ELSEVIER

European Journal of Pharmacology 319 (1997) 279–285

ejp

Effects of atrial natriuretic peptide on phagocytosis and respiratory burst in murine macrophages

Angelika M. Vollmar [a,*], Reinhold Förster [b,1], Rüdiger Schulz [a]

[a] *Institute of Pharmacology, Toxicology and Pharmacy, University of Munich, Königinstrasse 16, D-80539 Munich, Germany*
[b] *Institute of Medical Microbiology, University of Munich, Veterinärstrasse 13, D-80539 Munich, Germany*

Received 25 July 1996; revised 18 October 1996; accepted 25 October 1996

Abstract

Atrial natriuretic peptide (ANP) is known to affect cardiovascular physiology displaying both hormone- and neurotransmitter characteristics. However, there is increasing evidence that ANP possesses additional biological activities refering to the immune system. To further strengthen this hypothesis the effect of ANP on two major functions of macrophages, i.e., phagocytosis and respiratory burst was tested. Both parameters were analyzed by flow cytometry employing bone marrow derived macrophages and the murine macrophage cell line J774. In both cell types preincubation with ANP dose dependently (10^{-10}–10^{-7} M) increases ingestion of opsonized fluorescent latex particles. The respiratory burst activity was monitored by oxidation of dihydrorhodamine-123 in cells stimulated either with phorbol-myristate (PMA, 10 ng/ml) or formyl-Met-Leu-Phe (fMLP, 1 μM). In both cases preexposure of cells to ANP (10^{-8}–10^{-6} M) for 2 h enhances reactive oxygen production. The data demonstrate an influence of ANP on important defense mechanisms of macrophages and thus extend the knowledge regarding the pharmacological profile of this natriuretic peptide.

Keywords: Oxidative burst; ANP (atrial natriuretic peptide); Fluorescent latex bead; Dihydrorhodamine-123; Immune function; Flow cytometry

1. Introduction

Atrial natriuretic peptide (ANP) is primarily synthesized by the heart atria and elicits profound changes in renal and cardiovascular functions such as diuresis, natriuresis, and hypotension (De Bold et al., 1981; Brenner et al., 1990; Rosenzweig and Seidman, 1991). ANP acts as circulating hormone as well as neuropeptide (Samson, 1985).

Besides these well-studied effects ANP appears to influence immune functions. The peptide stimulates migration of human neutrophils (Elferink and De Koster, 1995), enhances natural cytoxicity (Moss and Golightly, 1991), primes polymorphonuclear neutrophils (Wiedermann et al., 1992) and interferes with nitric oxide (NO) synthesis of macrophages (Vollmar and Schulz, 1995b). Furthermore, ANP and ANP receptors are expressed by immune-related tissues and cells (Agui et al., 1995; Houdijk et al., 1990; Throsby et al., 1993; Vollmar and Schulz, 1990, 1994; Vollmar et al., 1995, 1996).

We have recently shown that mouse macrophages synthesize and secrete ANP. Production of the peptide is differentially controlled by various immunomodulatory substances including lipopolysaccharides and zymosan (Vollmar and Schulz, 1994, 1995a). Aim of the present study was to investigate whether ANP is not only synthesized by macrophages, but also affects functions of these cells. Phagocytosis as well as production of reactive oxygen radicals reflect the most representative functions of these cells (Adams and Hamilton, 1984). Phagocytotic activity is the primary mechanism through which the immune system eliminates most extracellular pathogenic microorganisms. The production of reactive oxygen intermediates referred as 'oxidative burst' or 'respiratory burst' is a critical step in the destruction of invading pathogenic bacteria (Adams and Hamilton, 1984). In the present study both parameters were assessed using flow cytometry. Opsonized coumarin-labeled microspheres were used for examining phagocytosis (Ichinose et al., 1994) and the respiratory burst was monitored by loading cells with dihydrorhodamine-123 to measure the fluorescence of oxidized dihydrorhodamine-123 (Emmendörffer et al., 1990). We show that ANP dose dependently increases both phago-

* Corresponding author. Tel.: (49-89) 2180-2663; Fax: (49-89) 342-316.
[1] Present address: Max-Delbrück-Centrum für Molekulare Medizin, Robert-Rössle-Strasse 10, D-13122 Berlin-Buch, Germany.

cytic capacity and production of reactive oxygen intermediates in murine macrophages.

