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We investigated the effects of recombinant human IL-1α, -1β, -2, -6 and TNF on the in vitro secretion of β-endorphin-immunoreactivity (βE-IR) by the rat anterior and neurointermediate lobes (AL and NIL, respectively) and of B by the rat adrenal gland. Isolated AL and NIL cells were incubated for 2 h with cytokines (1 pg/ml–1 µg/ml), CRH (5.10⁻¹⁰ M) or with cytokines in combination with CRH (AL cells), isolated adrenal cells were incubated for 2 h with cytokines, ACTH (25 pg/ml) or with cytokines in combination with ACTH. Furthermore, AL, NIL and adrenal tissue fragments were superfused for 30 or 60 min with cytokines (10 and/or 100 ng/ml). Incubation of AL, NIL and adrenal cells and superfusion of these tissues with cytokines had no significant effect on βE-IR and B release. However, there are some exceptions: incubation of AL cells with IL-2 increased CRH-induced βE-IR release, incubation of NIL cells with IL-2 induced an increase of basal βE-IR release, ACTH-induced B secretion was reduced after co-incubation of adrenal cells with TNF and after prolonged (6 h) superfusion of adrenal tissue with TNF, and finally, prolonged (6 h) superfusion of adrenal fragments with IL-1β increased basal B release. Taken together, these data suggest that the acute activation of the pituitary-adrenal axis of rats by administration of cytokines (at least IL-1, IL-6 and TNF) in vivo is not mediated by a direct action of these cytokines at the level of the pituitary and/or adrenal gland.

The concept that there is a functional relationship between the immune system and the (neuro-)endocrine system is now widely accepted. It has been postulated that cytokines—polypeptides synthesized and released by cells of the immune system—may function as messengers of the immune system to activate the pituitary-adrenal axis (PA-axis). There is ample evidence that in rodents, after intravenous or intracerebroventricular bolus injection of interleukin 1 (IL-1), plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (B) rise within 10–30 min. Besides IL-1 also interleukin 2 (IL-2), interleukin 6 (IL-6) and tumour necrosis factor-α (TNF) have been reported to stimulate the activity of the PA-axis when injected into laboratory animals or humans. There is increasing consensus that the central nervous system is the main site of action of IL-1 in activating the PA-axis. Whether IL-1 can directly stimulate ACTH and β-endorphin (βE) secretion by the anterior lobe (AL) of the pituitary gland and/or B secretion by the adrenal gland is controversial. Data concerning direct effects of IL-2 and TNF on hormone release by the AL in vitro are also conflicting. Positive effects of IL-2 on ACTH release by cultured murine AtT-20 cells and of TNF on ACTH release by rat anterior pituitary cells or tissue have been described. However, other researchers found no effect of IL-2 on ACTH release by cultured AtT-20 cells and of TNF on ACTH release by rat AL cells in vitro. Regarding the effects of IL-6, positive effects on ACTH release by rat anterior pituitary tissue or on murine AtT-20 cells have been described. With respect to the effects of IL-2, IL-6 and TNF on B release by adrenal cells in vitro only few data are available, whereas there are no data at all concerning direct effects of IL-1 and other cytokines on hormone release by the intermediate lobe of the pituitary gland.

In the light of these controversial data, we systematically investigated in the present study the effects of a number of cytokines (IL-1α, IL-1β, IL-2, IL-6 and TNF) in a broad range of concentrations, on the secretion of β-endorphin-immunoreactivity (βE-IR) by the AL and the NIL of the rat pituitary gland and on the...
secretion of βE by the rat adrenal gland. We used two supplementary in vitro methods: static incubation of freshly prepared cell suspensions and superfusion of the tissues.

RESULTS

Cell suspension experiments

AL cells. Figure 1 shows the effects of incubation of freshly isolated AL cells with IL-1α, IL-1β, IL-2, IL-6 or TNF alone, or with these cytokines in combination with CRH on the release of βE-IR in vitro. In all experiments incubation of AL cells with 5.10^{-10} M CRH induced a significant (P < 0.001) increase in βE-IR release (+114 ± 8%; n = 38) as compared to βE-IR release by cells incubated with EBSS (1.5 ± 0.1 ng/2 h; n = 40). Incubation of cells with each of the cytokines (1 pg/ml-1 μg/ml) alone had no significant effect on βE-IR release. Incubation of cells with IL-1α, IL-1β, IL-6 or TNF in combination with CRH had no significant effect on βE-IR release, whereas incubation of cells with IL-2 in

![Graphs showing the effects of cytokines on βE-IR release from AL cells.](image)

**Figure 1.**

Release of βE-IR from isolated rat AL cells in vitro incubated with IL-1α, IL-1β, IL-2, IL-6 or TNF (1 pg/ml-1 μg/ml; □) or with these cytokines in combination with 5.10^{-10} M CRH (■). After 2 h of incubation AL cells and medium were separated. βE-IR content in the medium was measured by a specific RIA. βE-IR release is expressed as percentage (mean ± SEM; n = 8) of hormone release by the control group. ND = not determined. ***: IL-2 induced a dose-dependent increase of CRH-induced βE-IR release (P < 0.0001).
combination with CRH induced a significant \( (P < 0.0001) \) dose-dependent increase in \( \beta E \)-IR secretion as compared to the release by cells incubated with CRH alone.

