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Regulation of interleukin-6 secretion in murine pituicytes

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Abstract

Pituicytes, the astrocytic glial cells of the neural lobe, are known to secrete interleukin-6 and nitric oxide upon stimulation with various inflammatory mediators, i.e. interleukin-1β. Nitric oxide is described to modulate the secretion of interleukin-6 in various cell types. The aim of the present study was to investigate the effect of nitric oxide on interleukin-1β induced interleukin-6 secretion. Furthermore the effect of interferon-γ on interleukin-6 and nitric oxide release was investigated. Cultures of pituicytes were prepared of neural lobes from male mice. The effect of interleukin-1β and interferon-γ on interleukin-6 and nitric oxide secretion was investigated in pituicytes cultured for 14 days. The secretion of interleukin-6 and nitric oxide was determined after 24 h of stimulation. Pituicytes secrete interleukin-6 upon stimulation with interleukin-1β dose dependently but did not induce any detectable nitric oxide release. Co-stimulation with interferon-γ and interleukin-1β induced a significant nitric oxide release. In addition interferon-γ inhibits interleukin-1β induced interleukin-6 secretion dose dependently. The observed effect of interferon-γ on interleukin-6 secretion was not affected by the specific inducible nitric oxide synthase inhibitor 1400W (*N*-(3-[aminomethyl]benzyl)acetamidine). Furthermore interferon-γ dose dependently inhibits unstimulated interleukin-6 secretion. Use of the nitric oxide releaser DETA/NO (2,2′-(hydroxynitrosohydrazono)*bis*-ethanimine) demonstrated that nitric oxide does not inhibit interleukin-1β induced interleukin-6 secretion.

These results demonstrated that nitric oxide has no influence on interleukin-1β induced interleukin-6 secretion in cultured pituicytes. However the results are showing that interferon-γ has an inhibitory effect on interleukin-6 secretion.

Keywords: Pituicytes; Interferon-γ; Interleukin-1β; Interleukin-6; Nitric oxide; Neural lobe; Neurophypophysis

1. Introduction

Oxytocin and vasopressin are neuropeptides which act as hormones in the periphery and as neurotransmitters in the central nervous system. Secretion of oxytocin and vasopressin is regulated both at the level of the hypothalamus and in the neural lobe through presynaptic innervations (Bicknell, 1988; Boersma and van Leeuwen, 1994; Pittman et al., 1983). The majority of glial cells in the neural lobe are modified astrocytes named pituicytes, which stain positive for glial fibrillary acidic protein. The pituicytes surround and engulf the axonal terminals and show remarkable morphological plasticity during periods of high neurohormone demand. The cytokine interleukin-1β increases electric stimulated secretion of both oxytocin and vasopressin from the isolated rat neurohypophysis (Christensen et al., 1990). Furthermore, interleukin-6 and interleukin-1β stimulate the release of oxytocin and vasopressin in rat hypothalamic explants (Yasin et al., 1994). Pituicytes released high amounts of interleukin-6 upon stimulation with interleukin-1β or lipopolysaccharide (Christensen et al., 1999; Hansen et al., 1999; Spangelo et al., 1994). Thus pituicytes are believed to be involved in the release of oxytocin and vasopressin. In consequence it is of particular interest to investigate how the pituicytes are regulated.

Murine pituicytes stimulated with lipopolysaccharide for 24 h release nitric oxide and interleukin-6. When the released nitric oxide and interleukin-6 in pituicytes are plotted after 24 h stimulation there is a weak correlation

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($r^2 = 0.84$) between the two substances (Kjeldsen et al., 2003). This indicates a connection between the release of interleukin-6 and nitric oxide. One may speculate whether nitric oxide might modulate the interleukin-6 secretion.