2. Materials and methods

2.1. Preparation and cultivation of cells

Bone marrow cells were flushed with RPMI 1640 medium from the femurs of BALB/c mice (female, 20–30 g). Cells were seeded at a density of 2×10^5 cells/ml (175 cm^2 flasks, Greiner, Solingen, Germany) and cultured (37°C, 5% CO_2) in RPMI 1640 medium (Gibco BRL, Peasley, UK) containing 20% (v/v) L-929 cell-conditioned medium as source of the macrophage growth factor (Stanley et al., 1978), 10% foetal calf serum, and penicillin (100 U/ml)/streptomycin (100 μg/ml) (both purchased from Gibco BRL). To eliminate contaminating fibroblasts, nonadherent bone marrow cells were transferred after 24 h to new flasks and grown in the above mentioned medium for 5–6 days. L-929 cell-conditioned medium was removed at least 12 h prior to the experiment. Preparations of bone marrow-derived macrophages were found > 95% pure as judged by flow cytometry using a fluorescent isothiocyanate (FITC)-labeled antiserum against the macrophage antigen F40/80 (Serotec Canon, Wiesbaden, Germany). In addition to bone marrow-derived macrophages, the murine macrophage cell line J774 was used. These cells were grown in Dulbecco's modified essential medium (DMEM, Gibco BRL) supplemented with antibiotics (pen/strep) and 10% foetal calf serum.

2.2. Phagocytosis assay

Coumarin-labeled latex particles (No. 098847, carboxylated, 2 μm diameter, Polysciences, Warrington, PA, USA) were opsonized by incubating beads (4×10^9 beads/ml) with 10% mouse serum (37°C, 30 min). Bone marrow-derived macrophages and J774 cells, respectively, were seeded in 12 well plates at a density of 5×10^5 cells/ml and incubated in the presence or absence of rat ANP 99–126 (10^{-6}–10^{-12} M, Calbiochem, Heidelberg, Germany) for 2 or 18 h. Latex beads were sonicated (5 s) before addition to the cells at a final ratio of 100 beads per cell. Incubation was conducted for various times (5–180 min; 37°C, 5% CO_2). Thereafter the monolayers were washed 4–5 times with phosphate-buffered saline (PBS) and the absence of considerable amounts of undigested latex particles was controlled microscopically. Cells were either harvested with a rubberpoliceman (J774) or by incubation with 0.25% trypsin (10 min, bone marrow-derived macrophages). Cell suspensions were transferred to plastic tubes (Falcon 2008, Becton-Dickenson, Heidelberg, Germany) and centrifuged at $250 \times g$ for 20 min. The cell pellet was thoroughly, but carefully resuspended in buffer (PBS without Ca^{2+} and Mg^{2+} containing 1 mM EDTA) and subjected to flow cytometric analysis. Cell viability was assessed by staining cells with propidium iodine and analyzing by flow cytometry. Only cell preparations with a viability > 80% were employed for experiments. Flow cytometry using a FACScan (Becton-Dickinson) was employed to measure the number of particles ingested per cell. Analysis was usually performed on 10^4 cells. The green fluorescence (FL1) from coumarin labelled latex beads was collected through a 530 nm bandpass filter. The data acquired were processed using Lysis II software. The fluorescence distribution was displayed in single histograms. The phagocytotic activity was assessed by monitoring the mean fluorescence of phagocytozing cells as well as by calculating the phagocytic index. The phagocytoc index was defined as the average number of particles ingested per macrophage and was calculated as described in detail (Ichinose et al., 1994). Briefly, the total number of ingested beads was divided by the number of total macrophages. Experiments were performed at least in triplicates and each experiment repeated 3–4 times.

2.3. Measurement of respiratory burst

Dihydrorhodamine-123 (Molecular Probes, Eugene, OR, USA) was dissolved in *N,N*-dimethylformamide (DMF, Sigma Deisenhofen, Germany) at a concentration of 43.3 mM. Phorbol-myristate (PMA, Sigma) was diluted in DMF at a stock concentration of 2 mg/ml. Stock solution of formylated Met-Leu-Phe (fMLP, 5 mM, Sigma) was prepared in water. Cells were incubated with ANP (10^{-12} M–10^{-6} M) for 2 and 18 h, respectively. Cells were collected in 5 mM Hepes buffered saline (0.15 M NaCl, pH 7.4) and adjusted to a concentration of 2×10^6/ml. For the induction of the respiratory burst 2×10^5 cells (100 μl) were placed in polypropylene tubes (Becton-Dickinson) and loaded with dihydrorhodamine-123 (final concentration 0.4 mM) for 15 min following stimulation of reactive oxygen intermediates production by either PMA (10 ng/ml) or fMLP (1 μM) for 15 min. For estimation of reactive oxygen production fluorescence intensity (FL1, 530 nm) of 10 000 cells was recorded. Cells incubated with dihydrorhodamine only served as negative control. In order to analyze cells treated with PMA or fMLP size (forward scatter (FSC)) and granularity (side scatter (SSC)) properties of each cell sample were recorded. Fluorescence intensity was obtained as histogram statistics. Experiments were run in triplicates and at least three independent experiments were performed.

