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Adrenaline influences the release of interleukin-6 from murine pituicytes: role of β_2-adrenoceptors

Jens D. Christensen *, Erik W. Hansen, Charlotte Frederiksen, Mette Mølris, Lise Moesby

Department of Pharmacology, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark

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Abstract

In this study, we examined the effect of adrenaline and interleukin-1β on interleukin-6 secretion from cultured murine neurohypophyseal cells. Cells were cultured from neurohypophyses of 3- to 5-week-old mice and experiments were performed after 13 days in culture. Interleukin-6 was measured in 24-h samples using a sandwich fluoroimmunoassay. Unstimulated cells released 19 ± 3 fmol interleukin-6/neurohypophysis/24 h (mean ± S.E.M., $n = 42$). Adrenaline and interleukin-1β increased the release of interleukin-6 from the cells in a concentration-dependent manner. Incubation with adrenaline (10^{-6} M) or interleukin-1β (11 pM) induced maximal secretion of interleukin-6, resulting in a 2.2-fold and 19.8-fold increase, respectively ($P < 0.01$). The action of adrenaline (10^{-6} M) and interleukin-1β (1.1 pM) was examined separately and together. The sum of the effect of the two compounds given alone was significantly less ($P < 0.05$) than the effect when adrenaline and interleukin-1β were given together. We examined the effect of the β-adrenoceptor antagonist propranolol (3.4×10^{-6} M), the β_2-adrenoceptor antagonist (±)-1-[2,3-(Dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI 118551) (10^{-7} M) and the β_1-adrenoceptor antagonist atenolol (10^{-7} M and 10^{-6} M) on the adrenaline-stimulated release of interleukin-6. Propranolol and ICI 118551 completely blocked the action of adrenaline, whereas atenolol was inactive. It is concluded that the stimulatory effect of adrenaline is mediated via β_2-adrenoceptors.

Keywords: Adrenaline; Interleukin-1β; Interleukin-6; β-Adrenoceptor antagonist; Pituicyte; Neurohypophysis

1. Introduction

Axonal terminals from the magnocellular cells in the hypothalamus are important neural elements in the neurohypophysis. However, the largest population of intact cells in the neural lobe are modified astrocytes (pituicytes) that stain positive for the astrocytic glial fibrillary acidic protein (GFAP) (Boersma and Van Leeuwen, 1994). Spangelo et al. (1994) reported that the neurointermediate pituitary lobe cells from rats are able to synthesise and release interleukin-6 after endotoxin and interleukin-1β stimulation. We have recently shown the same effect of endotoxin on primary cultures of pituicytes from the murine neurohypophysis (Hansen et al., 1999). Besides modulation from the hypothalamus, the secretion of the neurohypophyseal hormones, oxytocin and vasopressin, can be regulated at the level of the neural lobe through presynaptic innervation (Pittman et al., 1983; Bicknell, 1988). These results and the anatomically close connection between the pituicytes and the axonal terminals make paracrine communication possible in this area. For this reason, it is of interest to further investigate how interleukin-6 secretion is regulated in pituicytes.

Norris and Benveniste (1993) reported that noradrenaline induces interleukin-6 mRNA and interleukin-6 secretion in primary neonatal rat astrocytes. Furthermore, their results suggested the involvement of β_2- and α_1-adrenoceptors in this effect. The neurohypophysis receives noradrenergic nerve fibres (Alper et al., 1980), indicating that they have a physiological role. The adrenergic binding sites in the neural lobe have been shown to be dominated by the β_2-adrenoceptor type (De Souza, 1985). In vitro stimulation of β-adrenoceptors in adult rat pituicytes provokes changes in pituicyte morphology (Bicknell et al., 1989). This effect is considered to be of great importance in the regulation of oxytocin and vasopressin secretion.

* Corresponding author. Tel.: +45-35-30-60-00; fax: +45-35-30-60-01; E-mail: jdc@mail.dfh.dk

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This naturally raises the question whether adrenergic stimulation of pituicytes might stimulate the secretion of interleukin-6. Hatton et al. (1991) have shown that adrenaline is a more potent agonist than noradrenaline in stimulating morphological changes in pituicytes. We wanted to examine if adrenaline was able to stimulate the secretion of interleukin-6 from primary murine pituicytes and, if so, to characterise the receptors involved.

