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*ACCELERATED COMMUNICATION*

# Induction of L-Arginine Transport Is Inhibited by Atrial Natriuretic Peptide: A Peptide Hormone as a Novel Regulator of Inducible Nitric-Oxide Synthase Substrate Availability

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## ABSTRACT

**Background:** The inducible nitric-oxide synthase (iNOS) synthesizes NO from L-arginine. Availability of L-arginine is maintained by a lipopolysaccharide (LPS)-induced induction of the CAT-2B amino acids transporter. Recently, we could show that the cardiovascular hormone atrial natriuretic peptide (ANP) inhibits the induction of iNOS in LPS-stimulated macrophages via its guanylate cyclase-coupled A-receptor. **Purpose:** To investigate whether ANP exerts an effect on LPS-induced L-arginine uptake. **Methods:** Murine bone marrow derived macrophages were activated with LPS (1 $\mu$g/ml, 20 h) in the presence or absence of ANP or C-type natriuretic peptide (CNP). L-Arginine transport was determined by measuring the uptake of L-[$^3$H]arginine. L-[$^3$H]Arginine influx was also determined in LPS-activated cells in the presence of $N^G$-monomethyl-L-arginine (L-NMMA), competitor amino acids, or ANP. Nitrite accumulation was determined in supernatants of LPS-activated cells cultured in the presence or absence of L-ornithine. **Results:** ANP dose dependently ($10^{-8}$–$10^{-6}$M) inhibited LPS-induced L-[$^3$H]arginine uptake when added simultaneously with LPS, whereas it showed no effect when added simultaneously with L-[$^3$H]arginine. The effect was abrogated by the A-receptor antagonist HS-142–1 (10 $\mu$g/ml). CNP ($10^{-6}$ M) did not influence L-arginine transport. Competitor amino acids ($10^{-2}$ M) inhibited L-[$^3$H]arginine uptake. An excess of unlabeled L-arginine ($10^{-2}$ M) as well as its analog L-NMMA ($10^{-3}$ M) also reduced L-[$^3$H]arginine influx. L-Arginine uptake was critical for production of NO because L-ornithine significantly decreased LPS-induced nitrite accumulation. **Conclusion:** This work demonstrates that ANP inhibits LPS-induced L-arginine uptake via its guanylate cyclase-coupled A-receptor. Besides its influence on the induction of iNOS, this effect may represent an important and unique mechanism by which ANP regulates NO production in macrophages.

The inducible nitric-oxide synthase (iNOS) synthesizes an important mediator of host defense, nitric oxide (NO), from the amino acid L-arginine (Förstermann et al., 1995). Many cell types, especially macrophages, express iNOS upon stimulation, such as exposure to bacterial lipopolysaccharide, cytokines, or viruses (Förstermann et al., 1995). NO exerts its role in host defense because of its antibacterial and virustatic properties. However, if NO production gets out of control, damage of host cells occurs because of the cytotoxic potential of NO (Wong and Billiar, 1995). Therefore, NO is discussed as a key regulator of inflammatory processes (Maeda and Akaike, 1998) such as septic shock (Titheradge, 1999), chronic inflammatory bowel disease (Kubes and McCafferty, 2000), ischemia/reperfusion injury (del Zoppo et al., 2000), or arthritis (Miyasaka and Hirata, 1997). The mechanisms regulating the production of NO is therefore of highest interest.

iNOS, in contrast to the constitutive NO synthase isoforms endothelial NOS and neuronal NOS, is mainly regulated on the transcriptional level (Wong and Billiar, 1995). However, increasing evidence is provided that the availability of the substrate L-arginine is also a crucial step for NO production (Mori and Gotoh, 2000). L-Arginine is actively transported into cells via a specific transporter system for cationic amino acids, the $y^+$ system (Deves and Boyd, 1998). The genes

