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# Effects of a Nitric Oxide Synthase Inhibitor on Vasopressin and Atrial Natriuretic Hormone Release, Thermogenesis and Cardiovascular Functions in Response to Interleukin-1$\beta$ in Rats

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YAMAMOTO, T., KIMURA, T., OTA, K., SHOJI, M., INOUE, M., OHTA, M., SATO, K., FUNYU, T. and ABE, K. *Effects of a Nitric Oxide Synthase Inhibitor on Vasopressin and Atrial Natriuretic Hormone Release, Thermogenesis and Cardiovascular Function in Response to Interleukin-1$\beta$ in Rats.* Tohoku J. Exp. Med., 1994, **174** (1), 59-69 —— To assess whether nitric oxide (NO) formed by IL-1$\beta$ affects vasopressin (AVP) and atrial natriuretic hormone (ANH) release and the regulation of blood pressure and body temperature, intravenous infusion of either $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME) alone (50 $\mu$g/kg • body weight • min for 135 min), human recombinant interleukin 1$\beta$ (IL-1$\beta$) alone (750 ng/kg • body weight • min for 120 min), or L-NAME (50 $\mu$g/kg • body weight • min for 135 min) with IL-1$\beta$ (750 ng/kg • body weight • min for 120 min), was performed following priming doses of L-NAME (2 mg/kg • body weight) and IL-1$\beta$ (7.5 $\mu$g/kg • body weight) into conscious rats ($n=6$ each). In the control group, saline alone was administered. Plasma AVP and ANH, mean arterial blood pressure (MABP), heart rate (HR) and rectal temperature (RT) were determined. In response to L-NAME, plasma AVP significantly increased, but plasma ANH did not change, despite increases in MABP and decreases in HR. In response to IL-1$\beta$, both plasma AVP and ANH increased with decreases in MABP and RT without any changes in HR. With L-NAME and IL-1$\beta$, both plasma AVP and ANH increased, and depressor response to IL-1$\beta$ was partly attenuated by L-NAME, without any changes in RT. With saline alone, none of these parameters changed during the study. These results suggest that NO may directly affect the release of AVP and ANH and the regulation of body temperature and blood pressure, but NO formed by IL-1$\beta$ may not have direct effects on the release of these hormones, and the regulation of blood pressure and temperature. ———— cytokines; fever; blood pressure; central nervous system; prostaglandins

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It is well known that cytokines in inflammatory states have multiple effects on the central nervous system (CNS) and cardiovascular function. They not only stimulate the release of hypophyseal hormones, including vasopressin (AVP), and thermogenesis, but also affect the peripheral circulation (Besedovsky et al. 1986; Bernton et al. 1987; Harris et al. 1987; Katsuura et al. 1988; Weinberg et al. 1988; Cannon et al. 1990; Naito et al. 1991).

It has been proposed that interleukins (ILs) and tumor necrotizing factor (TNF) play a pivotal role in bacterial septic shock via the formation of nitric oxide (NO), prostaglandins, platelet activating factor and bradykinin (Okusawa et al. 1988; Kilbourn et al. 1990; Billiau and Vandekerckhove 1991). Thus, it has been reported that NO produced by NO synthase in the vascular endothelium and smooth muscle in response to ILs may not only participate in the relaxation of vascular smooth muscle in vitro, but also in the circulatory collapse that occurs in vivo study (Kilbourn et al. 1992). Moreover, neurons in the CNS have been reported to contain NO synthase and L-arginine, a precursor of NO (Bredt et al. 1990; Garthwaite 1991), which suggests that NO may play an important role in the release of hypothalamo-hypophyseal hormones. Thus, it is possible that NO produced by ILs may take part in the release of AVP and atrial natriuretic hormone (ANH), affecting cardiovascular function and water and electrolytes metabolisms, and in the regulation of thermogenesis and blood pressure in septic shock.

In the present study, therefore, in order to examine possibility that NO formation in response to IL-1$\beta$ may affect the release of these hormonal release, thermogenesis and blood pressure, effects of a NO synthase inhibitor, $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME), on AVP and ANH release, blood pressure, heart rate and rectal temperature in response to IL-1$\beta$ were determined in conscious rats.