3. Results

3.1. Flow cytometric measurement of phagocytosis

Experiments assessing optimal conditions for analysis of phagocytosis are shown in Fig. 1. Histograms demon-

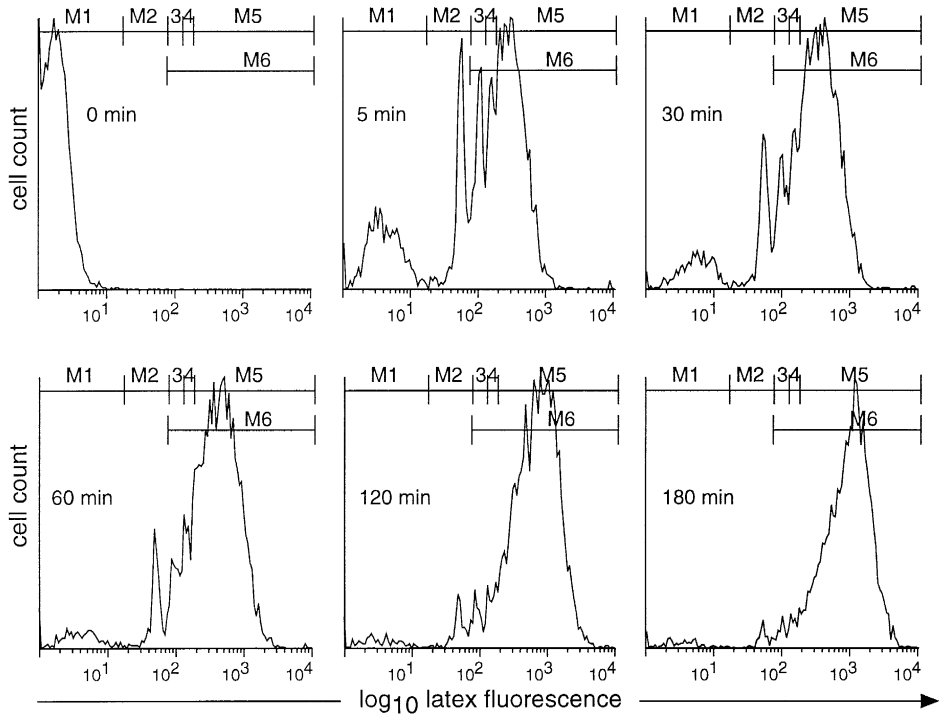

Fig. 1. Time course of phagocytic activity in J774 cells. Cells were incubated with opsonized coumarin-labeled latex particles (100 beads per cell). Fluorescence intensity (FL 1) after various periods of time was recorded and illustrated by histograms. The non-phagocytosing cell population and cells having ingested one, two, three, and more than three beads were evaluated by setting the markers M 1, M 2, M 3, M 4 and M 5, respectively. Mean fluorescence of total ingested particles was determined by recording FL 1 within the region M 6.

strate the ability of cells (J774) to ingest latex beads in a time-dependent manner. At time 0 the non-ingesting cell population (M 1) elicits low fluorescence. After 5 min a considerable amount of cells has been engulfed one (M 2), two (M 3), three (M 4) or more particles (M 5) as seen by increased fluorescence intensity. With increasing time, i.e., 30 min the number of noningesting cells is decreasing while the number of ingesting cells is increasing. After 120 min incubation time the number of beads ingested by the cells increased to high levels which prevent proper calculation of the phagocytic index. Therefore, an incubation time of 60 min was chosen for the forthcoming experiments. Furthermore, as $>95\%$ of all macrophages had ingested at least one bead, the mean fluoresence intensity of those cells (Fig. 1, M 6) was calculated as well and used as a parameter for phagocytosis. In addition, experiments were performed to find the optimum number of beads added per cell. A cells/beads ratio of 1:100 was found most suitable (data not shown). Furthermore it was demonstrated that exposure of cells to ANP at the concentration range used (10^{-6}–10^{-11} M) did not alter the cellular parameter for size (FSC) and for the intracellular structure (SSC) (data not shown). Since it is known that bacterial lipopolysaccharides prime macrophages to enhance phagocytic activity (Cooper et al., 1984), cells were incubated with lipopolysaccharides (0.5–20 μg/ml, 18 h) for positive control. Fig. 2 demonstrates a shift of fluorescence intensity upon exposure of cells to lipopolysaccharides (0.5 μg/ml) indicating an augmented phagocytosis of latex beads.