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**ABBREVIATIONS:** iNOS, inducible nitric-oxide synthase; NOS, nitric-oxide synthase; ANP, atrial natriuretic peptide; LPS, lipopolysaccharide; NF-$\kappa$B, nuclear factor-$\kappa$B; NP, natriuretic peptide; NPR, natriuretic peptide receptor; CNP, C-type natriuretic peptide; BMM, bone marrow macrophages; PBS, phosphate-buffered saline; NMMA, $N^G$-monomethyl-L-arginine.

coding for this $y^+$ system were identified as the four different isoforms CAT-1, CAT-2A, CAT-2B, and CAT-3 (Deves and Boyd, 1998). The CAT transmembrane protein isoforms differ in their tissue distribution and regulatory properties (Deves and Boyd, 1998). It is known that induction of iNOS often parallels an increased uptake of L-arginine in LPS-activated cells, as described for smooth muscle cells (Wileman et al., 1995) and macrophages (Baydoun and Mann, 1994). The CAT-2B gene has been reported to be the isoform up-regulated in LPS-activated macrophages (Caivano, 1998; Messeri et al., 2000).

We could demonstrate that a cardiovascular hormone, the atrial natriuretic peptide (ANP), inhibits NO production in lipopolysaccharide (LPS)-activated macrophages (Kiemer and Vollmar, 1997). A destabilization of iNOS mRNA, a reduced activation of NF-$\kappa$B (Kiemer and Vollmar, 1998), and the elevation of free intracellular calcium (Kiemer and Vollmar, 2001) contribute to this effect exhibited by ANP.

ANP, a member of the natriuretic peptide (NP) family, is a cyclic 28-amino-acid peptide secreted mainly by heart atria (Levin et al., 1998). Most investigations deal with the diuretic, natriuretic, and vasodilating action of ANP regarding the regulation of volume-pressure homeostasis (Levin et al., 1998). However, the functions of NP are not restricted to the regulation of volume homeostasis. NP and their receptors were demonstrated to be expressed in diverse tissues besides the cardiovascular system (Gutkowska and Nemer, 1989). Our previous work drew attention to a new aspect in the biological profile of ANP (i.e., its role in the immune system) (Vollmar, 1996). In this context, we could demonstrate that ANP and its receptors are expressed and differentially regulated in macrophages (Vollmar and Schulz, 1994), cells that play a pivotal role in inflammatory processes. These observations led us to investigate macrophages as target cells for ANP (Vollmar and Schulz, 1995; Kiemer and Vollmar, 1997). We demonstrated that ANP, besides its inhibitory action on iNOS, stimulates phagocytosis and respiratory burst in macrophages (Vollmar et al., 1997) and that it inhibits LPS-induced tumor necrosis factor-$\alpha$ secretion (Kiemer et al., 2000a).

ANP mediates most of its effects via its guanylate cyclase-coupled A-receptor NPR-A (Garbers, 1992). The C-type natriuretic peptide (CNP) is the specific ligand for the B-receptor (NPR-B) (Maack, 1996). CNP was originally isolated in the brain (Sudoh et al., 1990) but was meanwhile also found in peripheral cells, such as endothelial cells (Nazario et al., 1995) and macrophages (Vollmar and Schulz, 1994, 1995). The effects of CNP are mainly vasoactive and less natriuretic and diuretic compared with ANP (Sudoh et al., 1990). Therefore, CNP is suggested to play a quite different physiological role compared with ANP (Nazario et al., 1995). This view is also supported by our data obtained by studying a role for CNP in regulating macrophage activation. Despite the expression of the NPR-B in macrophages (Kiemer and Vollmar, 1997) CNP exerted none of the effects observed for ANP (Kiemer and Vollmar, 1997; Vollmar et al., 1997; Kiemer et al., 2000a).

Based on data for a role of ANP as a specific autocrine regulator of iNOS in macrophages (Kiemer and Vollmar, 1998), we were interested in whether this cardiovascular hormone influences L-Arginine transport in LPS-activated macrophages as one central step in macrophage NO production.