## Materials and Methods

Twenty-four male Sprague-Dawley rats weighing about 300 g were used in this study. All were carried out according to the guiding principles in the care and use of animals approved by the Council of Tohoku University School of Medicine. The rats were housed in cages in a room with a 12 hr-light and 12 hr-dark cycle, and food (rat chow) and tap water were available ad libitum.

Twenty four hours before experiments, the right jugular vein and left femoral artery and vein were cannulated with PE-50 tubing under ether anesthesia. The cannulae were filled with heparinized (100 U/ml) saline, sealed, exteriorized, and secured at the back of the neck. The rats were kept in an individual cage and given food and water ad libitum. Twenty ml of 5% dextrose were provided in a separate bottle after surgery to prevent dehydration due to reduced water intake.

On the day of the experiments, the rats were placed in a plastic cage ($18\times 18\times 15$ cm) where the rats could move freely. The arterial line was connected to

a blood pressure transducer (Statham P23ID; Oxnard, CA, USA) and recorder (Sanei-Sokki, Tokyo). Rectal temperature (RT) was measured with a thermometer (PB 101; Unique Medical, Tokyo).

*Experimental protocols*

The rats were divided into 4 groups ($n=6$ in each). In the IL-1B (IL) group, 0.9% NaCl was infused from $-30$ to 0 min at a rate of 100 $\mu$l/min into the jugular vein, 7.5 $\mu$g/kg • body weight of IL-1$\beta$ (human recombinant IL-1$\beta$; Otsuka Pharmaceutical Company Ltd., Osaka) dissolved in 200 $\mu$l of saline was injected as a bolus at 0 min, and 2 separate infusions of 0.9% NaCl alone and the maintenance dose of 750 ng/kg • body weight • min of IL-1$\beta$ dissolved in saline were carried out at the respective rates of 50 $\mu$l/min from 0 to 120 min into the jugular vein. In the L-NAME (NAME) group, 0.9% NaCl was infused from $-30$ to $-15$ min, L-NAME (Sigma Chemical, Co., St. Louis, MO, USA), 2 mg/kg • body weight dissolved into 0.9% NaCl was injected as a bolus at $-15$ min and 2 separate infusions of 0.9% NaCl alone and 50 $\mu$g/kg • BW • min of L-NAME dissolved in saline were undertaken from $-15$ to 120 min, each at a rate of 50 $\mu$l/min in a manner similar to the IL group. In the L-NAME plus IL-1$\beta$ (NAME+IL) group, the procedures and doses of administration of L-NAME and IL-1$\beta$ were the same as those for each drugs used in IL and NAME groups; both drugs were simultaneously administered into the jugular vein. In the control (CON) group, saline alone was administered from $-30$ to 120 min at a rate of 100 $\mu$l/min.

Mean arterial blood pressure (MABP), heart rate (HR) and rectal temperature (RT) were measured at $-15$, 0, 15, 30, 45 and 60 min, except that rectal temperature was not measured at 45 min. Blood samples were collected at 0 (3 ml), 15 (2.5 ml), 30 (2.5 ml), 60 (2.5 ml) and 120 min (3.0 ml) from the arterial line and replaced simultaneously via the femoral vein with an equal volume of heparinized donor blood. RT, MABP and HR, calculated from the simultaneously recorded arterial pulse pressure, were determined before each blood sampling.

*Measurement of AVP and ANH*

The RIAs for AVP and ANH were reported previously (Iitake et al. 1989). Briefly, AVP and ANH were extracted using octadecyl silane packed in a cartridge (Sep-Pak C18 cartridge; Waters Associates, Milford, MA, USA) and were assayed using specific antibodies (Mitsubishi Petrochemical, Tokyo) to AVP and ANH. The recovery rates of added AVP and ANH were $72.4\pm6.8$% and $68.1\pm9.7$% (mean$\pm$S.D.), respectively. Inter- and intra-assay coefficients of the variation in AVP and ANH were 9.4 and 3.7% and 14.2 and 9.5%, respectively.

*Other measurements*

Plasma osmolality (Posm) was measured with an Advanced Instruments Osmometer (3D2; Needham Heights, MA, USA). Plasma sodium (Na) and potas-

sium (K) concentrations were determined by flame photometry with a Hitachi flame photometer (205D; Hitachi Ltd., Tokyo). Hematocrit (Hct) was determined with a microcapillary method.