3.2. Effect of ANP on phagocytosis

Preincubation of J774 as well as bone marrow-derived macrophages with ANP for 18 h increases their phagocytic activity as seen in the fluorescence histograms of Fig. 3A,B. A significant effect of ANP on phagocytosis was observed at a concentration of 10^{-10} M. The effect was maximal at 10^{-9} M expressed both as phagocytic index or

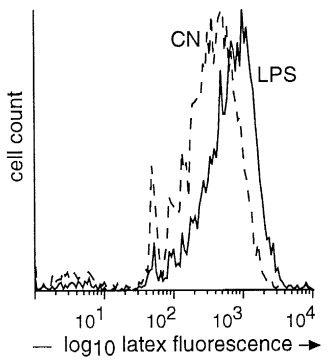

Fig. 2. The histogram (FL 1 versus number of cells) shows the stimulatory effect of lipopolysaccharides (0.5 μg/ml, 18 h) on uptake of fluorescent latex particles by J774 cells. Fluorescence intensity is shifted to the right in lipopolysaccharide-exposed cells (solid line). CN: control cells (dashed line).

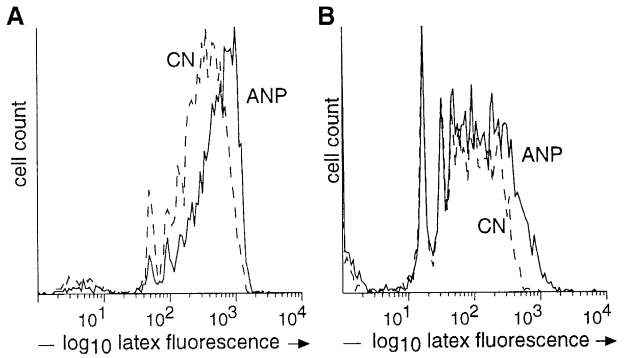

Fig. 3. Effect of ANP on phagocytosis of latex beads by J774 cells (panel A) and bone marrow derived macrophages (panel B). Representative histograms display a fluorescence shift to the right of ANP (10^{-9} M, 18 h) exposed cells (ANP, solid line) in comparison to control cells (CN, dashed line).

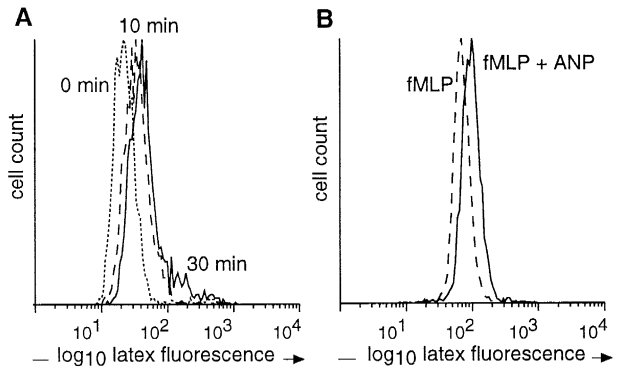

Fig. 5. (A) Kinetic measurement of the respiratory activity of J774 cells incubated with PMA (10 ng/ml). Data are given as histogram of green fluorescence (FL1, abscissa) and relative number of cells (arbitrary units, ordinate). 0 min (dotted line), 10 min (dashed line), 30 min (solid line). (B) Representative histogram (FL 1, number of cells) showing the stimulatory effect of ANP (10^{-8} M) (solid line) on reactive oxygen production of J774 cells induced by fMLP (10^{-6} M) (dashed line). Cells were preincubated with ANP for 2 h and further processed for measurement of respiratory burst as described in Section 2.

as mean fluorescence intensity of the entire cell population (Fig. 4A,B). The extent of increase recorded though was higher using mean fluorescence as parameter (up to 180% increase) as compared to phagocytic index (up to 135%) (Fig. 4A,B). The effect of ANP on phagocytic activity progressively decreased with increasing ANP concentrations and reached basal levels at a concentration of 10^{-6} M ANP (Fig. 4A,B). An exposure time of cells to ANP of 2 h resulted in a similar course of the experiment although the elevation of phagocytosis was less pronounced (data not shown).