NIL cells. The effects of incubation of freshly isolated NIL cells with IL-1\( \alpha \), IL-1\( \beta \), IL-2, IL-6 or TNF in concentrations of 1 pg/ml-1 \( \mu \)g/ml on \( \beta E \)-IR release are listed in Table 1. Incubation of NIL cells with dopamine \( (10^{-9} \text{M}) \) induced a significant \( (P < 0.01) \) reduction of \( \beta E \)-IR release \((-43 \pm 5\%; n = 12) \) as compared to hormone release by cells incubated with buffer alone \( (3.0 \pm 0.2 \text{ng/2 h}; \text{data not shown}) \). Incubation of cells with increasing doses of IL-1\( \alpha \), IL-1\( \beta \) or IL-6 had no significant effect on \( \beta E \)-IR release. Although \( \beta E \)-IR release by NIL cells in response to incubation with TNF at a dose of 10 pg/ml was markedly increased, TNF had no significant overall effect on \( \beta E \)-IR release. Incubation with IL-2, however, induced a statistically significant \( (P < 0.001) \) dose-dependent increase in \( \beta E \)-IR release.

Adrenal cells. Table 2 lists the effects of incubation of freshly isolated adrenal cells with IL-1\( \alpha \), IL-1\( \beta \), IL-2, IL-6 or TNF alone or with these cytokines in combination with ACTH on corticosterone release. Incubation of cells with 25 pg/ml ACTH induced a strong increase in B release \((+1672 \pm 71\%; n = 53) \) as compared to B release by the control group \((6.1 \pm 0.4 \text{ng/2 h}; n = 39; \text{data not shown}) \). Incubation of adrenal cells with cytokines \((1 \text{pg/ml}-1 \mu \text{g/ml}) \) alone had no significant effect on B release. Incubation of cells with IL-1\( \alpha \), IL-1\( \beta \), IL-2 or IL-6 in combination with ACTH had no significant effect on B release as compared to the release induced by ACTH alone, whereas TNF induced a significant \( (P < 0.01) \) dose-dependent decrease of the ACTH-induced stimulation of B secretion by rat adrenal cells as compared to the stimulation of B secretion by ACTH alone.

Superfusion experiments

AL tissue. Figure 2 (upper panel) shows the effects of superfusion of AL fragments with IL-1\( \alpha \), IL-1\( \beta \), IL-2, IL-6, TNF or CRH on \( \beta E \)-IR release. Superfusion of ALs for 30 min with medium containing CRH \((5.10^{-10}\text{M}) \) respectively \( 5.10^{-9} \text{M} \) induced a rapid, sustained, and significant \( (P < 0.001) \) dose-dependent increase in the

### Table 1. Effects of incubation of isolated NIL-cells for 2 h with cytokines on the release of \( \beta E \)-IRa

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>1 pg/ml</th>
<th>10 pg/ml</th>
<th>100 pg/ml</th>
<th>1 ng/ml</th>
<th>10 ng/ml</th>
<th>100 ng/ml</th>
<th>1 ( \mu )g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1( \alpha )</td>
<td>90.3 ± 7.5</td>
<td>88.3 ± 6.8</td>
<td>108.6 ± 9.5</td>
<td>105.0 ± 12.8</td>
<td>115.1 ± 7.4</td>
<td>95.7 ± 4.3</td>
<td>ND</td>
</tr>
<tr>
<td>IL-1( \beta )</td>
<td>120.0 ± 10.1</td>
<td>106.5 ± 8.3</td>
<td>94.1 ± 5.8</td>
<td>95.3 ± 8.9</td>
<td>92.9 ± 7.3</td>
<td>100.3 ± 10.0</td>
<td>ND</td>
</tr>
<tr>
<td>IL-2( ** )</td>
<td>107.3 ± 6.2</td>
<td>102.2 ± 4.4</td>
<td>104.1 ± 5.0</td>
<td>102.6 ± 4.3</td>
<td>110.0 ± 5.0</td>
<td>118.0 ± 5.1</td>
<td>126.0 ± 6.8</td>
</tr>
<tr>
<td>IL-6</td>
<td>96.2 ± 6.2</td>
<td>108.9 ± 14.3</td>
<td>93.2 ± 11.4</td>
<td>83.8 ± 6.4</td>
<td>96.0 ± 6.7</td>
<td>98.1 ± 5.2</td>
<td>97.7 ± 10.3</td>
</tr>
<tr>
<td>TNF</td>
<td>114.3 ± 6.5</td>
<td>153.1 ± 7.8</td>
<td>108.2 ± 6.5</td>
<td>115.3 ± 8.6</td>
<td>100.0 ± 8.3</td>
<td>101.4 ± 4.8</td>
<td>111.1 ± 14.8</td>
</tr>
</tbody>
</table>

a\( \beta E \)-IR release is expressed as percentage \((\text{mean} \pm \text{SEM}; n = 8-12) \) of the basal release.