Statistical analyses of the data were performed by one- or two-way analyses of variance for repeated measurements. Statistically significant differences were isolated by Dunnett's test (within group) and by the unpaired $t$-test (between groups). The value of $p<0.05$ was considered statistically significant. The value at 0 min in each group was used as the baseline value.

## Results

In the NAME and NAME+IL groups, MABP at −15 min were significantly lower than the values at 0 min ($p<0.01$). In the former group, MABP increased approximately to a plateau at 15 min that was maintained until 60 min, followed by a decrease in MABP at 120 min ($p<0.05$ or 0.01). In the NAME+IL group, MABP tended to increase at 15 min, decreased significantly at 30 min and increased at 60 min ($p<0.05$). In the IL group, MABP tended to increase at 15 min and decreased at 30 to 45 min followed by a gradual recovery to the base line ($p<$ 0.05 or 0.01). MABP did not change throughout the study in the CON group. Significant differences were observed between the CON and NAME or NAME+IL at 0 to 120, between NAME+IL and IL at 0 to 120 and between NAME+IL and NAME groups at 15 to 45 min ($p<0.05$ or 0.01) (Fig. 1A).

HR at −15 min in the NAME and NAME+IL groups was significantly higher than the respective values at 0 min ($p<0.05$ or 0.01), but HR did not change significantly throughout the studies except for a significant increase at 120 min in both groups ($p<0.01$). In the IL group, HR gradually increased during the study and reached the significant higher levels at 30 and 120 min ($p<0.05$ or 0.01). There were no differences between the CON and NAME or IL as well as between NAME and NAME+IL groups, but there were differences between CON and NAME+IL as well as between IL and NAME+IL groups from 0 to 120 min ($p<0.01$) (Fig. 1B).

In the NAME and NAME+IL groups, RT decreased significantly at 60 min and thereafter ($p<0.01$). In the IL group, RT decreased significantly at 30 min and reached a nadir at 60 to 120 min ($p<0.05$ or 0.01). RT did not change throughout the study in the CON group. Significant differences were found between the CON and IL groups at 60 min ($p<0.05$), but there were no differences among the other groups throughout the studies (Fig. 2).

In the NAME group, plasma AVP increased significantly at 60 min and thereafter ($p<0.01$). In the IL and NAME+IL groups, plasma AVP increased significantly at 15 min and thereafter ($p<0.05$ or 0.01). In the CON group, plasma AVP did not change significantly during the study. There were significant differences between the CON and NAME+IL groups at 15 to 120 min as well as between the CON and the other groups at 30 to 120 min ($p<0.05$ or