3.3. Measurement of reactive oxygen intermediate production using flow cytometry

First, basal conditions for measurement of reactive oxygen intermediates in PMA-treated cells were established by assessing the effect of PMA on cell size and granularity. PMA induces changes in FSC as well as SSC (data not shown). Therefore, no FSC or SSC limiting gate was set for the following assays. Fig. 5A shows kinetic measurements (0-10-30 min) of respiratory activity with cells exposed to PMA. Production of reactive oxygen molecules increases by time as seen by the shift of fluorescence intensity. Panel B demonstrates the stimulatory effect of a 2 h preincubation of ANP (10^{-8} M) on reactive oxygen production induced by fMLP monitored as an increase of mean fluorescence intensity of dihydrorhodamine-123 loaded cells (J774). ANP itself did not elicit any effect (data not shown). Fig. 6 summarizes the dose-dependent

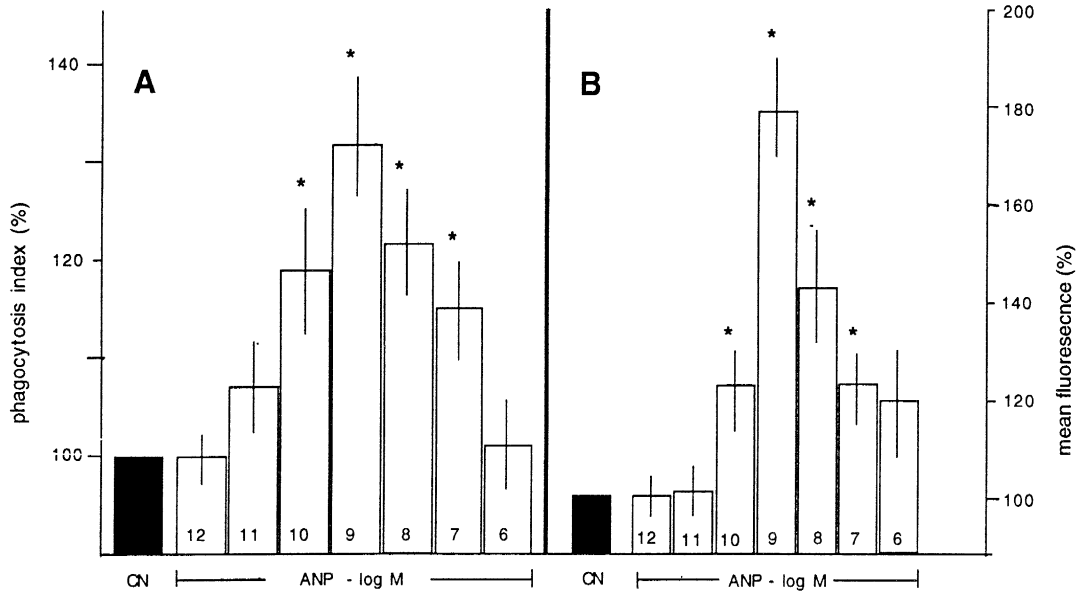

Fig. 4. Concentration-response curves showing enhancement of phagocytosis in J774 cells by ANP incubated for 18 h. Left and right graphs display the increase of phagocytotic indexes and mean fluorescence intensities, respectively. Data are means ± S.E. ($n = 4$) given as percentage of phagocytosis activity of control cells (CN, 100%). * $P < 0.05$ (t-test).