In pituicytes inducible nitric oxide synthase (iNOS, NOS2) expression and activity can be induced by lipopolysaccharide (Kjeldsen et al., 2003). The inducible nitric oxide synthase can generate large amounts of nitric oxide for a long period of time. Several studies indicate that nitric oxide modulates the secretion of oxytocin and vasopressin both in vitro in rat neural lobes (Lutz-Bucher and Koch, 1994) and in vivo (Kadekaro, 2004; Kadekaro et al., 1997; Liu et al., 1998). Nitric oxide inhibits interleukin-6 release in a murine macrophage cell line (Deakin et al., 1995). In intact rats blockade of the synthesis of nitric oxide increases lipopolysaccharide induced plasma interleukin-6 levels (Kim and Rivier, 1998). Besides, a weak correlation between nitric oxide and interleukin-6 release in pituicytes after stimulation with lipopolysaccharide has been found, indicating that nitric oxide might modulate the interleukin-6 secretion in pituicytes (Kjeldsen et al., 2003).

It is known, that interferon-γ in combination with interleukin-1β induces nitric oxide in murine astrocytes, whereas interferon-γ or interleukin-1β alone fails to induce nitric oxide production (Falsig et al., 2004; Hewett et al., 1993).

The aim of this study was to investigate the ability of interleukin-1β and interferon-γ individually and in combination to induce interleukin-6 and nitric oxide in murine pituicytes and to unravel whether nitric oxide has a modulating effect on the interleukin-6 synthesis in murine pituicytes.

2. Materials and methods

2.1. Materials

The cells were maintained in RPMI 1640 with L-glutamine and HEPES (25 mM) (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 5% fetal calf serum (Biological Industries), GlutaMAX™ I (2 mM) (Gibco, Carlsbad, CA, USA), benzylpenicillin sodium (100 IU/ml), streptomycin sulfate (100 μg/ml) and 2-mercaptoethanol (52.5 μM). Ascorbic acid (0.1 mM) (Struers, Copenhagen, Denmark) was added to the supplemented RPMI 1640 prior stimulation to prevent oxidation. All solutions of low molecular substances were passed through filters with 20-kDa cut off (Ultrasart D20, Sartorius). All laboratory glassware used in handling cell cultures were rendered pyrogen free through dry heat treatment at 180 °C for 6 h.

In order to stimulate the cells the following substances were used: Recombinant murine interleukin-1β (17 393 kDa; 401-ML; R&D Systems, UK) dissolved in supplemented RPMI 1640, recombinant mouse interferon-γ (485-MI; R&D Systems, UK) dissolved in supplemented RPMI 1640, *N*-(3-[aminomethyl]benzyl)acetamidine (1400W) (ALX-270-073; ALEXIS, Lausen, Switzerland) dissolved in pyrogen-free water, 2,2′-(hydroxynitrosohydrazono)*bis*-ethanimine (DETA/NO) (D185; Sigma, St. Louis, MO, USA) dissolved in NaOH (10 μM) and stored under nitrogen at −80 °C. All subsequent dilutions of stock solutions were made in supplemented RPMI 1640.

2.2. Primary cell culture from the neural lobe

This work with pituitary cell cultures was made in accordance to a previously described procedure, which provides cultures without contamination.

3–5 weeks old male albino mice of the NMRI strain were used for the experiments. The mice were acquired from Taconic M&B, Ry, Denmark or from the animal breeding facilities at The Danish University of Pharmaceutical Sciences, Copenhagen. The mice were decapitated and the brains and the intact pituitaries were rapidly removed aseptically. The neural lobe was separated from the anterior and the intermediate lobe under a stereo microscope. Neural lobes from 9 to 15 mice were collected in 10 ml supplemented RPMI 1640 kept on ice. Enzymatic digestion of the tissue was accomplished in two steps. At first, the tissue was exposed to trypsin (2 mg/ml) (Fluka 93610) in unsupplemented RPMI 1640 for 25 min at 37 °C and 5% CO_2. The trypsin was removed after the solution was centrifuged at $125 \times g$ for 5 min. Secondly; the pellet was resuspended in pancreatin (2.5 mg/ml) (P-7545; Sigma) in unsupplemented RPMI 1640 and incubated for 5 min at 37 °C and 5% CO_2. The pancreatin was removed after centrifugation ($125 \times g$ for 5 min). The trypsin and pancreatin solutions were passed through 0.20 μm filters (Minisart 0.2 μm, Sartorius) prior use. The pellet was resuspended in 5 ml Minimal Essential Medium Eagle (M4767; Sigma) containing 10% fetal calf serum (Biological Industries) and incubated for 55 min at 37 °C and 5% CO_2 to stop the enzymatic reaction. Subsequent centrifugation for 5 min ($125 \times g$ for 5 min) the tissue was resuspended in 300 μl unsupplemented RPMI 1640 and mechanically disrupted to single cells using a plastic pipette tip. Finally the cell suspension was plated in 24-well culture plates (Nunc, Roskilde, Denmark) obtaining 0.4 neural lobe/1500 μl in each well. The dilutions were made in supplemented RPMI 1640. The cells were cultured at 37 °C and 5% CO_2 in a humidified atmosphere. The medium was first replaced after 7 days and thereafter twice a week.

