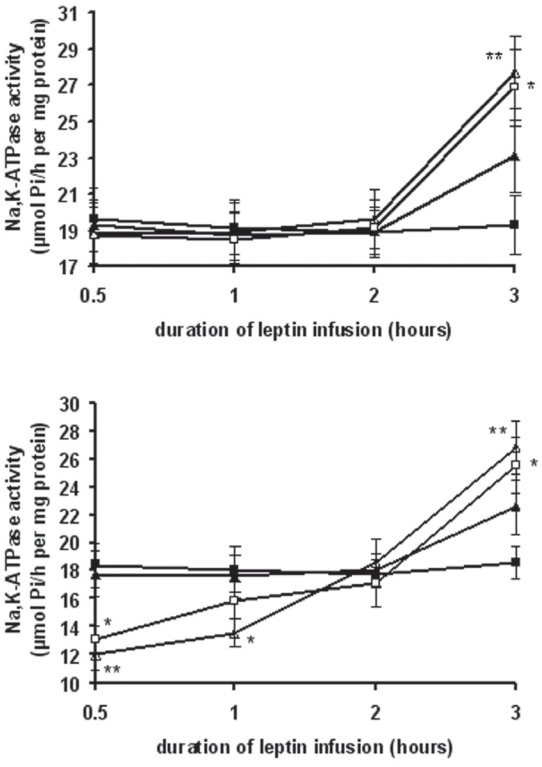

Figure 1. Time-dependent effect of leptin on Na^+,K^+-ATPase activity in the renal cortex (top panel) and medulla (bottom panel).
Control animals (closed squares) received 0.9% NaCl infusion for 30 min, followed by leptin vehicle solution for additional 0.5, 1, 2 or 3 h. Other groups received leptin at doses of 0.1 (closed triangles), 1 (open squares) or 10 (open triangles) μg/min per kg. Na^+,K^+-ATPase was assayed in isolated microsomal fraction and is expressed in μmole of inorganic phosphate liberated by the enzyme contained in 1 mg of microsomal protein in 1 h (μmol P_i/h per mg protein). N = 8 rats in each group. *$P < 0.05$, **$P < 0.01$ *vs.* time-matched control group.

The inhibitory effect of leptin on medullary Na^+,K^+-ATPase was abolished by treatment with either wortmannin (10^{-8} mol/min per kg) or LY 294002 (10^{-6} mol/min per kg), two structurally different inhibitors of PI3K (leptin alone: 13.1 ± 1.0 μmol/min per kg; leptin + wortmannin: 18.1 ± 1.4 μmol/min per kg, leptin + LY 294002: 17.8 ± 1.3 μmol/min per kg). However, wortmannin and LY 294002 did not abolish the stimulatory effect of leptin. These PI3K inhibitors had no effect on the Na^+,K^+-ATPase activity in animals not treated with leptin.

Upon binding to plasma membrane receptor, leptin activates cytosolic protein tyrosine kinases belonging to the JAK family (Hegyi *et al.*, 2004). In the present study the specific JAK inhibitor, tyrphostin AG490, blocked both the stimulatory and the inhibitory effect of leptin (Fig. 2).

Effect of apocynin and tempol on Na^+,K^+-ATPase stimulation by leptin

Nitric oxide decreases renal Na^+,K^+-ATPase activity, however, its effect may be curtailed by intrarenally produced superoxide anion radical ($O_2^{\bullet-}$). NAD(P)H oxidase is a main source of $O_2^{\bullet-}$ in the cardiovascular system and kidney (Kalinowski & Maliński, 2004). Previously we have demonstrated that an NAD(P)H oxidase inhibitor, apocynin, and a superoxide dismutase mimetic, tempol, decrease renal medullary Na^+,K^+-ATPase activity in an NO-dependent manner, suggesting that endogenous $O_2^{\bullet-}$ tonically stimulates the Na^+ pump by scavenging NO (Bełtowski *et al.*, 2004c). Leptin stimulates reactive oxygen species (ROS) formation in various cell types (Boulomie *et al.*, 1999; Yamagishi *et al.*, 2001). In addition, up-regulation of renal Na^+,K^+-ATPase in rats treated with leptin for 7 days was prevent-