2. Materials and methods

2.1. Materials

All solutions and media used for cell cultures were passed through a 20 kDa cut off ultrafilter (Ultrasart D 20, Sartorius) immediately after they were prepared. After filtration the content of endotoxin was below the detection limit in our Limulus amoebocyte lysate assay (<1 pg endotoxin/ml). All laboratory glassware used in the handling of cells was rendered pyrogen-free through heating at 180°C for 6 h.

The cells were maintained in RPMI 1640 (Gibco, NY, USA) supplemented with HEPES (10 mM), 5% Myoclone Super Plus fetal bovine serum (Gibco, NY, USA), penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and 2-mercaptoethanol (5×10^{-5} M). For stimulation of the cells the following substances were used: recombinant murine interleukin-1β (specific activity 50 U/ng) from Boehringer Mannheim, adrenaline (adrenaline bistartrate, Rhone Poulenc), ascorbic acid (Merck, Darmstadt). Ascorbic acid (10^{-4} M) was added to avoid oxidation of adrenaline. The β-adrenoceptor antagonists used were propranolol (Inderal 1 mg/ml, ICI-pharma), (±)-1-[2,3-(Dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol (ICI 118551; ICI PLC, Macclesfield, GB) and atenolol (ICI PLC, Macclesfield, GB). Stock solutions were made in pyrogen-free water and purified by ultrafiltration. Subsequent dilutions were made in RPMI 1640 supplemented.

2.2. Primary neurohypophyseal cell cultures

Male albino mice of the NMRI strain 3–5-weeks-old were used for the experiment. The mice were acquired from the animal facilities at the Panum Institute, University of Copenhagen. The animals were decapitated and the brains with intact hypophyses were rapidly removed. Under a stereo microscope, the neural lobe was separated from the anterior and intermediate lobes. The neural lobes from 5 to 10 mice were placed in 2 ml RPMI 1640 supplemented. Enzymatic digestion was accomplished in two steps. First, the tissue was exposed to trypsin Sigma T-8918 (2.0 mg/ml) in RPMI 1640 without serum for 25 min at 37°C. The suspension was centrifuged at $155\times g$ for 5 min. The pellet was resuspended in 2 ml pancreatin Sigma P-7545 (2.5 mg/ml) in RPMI 1640 (without serum) and incubated for 5 min at 37°C. Pancreatin was removed by centrifugation ($155\times g$ for 5 min). The pellet was resuspended in Minimal Essential Medium Eagle (Sigma M-4767) containing 10% Myoclone Super Plus fetal bovine serum and incubated for 60 min at 37°C to stop enzymatic digestion. Subsequently, the tissue was disrupted to single cells in serum-free Minimal Essential Medium Eagle using a plastic pipette tip and cells were pelleted by centrifugation ($155\times g$ for 5 min). Finally, the cells were resuspended in RPMI 1640 supplemented and plated out in 96-well tissue culture plates (Nunc, Roskilde, Denmark) with 0.5 neural lobe/250 μl/well. The cells were cultured in a humidified atmosphere of 5% CO_2–95% air at 37°C. The medium (300 μl RPMI 1640 supplemented) was replaced twice weekly.

2.3. Stimulation of primary neurohypophyseal cell cultures

After 14 days in culture the cells were confluent. Cross-contamination of released interleukin-6 was minimised by aspirating the medium and by adding 300 μl fresh RPMI 1640 supplemented after 13 days in culture. On day 14, the culture medium was aspirated and discarded. 300 μl fresh RPMI 1640 supplemented was added to the cells and after 24 h, the supernatant (300 μl) was removed and analysed for interleukin-6 to estimate the unstimulated release. Subsequently, the cell cultures were stimulated for 24 h with adrenaline, interleukin-1β or both in combination. The drugs had been dissolved in 300 μl RPMI 1640 supplemented. The culture supernatants were collected and analysed for interleukin-6 to estimate the stimulated release. The results are expressed as the ratio of interleukin-6 release on day 15 (stimulated release) to interleukin-6 release on day 14 (unstimulated release).