Contamination of the cultures with other cell types than pituicytes were evaluated through microscopic observations and marking for the surface glycoprotein CD11b which is a marker for microglia and macrophages among others. These evaluations did not reveal any contaminations compared to a positive control (murine microglia cells, BV-2).

2.3. Stimulation of primary cell cultures

After 13 days in culture, the cells were confluent. The medium was aspired, discarded and the cells were gently washed with 1 ml supplemented RPMI 1640 to remove any accumulated interleukin-6 and nitrite (nitric oxide). Afterwards 1000 μl/well supplemented RPMI 1640 was added, and after 24 h the supernatants were aspired and collected for analysis of unstimulated release of interleukin-6 and nitric oxide. Subsequently (on day 14) the cells were washed with 1 ml supplemented RPMI 1640 and incubated for 24 h in the presence or absence of the test substances. The culture supernatants were

collected and analysed for interleukin-6 and nitric oxide. The interleukin-6 secretion was expressed as a ratio of interleukin-6 on day 14 (stimulated) to interleukin-6 on day 13 (unstimulated). The nitric oxide release was expressed as nitrite accumulation on day 14.

2.4. Assay for interleukin-6

The interleukin-6 concentration was determined using a time resolved dissociation-enhanced lanthanide fluoro immunoassay (DELFIA) which is a noncompetitive sandwich immunoassay as previously described (Moesby et al., 1997). Fluoronunc™ microtiter plates (Nunc, Denmark) were coated with monoclonal anti-mouse interleukin-6 antibody (2 μg/ml; 100 μl/well) (MAB406; R&D Systems, UK) in phosphate-buffered solution pH 8.0. After 30 min in a DELFIA® plateshake (Wallac, Turku, Finland) (950 r.p.m.) the plates were incubated overnight at room temperature. After three washes (DELFIA® Columbus M8/R2 platewasher, Wallac) in wash buffer (0.05% Tween 20 in phosphate buffered saline pH 7.4), non-specific binding was blocked with 300 μl blocking buffer (1% bovine serum albumin (A9418; Sigma), 5% sucrose in phosphate-buffered solution pH 8.0). The plates were shaken (950 r.p.m.) for at least 1 h at room temperature. The wells were washed three times with wash buffer. Standards prepared from recombinant mouse interleukin-6 (406-ML, R&D Systems, UK) and test solutions were diluted in supplemented RPMI 1640. Standard and test solution were added (100 μl/well) to the wells and shaken (950 r.p.m.) for 2 h at room temperature. After three washes biotinylated polyclonal goat anti-mouse interleukin-6 antibody (100 ng/ml; 100 μl/well) (BAF406, R&D Systems, UK) in Tris-buffered sodium chloride pH 7.4 with 0.1% bovine serum albumin was added to each well. The plates were shaken (950 r.p.m.) for 2 h at room temperature. The plates were washed three times with wash buffer and next DELFIA® europium-labelled streptavidin (100 ng/ml; 100 μl/well) diluted in DELFIA® assay buffer was added. After shaking (950 r.p.m.) the plates 30 min at room temperature the plates were washed six times with wash buffer. The europium was rendered fluorescent with DELFIA® enhancement solution (100 μl/well) and the plates were shaken (1150 r.p.m.) for 10 min. The fluorescence was detected in a 1234 DELFIA® fluorometer (Wallac).