## Experimental Procedures

**Materials.** Rat ANP 99–126 (i.e., ANP) was purchased from Calbiochem/Novabiochem (Bad Soden, Germany) and CNP from Saxon Biochemicals (Hannover, Germany). HS-142–1 was a gift from Dr. Matsuda (Kyowa Hakko Kogyo, Mishima, Japan). Antiserum against the macrophage antigen F4/80 was from Serotec LTD (Wiesbaden, Germany); cell culture medium (RPMI 1640), fetal calf serum, and penicillin/streptomycin were from Life Technologies (Karlsrühe, Germany) and Biochrom (Berlin, Germany), and L-[2,3,4,5-$^3$H]-arginine (60 Ci/mmol) was from Amersham Pharmacia Biotech (Freiburg, Germany). Bradford protein assay was from Bio-Rad (Munich, Germany). Rotiszint was purchased from Roth (Karlsruhe, Germany). All other materials were purchased from either Sigma (Deisenhofen, Germany) or ICN Biomedicals (Eschwege, Germany).

**Cell Culture.** Mouse bone marrow macrophages (BMM) were prepared as described previously (Vollmar and Schulz, 1994), seeded at a density of $2 \times 10^5$ cells/ml in 24-well tissue plates and grown for 5 d (5% $CO_2$, 37°C) in RPMI 1640 medium supplemented with 20% L-929 cell-conditioned medium, 10% heat-inactivated fetal calf-serum, 100 U/ml penicillin, and 100 $\mu$g/ml streptomycin. L-929 cell-conditioned medium was removed at least 12 h before experiments. BMM were found >95% pure as judged by fluorescence-activated cell sorting analysis (FACScan; Becton Dickenson, Heidelberg, Germany), using an antiserum against the macrophage antigen F4/80 (Lee et al., 1985).

**Measurement of L-Arginine Transport.** Unidirectional transport of L-arginine was measured in BMM (24-well plates, 200 $\mu$l) untreated, treated with ANP ($10^{-6}$ M), or treated with lipopolysaccharide (LPS, *Escherichia coli*, serotype 055:B5; 0.1, 1, or 10 $\mu$g/ml) for 20 h. The effect of ANP ($10^{-9}$-$10^{-6}$ M), CNP ($10^{-6}$ M), HS-142–1 (10 $\mu$g/ml) (Morishita et al., 1992), L-ornithine ($10^{-2}$ M), and L-NMMA ($10^{-3}$ M) on L-arginine uptake was determined. As shown previously, none of the substances in the used concentrations possesses cytotoxic activity on BMM (Kiemer and Vollmar, 1997). Unless otherwise stated, substances were added to the cells simultaneously with LPS. After 20 h, cells were rinsed twice with phosphate-buffered saline (PBS) (with 0.01% $CaCl_2$, 0.1% D-glucose, and 0.01% $MgCl_2$) kept at 37°C. Uptake was measured by adding 200 $\mu$l of PBS (with 0.01% $CaCl_2$, 0.1% D-glucose, and 0.01% $MgCl_2$ at 37°C) containing L-[$^3$H]arginine (300,000 cpm) and 40 $\mu$M unlabeled L-arginine to each well. Transport was linear for up to 3 min (data not shown) and influx was measured over 30, 60, or 120 s. Plates were then placed on ice and cells rinsed three times with 200 $\mu$l of ice-cold PBS (with 0.01% $CaCl_2$, 0.1% D-glucose, and 0.01% $MgCl_2$) containing 10 mM unlabeled L-arginine. Cell protein was determined with Bio-Rad, and radioactivity (cpm) in cells lysed with 500 $\mu$l of 0.5 N NaOH (80°C) was measured by liquid scintillation counting.

**Selectivity of L-Arginine Transport.** Inhibition of L-arginine transport by competitor amino acids (L-ornithine, L-leucine, L-glycine, L-arginine), an excess of unlabeled L-arginine, and the L-arginine analog $N^G$-monomethyl-L-arginine (L-NMMA) was examined by incubating cells with PBS (with 0.01 M $CaCl_2$, 0.1% D-glucose, and 0.01 M $MgCl_2$) containing L-[$^3$H]arginine (300,000 cpm) in the absence or presence of L-ornithine, L-leucine, L-glycine, L-arginine ($10^{-2}$ M), or L-NMMA ($10^{-3}$ M). This method does not allow a distinction between the $y^+$ and $y^+$L transport system.