** Indicates that there was a significant dose-response relationship \( (P < 0.001) \).

### Table 2. Effects of incubation of isolated adrenal cells for 2 h with cytokines and/or ACTH on B releaseb

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>1 pg/ml</th>
<th>10 pg/ml</th>
<th>100 pg/ml</th>
<th>1 ng/ml</th>
<th>10 ng/ml</th>
<th>100 ng/ml</th>
<th>1 ( \mu )g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1( \alpha )</td>
<td>0</td>
<td>91.4 ± 9.5</td>
<td>98.9 ± 5.1</td>
<td>100.3 ± 8.3</td>
<td>70.0 ± 17.5</td>
<td>94.7 ± 10.1</td>
<td>107.8 ± 13.0</td>
</tr>
<tr>
<td>25</td>
<td>101.5 ± 3.7</td>
<td>101.6 ± 5.7</td>
<td>102.6 ± 6.7</td>
<td>104.4 ± 5.6</td>
<td>93.7 ± 4.2</td>
<td>88.3 ± 2.7</td>
<td>ND</td>
</tr>
<tr>
<td>IL-1( \beta )</td>
<td>0</td>
<td>85.3 ± 2.9</td>
<td>109.6 ± 8.8</td>
<td>109.6 ± 10.0</td>
<td>91.9 ± 9.7</td>
<td>88.5 ± 12.5</td>
<td>112.9 ± 14.2</td>
</tr>
<tr>
<td>25</td>
<td>99.2 ± 5.1</td>
<td>98.5 ± 7.5</td>
<td>104.5 ± 5.2</td>
<td>111.0 ± 6.6</td>
<td>99.4 ± 5.3</td>
<td>100.7 ± 3.4</td>
<td>ND</td>
</tr>
<tr>
<td>IL-2</td>
<td>0</td>
<td>105.0 ± 11.2</td>
<td>81.3 ± 3.3</td>
<td>90.7 ± 3.1</td>
<td>81.7 ± 7.3</td>
<td>83.3 ± 6.4</td>
<td>90.7 ± 3.1</td>
</tr>
<tr>
<td>25</td>
<td>106.2 ± 6.3</td>
<td>106.6 ± 6.8</td>
<td>106.2 ± 6.1</td>
<td>103.3 ± 5.8</td>
<td>96.4 ± 4.8</td>
<td>108.6 ± 4.6</td>
<td>99.5 ± 8.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>0</td>
<td>98.9 ± 12.3</td>
<td>100.0 ± 5.3</td>
<td>123.3 ± 14.5</td>
<td>97.6 ± 13.4</td>
<td>85.9 ± 5.9</td>
<td>96.8 ± 9.0</td>
</tr>
<tr>
<td>25</td>
<td>98.2 ± 5.1</td>
<td>101.3 ± 6.0</td>
<td>99.0 ± 3.7</td>
<td>98.9 ± 3.9</td>
<td>87.0 ± 1.8</td>
<td>106.0 ± 3.9</td>
<td>107.9 ± 6.8</td>
</tr>
<tr>
<td>TNF</td>
<td>0</td>
<td>122.2 ± 11.1</td>
<td>110.4 ± 14.2</td>
<td>93.1 ± 10.4</td>
<td>104.6 ± 10.7</td>
<td>104.9 ± 12.1</td>
<td>98.6 ± 14.4</td>
</tr>
<tr>
<td>25*</td>
<td>107.0 ± 3.6</td>
<td>92.1 ± 5.0</td>
<td>91.0 ± 4.9</td>
<td>85.7 ± 9.0</td>
<td>80.7 ± 3.8</td>
<td>79.5 ± 9.2</td>
<td>80.0 ± 4.1</td>
</tr>
</tbody>
</table>

bB release is expressed as percentage \((\text{mean} \pm \text{SEM}; n = 5-6) \) of B release by cells incubated with only buffer or with buffer containing 25 pg/ml ACTH respectively.

* Indicates that there was a significant dose-response relationship \( (P < 0.01) \).
Figure 2.

**Upper panel:** Release of βE-IR from superfused rat AL tissue in vitro. ALs were superfused twice (fr. 7–9 and 17–19; black bars) for 30 min with medium containing IL-1α (●), IL-1β (○), IL-2 (■), IL-6 (□) or TNF (▲) (1st pulse: 10 ng/ml, 2nd pulse: 100 ng/ml) or CRH (5.10^{-10} M respectively 5.10^{-8} M, ▼). After each pulse, superfusion was continued with initial medium. Flow rate was 0.1 ml/min and 10 min fractions were collected for measurement of βE-IR content. Data are expressed as the mean (+ SEM) βE-IR release of 4–7 observations.

**Lower panel:** Release of B from superfused rat adrenal tissue in vitro. Tissue was superfused for 60 min (fr. 11–14; black bar) with medium containing IL-1α (●), IL-1β (○), IL-2 (■), IL-6 (□), TNF (▲) (100 ng/ml) or ACTH (125 pg/ml, ▼). Thereafter superfusion was continued with initial medium for 120 min (fr. 15–22). Flow rate was 0.1 ml/min and 15 min fractions were collected for B determination. Data are presented as the mean (+ SEM) B release of 6 observations.

**TABLE 3. Effects of superfusion of NILs with cytokines on the release of βE-IR**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>fr. 7–13</th>
<th>fr. 17–23</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>93.5 ± 1.6</td>
<td>95.5 ± 1.2</td>
</tr>
<tr>
<td>IL-1β</td>
<td>92.5 ± 2.9</td>
<td>99.4 ± 1.9</td>
</tr>
<tr>
<td>IL-2</td>
<td>90.8 ± 2.3</td>
<td>106.8 ± 2.9</td>
</tr>
<tr>
<td>IL-6</td>
<td>103.5 ± 2.4</td>
<td>96.9 ± 1.9</td>
</tr>
<tr>
<td>TNF</td>
<td>97.6 ± 3.4</td>
<td>89.4 ± 5.0</td>
</tr>
</tbody>
</table>

*βE-IR release is expressed as percentage (mean ± SEM; n = 4) of the computed basal release as described in materials and methods. Cytokines were added to the medium during pulse 1 (10 ng/ml) and pulse 2 (100 ng/ml).