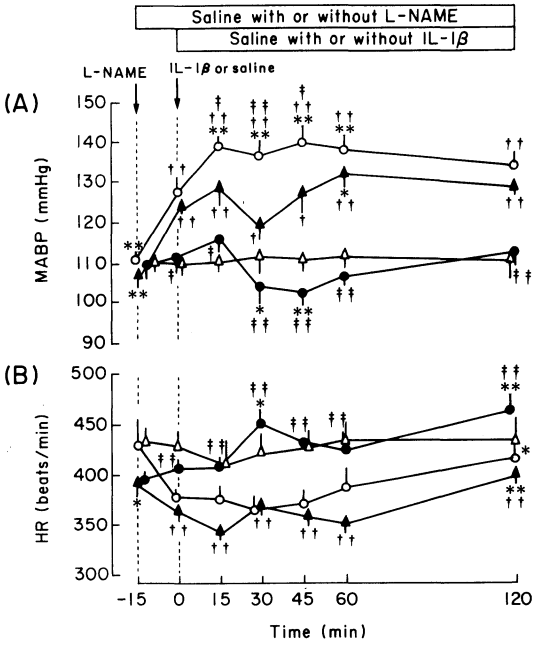


Fig. 1. Changes in mean arterial blood pressure (MABP, A) and heart rate (HR, B) in response to interleukin-1$\beta$ (IL-1$\beta$, closed circle) alone, $N^{\omega}$-nitro-L-arginine methyl ester (L-NAME, open circle) alone, L-NAME along with IL-1$\beta$ (closed diamond) and saline alone (CON, open triangle). All values were expressed by the differences from the value at 0 min. At $-15$ min, the priming dose of L-NAME (2 mg $\cdot$ kg$^{-1}$ $\cdot$ body weight, iv) was one bolusly administered and its maintenance dose (50 $\mu$g $\cdot$ kg$^{-1}$ $\cdot$ body weight $\cdot$ min$^{-1}$) was infused from $-15$ through 120 min in the groups except for the CON and IL groups. The priming dose of IL-1$\beta$ (7.5 $\mu$g $\cdot$ kg$^{-1}$ $\cdot$ body weight) was given iv at 0 min and its maintenance dose (750 ng $\cdot$ kg$^{-1}$ $\cdot$ body weight $\cdot$ min$^{-1}$) was infused from 0 through 120 min in the IL-1$\beta$ and L-NAME along. with IL-1$\beta$ groups, but saline alone was administered in the other groups. *($p<0.05$) and **($p<0.01$) show the significant differences from the value at 0 min, †($p<0.05$) and ††($p<0.01$) differences from the CON group, and ‡($p<0.05$) and ‡‡($p<0.01$) differences from the L-NAME along with IL-1$\beta$ group.

0.01). Significant differences were found between the NAME+IL and NAME groups at 30 to 120 min as well as between the NAME+IL and IL groups at 120 min ($p<0.05$ or 0.01) (Fig. 3A). In the IL and NAME+IL groups, Plasma ANH increased significantly at 60 min and thereafter ($p<0.05$ or 0.01). In the CON and NAME groups, plasma ANH did not change during the study. There were differences between the CON and NAME+IL groups at 30 to 120 min as well as between the CON and IL groups at 60 to 120 min ($p<0.05$ or 0.01).

As shown in Table 1, plasma Na concentrations and Hct did not change throughout the studies in any of the groups, and there were no significant differences among the groups. Plasma K concentrations significantly decreased at 15 min and thereafter in the IL group, at 30 min and thereafter in the NAME and

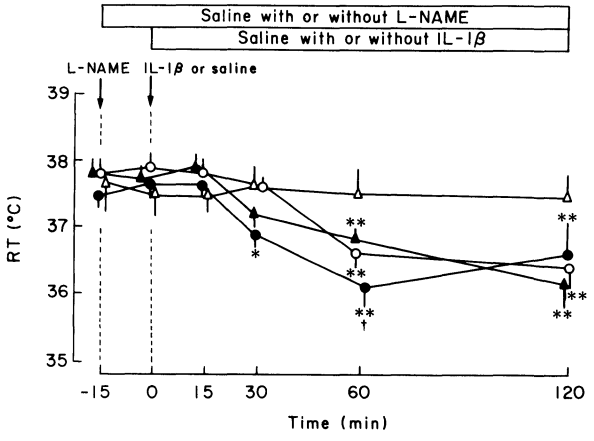


Fig. 2. Changes in rectal temperature in response to IL-1$\beta$ alone, L-NAME alone, L-NAME along with IL-1$\beta$ and saline alone. *($p<0.05$) and **($p<0.01$) show the significant differences from the value at 0 min and †($p<0.05$) the significant differences from the CON group. All abbreviations and explanations were the same as them in Fig. 1.

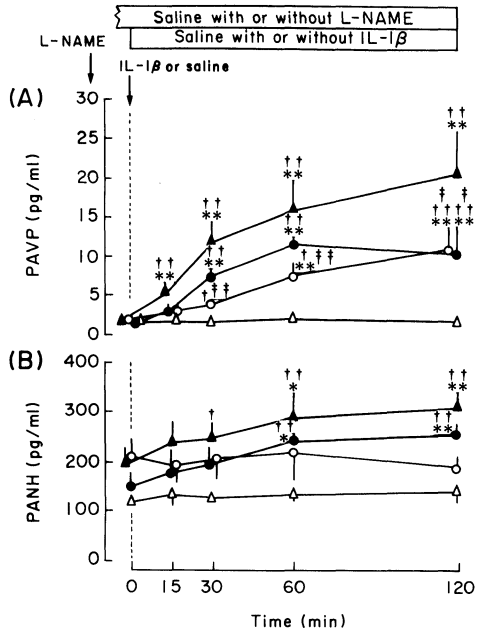


Fig. 3. Changes in plasma vasopressin (AVP) and atrial natriuretic hormone (ANH) in response to IL-1$\beta$ alone, L-NAME alone, L-NAME along with IL-1$\beta$ and saline alone.
*($p<0.05$) and **($p<0.01$) were the significant differences from the value at 0 min, †($p<0.05$) and ‡($p<0.01$) from the L-NAME along with IL-1$\beta$. All abbreviations and explanations were the same as them in Fig. 1.