2.4. Assay for interleukin-6

Recombinant murine interleukin-6 was purchased from R&D Systems, UK. Monoclonal anti-mouse interleukin-6 antibody and biotinylated polyclonal goat anti-mouse interleukin-6 antibodies were obtained from R&D Systems, UK. The manufacturer recommended the antibodies as a capturing and detection pair for sandwich enzyme-linked immunosorbent assays (ELISA).

The interleukin-6 concentration was determined using a non-competitive immunoassay as previously described (Moesby et al., 1997). Europium-labelled streptavidin (0.1 mg/ml), DELFIA® assay buffer, DELFIA® wash concentrate and DELFIA® enhancement solution were all obtained from Wallac (Turku, Finland).

FluoroNunc™ microtiter plates (Nunc, Roskilde, Denmark) were coated with monoclonal anti-mouse interleukin-6 antibody (2 μg/ml; 100 μl/well) in phosphate-

buffered saline pH 7.4. The plates were incubated overnight at room temperature. After three washes with DELFIA® wash buffer non-specific binding was blocked with 300 μl blocking buffer (1% bovine serum albumin, 5% sucrose, 0.05% NaN_3 in phosphate-buffered saline pH 7.4) per well and the plates were shaken in a DELFIA® plate shake (slow) for at least 1 h at room temperature. The plates were washed three times with DELFIA® wash buffer. Standards and test solutions diluted in supplemented RPMI 1640 and water (1:1) were added to the wells (100 μl/well) and shaken (slow) for 2 h at room temperature. The wells were washed three times and 100 μl biotinylated polyclonal goat anti-mouse interleukin-6 antibody (100 ng/ml) was added to each well and shaken (slow) for 2 h at room temperature. After the wells were washed three times, 100 μl europium-labelled streptavidin (100 ng/ml) diluted in DELFIA® assay buffer was added to each well. The plate was shaken for 30 min at room temperature and afterwards the wells were washed six times with DELFIA® wash buffer. Finally, the europium was rendered fluorescent with DELFIA® enhancement solution (100 μl); the plate was shaken for 5–10 min. Fluorescence was detected in a 1234 DELFIA® fluorometer (Wallac, Turku, Finland).

The detection limit of the assay was 12.5 pg/ml. The inter-assay variation was CV% = 9.4 ($n = 9$) and the intra-assay variation was CV% = 5.1 ($n = 9$).

2.5. Glial fibrillary acidic protein immunocytochemistry

Cells were grown in 96-well culture plates for 13 days. The cells were fixated (0.1% glutaraldehyde) and washed in phosphate-buffered saline (pH 7.4). The cells were incubated for 24 h in diluted (1:100) rabbit anti-cow GFAP (DAKO) containing 0.1% Triton X-100, rinsed with phosphate buffered saline (pH 7.4) and incubated for 90 min with diluted (1:40) fluorescein isothiocyanate conjugated swine anti-rabbit immunoglobulin (DAKO). Labelled cultures were examined using a Leica fluorescence microscope. After 13 days in culture, the cells were confluent. Therefore, we are unable to count the cells and give an exact percentage of GFAP positive cells.

2.6. Statistical analysis

Unless otherwise stated, the Mann–Whitney Rank Sum Test was used.

3. Results

3.1. Characterisation of cell cultures

In cultures grown for 13 days, the majority of cells were immunoreactive for GFAP. Omission of the primary antibody abolished the immunoreactivity, indicating a specific staining for GFAP.

3.2. Adrenaline-induced interleukin-6 release from neurohypophyseal cells in vitro: effect of adrenaline concentration

After 13 days in culture, the unstimulated release of interleukin-6 was 19 ± 3 fmol/neurohypophysis/24 h (mean ± S.E.M.; $n = 42$). In the presence of adrenaline, the release of interleukin-6 was concentration dependently increased (Fig. 1). After incubation with adrenaline (10^{-6} M), the release of interleukin-6 was significantly different from control ($P < 0.01$) and a 2.2-fold increase was seen, which was maximal.