**Nitrite Accumulation.** BMM (24-well plates, 200 $\mu$l) were either untreated or treated with LPS in the presence or absence of $N^G$-monomethyl-L-arginine (L-NMMA) ($10^{-3}$ M) or L-ornithine ($10^{-2}$ M). After 20 h, the stable metabolite of NO, nitrite, was measured in the medium by the Griess reaction (Green et al., 1982). Cell culture supernatant (100 $\mu$l) was removed, and 90 $\mu$l of 1% sulfanilamide in 5% $H_3PO_4$ and 90 $\mu$l of 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride in $H_2O$ was added, followed by spectrophotometric mea-

surement at 550 nm (reference wavelength, 620 nm). A standard curve was prepared using sodium nitrite dissolved in medium.

**Statistical Analysis.** All experiments were at least performed three times in independent experiments. Within each independent experiment, experiments were performed at least three times. Data are expressed ± S.E.M. Values with $p < 0.01$ were considered significantly different compared with 100% (LPS-treated cells only) by one sample *t* test.

## Results

**ANP Reduced LPS-Induced L-[$^3$H]Arginine Uptake.** Murine bone marrow-derived macrophages were stimulated with LPS in different concentrations (0.1, 1, or 10 μg/ml) for 20 h to evoke an elevation of L-arginine uptake. Treatment with LPS lead to a dose-dependent increase in L-arginine uptake (Fig. 1A). Because previous investigations of the regulation of ANP on iNOS were performed in macrophages treated with 1 μg/ml LPS, this concentration was used for all further studies. Coincubation of BMM with ANP ($10^{-8}$-$10^{-6}$ M) and LPS (1 μg/ml) resulted in a dose-dependent, significant reduction of L-arginine uptake compared with LPS-treatment only (Fig. 1B). When ANP ($10^{-6}$ M) was added simultaneously with L-[$^3$H]arginine to untreated or LPS-activated cells (20 h, 1 μg/ml) it did not influence L-[$^3$H]arginine influx (data not shown). When cells were treated with ANP ($10^{-6}$ M) alone for 20 h followed by measurement of L-arginine uptake, no significant changes were observed compared with untreated cells (Fig. 1C).

To evaluate a time-dependence of the observed effect of ANP on L-arginine uptake, the measurements were done over a time frame of 120 s. At each time point, ANP elicited a significant decrease in L-arginine uptake (Fig. 1D).

BMM were previously shown to express all types of natriuretic peptide receptors (Kiemer and Vollmar, 1997) and therefore may represent target cells for all types of natriuretic peptides. To elucidate whether the inhibitory effect of L-arginine uptake is specific for ANP, we examined the ligand for the B receptor (NPR-B), CNP. As shown in Fig. 1, CNP even at a concentration of $10^{-6}$ M did not affect LPS-induced uptake of L-arginine.

To make sure that differences in L-arginine uptake were not due to differences in cell/protein content, protein measurement was performed. The differences in protein content between different wells in one experiment was always less than 5% (data not shown).

**Competition Studies.** Because the induction of nitric oxide production in macrophages is known to depend critically on CAT2 (Bogle et al., 1996; Nicholson et al., 2001), competition studies were performed without distinguishing between the $y^+$ and the $y^+L$ transport system. The latter represents a broad scope transport system in erythrocytes, thrombocytes, fibroblasts, or placenta. The $y^+L$ system, however, has not been described in macrophages (Deves et al., 1998). The specificity of the L-arginine uptake in our assay system was determined by competitor studies performed in the presence of unlabeled L-cysteine, L-ornithine, L-lysine, and L-NMMA, which are known to reduce L-arginine uptake (Bogle et al., 1996; Deves and Boyd, 1998) (Fig. 2A). An excess of unlabeled L-arginine also significantly reduced L-[$^3$H]arginine influx (Fig. 2A).