TABLE 1. *Changes in plasma Na ($P_{Na}$), K ($P_K$), osmolality ($P_{osm}$) and hematocrit ($H_{ct}$) in $N^{\omega}$-nitro-L-arginine methyl ester (NAME), interleukin (IL), NAME + IL, and control groups (CON)*

| | Time (min) | | | | |
|---|---|---|---|---|---|
| | 0 | 15 | 30 | 60 | 120 |
| $P_{Na}$ (mEq/liter) | | | | | |
| NAME | 139.4±1.0 | 140.4±0.7 | 140.5±1.2 | 140.3±0.9 | 141.0±1.0 |
| IL | 138.9±0.9 | 139.5±1.3 | 139.6±0.7 | 140.2±0.7 | 140.2±1.1 |
| NAME+IL | 139.1±0.6 | 139.5±0.7 | 139.9±0.9 | 139.3±0.8 | 140.3±0.9 |
| CON | 141.7±1.2 | 141.7±0.7 | 141.6±1.1 | 141.8±0.7 | 142.6±1.0 |
| $P_K$ (mEq/liter) | | | | | |
| NAME | 5.2±0.2 | 4.9±0.2 | 4.8±0.2* | 4.5±0.3** | 4.2±0.3** |
| IL | 5.6±0.4 | 5.1±0.4* | 4.9±0.5** | 4.6±0.4** | 4.5±0.5** |
| NAME+IL | 5.6±0.3 | 5.3±0.2 | 5.0±0.3** | 4.6±0.3** | 4.4±0.3** |
| CON | 5.9±0.3 | 5.5±0.3 | 5.4±0.4 | 5.3±0.3* | 4.9±0.3** |
| Posm (mOsm/kg) | | | | | |
| NAME | 289±1 | | | | 288±1 |
| IL | 289±1 | | | | 286±1* |
| NAME+IL | 289±1 | | | | 290±1 |
| CON | 291±2 | | | | 289±1* |
| Hct (%) | | | | | |
| NAME | 45±1 | 44±0 | 44±1 | 45±1 | 44±1 |
| IL | 45±1 | 46±1 | 45±1 | 45±1 | 44±1 |
| NAME+IL | 44±1 | 44±1 | 44±1 | 43±1 | 43±2 |
| CON | 43±2 | 43±2 | 44±2 | 44±1 | 43±1 |

*$p<0.05$ and **$p<0.01$ indicate significant differences compared with the value at 0 min.

NAME+IL groups, and at 60 min and thereafter in the CON group. No significant differences were found among the groups. Posm decreased significantly at 120 min in the IL and CON groups ($p<0.05$), but never changed in the other groups. No significant differences were found among the groups. Hct did not change within and among the groups.

## Discussion

The present study clearly showed that IL-1$\beta$ given iv increased plasma AVP and ANH and decreased MABP and RT. L-NAME given iv increased plasma AVP and MABP with decreased RT, but did not affect plasma ANH. In the presence of L-NAME, IL-1$\beta$ increased plasma AVP and ANH but failed to decrease MABP. However, increases in MABP in response to L-NAME following IL-1$\beta$ were apparently smaller than increases in MABP produced by L-NAME alone. The extent of decreases in RT produced both by L-NAME alone and by IL-1$\beta$ with L-NAME was similar to that of its decreases in response to IL-1$\beta$ alone.

These results obviously showed that constitutive NO formation derived from the vascular endothelial cell, smooth muscle and neurons in the brain might take part in the regulation of AVP release, cardiovascular function and thermogenesis under physiological states, regardless of the presence or absence of IL-1$\beta$. On the other hand, IL-1$\beta$-induced NO might not play an essential role in the release of these hormones for the relatively short periods, but might partly participate in the regulation of blood pressure. Indeed, Corbett et al. (1993) recently showed that the impaired insulin response to glucose by IL-1$\beta$ becomes normal less than 1 hr following L-NAME administration. However, the possibility is not ruled out that the doses of IL-1$\beta$ used in the present experiments may not have produced a large enough amount of NO to affect the release of AVP and ANH and cardiovascular functions. This is not the case, because the dose of IL-1$\beta$ used in the present study was enough to produce decreases in blood pressure and temperature, which were mimic to the septic shock state. Moreover, we have already reported that the present dose of IL-1$\beta$ brought about plasma IL-1$\beta$ level similar to patients with septic shock (Yamamoto et al. 1994).