3.3. Interleukin-1β-induced interleukin-6 release from neurohypophyseal cells in vitro

Interleukin-1β stimulated the release of interleukin-6 in a concentration-dependent manner (Fig. 2). 1.1 pM and 11 pM of interleukin-1β increased the release of interleukin-6 by 9.1- and 19.8-fold ($P < 0.01$), respectively. A higher concentration of interleukin-1β, 111 pM, did not increase the release of interleukin-6 further.

3.4. Action of interleukin-1β, adrenaline and combinations on interleukin-6 release from cultured neurohypophyseal cells

After incubation with adrenaline (10^{-6} M) or interleukin-1β (1.1 pM), the release of interleukin-6 increased significantly ($P < 0.01$) by 2.2- and 9.1-fold, respectively. When cells were cultured in the presence of a combination of adrenaline (10^{-6} M) and interleukin-1β (1.1 pM), a 16.9-fold increase in interleukin-6 release was seen (Fig. 3). The simple sum of the effects of adrenaline and interleukin-1β given alone was significantly less ($P < 0.05$,

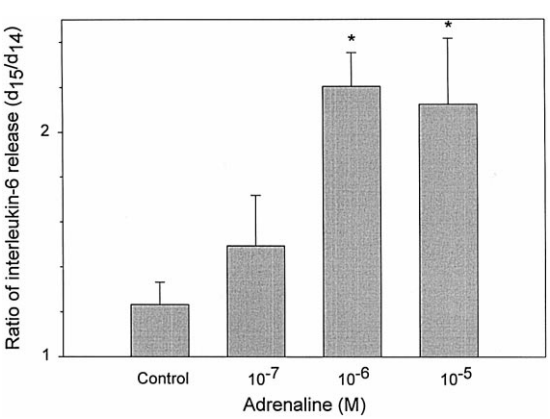

Fig. 1. Effect of adrenaline on interleukin-6 release from cultured murine neurohypophyseal cells. Cells were cultured for 13 days and incubated with RPMI 1640 supplemented for 24 h followed by incubation with adrenaline or RPMI 1640 supplemented for 24 h. The results are expressed as the ratio of interleukin-6 release on day 15 to interleukin-6 release on day 14. Each value is the mean ± S.E.M. of 6–12 observations. * $P < 0.01$ compared to control (Mann–Whitney Rank Sum Test).

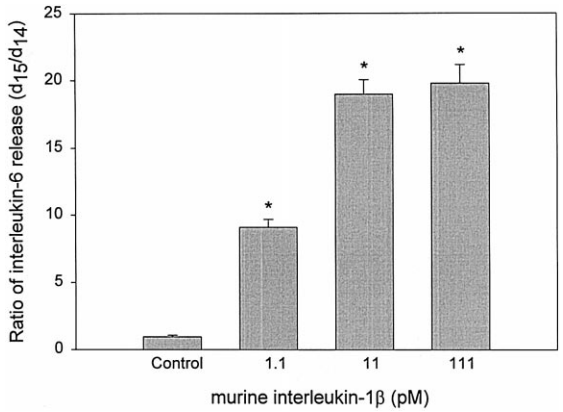

Fig. 2. Effect of interleukin-1β on interleukin-6 release from cultured murine neurohypophyseal cells. Cells were cultured for 13 days and incubated with RPMI 1640 supplemented for 24 h followed by incubation with interleukin-1β or RPMI 1640 supplemented for 24 h. The results are expressed as the ratio of interleukin-6 release on day 15 to interleukin-6 release on day 14. Each value is the mean ± S.E.M. of six observations. $^{*}P < 0.01$ compared to control (Mann-Whitney Rank Sum Test).

Student's *t*-test) than the effect seen when both compounds were present together.