Linearity between fluorescence and the interleukin-6 concentrations was observed within the concentration range (100–11 000 pg/ml). Each standard and test was determined in triplicate.

2.5. Assay for nitrite

Nitric oxide release was determined as the accumulation of nitrite in the cell culture supernatants using a colorimetric method. The assay used was based on a modified Griess reaction as previously described (Marzinzig et al., 1997). Standard solutions were prepared from sodium nitrite (Sigma) diluted in supplemented RPMI 1640.

In a microtiter plate (Nuclon™ delta surface, Nunc) 140 μl test or standard solution was mixed with 70 μl cold 4,4′-diaminodiphenylsulphone (14 mM) (Sigma) in HCl (2 M) and 70 μl *N*-(1-naphtyl)-ethylenediamine) (4 mM) (Sigma) in H_2O. The mixture was incubated for 10 min at 25 °C and the absorbance at 546 nm was measured with a VERSAmax™ Tunable microplate reader (Molecular Devices, Sunnyvale, USA). The standard curve was linear in the concentration range 0.31–50 μM, with 0.31 μM being the lowest standard used.

2.6. Statistical analysis

The non-parametric Mann–Whitney Rank Sum test was used on all data. $P<0.05$ is considered significant. The results are displayed as medians with 25th and 75th percentiles.

3. Results

3.1. Effect of interleukin-1β and interferon-γ on nitric oxide release

Pituicyte cell cultures were stimulated with various concentrations of interleukin-1β or co-stimulated with interleukin-1β and interferon-γ. Stimulation with interleukin-1β ($0.058–1.45\times10^4$ pM) did not result in any significant nitric oxide release compared to control (supplemented RPMI) (Fig. 1). Stimulation with interleukin-1β (0.58–57.9 pM) in combination with interferon-γ (100 U/ml) resulted in a dose dependent release of nitric oxide. The nitric oxide released upon co-stimulation with interleukin-1β (0.58 pM) and interferon-γ (100 U/ml) was significantly higher compared to stimulation with interferon-γ alone (100 U/ml) ($P=0.004$). Stimulation with interleukin-1β concentrations above 57.9 pM in combination with interferon-γ (100 U/ml) did not result in any significant changes in nitric oxide release.

Stimulation with interferon-γ (100 U/ml) alone did not result in a significant nitric oxide release compared to control (supplemented RPMI 1640).

3.2. Effect of interleukin-1β and interferon-γ on interleukin-6 secretion

After incubation with interleukin-1β a dose dependent secretion of interleukin-6 was observed (0.058–57.9 pM) (Fig. 2). Stimu-

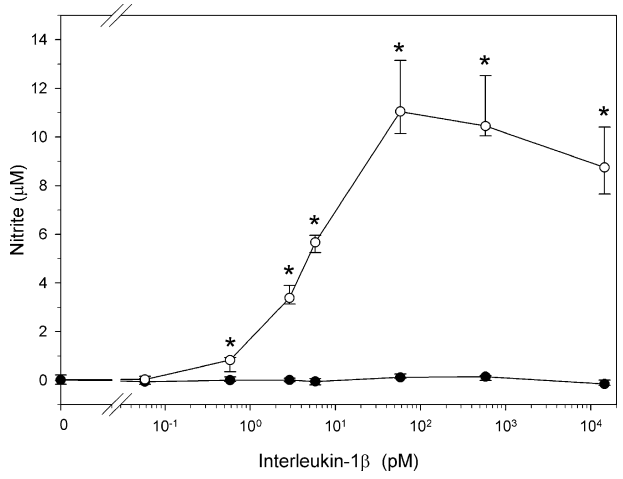

Fig. 1. Effect of interferon-γ (100 U/ml) on the interleukin-1β stimulated nitric oxide release. Pituicytes were cultured for 14 days and incubated with interleukin-1β or interleukin-1β+interferon-γ for 24 h. Culture supernatants were collected and assayed for nitrite. Each value represents the median with 25th and 75th percentiles of 6–20 observations. * $P\leq0.004$ compared to control (Mann–Whitney Rank Sum Test). Interleukin-1β (⋆) and interleukin-1β+interferon-γ (100 U/ml)(>). Control=supplemented RPMI 1640.