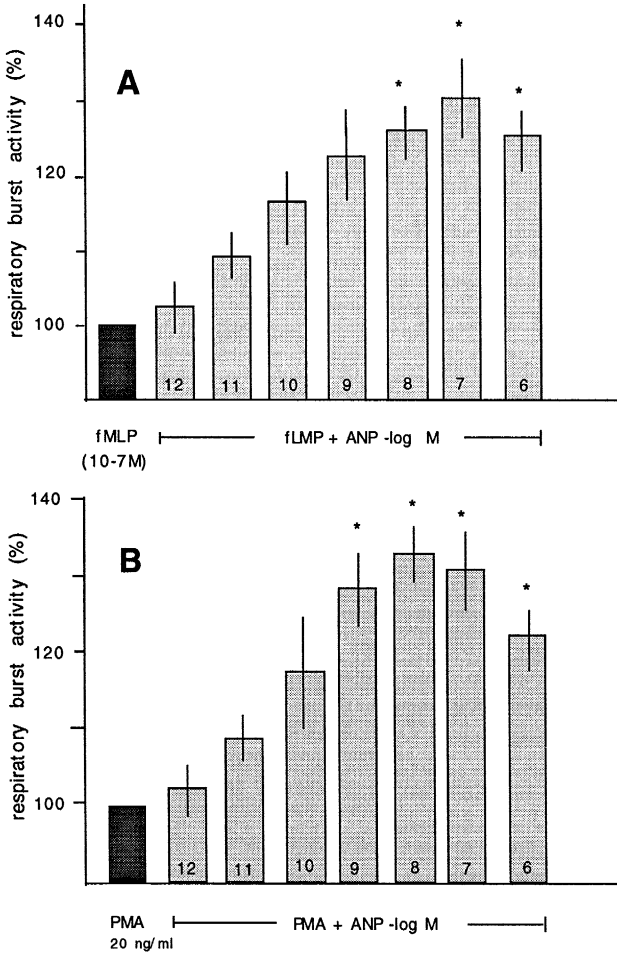

Fig. 6. Dose-response effect of ANP on respiratory activity of J774 cells stimulated with either fMLP (10^{-6} M) (panel A) or PMA (10 ng/ml) (panel B). Cells were incubated with increasing amounts of ANP for 2 h and further processed as described in detail in Section Section 2. Response is given as percentage of respiratory activity of cells which were not exposed to ANP. Bars represent means ± S.E. of four independent experiments. * $P < 0.05$ (*t*-test).

effects of ANP on respiratory burst activity of J774 cells either stimulated with fMLP (panel A) or with PMA (panel B). In a limited number of experiments ($n = 2$) exposure of cells for 18 h was shown to be less effective to augment respiratory burst as compared to the 2 h incubation period (data not shown). ANP elicited similar effects on bone marrow-derived macrophages treated with PMA (up to 125% increase at 10^{-8} M). However, fMLP was shown to cause a negligible increase of oxygen radicals on bone marrow-derived macrophages, and incubation of ANP did not result in a significant enhancement of respiratory activity (data not shown).

4. Discussion

The present study provides evidence that ANP influences two important functions of macrophages, i.e., phagocytosis and oxidative burst activity. ANP stimulates uptake of opsonized latex particles by mouse bone marrow-derived macrophages as well as of J774 cells, a mouse macrophage cell line. The effects were found at concentrations as low as 10^{-10} M, levels of ANP which can be found in the plasma (Gerbes and Vollmar, 1988). The decreased response to higher concentrations of the peptide might be explained by downregulation of the number of ANP receptors by exposure to ANP, a phenomenon previously described (Cao et al., 1995; Kato and Currie, 1992). ANP binds to two different types of receptors, i.e., the ANP_A, a guanylate cyclase coupled receptor and the so called ANP_C, which lacks guanylate cyclase activity (Nakao et al., 1992). Macrophages seem indeed to express ANP_A receptors, since elevated cGMP accumulation could be monitored for J774 cells, as well as for human peritoneal macrophages after exposure to ANP (Mattana and Singhal, 1993; Houdijk et al., 1990). No information exists for the presence of the ANP_C receptor on macrophages. Bell shaped dose response curves as seen here have been decribed for ANP and other neuropeptides in various immunological test such as migration or hydrogen peroxide production (Elferink and De Koster, 1995; Wiedermann et al., 1992; Biselli et al., 1996; De La Fuente et al., 1994; Chao et al., 1995). Even inhibitory effects on migration have been described for ANP at high concentrations (μM; Elferink and De Koster, 1995; Wiedermann et al., 1992). Interestingly, it has been reported that ANP at concentrations higher than 1 μM inhibits phagocytosis of immunoglobulin G (IgG) complexes by J774 cells (Mattana and Singhal, 1993). These authors, however, did not observe stimulation of particle uptake at lower ANP concentrations. This discrepancy may be related to the different assay conditions, since the authors looked specifically at fragment crystallisable (Fc)-receptor mediated phagocytosis employing IgG particles for ingestion. Furthermore, preincubation time of ANP in their experiment was only 10 min. We used an overnight incubation of ANP, since we observed lower stimulatory activities of the peptide with shorter incubation times (2 h and less, data not shown).

ANP also primes macrophages for increased production of reactive oxygen intermediates. Cells were stimulated with PMA as well as the more physiological stimulant fMLP. The enhancement is dependent on the concentration of ANP and the time of preincubation. 2 h incubation of ANP at concentrations greater than 10^{-9} M elicits significant increases in H_2O_2 production (up to 135%). Similar results have been reported for human polymorphonuclear neutrophils using the nitroblue tetrazolium reduction method (Wiedermann et al., 1992). Recently the data have been supported and extended by another flowcytometric study (Biselli et al., 1996). Coincubation of human polymorphonuclear neutrophils with ANP and fMLP or ANP and the eicosanoid chemoattractant leukotriene B4 resulted in a potentiated peroxide production at ANP concentrations as low as 10^{-14} and 10^{-12} M. The discrepancy in

ANP concentration necessary for maximal effect might be explained by differences in exposure modus (i.e., pre- or coincubation) and/or incubation time of cells to ANP.