The amount of βE-IR in the superfusates. Upon removal of CRH from the medium, the concentration of βE-IR in the superfusates rapidly decreased and returned to basal levels. Superfusion of AL tissue with medium containing cytokine (1st pulse: 10 ng/ml, 2nd pulse: 100 ng/ml) had no significant effect on βE-IR release.

**NIL tissue.** Superfusion of NILs for 30 min with medium containing the β-adrenoceptor agonist isoproterenol (10^{-7} M respectively 10^{-6} M) induced a significant (P < 0.01) dose-dependent increase of βE-IR release (1st pulse: +62 ± 15%, 2nd pulse: +122 ± 36%; data not shown). Table 3 shows that superfusion of NILs for 30 min with 10 respectively 100 ng/ml of IL-1α, IL-1β, IL-2, IL-6 or TNF had no significant effect on βE-IR release.

**Figure 3.**

**Upper panel:** B release from rat adrenal tissue superfused for 6 h (black bar) with medium (□) or with medium containing 100 ng/ml IL-1β (●), IL-2 (○) or TNF (▲). Thereafter superfusion was continued with initial medium for another 16 h. *P < 0.01. IL-1β vs. medium, from 8–24 h.

**Lower panel:** Continued superfusion of the same rat adrenal tissues as shown in the upper panel. These tissues were superfused for 30 min (28–28.5 h; black bar) with 250 pg/ml ACTH and subsequently with medium for another 3 h. Data are presented as the mean (+ SEM) B release of 11–12 observations.
Adrenal tissue. The effects of superfusion of adrenal tissue with IL-1α, IL-1β, IL-2, IL-6, TNF or ACTH on B release are shown in Figure 2 (lower panel). B content in the superfusates was found to be increased (+195 ± 5% vs. basal release) after superfusion of adrenal tissue for 60 min with medium containing 125 pg/ml ACTH. Superfusion of adrenal tissue for 1 h with each of the cytokines in a dose of 100 ng/ml, had no effect on B release. Prolonged superfusion (6 h) of adrenal tissue with cytokines did not induce an immediate increase in B release, therefore total B release was calculated from 8–24 h by summation of the B content of each of the fractions, and subsequently compared to the total B release (8–24 h) after superfusion of adrenal tissue with medium alone. Figure 3 shows that superfusion of adrenal tissue for 6 h with IL-1β resulted in a significantly higher (P < 0.01) B release as compared to the release by the control tissues. IL-2 and TNF had no significant effect on basal B release. Superfusion of these adrenals was continued, and Figure 3 (lower panel) shows that exposure of the tissues for 30 min to 250 pg/ml ACTH induced a significant (all P < 0.001 vs. basal release) increase in B release. Superfusion of adrenal tissue with IL-1β or IL-2 prior to ACTH had no effect on ACTH-induced B release 16 h later, whereas superfusion with TNF resulted in a reduced (−26.5 ± 10.3%; 0.01 < P < 0.05) response of this tissue to ACTH as compared to ACTH-induced B release by tissue superfused with only medium.

**DISCUSSION**

The present study shows that short-term (2 h) incubation of rat AL cells with recombinant human (rh) IL-1α or IL-1β in concentrations up to 1 μg/ml did not significantly modify basal βE-IR secretion. A review of the effects of rhIL-1 on ACTH and or βE release by the rat anterior pituitary in vitro is given in Table 4. Our results are in agreement with those in two other studies, which demonstrated that IL-1 has no effect on ACTH release by cultured rat AL cells after an incubation period of 2–3 h. In contrast with the lack of a stimulatory effect during short-term incubation, it seems that IL-1 stimulates ACTH or βE secretion by rat AL cells or mouse anterior pituitary tumour cells (AtT-20 cells) during incubation for a more prolonged time (4–8 h for rat AL cells) and 23–24 h for murine AtT-20 cells). In our study IL-1 did not modulate CRH-induced βE-IR release by AL cells, which is in agreement with data from Berkenbosch et al.6 We showed that IL-2, IL-6 and TNF were unable to modify basal βE-IR release by rat AL cells during a 2 h incubation period. Other researchers have demonstrated that rhIL-2β and rhIL-6 increase basal ACTH release by murine AtT-20 cells, but only after 24 h of incubation. With regard to TNF our results are in agreement with the results of other studies.6,31 Even during a more prolonged incubation period (24 h), it seems that rhTNF is still not capable of modifying basal ACTH release by rat AL cells.6,32 We found that IL-2 induced