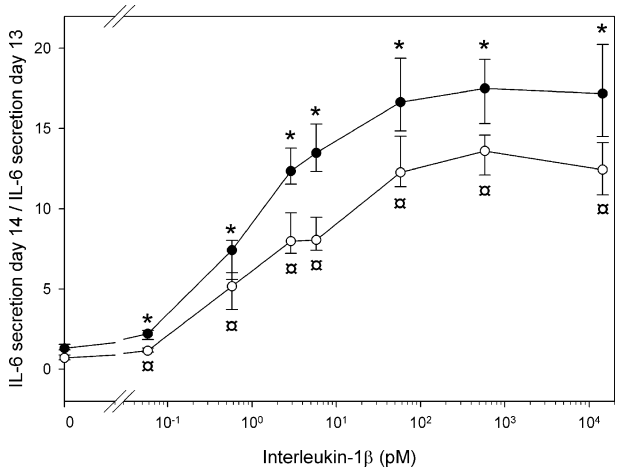

Fig. 2. Effect of interferon-γ (100 U/ml) on the interleukin-1β stimulated interleukin-6 secretion. Pituicytes were cultured for 13 days and incubated with supplemented RPMI 1640 for 24 h (unstimulated) followed by incubation with interleukin-1β or interleukin-1β+interferon-γ for 24 h (stimulated). The supernatants were collected and assayed for interleukin-6. The results are expressed as the ratio of interleukin-6 secretion on day 14 to interleukin-6 secretion on day 13. Each value represents the median with 25th and 75th percentiles of 6–27 observations. Interferon-γ significantly reduced the interleukin-1β induced interleukin-6 secretion at all tested concentrations ($P \leq 0.031$). *$P \leq 0.001$ compared to control. ¤ $P \leq 0.02$ compared to interferon-γ (100 U/ml) induced interleukin-6 secretion (Mann–Whitney Rank Sum Test). Interleukin-1β (⋆) and interleukin-1β+interferon-γ (100 U/ml) (>). Control=supplemented RPMI 1640. IL-6=interleukin-6.

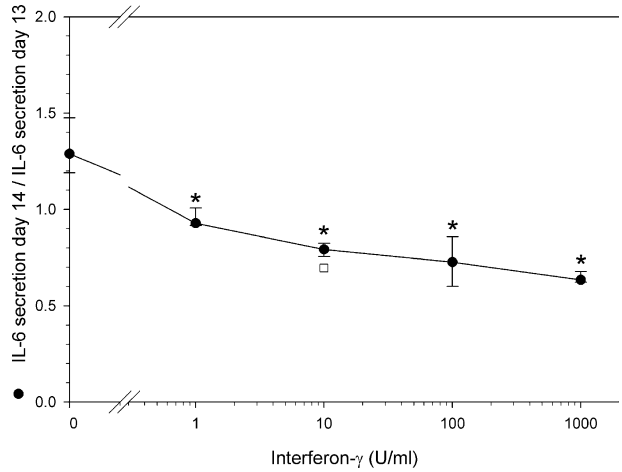

Fig. 3. Effect of interferon-γ on the unstimulated interleukin-6 secretion. Pituicytes were cultured for 13 days and incubated with supplemented RPMI 1640 for 24 h (unstimulated) followed by incubation with interferon-γ for 24 h (stimulated). The supernatants were collected and assayed for interleukin-6. The results are expressed as the ratio of interleukin-6 secretion on day 14 to interleukin-6 secretion on day 13. Each value represents the median with 25th and 75th percentiles of 4–33 observations. *$P \leq 0.001$ compared to control. □ $P=0.001$ compared to interferon-γ (1 U/ml) induced interleukin-6 secretion (Mann–Whitney Rank Sum Test). (⋆) Interleukin-6 secretion day 14/Interleukin-6 secretion day 13. IL-6=interleukin-6.

lation with interleukin-1β (0.058 pM) resulted in a significant higher interleukin-6 secretion compared to control ($P \leq 0.001$). Stimulation with interleukin-1β (0.058 pM)+interferon-γ (100 U/ml) resulted in a significantly higher interleukin-6 secretion compared to stimulation with interferon-γ alone (100 U/ml) ($P=0.02$). Co-stimulation with interleukin-1β and interferon-γ (100 U/ml) significantly reduced the interleukin-1β stimulated interleukin-6 secretion at all the tested concentrations of interleukin-1β ($P \leq 0.031$).