In our study both macrophage parameters were measured by flow cytometry. Flowcytometric measurement of phagocytosis exceeds commonly used methods such as microscopic counting of ingested particles. More than 100 times as many cells and, more importantly, individual cells can be analyzed which leads to more objective conclusion in less time (Ichinose et al., 1994). These general advantages also apply for evaluation of oxidative burst by flow cytometry (Emmendörffer et al., 1990). However, it has to be mentioned that strongly adherent and stickly cells such as bone marrow macrophages may cause considerable problems in applying to the flow cytometer since only single cell suspensions can be analyzed correctly.

Although the mechanisms involved in activation of phagocytosis and respiratory burst by ANP are not defined, previous work on the expression of natriuretic peptides in macrophages suggests an autocrine mechanism of action. Bone marrow-derived macrophages express and secrete ANP as well as the other natriuretic peptide, brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP) (Vollmar and Schulz, 1994, 1995a). Immunostimulatory substances such as bacterial lipopolysaccharides or zymosan were able to increase expression and release of ANP (Vollmar and Schulz, 1994). Macrophage derived ANP may bind to its receptors and affect functions of the cells. In this respect ANP affects another important immunological parameter of macrophages, i.e., synthesis of NO. The peptide has been shown to inhibit NO production in lipopolysaccharide-activated bone marrow derived macrophages (Vollmar and Schulz, 1995b) as well as the cytokine-stimulated NO synthesis in primary cultures of proximal tubular cell (McLay et al., 1995).

ANP therefore seems to be able to modulate the balance between nitric oxide and superoxide, which is argued to be a critical determinant in various diseases (Darley-Usmar et al., 1995). In this respect it is important to know that NO has dual effects on O_2^- generation. NO at low concentration enhances, whereas at high concentrations it suppresses O_2^- production by polymorphonuclear leukocytes (Morikawa et al., 1995).

We conclude that ANP plays a role in two important functions of macrophages, i.e., phagocytosis and respiratory burst. Thus, the peptide may influence cellular defense mechanisms against invading microorganisms. The data further support the link between natriuretic peptides, originally described as cardiovascular hormones, and the immune system and thus extend the knowledge of the pharmacological activities of ANP.

Acknowledgements

We like to thank Ms. C. Siegl for excellent technical assistance. Dr. G. Hültner (GSF, Munich) is thanked for providing the J774 cells. This work was supported by the Deutsche Forschungsgemeinschaft (Vo-376/6-1).

References

Adams, D.O. and T.A. Hamilton, 1984, The cell biology of macrophage activation, Annu. Rev. Immunol. 2, 283.

Agui, T., X. Xin, Y. Cai, G. Shim, Y. Muramatsu, T. Yamada, H. Fujiwara and K. Matsumoto, 1995, Opposite actions of transforming growth factor-β1 on the gene expression of atrial natriuretic peptide biological and clearance receptors in a murine thymic stromal cell line, J. Biochem. 118, 500.

Biselli, R., S. Farrace, C. DeSimone and A. Fattorossi, 1996, Potentiation of human polymorphonuclear leucocyte activation by atrial natriuretic peptide. Inhibitory effect of carnitine congeners, Inflammation 20, 33.

Brenner, B.M., B.J. Ballermann, M.E. Gunning and M.L. Zeidel, 1990, Diverse biological actions of atrial natriuretic peptide, Physiol. Rev. 70, 665.

Cao, L., J. Wu and D.G. Gardner, 1995, Atrial natriuretic peptide suppresses the transcription of its guanylyl cyclase-linked receptor, J. Biol. Chem. 270, 24891.

Chao, T.C., H.P. Cheng and R.J. Walter, 1995, Somatostatin and macrophage function: modulation of hydrogen peroxide, nitric oxide and tumor necrosis factor release, Regul. Pept. 58, 1.

Cooper, P.H., P. Mayer and M. Baggiolini, 1984, Stimulation of phagocytosis in bone marrow-derived mouse macrophages by bacterial lipopolysaccharide: correlation with biochemical and functional parameters, J. Immunol. 133, 913.

Darley-Usmar, V., H. Wiseman and B. Halliwell, 1995, Nitric oxide and oxygen radicals: a question of balance, FEBS Lett. 369, 131.

De Bold, A.J., H.B. Borenstein, A.T. Veress and H. Sonnenberg, 1981, A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats, Life Sci. 28, 89.

De La Fuente, M., M. Delgado, M. Del Rio, E. Garrido, J. Leceta, A. Hernanz and R.P. Gomariz, 1994, Vasoactive intestinal peptide modulation of adherence and mobility in rat peritoneal lymphocytes and macrophages, Peptides 15, 1157.