The crucial role for L-arginine uptake for NO production was demonstrated by measuring NO production of LPS-activated BMM over 20 h in the presence of the iNOS inhibitor L-NMMA ($10^{-3}$ M) or L-ornithine ($10^{-2}$ M), which had been shown to competitively inhibit L-arginine uptake (Fig. 2A). Both L-NMMA and L-ornithine significantly reduced LPS-induced NO production (Fig. 2B).

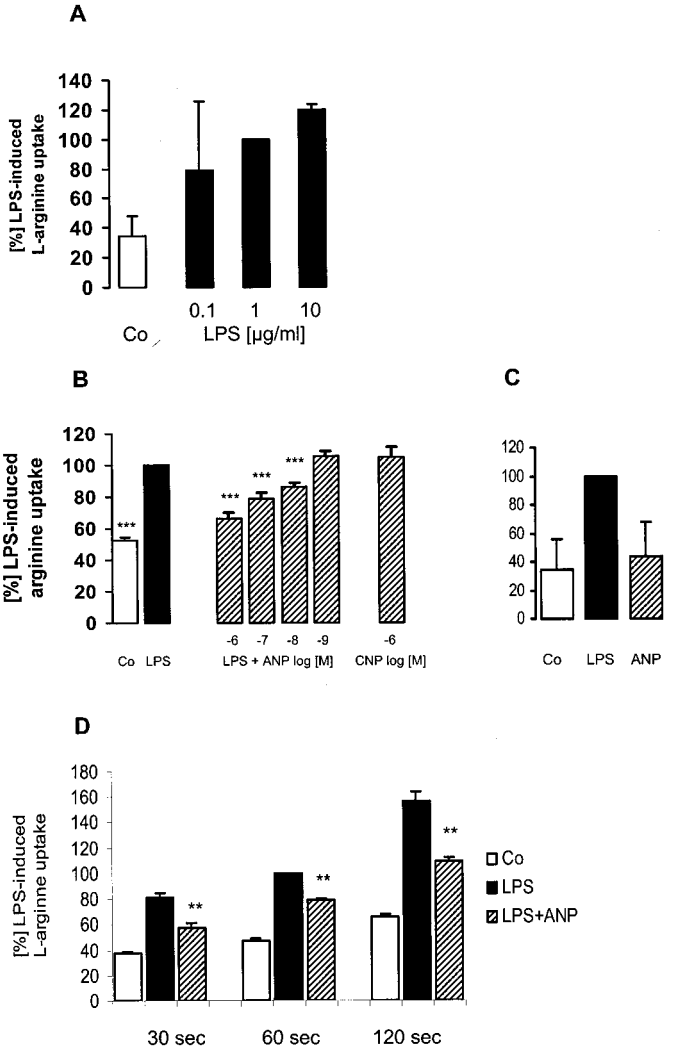


**Fig. 1.** ANP dose-dependently inhibits LPS-induced L-arginine uptake in BMM. A, cells were cultured for 20 h in either medium alone (Co) or in medium containing LPS (0.1, 1, or 10 μg/ml). L-arginine transport was determined as described under *Experimental Procedures* over 60 s. Data are expressed as percentage of L-arginine uptake in macrophages activated with 1 μg/ml LPS (100%). B, L-arginine transport of cells treated for 20 h with LPS (1 μg/ml) or a combination of LPS (1 μg/ml) and various concentrations of ANP ($10^{-9}$-$10^{-6}$ M) or CNP ($10^{-6}$ M) was measured over 60 s as described under *Experimental Procedures*. Data are expressed as percentage of L-arginine uptake in LPS-activated (1 μg/ml) macrophages (100%) and represent the mean ± S.E.M. of 4 to 10 independent experiments performed in triplicate. ***$p < 0.0001$ represent significant differences compared with the values seen in LPS activated cells (one sample *t* test). C, cells were left untreated or treated with LPS (1 μg/ml) or ANP ($10^{-6}$ M) for 20 h. L-arginine transport was determined as described under *Experimental Procedures* over 60 s. Data are expressed as percentage of L-arginine uptake in LPS-activated macrophages (100%). D, L-arginine transport of cells treated for 20 h with LPS (1 μg/ml) or a combination of LPS (1 μg/ml) and ANP ($10^{-6}$ M) was measured over 30, 60, and 120 s as described under *Experimental Procedures*. Data are expressed as percentage of L-arginine uptake in LPS-activated (1 μg/ml) macrophages (100%) measured over 60 s. Data represent means ± S.E.M. **$p < 0.01$ represent significant differences compared with the values seen in LPS activated cells of the respective time point (one sample *t* test).