3.3. Dose dependent effect of interferon-γ on interleukin-6 secretion

Interferon-γ dose dependently inhibited the unstimulated interleukin-6 secretion significantly (Fig. 3). The interleukin-6 secretion was significantly inhibited at all tested concentrations of interferon-γ compared to control ($P \leq 0.001$). Furthermore interferon-γ dose dependently inhibited the interleukin-1β induced interleukin-6 secretion and potentiated the nitric oxide release (Fig. 4). Stimulation with interferon-γ (1 U/ml)+interleukin-1β (5.8 pM) resulted in a significant lower interleukin-6 secretion ($P=0.004$) and a significant higher nitric oxide release ($P=0.001$) compared to stimulation with interleukin-1β alone (5.8 pM).

3.4. Effect of 1400 W on stimulated interleukin-6 and nitric oxide release

The specific iNOS inhibitor 1400W (0.5–50 μM) dose dependently inhibited the interleukin-1β and interferon-γ induced nitric oxide release but did not influence the interleukin-6 secretion significantly (Fig. 5). A significant decrease in nitric oxide release was observed upon stimulation with 1400W (0.5 μM)+interleukin-1β (5.8 pM)+interferon-γ (100 U/ml) ($P \leq 0.001$).

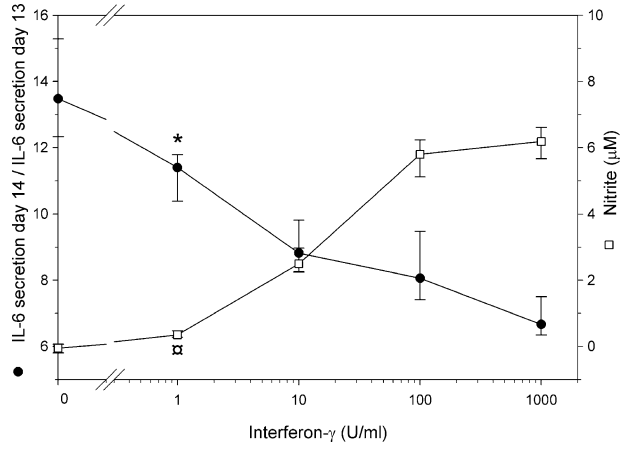

Fig. 4. Effect of interferon-γ on the interleukin-1β (5.8 pM) induced interleukin-6 secretion and nitric oxide release. Pituicytes were cultured for 13 days and incubated with supplemented RPMI 1640 for 24 h (unstimulated) followed by incubation with interleukin-1β or interleukin-1β+interferon-γ for 24 h (stimulated). The supernatants were collected and assayed for interleukin-6 and nitrite. The interleukin-6 results are expressed as the ratio of interleukin-6 secretion on day 14 to interleukin-6 secretion on day 13. Each value represents the median with 25th and 75th percentiles of 9–19 observations. *$P=0.004$ compared to interleukin-1β (5.8 pM) induced interleukin-6 secretion. ¤ $P=0.001$ compared to interleukin-1β (5.8 pM) induced nitrite accumulation (Mann–Whitney Rank Sum Test). (⋆) Interleukin-6 secretion day 14/Interleukin-6 secretion day 13; (□) nitrite (μM). IL-6=interleukin-6.