It is well known that NO formed by NO synthase from L-arginine enhances not only the generation of cGMP in vascular smooth muscle, inducing a vasorelaxation, but also its formation in neurons, astrocytes and cerebrovascular endothelium. NO in the CNS can lead to attenuation of sympathetic outflow (Ignarro 1989; Knowles et al. 1989; Southam and Garthwaite 1991; Sakuma et al. 1992; Togashi et al. 1992). Indeed, Elsner et al. (1992) reported that NO synthase inhibitors given iv elevated arterial blood pressure due to increases in total peripheral resistance, despite a decrease in cardiac output. On the other hand, Sakuma et al. (1992) showed that iv $N^G$-methyl-L-arginine (NMA) inhibited the formation of NO in the brain and increased sympathetic outflow, including renal

sympathetic nerve activity. In the present study, the peripheral effect of L-NAME might have played a more important role in increased blood pressure because HR decreased following L-NAME administration. However, the possibility is not ruled out that simultaneous increases in plasma AVP also may participate in the pressor response in the late phase (after 60 min).

In the present study, L-NAME increased plasma AVP despite a marked increase in blood pressure, which would otherwise tend to suppress the release of AVP via the baroreceptor reflex (Kimura et al. 1980). These results suggest that the impaired formation of NO may directly or indirectly stimulate AVP release. The exact mechanisms whereby L-NAME increases plasma AVP are uncertain, but the following may be potential explanations. First, it is possible that the impaired formation of NO in response to L-NAME interfered with the production of cGMP in the vasopressinergic neurons and/or in the posterior pituitary gland, which, in turn, resulted in a increase in cytosol $Ca^{++}$ concentration with a subsequent increase in AVP release. Indeed, Bredt et al. (1990) demonstrated immunohistologically that NO synthase was concentrated in the neural innervation of the posterior pituitary gland. Moreover, L-NAME and NMA given iv have been reported to cross the blood brain barrier, thereby resulting both in increased sympathetic outflow and in the impairment of memory process (Chapman et al. 1992; Togashi et al. 1992). However, Ohta et al. (1993) have recently found that L-arginine given intracerebroventricularly, a precursor of NO formation, increases blood pressure and AVP release. Second, it is also possible that L-NAME-induced cerebrovascular constriction might impair the circulation in the hypothalamohypophyseal system with a subsequent AVP release via the hypoxemia. However, Sakuma et al. (1992) found that NMA did not change cerebral blood flow and oxygenation. Third, renal and hepatic vasoconstriction due to L-NAME may have decreased the metabolic clearance rate (MCR) of plasma AVP, resulting in increased plasma AVP. Taken together, it remains to be clarified in the future whether NO formation in vasopressinergic neurons may act to regulate directly the release of AVP under physiological states.

Increases in both arterial blood pressure and plasma AVP have been reported to elevate ANH release (Manning et al. 1985; Inoue et al. 1988). In the present study, however, plasma ANH did not change despite increases in plasma AVP and blood pressure induced by L-NAME. This suggests that the formation of NO might play an improtant role in the release of ANH in response to increased blood pressure.

It is well known that cytokines and $PGE_2$ centrally stimulate the sympathetic nervous system, thereby resulting in hyperthermia due to the vasoconstiriction in the skin as well as to the activation of $\beta$-adrenoceptors in brown adipose tissue (Blaak et al. 1993; Nakamori et al. 1993). The exact mechanisms by which L-NAME and IL-1$\beta$ cause hypothermia are unclear from the present study. However, these results suggest the possibility that thermogenesis may not occur by

only vasoconstriction and that other factors such as adipose tissue metabolism and sympathetic outflow from the CNS may play an important role in it.

In conclusion, the results indicate that IL-1$\beta$ stimulates the release of AVP and ANH, and that L-NAME pretreatment per se increases AVP, but not ANH, thus potentiating the effects of IL-1 on AVP release.

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