Elferink, J.G.R. and B.M. De Koster, 1995, Atrial natriuretic factor stimulates migration by human neutrophils, Eur. J. Pharmacol. Mol. Pharmacol. Sect. 288, 335.

Emmendörffer, A., M. Hecht, M.-L. Lohmann-Matthes and J. Roesler, 1990, A fast and easy method to determine the production of reactive oxygen intermediates by human and murine phagocytes using dihydrorhodamine 123, J. Immunol. Methods 131, 269.

Gerbes, A.L. and A.M. Vollmar, 1988, Water immersion increases the concentrations of the immunoreactive N-terminal fragment of proatrial natriuretic factor in human plasma, Biochem. Biophys. Res. Commun. 156, 228.

Houdijk, A.P., M.J. Adolfs, I.L. Bonta and H.R. DeJonge, 1990, Atriopeptins and nitroprusside provoke opposite changes in cGMP and cAMP levels in human macrophages, Eur. J. Pharmacol. 179, 413.

Kato, J. and M.G. Currie, 1992, Suppression of atrial natriuretic peptide (ANP) receptor recovery from homologous down-regulation by 8-bromo-cGMP in endothelial cells, Eur. J. Pharmacol. 225, 113.

Ichinose, M., N. Hara, M. Sawada and T. Maeno, 1994, A flow cytometric asssay reveals an enhancement of phagocytosis by platelet activating factor in murine peritoneal macrophages, Cell. Immunol. 156, 508.

Mattana, J. and P.C. Singhal, 1993, Effects of atrial natriuretic peptide and cGMP on uptake of IgG complexes by macrophages, Am. J. Physiol. 265, C92.

McLay, J.S., P.K. Chatterjee, A.G. Jardine and G.M. Hawksworth, 1995, Atrial natriuretic factor modulates nitric oxide production: an ANF-C receptor-mediated effect, J. Hypertension 13, 625.

Morikawa, M., M. Inoue, S. Tokumaru and H. Kogo, 1995, Enhancing and inhibitory effects of nitric oxide on superoxide anion generation in human polymorphonuclear leukocytes, Br. J. Pharmacol. 115, 1302.

Moss, R.B. and M.G. Golightly, 1991, In vitro enhancement of natural cytotoxicity by atrial natriuretic peptide fragment 4-28, Peptides 12, 851.

Nakao, K., S. Ogawa, S. Suga and H. Imura, 1992, Molecular biology and biochemistry of the natriuretic peptide system. II. Natriuretic peptide receptors, J. Hypertens. 10, 111.

Rosenzweig, A. and C.E. Seidman, 1991, Atrial natriuretic factor and related peptide hormones, Annu. Rev. Biochem. 60, 229.

Samson, W.K., 1985, Atrial natriuretic factor inhibits dehydration- and hemorrhage-induced vasopressin release, Neuroendocrinology 40, 277.

Stanley, E.R., D.M. Chen and H.S. Lin, 1978, Induction of macrophage production and proliferation by a purified colony stimulating factor, Nature 274, 168.

Throsby, M., Z. Yang, D. Lee, W. Huang, D.L. Copolov and A.T. Lim, 1993, In vitro evidence for atrial natriuretic factor-(5-28) production by macrophages of adult rat thymi, Endocrinology 132, 2184.

Vollmar, A.M. and R. Schulz, 1990, Atrial natriuretic peptide is synthesized in the human thymus, Endocrinology 126, 2277.

Vollmar, A.M. and R. Schulz, 1994, Gene expression and secretion of atrial natriuretic peptide in murine macrophages, J. Clin. Invest. 94, 539.

Vollmar, A.M. and R. Schulz, 1995a, Expression and differential regulation of natriuretic peptides in mouse macrophages, J. Clin. Invest. 95, 2442.

Vollmar, A.M. and R. Schulz, 1995b, Atrial natriuretic peptide inhibits nitric oxide synthesis in mouse macrophages, Life Sci. (Pharm. Lett.) 56, 149.

Vollmar, A.M., R. Wolff and R. Schulz, 1995, Co-expression of the natriuretic peptides (ANP, BNP, CNP) and their receptors in normal and acutely involuted thymus, J. Neuroimmunol. 57, 117.

Vollmar, A.M., K.-N. Schmidt and R. Schulz, 1996, Natriuretic peptide receptors on rat thymocytes: inhibition of proliferation by atrial natriuretic peptide, Endocrinology 137, 1706.

Wiedermann, C.J., M. Niedermühlbichler and H. Braunsteiner, 1992, Priming of polymorphonuclear neutrophils by atrial natriuretic peptide, J. Clin. Invest. 89, 1580.