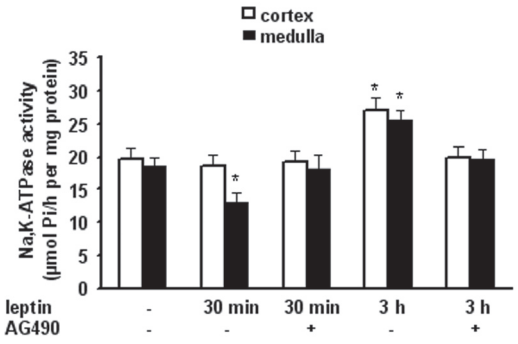

Figure 2. The effect of JAK inhibitor, tyrphostin AG490, on Na^+,K^+-ATPase regulation by leptin.
Leptin was infused at a dose of 1 μg/min per kg for either 30 min or 3 h. In separate groups of animals, AG490 (10^{-7} mol/min per kg) was administered for 60 min, i.e. for 30 min before and throughout leptin infusion in animals treated with leptin for 30 min, or for the last 60 min of leptin infusion in rats receiving this hormone for 3 h. *$P < 0.05$ *vs.* control group not treated with either leptin or AG490.

ed by tempol and apocynin (Bełtowski *et al.,* 2005). Therefore, we hypothesized that the stimulation of Na^+,K^+-ATPase observed in the present study could also result from $O_2^{\bullet-}$ dependent scavenging of NO.

Apocynin or tempol administered alone decreased the Na^+,K^+-ATPase activity in the renal medulla but had no effect on the cortical enzyme (Fig. 3). However, whereas apocynin abolished the effect of leptin in both cortex and medulla, tempol even augmented the leptin-induced stimulation of cortical and medullary Na^+,K^+-ATPase (Fig. 3). Polyethylene glycol-coupled superoxide dismutase infused at a dose of 500 U/min per kg had a similar effect, whereas 3-carbamoyl-proxyl, a compound structurally related to tempol but devoid of $O_2^{\bullet-}$ scavenging properties, failed to augment the effect of leptin (not shown). These results indicate that the effect of tempol results from the scavenging of $O_2^{\bullet-}$.

Leptin stimulated Na^+,K^+-ATPase also in animals in which the activity of the NO-cGMP pathway was artificially stimulated by the administration of 8-bromo-cGMP. As previously reported (Bełtowski *et al.,* 2004c), 8-bromo-cGMP (100 nmol/min per kg for 60 min) decreased the Na^+,K^+-ATPase activity in the renal medulla to 13.1 ± 0.8 μmol/min per kg ($P < 0.01$ *vs.* control) but not in the renal cortex (17.9 ± 1.6 μmol/min per kg). However, the Na^+,K^+-ATPase activity in animals receiving leptin (1 μg/min per kg for 3 h) and 8-bromo-cGMP (for the last hour of leptin infusion) was higher than in rats treated with 8-bromo-cGMP alone (cortex: 24.7 ± 1.8 μmol/min per mg, medulla: 18.8 ± 1.6 μmol/min per mg, both $P<0.05$ *vs.* 8-bromo-cGMP alone). Taken together, these results suggest that NAD(P)H oxidase-derived ROS other than superoxide mediate the stimulatory effect of leptin and that scavenging of NO is not involved.

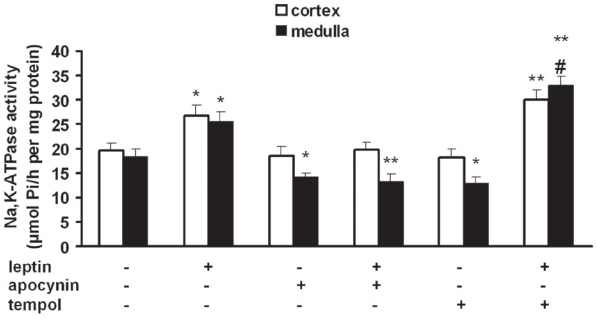

Figure 3. The effect of NAD(P)H oxidase inhibitor, apocynin, and superoxide scavenger, tempol, on leptin-induced stimulation of renal Na^+,K^+-ATPase.
Leptin was infused at a dose of 1 μg/min per kg for 3 h. In separate groups of rats, apocynin (1 μmol/min per kg) or tempol (100 μmol/min per kg) were infused for the last 60 min of leptin/vehicle administration. $^*P < 0.05$, $^{**}P < 0.01$ *vs.* control group, $^{\#}P < 0.05$ *vs.* leptin-treated group without tempol.