The LPS-induced induction of L-arginine transport was not dependent on intracellular consumption of L-arginine. When cells were activated with LPS (1 $\mu$/ml) for 20 h and when iNOS activity was inhibited by the addition of L-ornithine or L-NMMA (see above), the following L-arginine uptake was not significantly altered (Fig. 2C) compared with L-arginine-consuming cells (only LPS-activated).

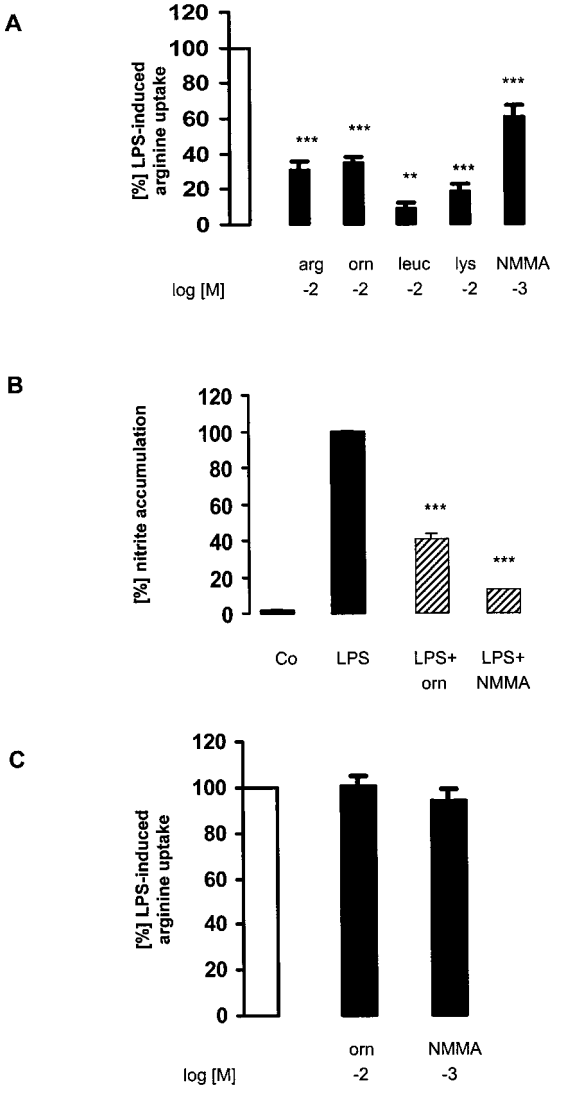


**Fig. 2.** A, competition of L-arginine uptake. Transport of 40 $\mu$M L-arginine was measured over 60 s in the absence or presence of a given inhibitor (arg, L-arginine; orn, L-ornithine; leuc, L-leucine; lys, L-lysine; NMMA, L-NMMA) in cells activated with LPS (1 $\mu$g/ml) for 20 h. Data are expressed as percentage of respective control influx in LPS-activated cells. Values are the means ± S.E.M. of two (L-leucine) to eight (L-ornithine) independent experiments performed in triplicate. $^{***}p < 0.0001$ and $^{**}p < 0.01$ represent significant differences compared with the values seen in LPS activated cells (one sample $t$ test). B, L-arginine uptake is critical for NO production. NO production was induced by treatment of the cells with LPS (1 $\mu$g/ml) in the presence or absence of L-NMMA ($10^{-3}$ M) or L-ornithine ($10^{-2}$ M). Nitrite accumulation was measuread by the Griess assay as described under *Experimental Procedures*. Data are expressed as percentage of nitrite accumulation in LPS-activated macrophages (100%) and represent the mean ± S.E.M. of three independent experiments performed in triplicate. $^{***}p < 0.0001$ represent significant differences compared with the values seen in LPS-activated cells (one sample $t$ test). C, L-arginine uptake is independent of intracellular consumption. Cells were treated with LPS (1 $\mu$g/ml) in the presence or absence of L-NMMA ($10^{-3}$ M) or L-ornithine ($10^{-2}$ M) for 20 h. Uptake of L-arginine was then measured as described under *Experimental Procedures* over 60 s. Data are expressed as percentage of the respective control influx in LPS-activated cells. Values are the means ± S.E.M. of four independent experiments performed in triplicates.