**TABLE 4. A survey of literature concerning direct effects of recombinant human IL-1 on pituitary ACTH/βE and adrenal B release**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Dose</th>
<th>Method</th>
<th>Effect</th>
<th>REF</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhIL-1α (HLR)</td>
<td>1 nM</td>
<td>ACTH (20/D16-16 mouse AP tumour cells)</td>
<td>BE ↑ and CRH↓βE↑ after 24 h</td>
<td>10</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>0.3–0.3–nM</td>
<td>Perfusion rat (SD) AP cells</td>
<td>ACTH ↑ DD after 20 min pulse</td>
<td>11</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Cultured rat (SD) AP cells</td>
<td>ACTH ↑ after 4 h from 10 × M</td>
<td>12</td>
</tr>
<tr>
<td>rhIL-1β (Otsuka)</td>
<td>10 × 10 × M</td>
<td>ACTH and DM mouse AP tumour cells</td>
<td>BE ↑ after 23 h from 10 × M</td>
<td>13</td>
</tr>
<tr>
<td>rhIL-1β 1 (Genzyme)</td>
<td>25–100 pg/ml</td>
<td>Perfusion fr. iso. rat (W) AP cells</td>
<td>ACTH ↑ after 24 h from 12 × M</td>
<td>14</td>
</tr>
<tr>
<td>rhIL-1β (Bio): Biogen</td>
<td>0.03–30 nM</td>
<td>Cultured rat (W) AP cells</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>15</td>
</tr>
<tr>
<td>rhIL-1β (MLR)</td>
<td>1 × M</td>
<td>ACTM-20 mouse AP tumour cells</td>
<td>ACTH ↑ after 24 h</td>
<td>16</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>0.01–100 nM</td>
<td>Cultured rat (CD) AP cells</td>
<td>ACTH ↑ after 10 min</td>
<td>17</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>0.01–100 nM</td>
<td>Perfusion rat (W) AP cells</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>18</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>0.01–100 nM</td>
<td>Cultured rat (SD) AP cells; AtT-20 cells</td>
<td>ACTH ↑ after 10 min</td>
<td>19</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>0.01–100 nM</td>
<td>Perfusion rat (W) AP cells</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>20</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>1–100 U/ml</td>
<td>Perfusion fr. iso. rat (W) AP cells</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>21</td>
</tr>
<tr>
<td>rhIL-1β (own product)</td>
<td>3.5–35 µg</td>
<td>In situ perfusion rat (F) adrenals</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>22</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>0.05–5.12µl</td>
<td>fr. iso. rat (W) AD cells</td>
<td>ACTH ↑ after 5 min pulse</td>
<td>23</td>
</tr>
<tr>
<td>rhIL-1β (HLR)</td>
<td>10 × 10 × M</td>
<td>Cultured rat (SD) AD cells; AD organ cultured</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>24</td>
</tr>
<tr>
<td>rhIL-1β (HLR)</td>
<td>10 × 10 × M</td>
<td>Perfusion rat (W) AD cells</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>25</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Cultured rat (SD) AD cells</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>26</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Cultured rat (W) AD cells</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>27</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Cultured rat (W) adrenocortical cells</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>28</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Rat (SD) AD organs</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>29</td>
</tr>
<tr>
<td>rhIL-1β (Cistron)</td>
<td>10 × 10 × M</td>
<td>Perfusion fr. iso. rat (AD) AD cells</td>
<td>ACTH ↑ after 24 h at 10 × M</td>
<td>30</td>
</tr>
</tbody>
</table>

Abbreviations: HLR: Hoffman-La Roche; RPR: Rhône-Poulenc Rorer; AP: anterior pituitary; AD: adrenal; fr. iso.: freshly isolated; SD: Sprague-Dawley; W: Wistar; F: Fischer 344; CD: corticovertebral; T: increase; ↓ decrease; x: no effect on hormone release; DD: dose-dependent; CRH: CRH-induced; ACTH: ACTH-induced.
a small but significant and dose-dependent increase in CRH-induced βE-IR release by AL cells, whereas IL-6 and TNF had no such a modulatory effect. Concerning IL-2 and IL-6, no comparable data are available in the literature. With respect to TNF, our results are in line with those of Sharp et al., who demonstrated an inhibitory effect of TNF on CRH-induced ACTH release by rat AL cells, but only after prolonged (8–24 h) and not after short-term (2 h) incubation.

In our study, incubation of NIL cells with IL-2 for 2 h induced a small but significant and dose-dependent increase in βE secretion, whereas the other cytokines tested were not effective in this respect. There are no data in literature regarding effects of cytokines on βE secretion by the NIL of the rat pituitary gland. Very recently, Stepien et al. demonstrated that IL-1β stimulated proliferation of rat intermediate lobe cells.

In the present study short-term (2 h) incubation of isolated adrenal cells with IL-1α, IL-1β, IL-2, IL-6 or TNF in concentrations up to 1 μg/ml had no effect on basal B release. Table 4 reviews the effects of rhIL-1 on B release by rat adrenal cells or tissue in vitro. Our results with respect to IL-1 are in agreement with the results of other studies. However, one of these studies did demonstrate a stimulatory effect of IL-1 on B secretion by rat adrenal slices after 24 h of incubation. Some of these researchers found evidence for an adrenergic or prostaglandin-dependent mechanism by which IL-1 stimulated the adrenal cells to release B. Our results concerning TNF are in line with those of Brennan et al., who found no effect of rhTNF on basal secretion of B by adrenal cells during short-term (90 min) incubation. Regarding IL-2, only prolonged (24 h) incubation experiments have been performed. In this study it was found that rhIL-2 had no effect on B release by rat adrenal cells, whereas rat IL-2 increased B release. With respect to rhIL-6 a stimulatory effect on B secretion by freshly isolated or cultured rat adrenal cells has been demonstrated after 24 h of incubation, though not after 3 or 12 h. In our study, IL-1 had no modulatory effect on ACTH-induced B release, which is in agreement with the results of a study performed by Andreis et al. We demonstrated in this study that neither IL-2 nor IL-6 had any modulatory effect on ACTH-induced B secretion. Other researchers have demonstrated that rhIL-2 and rhIL-6 increased ACTH-induced B release by rat adrenal cells after 24 h of incubation. In the present study it is shown that TNF dose-dependently decreased ACTH-induced B secretion during 2 h of incubation.

Such an inhibitory effect was also demonstrated by Brennan et al.