Role of H_2O_2 in leptin-induced stimulation of Na^+,K^+-ATPase

Tempol catalytically converts $O_2^{\bullet-}$ to hydrogen peroxide and increases its tissue concentration and urinary excretion (Chen *et al.,* 2003). Although the effect of H_2O_2 on renal Na^+ pump has not been studied so far, H_2O_2 infused into the renal medullary interstitium reduces natriuresis without affecting glomerular filtration, which suggests a predominantly tubular effect (Makino *et al.,* 2003). Thus we hypothesized that leptin could stimulate Na^+,K^+-ATPase in a H_2O_2-dependent manner. Exogenous H_2O_2 (200 nmol/min per kg) infused for only 30 min stimulated cortical and medullary Na^+,K^+-ATPase by 41.8% and 58.7%, respectively, which was accompanied by about 4-fold increase in urinary H_2O_2 excretion (Fig. 4). Moreover, leptin administered at a dose of 1 μg/min per kg increased H_2O_2 excretion between 2 and 3 h of infusion (baseline: 2.4 ± 0.6 μmol/g creatinine, 2–3 h of leptin infusion: 6.1 ± 1.4 μmol/g creatinine, $P < 0.05$) but not in earlier time periods (0–0.5 h of leptin infusion: 2.6 ± 0.5 μmol/g creatinine, 1–2 h of leptin infusion: 3.5 ± 0.8 μmol/g creatinine, both NS *vs.* baseline). Tempol (100 μmol/min per kg) infused for 60 min produced a modest insignificant increase in H_2O_2 excretion (3.7 ± 0.8 μmol/g creatinine). However, if tempol was infused for the last 60 min of leptin administration, urinary H_2O_2 excretion was even higher than in rats treated with leptin alone (10.2 ± 2.1 μmol/g creatinine, $P < 0.05$). In contrast, apocynin (1 μmol/min per kg) tended to reduce, although not significantly, basal H_2O_2 excretion (1.3 ± 0.4 μmol/g creatinine, NS *vs.* control), and attenuated the leptin-induced increase in H_2O_2 excretion (3.3 ± 0.5 μmol/g creatinine). Catalase (1 mg/min per kg) administered between 2 and 3 h of leptin infusion abolished the increase in Na^+,K^+-ATPase activity and H_2O_2 excretion (cortical Na^+,K^+-ATPase:

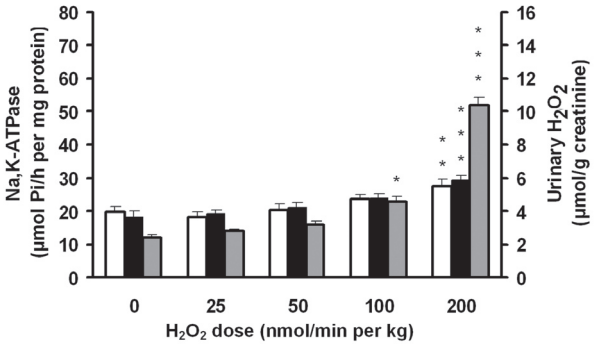

Figure 4. The effect of H_2O_2 on cortical (white bars) and medullary (black bars) Na^+,K^+-ATPase activity and urinary excretion of H_2O_2 (grey bars).
After 30-min baseline period H_2O_2 was infused for another 30 min. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* control group.

20.8 ± 1.7 μmol/h per mg protein, medullary Na^+,K^+-ATPase: 19.6 ± 1.5 μmol/h per mg protein, H_2O_2 excretion: 3.6 ± 0.7 μmol/g creatinine). Catalase administered alone had no effect on Na^+,K^+-ATPase and urinary H_2O_2. Taken together, these results suggest that H_2O_2 is involved in the leptin-induced increase in Na^+,K^+-ATPase activity.

Role of mitogen-activated protein kinases in Na^+,K^+-ATPase regulation by leptin

H_2O_2 stimulates mitogen-activated protein kinases (MAPKs) in many cell types and leptin has been demonstrated to activate MAPKs in an H_2O_2-dependent manner (Cao *et al.,* 2004). Therefore, we investigated if MAPKs are involved in the regulation of renal Na^+,K^+-ATPase by leptin. The specific inhibitor of extracellular signal-regulated kinases (ERK1/p44MAPK and ERK2/p42MAPK), PD98059, abolished the leptin-induced as well as the H_2O_2-induced increase in Na^+,K^+-ATPase activity (Fig. 5). A similar effect was observed if a structurally unrelated ERK inhibitor, U0126, was infused with leptin. In contrast, a specific inhibitor of p38 MAPK, SB203580, failed to attenuate the leptin- or H_2O_2-induced increase in Na^+,K^+-ATPase activity (Fig. 5). Neither of the MAPK inhibitors had any effect on Na^+,K^+-ATPase in animals not treated with leptin. PD98059 and U0126 did not affect H_2O_2 excretion in rats infused with leptin (not shown), suggesting that ERKs are downstream to H_2O_2 in the Na^+,K^+-ATPase-stimulating signaling cascade. PD98059 did not attenuate the increase in cortical Na^+,K^+-ATPase activity induced by either dibutyryl-cAMP or phorbol 12,13-dibutyrate, activators of protein kinase A and C, respectively, indicating that its effect is not due to nonspecific toxicity or inhibition of other protein kinases (not shown).