**Inhibition of L-Arginine Uptake Is Mediated via the NPR-A.** To determine whether the NPR-A mediates the inhibitory effect of ANP on L-arginine uptake, an antagonist of the guanylate cyclase-coupled NPR-A, HS-142–1 (Morishita et al., 1992) (10 $\mu$g/ml), was employed. As shown in Fig. 3, HS-142–1 abrogated the uptake-reducing effect of ANP ($10^{-6}$ M) in LPS-stimulated BMM.

## Discussion

The induction of iNOS in macrophages represents an important pathomechanism in diverse inflammatory processes (Evans, 1995; Wong and Billiar, 1995; Maeda and Akaike, 1998; Kubes and McCafferty, 2000). Therefore, special interest focuses on the regulatory mechanism of NO production and on tools for potential pharmacological intervention (Southan and Szabo, 1996). Most work on research for inhibitors of NO production focuses on the characterization of L-arginine analogs acting as competitive inhibitors for L-arginine uptake and iNOS activity, respectively (Southan and Szabo, 1996). By characterization of a novel endogenous substance (i.e., ANP) regulating L-arginine transport, the study presented here might therefore be of special interest.

The increased L-arginine transport activity in LPS-activated macrophages was originally thought to be initiated by elevated L-arginine consumption caused by nitric oxide production (Sato et al., 1992). The differential induction of iNOS and L-arginine transport in smooth muscle cells (Durante et al., 1996), however, showed that the two mechanisms represent two processes that are initiated independently. In addition, our data confirm that L-arginine uptake is not influenced by iNOS inhibitors and therefore by altered L-arginine consumption. In LPS-activated macrophages, it is established that the induction of the CAT-2B transporter is one crucial step for efficient NO production (Bogle et al., 1996; Chou et al., 1998; Deves and Boyd, 1998; Kakuda et al., 1998; Messeri et al., 2000; Nicholson et al., 2001). In contrast to the constitutively expressed CAT-1 gene, CAT-2B is specifically up-regulated in LPS-treated macrophages (Caivano, 1998; Deves and Boyd, 1998; Closs et al., 2000; Hammermann et

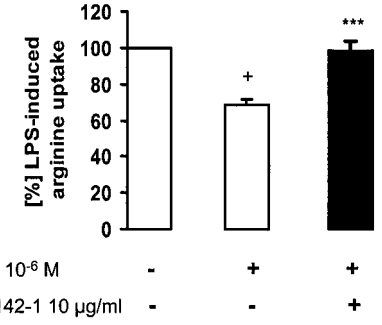


**Fig. 3.** NPR-A mediated inhibitory action of ANP on L-arginine influx. Cells were treated with LPS (1 $\mu$g/ml, 204 h), L-arginine uptake was measured as described under *Experimental Procedures* over 60 s and referred to as 100%. ANP ($10^{-6}$ M) alone or in addition with the NPR-A antagonist HS-142–1 (10 $\mu$g/ml) were added simultaneously with LPS. Means ± S.E.M of five independent experiments performed in triplicates. $^{+}p < 0.0001$ represents significant difference compared with the values seen in LPS-activated cells (one sample $t$ test). $^{***}p < 0.0001$ represents significant difference compared with LPS + ANP treatment (unpaired $t$ test).