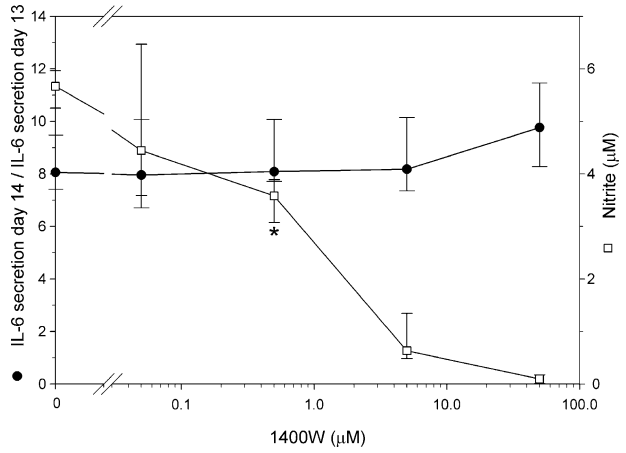

Fig. 5. Effect of the specific iNOS inhibitor 1400 W on the interleukin-1β and interferon-γ stimulated interleukin-6 and nitric oxide release. Pituicytes were cultured for 13 days and incubated with supplemented RPMI 1640 for 24 h (unstimulated) followed by incubation with interleukin-1β (5.8 pM), interferon-γ (100 U/ml) and 1400W for 24 h (stimulated). The supernatants were collected and assayed for interleukin-6 and nitrite. The interleukin-6 results are expressed as the ratio of interleukin-6 secretion on day 14 to interleukin-6 secretion on day 13. Each value represents the median with 25th and 75th percentiles of 6–17 observations. *$P \leq 0.001$ compared to interleukin-1β (5.8 pM) and interferon-γ (100 U/ml) induced nitrite accumulation (Mann–Whitney Rank Sum Test). (⋆) Interleukin-6 secretion day 14/Interleukin-6 secretion day 13; (□) nitrite (μM). IL-6=interleukin-6.

3.5. Effect of DETA/NO on stimulated interleukin-6 secretion

As previously shown interleukin-6 secretion was increased upon stimulation with interleukin-1β (Fig. 6). Co-stimulation with interleukin-1β (5.8 pM) and the nitric oxide releaser DETA/NO (10 μM) does not decrease interleukin-6 secretion. The concentration of DETA/NO used resulted in a nitrite accumulation equal to the accumulation upon stimulation with interleukin-1β (57.9 pM)+interferon-γ (100 U/ml).

4. Discussion

The results presented in this paper show that interleukin-1β and interferon-γ alone did not induce nitric oxide release in cultured pituicytes but in combination they induced a significant amount of nitric oxide. This is in accordance to the results presented by Hewett et al. (1993) and Falsig et al. (2004) based on work accomplished with murine astrocytes. Lee et al. (1993), however, did observe a marginal increase in nitric oxide release upon stimulation with interleukin-1β in humane astrocytes, although the cells were incubated for several days before a significant release of nitric oxide was observed. The observed increase in nitric oxide release upon stimulation with interleukin-1β and interferon-γ in combination can be explained by the signal transduction mechanisms suggested by Schroder et al. (2004). The gene for iNOS contains binding sites for the transcription factors Stat1 (signal transducers and activators of transcription 1) and nuclear factor κB (NF-κB) which are activated by interferon-γ and interleukin-1β, respectively. However, maximal transcription requires both signals. This hypothesis is confirmed through stimulation with lipopolysaccharide and interferon-γ (Schroder et al., 2004) but it is well known that interleukin-1β and lipopolysaccharide in part shares the same signal transduction pathway through NF-κB (Subramaniam et al., 2004).

Both in vivo (Kim and Rivier, 1998) and in vitro (Deakin et al., 1995) studies have indicated nitric oxide as a possible inhibitor of the lipopolysaccharide induced interleukin-6 secretion. In contrary to these studies the present results reveal that nitric oxide does not inhibit the interleukin-1β and interferon-γ induced interleukin-6 secretion. This is strongly supported by the results using the specific iNOS inhibitor 1400W and the nitric oxide releaser DETA/NO. The concentration of DETA/NO used resulted in a significant higher nitric oxide concentration than the nitric oxide concentration in the corresponding interleukin-1β stimulation (5.9 pM) and therefore any direct inhibitory effect of nitric oxide on interleukin-6 secretion should be observed. Stimulation with interleukin-1β concentrations above 58.9 pM and interferon-γ (100 U/ml) does not result in a significant decline in nitric oxide release although a tendency is observed. This tendency could be explained by a negative feed back mechanism as previously described in macrophages (Griscavage et al., 1993). In previous work on murine astrocytes a decrease in viable cell count upon stimulation with interleukin-1β and interferon-γ has been observed (Falsig et al., 2004). The observed tendency to a declining nitric oxide release does not seem to be a result of a decrease in viable cells since the corresponding interleukin-6 secretion is not reduced.