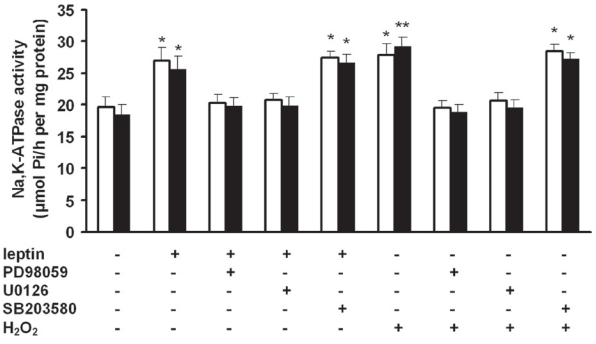

Figure 5. Effect of ERK1/2 inhibitors, PD98059 and U0126, and p38 MAPK inhibitor, SB203580, on Na^+,K^+-ATPase regulation by leptin (1 μg/min per kg for 3 h) and H_2O_2 (200 nmol/min per kg for 30 min).
MAPK inhibitors (100 nmol/min per kg) were administered for the last 1 h of leptin infusion or for 30 min before and during H_2O_2 infusion. **P*<0.05, ***P*<0.01 *vs*. control group.

DISCUSSION

The results of this study indicate that the decrease in the Na^+,K^+-ATPase activity in the renal medulla induced by short-term leptin infusion is only transient and is replaced by stimulation of the cortical and medullary enzyme when leptin is administered for >2 h. The stimulatory effect is mediated by JAKs, H_2O_2 and MAPK belonging to the ERK subfamily. This is not the first study demonstrating opposite short- and long-term effects of leptin. For example, in cultured rat cardiomyocytes leptin stimulates or inhibits adenylate cyclase after 30-min or 18-h exposure, respectively (Iliano *et al.*, 2002).

Previously, we have reported that chronic hyperleptinemia leads to an increase in renal Na^+,K^+-ATPase activity and that this effect is abolished by apocynin and tempol (Bełtowski *et al.*, 2005). Although apocynin blocked the stimulatory effect of leptin also in the present study, the mechanism downstream to NAD(P)H oxidase seems to be different in both cases. Since leptin reduced and tempol restored urinary excretion of nitric oxide metabolites and cGMP in rats treated with leptin for 7 days (Bełtowski *et al.*, 2005), one may suggest that hyperleptinemia stimulated renal Na^+,K^+-ATPase by $O_2^{\bullet-}$-dependent scavenging of NO. In contrast, in the present study tempol augmented the stimulatory effect of leptin, indicating that H_2O_2 rather than $O_2^{\bullet-}$ is involved. In addition, the effect of leptin was preserved in animals treated with 8-bromo-cGMP, suggesting that the increase in the Na^+,K^+-ATPase activity was not associated with inhibition of the NO-cGMP pathway.

At present we can not explain why the mechanisms involved in the up-regulation of Na^+,K^+-ATPase are differed in both studies. One may speculate that in the present 3-h experiment superoxide was efficiently scavenged to H_2O_2 by SOD, which shifted the Na^+,K^+-ATPase-stimulatory mechanism from an $O_2^{\bullet-}$-NO-dependent toward an H_2O_2-ERK dependent one, whereas in 7-day hyperleptinemia the $O_2^{\bullet-}$-scavenging system became ineffective. Reduced expression and/or activity of various SOD isoforms was reported in models of hypertension associated with chronic intrarenal oxidative stress such as spontaneously hypertensive rat (Zhan *et al.*, 2004), 5/6 nephrectomy (Vaziri *et al.*, 2003) or angiotensin II-induced hypertension (Chabrashvili *et al.*, 2003). Moreover, H_2O_2 metabolizing enzymes, glutathione peroxidase and catalase, are upregulated in the kidney of spontaneously hypertensive rat. Thus, chronic intrarenal oxidative stress may favour a higher $O_2^{\bullet-}$/H_2O_2 ratio. In addition, chronic hyperleptinemia is associated with hypertension and positive Na^+ bal-

ance (Bełtowski *et al.,* 2005), conditions which *per se* reduce renal SOD expression (Kitiyakara *et al.,* 2003). It remains to be established if chronic hyperleptinemia induces changes of the expression of antioxidant enzymes similarly to other oxidative stress-dependent forms of hypertension, however, such changes are unlikely to occur in animals receiving leptin for only 3 h.