3.5. Effect of propranolol, ICI 118551 and atenolol on adrenaline-stimulated release of interleukin-6 from neurohypophyseal cells

From Fig. 4 it can be seen that propranolol (3.4×10^{-6} M) and ICI 118551 (10^{-7} M) completely and significantly ($P < 0.01$ compared to adrenaline) blocked the stimulatory effect of adrenaline on interleukin-6 release from cultured neurohypophyseal cells. In contrast, atenolol (10^{-7} M) was without effect and even a 10-fold increase in atenolol concentration had no effect (not shown).

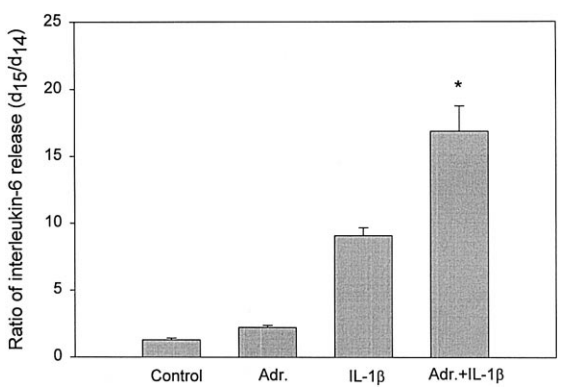

Fig. 3. Effect of adrenaline, interleukin-1β and combinations on interleukin-6 release from murine neurohypophyseal cells. Cells were cultured for 13 days and incubated with RPMI 1640 supplemented for 24 h followed by incubation with test substance or combinations for 24 h. The results are expressed as the ratio of stimulated interleukin-6 release (day 15) to unstimulated interleukin-6 release (day 14). Each value is the mean ± S.E.M. of 6–12 observations. $^{*}P < 0.05$ compared to the sum of responses after adrenaline and interleukin-1β (Student's *t*-test). Control = RPMI 1640 supplemented; Adr = adrenaline, 10^{-6} M; IL-1β = interleukin-1β, 1.1 pM.

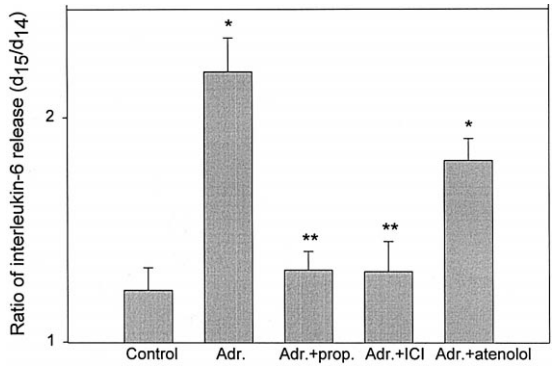

Fig. 4. Effect of adrenaline, propranolol, ICI 118551, atenolol and combinations on interleukin-6 release from cultured murine neurohypophyseal cells. Cells were cultured for 13 days and incubated with RPMI 1640 supplemented for 24 h followed by incubation with test substance or combinations of test substances for 24 h. The results are expressed as the ratio of interleukin-6 release in the presence of test substance/test substances (day 15) to unstimulated interleukin-6 release (day 14). Each value is the mean ± S.E.M. of 6–12 observations. $^{*}P < 0.01$ compared to control, $^{**}P < 0.01$ compared to adrenaline (Mann–Whitney Rank Sum Test). Control = RPMI 1640 supplemented; Adr = adrenaline, 10^{-6} M; Prop = propranolol, 3.4×10^{-6} M; ICI = ICI 118551, 10^{-7} M and atenolol = atenolol, 10^{-7} M.