Since paracrine effects can be missed in a cell suspension system, the present study also describes the effects of superfusion of AL, NIL and adrenal tissue with cytokines on the release of βE-IR and B. We demonstrated that superfusion of rat AL or NIL tissue with 10 respectively 100 ng/ml of IL-1α, IL-1β, IL-2, IL-6 or TNF for 30 min had no significant effect on the secretion of βE-IR. There are no other reports on effects of cytokines on the secretion of βE-IR by the NIL using the superfusion technique. With respect to the AL, our results concerning IL-1 are in line with those of Parsadaniantz et al., who showed that neither rhIL-1α nor rhIL-1β was able to modify basal or CRH-induced ACTH and βE release after 50 min of perfusion of rat anterior pituitary tissue, and are in contrast with those of Beach et al., who demonstrated that perfusion of rat anterior pituitary tissue with IL-1 for 20 min induced a dose-dependent increase in ACTH release. With respect of IL-6 and TNF, our results conflict with those of other investigators, who found a stimulatory effect of rhIL-6 and rhTNF on ACTH release by rat anterior pituitary tissue during 2 h of incubation. However, these researchers performed static incubation experiments using hemi-(anterior) pituitaries.

In our experiments, superfusion of rat adrenal tissue with IL-1α, IL-1β, IL-2, IL-6, or TNF for 60 min had no effect on basal B release. Our in vitro results are in line with in vivo data by Gwosdow et al., who demonstrated that plasma B levels were not increased after administration of IL-1β to hypophysectomized rats, implying that the cytokine had no detectable direct effect on the adrenal gland. On the contrary, Roh et al. found that acute administration of IL-1β into the renal artery of dissected and perfused rat adrenal glands increased adrenocortical activity as measured by increased output of B. It has to be noted, however, that these authors demonstrated stimulation of B secretion after a bolus injection in the renal artery of a high dose (3.5 μg) of IL-1. In our study, prolonged superfusion of adrenal tissue for 6 h with medium containing IL-1 significantly increased B secretion as compared to superfusion of adrenal tissue with only medium, whereas IL-2 and TNF were not effective in this respect. ACTH-induced B release was not significantly affected after prolonged superfusion of adrenal tissue with IL-1 or IL-2. Prolonged superfusion with TNF, however, seems to inhibit ACTH-induced B release. This result is in agreement with the data on simultaneous incubation of isolated rat adrenal cells with both TNF and ACTH (this study). In this respect, it is of interest that Keri et al. demonstrated that plasma from patients with septic shock contains (a) factor(s) which attenuate the responsiveness of adrenocortical cells to ACTH and that Mathison et al. observed that lipopolysaccharide
(LPS)-stimulated macrophages release a product that suppresses the steroidogenic response of adrenocortical cells to ACTH. We now know that one of the substances, produced and secreted by macrophages in response to LPS, is TNF. So the observations of Keri et al. and Mathison et al. might be mediated by TNF.

In summary, the present study shows: (1) prolonged superfusion (6 h) of rat adrenal tissue with IL-1 increased basal B secretion; (2) short-term (2 h) incubation with IL-2 increased CRH-stimulated B secretion by AL cells and basal B secretion by NIL cells; (3) short-term (2 h) incubation of adrenal cells with TNF inhibited ACTH-induced B release and prolonged (6 h) superfusion of adrenal tissue with TNF also reduced ACTH-induced B release; (4) in all other experiments IL-1α, IL-1β, IL-2, IL-6 and TNF did not modify basal or CRH-induced secretion of B and basal or ACTH-induced secretion of B. It has to be stressed, however, that the effects of cytokines on hormone secretion by the pituitary or adrenal gland were small and only found during incubation with high doses of cytokines and/or prolonged incubation. Our data make it unlikely that the acute elevation of plasma ACTH and B levels, seen after bolus injections of these cytokines in vivo, are mediated by a direct action of these cytokines on the pituitary and/or adrenal gland.

MATERIALS AND METHODS

Test materials

Human adrenocorticotropic hormone (ACTH) was obtained from the National Institute for Biological Standards and Control (NIBSC, Potters Bar, UK; MRC standard 74/555). Rat corticotropin-releasing hormone (CRH) was obtained from Byk (Zwanenburg, The Netherlands) and isoprotrenol (isoprenalini sulfas) was obtained from OPG (Utrecht, The Netherlands). Dopamine HCl was obtained from Sigma Chemical Company (St. Louis, MO). Recombinant human interleukin 1α (IL-1α; specific activity (S.A.) 3.10^8 U/mg protein, endotoxin contamination (E.C.) <1 endotoxin unit/mg protein) was kindly provided by Dr. P. Loomedic (Hoffman-La Roche, Nutley, NJ), recombinant human interleukin-1β (IL-1β; S.A. 2.5.10^7 U/mg protein, E.C. <1.2 ng/mg protein) was kindly provided by Dr. A. Shaw (Biogen, Geneva, Switzerland), recombinant human tumour necrosis factor-α (TNF; S.A. 6.10^7 U/mg protein, E.C. <1 endotoxin unit/mg protein) was obtained from Boehringer (Ingelheim, Germany), recombinant human interleukin 2 (IL-2; S.A. 1.5.10^9 U/mg protein, E.C. <7 endotoxin units/mg protein) was kindly provided by Dr. A. Galazka (Glaxo, Geneva, Switzerland) and recombinant human interleukin 6 (IL-6; S.A. 1.0.10^6 U/mg protein, E.C. <1.2 ng/mg proin) was generously donated by Dr. L. Aarden (Central Laboratory of the Blood Transfusion Service of The Netherlands Red Cross, Amsterdam, The Netherlands). All chemicals used were of analytical grade.