The second possibility is that NO availability contributes to the different mechanisms involved in the stimulation of renal Na^+,K^+-ATPase. In particular, 7-day hyperleptinemia is associated with NO deficiency (Bełtowski *et al.,* 2005). Although this may result from scavenging of NO by superoxide, other mechanisms such as altered expression of nitric oxide synthase can not be excluded. NO deficiency will render hyperleptinemic animals more sensitive to further NO shortage by excess superoxide. In contrast, acutely administered leptin stimulates NO production (Fruhbeck, 2004), therefore, scavenging of NO might not be rate-limiting for the regulation of renal Na^+,K^+-ATPase in the present study.

Another interesting finding of the present study is that H_2O_2 increases renal Na^+,K^+-ATPase activity. The role of H_2O_2 in blood pressure regulation is controversial. Most studies indicate that H_2O_2 dilates blood vessels and may serve as an endothelium-derived hyperpolarizing factor in some vascular beds (Shimokawa & Matoba, 2004). On the other hand, increased plasma H_2O_2 level has been reported in animal and human hypertension (Swei *et al.,* 1997; Lacy *et al.,* 2000), and mice overexpressing catalase are characterized by reduced pressor response to various vasoconstrictors (Yang *et al.,* 2003). These findings suggest that *in vivo* H_2O_2 might be prohypertensive. Infusion of H_2O_2 into the renal medullary interstitium reduces natriuresis and diuresis and elevates blood pressure in the long run (Makino *et al.,* 2003; Chen *et al.,* 2003). The results presented here suggest that increased Na^+,K^+-ATPase-dependent tubular Na^+ reabsorption may contribute to the hypertensive effect of H_2O_2.

H_2O_2 stimulates various MAPKs, including ERKs (Lee & Esselman, 2002). Several recent studies suggest a role of ERKs in the regulation of renal Na^+,K^+-ATPase. ERK1/2 are involved in the stimulation of Na^+,K^+-ATPase by dopamine D_2 receptor agonists in the rat proximal tubules (Narkar *et al.,* 2002), by vasopressin and aldosterone in the principal cells of mouse cortical collecting duct (Michlig *et al.,* 2004) and by proinsulin C-peptide in cultured human renal tubular cells (Zhong *et al.,* 2004). Outside the kidney, ERKs mediate the stimulatory effect of angiotensin II on the Na^+ pump in vascular smooth muscle cells (Isenovic *et al.,* 2004). In addition, ERK1/2 are involved in the stimulatory effect of calcitonin and β-adrenergic agonists on a related enzyme, ouabain-resistant H^+,K^+-ATPase, in α- and β-intercalated cells of the cortical collecting duct, respectively (Laroche-Joubert *et al.,* 2002; 2003). This study indicates that leptin may be another mediator which utilizes ERKs for the stimulation of Na^+,K^+-ATPase. ERKs have been demonstrated to phosphorylate the α_1 subunit of Na^+,K^+-ATPase (Al-Khalili *et al.,* 2004), however, the precise mechanism of ERK-dependent regulation of its activity remains to be established.

Because the stimulatory effect of leptin on Na^+,K^+-ATPase was observed only after 3 h of hormone infusion, one can not exclude that it was caused by an increase in enzyme expression. Further studies are needed to clarify whether leptin stimulates gene expression of Na^+,K^+-ATPase subunits. However, because the stimulatory effect of leptin was mimicked by short-term H_2O_2 infusion, and the effect of the latter was abolished by PD98059, one may conclude that the increase in the Na^+,K^+-ATPase activity mediated by the H_2O_2-ERK pathway can not be attributed solely to a genomic mechanism.

In conclusion, we have demonstrated that leptin has a short-term PI3K-mediated inhibitory effect on renal medullary Na^+,K^+-ATPase followed by PI3K-independent, H_2O_2 and ERK-dependent stimulation of the enzyme when the hormone is infused for >2 h. Both the inhibitory and the stimulatory effect are mediated by JAKs. These results suggest that prolonged hyperleptinemia associated with obesity or diabetes mellitus may contribute to the development of arterial hypertension by increasing renal Na^+,K^+-ATPase activity and promoting Na^+ retention.

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