4. Discussion

Pituicytes are defined as astrocytic glial cells of the neural lobe and possess a number of astrocytic characteristics. Since most of the cells in this study were GFAP immunoreactive, we consider them to be pituicytes. It has been known for some time that pituicytes from explants of adult rat neurohypophysis undergo morphological changes in response to adrenergic stimulation of β-adrenoceptors (Bicknell et al., 1989). These receptors have been further characterised to be of the β_2-adrenoceptor type (Hatton et al., 1991). In this study, we analysed the effect of adrenaline on interleukin-6 secretion from primary murine pituicytes. We believe this to be the first study to show that adrenaline stimulates the release of interleukin-6 in a dose-dependent manner. The present data strongly suggest that this action of adrenaline is connected with β_2-adrenoceptor activation since both propranolol (3.4×10^{-6} M) and ICI 118551 (10^{-7} M), a specific β_2-adrenoceptor antagonist, completely blocked the effect of adrenaline on interleukin-6 secretion. In contrast, atenolol, a specific β_1-adrenoceptor antagonist, up to 10^{-6} M was ineffective in this respect.

Norris and Benveniste (1993) have shown that noradrenaline induces interleukin-6 mRNA and interleukin-6 secretion in neonatal rat cortical astrocytes. They found evidence for the involvement of α_1- and β_2-adrenoceptors in this effect of noradrenaline. Since the specific β_2-adrenoceptor antagonist in our study completely blocked the

effect of adrenaline on interleukin-6 secretion, we concluded that α_1-receptors were not involved in this response in murine pituicytes. Apart from the fact that the cells used in the above-mentioned study were of rat origin, another explanation for the discrepant findings is that pituicytes and astrocytes have different characteristics.

The present data support earlier findings (Spangelo et al., 1994) that interleukin-1β stimulates the secretion of interleukin-6 from rat neurointermediate pituitary lobe. In the present study, the maximal response after interleukin-1β was nine times higher than that of adrenaline. The reason for this might be differences in the intrinsic activity of the two ligand receptor complexes and/or the number of the two receptor types.

The effect of the concomitant addition of adrenaline and interleukin-1β was greater than the simple sum of the effects of the two compounds given alone, indicating that a co-operative mechanism may be activated when both agents are present. It has been shown that β-adrenergic-stimulated release of vasopressin in the neural lobe is accompanied by an increase in cAMP formation (Al-Zein et al., 1984). The biological effects of interleukin-1β stimulation are also known to be followed by an increase in cAMP level (Maimone et al., 1993). Other compounds known to increase cAMP are reported to have a synergistic action with interleukin-1β on interleukin-6 secretion in rat astrocytes, i.e., norepinephrine (Norris and Benveniste, 1993), pituitary adenylate cyclase-activating polypeptide and vasoactive intestinal peptide (Gottschall et al., 1994). A similar synergistic effect of noradrenaline and interleukin-1β on the interleukin-6 levels in rat spleen lymphocyte culture medium has recently been reported by Huang et al. (1997). The nature of the synergistic mechanism is for the present not known.

The β-adrenoceptor ligand isoprenaline has been shown to increase the electrically evoked release of vasopressin from the isolated neurointermediate lobe (Racke et al., 1982). Zhao et al. (1988) found similar results for the neural lobe concerning oxytocin and vasopressin. In the rat hypothalamic explant model, Yasin et al. (1994) have shown a stimulatory effect of interleukin-6 on vasopressin and oxytocin secretion. Although the nature of this experiment does not indicate whether interleukin-6 acted at the hypothalamic or the hypophyseal level, one might speculate that the stimulatory action of isoprenaline on oxytocin and vasopressin secretion is mediated via pituicyte β_2-adrenoceptor stimulation followed by interleukin-6 secretion. This might reflect a physiological mechanism because the neurohypophysis is known to contain noradrenergic nerve fibres that are in close contact with axon terminals and pituicytes (Alper et al., 1980). At present, it is not known whether the neural lobe contains adrenergic nerves. However, this does not exclude that noradrenergic nerves may stimulate β_2-adrenoceptors although it is well known that β_2-adrenoceptors have a higher affinity for adrenaline than for noradrenaline. Another source for β_2-adrenergic activation is blood-borne adrenaline. Beagley and Hatton (1994) have shown that catecholamines from the adrenal medulla are able to penetrate to the neural lobe and induce changes in pituicyte morphology.

In summary, these results demonstrate that adrenaline stimulates the release of interleukin-6 from isolated murine pituicytes. Furthermore, this response was mediated by the action of adrenaline on β_2-adrenoceptors.

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