Animals

Male albino Wistar rats (Cpb: WU) weighing 180–240 g were obtained from the local breeding facility on the day before the experiment. The animals were then housed overnight, four to a cage, in a room adjacent to the animal home in which the decapitations were to be performed. In all experiments the rats were killed by decapitation between 0930 and 1030 h.

Cell suspension experiments

Pituitary cells. Immediately after decapitation the skull was opened, the brain taken out and the pituitary quickly removed from the cranium. The anterior lobe (AL) and the neurointermediate lobe (NIL) were carefully separated from each other and the AL was cut in about 8–10 pieces. AL and NIL tissues were separately placed in a flask filled with 10 ml Earle’s Balanced Salt Solution (EBSS, 116 mM NaCl, 5.36 mM KCl, 1.15 mM NaH₂PO₄, 2H₂O, 0.81 mM MgSO₄·7H₂O, 26 mM NaHCO₃, 1.79 mM CaCl₂·2H₂O and 5.0 mM glucose. H₂O) containing benzylpenicillin (15 μg/ml; Sigma), streptomycin (25 μg/ml; Merck, Darmstadt, Germany), ascorbic acid (30 μg/ml; Merck) and 0.25% (w/v) trypsin (type II; Sigma). The cells were dispersed at 37°C by mild mechanical agitation in a shaking waterbath. During dispersion the cells were gassed with carbogen (95% O₂/5% CO₂). After 20 min incubation the supernatant was decanted into a 10 ml plastic centrifuge tube and retained at room temperature. A further 10 ml of the EBSS/trypsin solution was added to the pituitary fragments. A total of 3 × 20 min cell harvests were collected. The supernatants were pooled and centrifuged at room temperature for 40 min (200 × g). The pellet, consisting of isolated cells, was resuspended and diluted in EBSS buffer containing bovine serum albumin (BSA, 0.25% (w/v); OHRD 20/21, Hoechst-Behring, Marburg, Germany), ascorbic acid (30 μg/ml), the antibiotics, aprotinin (100 KIE/ml; Bayer AG, Leverkusen, Germany), bacitracin (100 μg/ml; Sigma) and heparin (30 μg/ml), the antibiotics, aprotinin (100 KIE/ml; Bayer AG, Leverkusen, Germany), bacitracin (100 μg/ml; Sigma) and lima bean trypsin inhibitor (66 μg/ml; type I-S, Sigma) (EBSS') to a concentration of about 50 000 cells per 800 μl medium. Cell suspensions were preincubated for 90 min at 37°C, and subsequently 200 μl of EBSS' solution containing CRH (5.10⁻¹⁰ M), cytokine (IL-1α, IL-1β, IL-2, IL-6, or TNF; 1 μg/ml-1 ng/ml) or CRH in combination with cytokines, was added. Incubation was continued for another 120 min. NIL cells were only incubated with cytokines (IL-1α, IL-1β, IL-2, IL-6, or TNF; 1 pg/ml-1 μg/ml) or CRH in combination with cytokines, was added. Incubation was terminated by cooling on ice and the supernatant was subsequently collected by centrifugation (15 min, 1000 × g) and the medium assayed for βE-IR within 24 h.

Adrenal cells. Rat adrenal cells were isolated and incubated according to a method extensively described by Goverde et al. Isolated rat adrenal cells were incubated for 2 h with different peptides (ACTH 25 pg/ml; cytokines 1 pg/ml-1 μg/ml; ACTH and cytokines) dissolved in Krebs-Ringer-Bicarbonate buffer containing 0.2% glucose, 0.5% BSA and 7.6 mM Ca²⁺ (KRBBGACa). Incubation was stopped by cooling on ice and all samples were stored at 4°C. Corticosterone production was measured the next day.
Cell viability. After preparing the cell suspensions, cell viability was tested by mixing an aliquot of the cell suspension with an equal volume of trypan blue (BDH, 0.1% (w/v) in 0.9% NaCl). In all experiments, more than 95% of the cells excluded the dye. Responsiveness of the cells was tested by incubation of AL cells with CRH (5.10^-10 M), NIL cells with dopamine (10^-8 M) and adrenal cells with ACTH (25 pg/ml).

Superfusion experiments

Pituitary tissue. Dissected ALs (quartered) and NILs (intact) were transferred to a superfusion apparatus (2 ALs or 2 NILs per chamber). The tissues were superfused continuously with carbogenated KRB-buffer (118 mM NaCl, 4.85 mM KCl, 1.15 mM KH2PO4, 1.15 mM MgSO4.7H2O, 25 mM NaHCO3 and 1.25 mM CaCl2.2H2O) supplemented with BSA (0.5%; Sigma fraction V), ascorbic acid (0.1 mM) and glucose (1.1 mM) (medium). The flow rate was kept constant at 0.1 ml/min using a Gilson Mini-puls 3 peristaltic pump (Meyvis, Bergen Op Zoom, The Netherlands). The temperature of the superfusion chambers and media was kept constant at 37°C. In these experiments, the tissues were first superfused with medium for 180 min to allow the release of BE-IR to reach a rather stable level. Thereafter superfusion was continued for 240 min and fractions (fr.) were collected in ice-chilled tubes every 10 min. Pituitary tissues were superfused twice for 30 min (1st pulse: fr. 7-9, 2nd pulse: fr. 17-19) with medium containing cytokines (IL-1α, IL-1β, IL-2, IL-6 or TNF; 1st pulse: 10 ng/ml; 2nd pulse: 100 ng/ml), CRH (ALs: 1st pulse: 5.10^-10 M; 2nd pulse: 5.10^-9 M) or isoproterenol (NILs: 1st pulse: 10^-7 M; 2nd pulse: 10^-6 M). 100 μl aliquots of the superfusates were taken for determination of BE-IR.