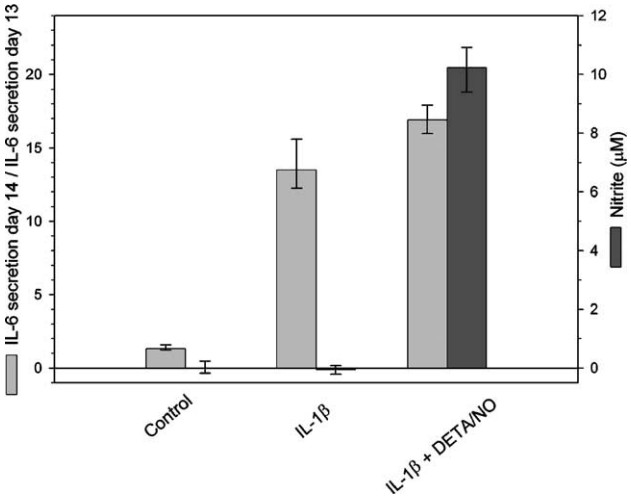

Fig. 6. Effect of the nitric oxide releaser DETA/NO on the interleukin-1β stimulated interleukin-6. Pituicytes were cultured for 13 days and incubated with supplemented RPMI 1640 for 24 h (unstimulated) followed by incubation with interleukin-1β (5.8 pM) and DETA/NO for 24 h (stimulated). The supernatants were collected and assayed for interleukin-6 and nitrite. The interleukin-6 results are expressed as the ratio of interleukin-6 secretion on day 14 to interleukin-6 secretion on day 13. Each value represents the median with 25th and 75th percentiles of 6–17 observations. IL-1β=interleukin-1β; IL-6=interleukin-6.

Interferon-γ was shown to be responsible for the inhibitory effect on the interleukin-6 secretion. This effect was observed on both unstimulated and interleukin-1β stimulated interleukin-6 secretion. This is in contrary to Falsig et al. (2004) who observed an increase in interleukin-6 secretion upon stimulation with interleukin-1β and interferon-γ in combination compared to stimulation with interleukin-1β alone. The present observations indicate that interferon-γ rather than nitric oxide is responsible for the inhibitory effect on interleukin-6 secretion since there was not any unstimulated nitric oxide release.

In conclusion, the results of this study show that nitric oxide does not have an inhibitory effect on interleukin-6 secretion, whereas interferon-γ possesses a significant inhibitory effect in murine pituicytes. This raises question whether circulating interferon-γ has an effect on the function of the pituicytes in vivo and thereby on the function of the neural lobe.

It is well known that the levels of the proinflammatory cytokines interferon-γ and interleukin-1β are raised during pathophysiological conditions such as certain infections and autoimmune diseases as a consequence of an induction of the immune system. In the posterior pituitary gland a raised level of these cytokines could lead to an induction of nitric oxide release from the pituicytes. Nitric oxide has an inhibitory effect on the secretion of vasopressin and oxytocin from the neurohypophysis (Kadowaki et al., 1994; Lutz-Bucher and Koch, 1994; Reid, 1994). This is contrary to interleukin-1β which has a stimulating effect on vasopressin and oxytocin secretion (Christensen et al., 1990). These oppositely orientated effects may establish a kind of balance in the regulation of vasopressin and oxytocin secretion from the neurohypophysis during pathophysiological conditions.

As mentioned both interleukin-6 and nitric oxide are indicated to have a modulating effect on the vasopressin and oxytocin secretion in the neurohypophysis. The present observations indicate that the effect of nitric oxide is not mediated through an inhibitory effect on the interleukin-6 secretion.

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