al., 2000; Messeri et al., 2000). Usually, the transporters for cationic amino acids are highly specific for the cationic amino acids L-arginine, L-lysine, and L-ornithine (Wileman et al., 1995; Deves and Boyd, 1998). Transport of L-arginine via CAT-2B, however, was reported to also be inhibited to a certain extent by the neutral amino acids homoserine, cysteine, and leucine (Deves and Boyd, 1998). Our data show that not only did an excess of unlabeled L-arginine and the basic amino acids L-lysine and L-ornithine significantly reduce L-[$^3$H]arginine uptake, but also L-leucine strongly attenuated L-arginine transport. This property is usually more characteristic for the broad scope $y^+$L system described, for example, in erythrocytes, thrombocytes, fibroblasts, and placenta (Deves et al., 1998). To our knowledge, however, the presence of the $y^+$L system in macrophages has not yet been described.

When LPS-activated BMM were cultured in the presence of ANP, the LPS-induced increase in L-arginine uptake was significantly and dose dependently reduced. The inhibitory effect of ANP on L-arginine uptake, however, was not caused by a direct competitive action. This was shown by the fact that when transport studies were performed in cells not pretreated with ANP, but in the presence of ANP, ANP exerted no effect on L-arginine uptake. This observation could almost be called unique, because data demonstrating a specific down-regulation of LPS-induced L-arginine influx that is not dependent on competitive action of the inhibitor are very rare. To our knowledge, only the work by Chou et al. (1998) shows action of dantrolene on L-arginine transport, which is dependent on a reduced velocity of L-arginine transport after dantrolene treatment.

The fact that inhibition of L-arginine influx by ANP is mediated via the NPR-A was demonstrated, because the microbial polysaccharide HS-142–1, which selectively blocks the guanylate cyclase-linked NP receptor and cGMP production (Morishita et al., 1992), reversed the ANP effect. This result is in concordance with our previous data demonstrating that the iNOS-inhibitory action of ANP is mediated via the guanylate cyclase-coupled NPR-A and could be completely abrogated by the NPR-A antagonist HS-142–1 (Kiemer and Vollmar, 1997).

Previous data demonstrated that macrophages express mRNA coding for all three NP receptor subtypes (i.e., NPR-A, NPR-B and NPR-C; Kiemer and Vollmar, 1997). The fact that macrophages express the NPR-B led us to hypothesize that they should be target cells for CNP action. The property to inhibit L-arginine uptake, however, seems to be specific for ANP, because CNP displayed no activity. This observation is in concordance with previous observations in macrophages showing a lack of effect of CNP on NO production (Kiemer and Vollmar, 1997), whereas ANP represents a potent inhibitor of this pro-inflammatory enzyme (Kiemer and Vollmar, 1998).

The induction of the cationic amino acid transporter is on the transcriptional level and involves elevated transcription of mCAT genes (MacLeod et al., 1994). This effect was reported to be mediated via the activation of mitogen-activated protein kinases (Caivano, 1998) and the activation of the transcription factor NF-$\kappa$B (Hammermann et al., 2000). Because our previously published work shows that ANP inhibits the activation of NF-$\kappa$B (Kiemer and Vollmar, 1998; Kiemer et al., 2000b), this regulatory action on transcriptional processes of ANP might be responsible for our observation of a reduced L-arginine transport in ANP-treated cells.

In summary, we could demonstrate a novel mechanism of action for ANP. The cardiovascular hormone interacts specifically with NO production of LPS-activated macrophages via an inhibition of L-arginine uptake. The limited capacity of LPS to stimulate L-arginine transport in ANP-treated cells may represent an important regulatory mechanism in controlling the release of NO at sites of inflammation. Modulation of NO production by ANP may have broad implications in inflammatory situations, such as endotoxic shock (Titheradge, 1999). The observation that ANP mediates macrophage activation is particularly interesting because ANP concentrations are highly elevated in septic shock (Aiura et al., 1995).

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