Adrenal tissue. Dissected adrenals were freed of fat, cut in about 16 pieces and transferred to a superfusion apparatus (1 adrenal/chamber). The adrenal tissue was first superfused for 30 min with medium to allow the release of BE-IR to reach a rather stable level. From then on 15 min fractions were collected in ice-chilled tubes for 5.5 h. During this period the adrenal tissue was superfused for 1 h (fr. 11-14) with medium containing cytokines (IL-1α, IL-1β, IL-2, IL-6 or TNF; 100 ng/ml) or ACTH (125 pg/ml). In order to investigate whether prolonged exposure of adrenal tissue to cytokines had any effect on basal or ACTH-induced B release, rat adrenal tissues were superfused in other experiments for 6 h with medium containing 100 ng/ml of IL-1β, IL-2 or TNF and later for 30 min with 250 pg/ml ACTH. Control tissues were superfused continuously with medium alone. During these prolonged adrenal superfusion experiments medium was supplemented with benzylpenicillin (100 μg/ml), streptomycin (100 μg/ml), aprotinin (20 KIE/ml) and bacitracin (30 μg/ml) (medium'). The flow rate was kept constant at 2 ml/h. B release was allowed to reach rather stable levels to 2 h and fractions were collected every 2 h for 28 h. Adrenal tissue was superfused for 6 h (fr. 4-6) with medium alone or with medium' containing 100 ng/ml of IL-1β, IL-2 or TNF. After 28 h of superfusion the responsiveness of the adrenal tissue was tested by superfusion for 30 min with medium' containing 250 pg/ml ACTH. From then on 30 min fractions were collected for 3.5 h. Total superfusate fractions were taken for B determination.

Hormone measurement

β-endorphin-like-immunoreactivity (βE-IR) was measured by radioimmunoassay (RIA) as described by Sweep et al. using an antiserum kindly provided by Dr V. M. Wiegant, Rudolf Magnus Institute (Utrecht, The Netherlands). B was measured fluorometrically, as described by Goverde et al.

Calculations and statistical analysis

Cell suspension experiments. βE-IR and B release is expressed as percentage of basal hormone release by AL and NIL cells incubated with EBSS solution (control) respectively by adrenal cells incubated with KRBGACa buffer (control). In case of incubation of adrenal cells with cytokines in combination with ACTH (25 pg/ml), B release is expressed as percentage of the release during incubation of cells with ACTH only. Data were statistically analysed for a dose-response relationship using a linear regression model. This model uses the logarithm of the dose of the used peptide.

\[
\% \text{ release} = \alpha_0 + \alpha_1 (\text{CRH}) + \alpha_2 (\log \text{cytokine}) + \alpha_3 (\text{CRH})(\log \text{cytokine})
\]

Here \( \alpha_0 \) is an estimate of the effect of incubation of AL or NIL cells with EBSS solution respectively incubation of adrenal cells with KRBGACa buffer or an estimate of the effect of incubation of adrenal cells with 25 pg/ml ACTH only. \( \alpha_1 \) is an estimate of the effect of incubation of AL cells with CRH alone. \( \alpha_2 \) is an estimate of the dose-response relationship for the effects of incubation of AL, NIL or adrenal cells with cytokines only or for the effects of incubation or adrenal cells with cytokines in combination with ACTH. \( \alpha_3 \) is an estimate of the dose-response relationship for the effects of incubation of AL cells with cytokines in combination with CRH. Testing for a significant dose-response implies testing whether the corresponding \( \alpha_3 \)'s are equal to zero. By way of precaution, differences are considered to be statistically significant if \( p < 0.01 \).

Superfusion experiments. The spontaneous rate of release of BE-IR and B was not identical for the different AL, NIL or adrenal tissues within the same experiments. To compare results between the superfusion chambers, the total amount of drug-induced BE-IR (AL and NIL): fr. 7-13 and fr. 17-23) or B (adrenal: fr. 12-21; prolonged adrenal superfusion: fr. 15-20 (28.5-31 h)) release was summed and expressed as percentage of the calculated basal release. The area under the line linking the means of the BE-IR or B content of the two fractions immediately preceding (BE-IR (AL and NIL): 1st pulse: fr. 5-6, 2nd pulse: fr. 15-16; B: adrenal: fr. 10-11; B (prolonged adrenal superfusion): 2nd pulse: fr. 13-14 (26-28 h)) and the first one or two fractions in which stabilization of the release to basal levels had occurred (BE-IR (AL and NIL): 1st pulse: fr. 14-15, 2nd pulse: fr. 24; B (adrenal); fr. 22; B (prolonged adrenal superfusion): 2nd pulse: fr. 21 (31.5 h)) was taken as basal release. Data concerning differences between the drug-induced hormone release and the computed basal release were statistically analysed by a paired t-test after logarithmic transformation of the doses. By way of precaution, differences are considered to be statistically significant if \( p < 0.01 \).
Acknowledgements

The authors wish to thank Mr G. Grutters and Mr H. Eikholt (Central Animal Laboratory Nijmegen, The Netherlands) for biotechnical assistance. Dr G. Borm (Dept. of Medical Statistics) is acknowledged for performing statistical analysis of the data. This work was supported by the Royal Netherlands Academy of Arts and Sciences.

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