ANTIPYRETIC ACTION OF DEXAMETHASONE ON EGTAZIC ACID INDUCED FEVER IN RABBITS

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ABSTRACT The purpose of the present study was to investigate whether the antipyretic effect of dexamethasone (DEX) delivered by intravenous injection (iv) on intracerebroventricularly (icv) administered egtazic acid2induced febrile response is relevant to the changes in cytosolic free calcium concentration of the hypothalamus. The colon temperatures were measured by a thermistor and the cytosolic free calcium concentration ([Ca2+]i) in dissociated brain cells was measured by Fura 2AM. The results demonstrated that the pyretic action of egtazic acid (0164mol, icv) was markedly inhibited by DEX (5 mg/ kg, iv), but DEX (60 ~ 120μmol/L) did not affect [Ca2+]i in dissociated hypothalamus cells. Actinomycin D, which interferes with gene transcription (3 nmol, icv), completely abolished the antipyretic action of DEX on egtazic acid2induced fever. These findings suggest that the antipyretic action of DEX on egtazic acid2induced fever is related to the activation of certain gene expression in the brain but not to the changes of transmembrane calcium ion current in hypothalamus neurons.

Key words: fever; egtazic acid; glucocorticoid; actinomycin D; calcium; dexamethasone

It is well established that glucocorticoids have an antipyretic action on fever induced by a variety of pyrogens[1][3]. However, cellular and molecular mechanisms by which glucocorticoids inhibit fever have not been precisely defined. Although reduction of fever by glucocorticoids has generally been attributed to inhibition of cytokine and prostaglandin (PG) synthesis[2][5], the antipyretic effects of these glucocorticoids on the responses to PGF2α and interleukin28 (IL28)[3,6] and IL28 induced fever is independent of the action of PGs[7]. It has been demonstrated that corticotropin releasing hormone (CRH) mediates the febrile responses to IL26, IL28, IL2β and PGF2α[8] and glucocorticoids effectively inhibit the synthesis and release of CRH[9], so that antipyretic action of glucocorticoid may result from inhibition of synthesis and/or release of CRH[3]. However, this hypothesis is unable to explain why lipocortin, which mediates many of the glucocorticoid actions, significantly attenuates the pyrogenic effect of CRH[7]. Furthermore, Coelho et al. showed that dexamethasone (DEX) has a significant antipyretic action even when this glucocorticoid is administered 1 h after the pyrogenic stimulation[3].
It is inferred that other mechanisms may be involved in DEX antipyresis. Previous studies indicate that the ratio of Ca\(^{2+}\) and Na\(^{+}\) ions in the hypothalamus is the physiological basis for maintenance of the level of body temperature\(^{10}\), but the effect of DEX on egtazic acid\(^{2}\)induced fever has not been investigated. Therefore, the present study was undertaken to investigate the effect of DEX on egtazic acid\(^{2}\)induced fever and cytosolic free calcium concentration ([Ca\(^{2+}\)]\(_{i}\)) in dissociated hypothalamus cells in rabbits.

1 MATERIALS AND METHODS

11 Animals New Zealand white rabbits of both sexes weighing 215 ±0.15 kg were used. Animals were caged individually and had access to food and water ad libitum. To minimize the stress response caused by handling during experimentation, animals were confined in stocks at room temperature of 20\(^{\circ}\) 22\(^{\circ}\)C. At the same time, a catheter mimicking the thermistor probe was inserted 10 cm into the colon of the rabbit and fixed on the root of the tail for 6 h/d of 3 d.

11 Intracerebroventricular cannula At least 5 d before experimentation, guide cannulae were stereotaxically implanted into the lateral ventricle of rabbits anesthetized with pentobarbitone sodium (iv, 30 mg/kg) as previously described\(^{11}\). The lateral ventricle infusion was controlled by a syringe pump (HL22 Type, Shanghai HuXi Instruments Factory).

11 Body temperature measurement Colon temperatures were measured in lightly restrained rabbits by insertion of catheter-mounted thermistor probe 10 cm beyond the anal sphincter, the thermistors were fed into a telethermometer (ST21 Type, Shanghai Medical Instruments Factory). Temperature records were taken at 10 min intervals until the experiment was over.

11 Chemicals The artificial cerebrospinal fluid (ACSF) was prepared routinely.\(^{12}\) Ethylene glycol bis (2aminoethyl2ether) tetraacetic acid (Egtazic acid, Feinbiochemica Heidelberg, USA) was dissolved in sterile ACSF at a concentration of 4 mmol/L (pH 7.135). Dexamethasone 21phosphate disodium (DEX) was dissolved in sterile pyrogenfree 0.9% sodium chloride (normal saline, NS) for injection. Actinomycin D (AM) was dissolved in ACSF at a concentration of 0.013 mmol/L. Fura 2 acetoxyethyl (Fura 2AM), triton X2100, trypan blue, DEX and AM were purchased from Sigma Chemical.

11 Measurement of [Ca\(^{2+}\)]\(_{i}\) in dissociated hypothalamus cells The procedure was similar to that of previous studies\(^{12,13}\). Rabbits were decapitated rapidly, the hypothalamus was removed and placed in 10 ml sterile icecold HEPES buffered Hanks, pH 7.14, containing in mmol/L: HEPES 20, NaCl 137, KCl 5.0, KH\(_{2}\)PO\(_{4}\) 0.14, Na\(_{2}\)HPO\(_{4}\) 0.16, NaHCO\(_{3}\) 310, CaCl\(_{2}\) 113, MgSO\(_{4}\) 0.14, MgCl\(_{2}\) 0.15, and glucose 516. Meninges and blood vessels were meticulously removed. The hypothalamus was transferred to a glass beaker and minced. The minced tissue was placed into glass tubes containing 1 ml of 0.1125% trypsin in D2Hanks and incubated at 37 \(^{\circ}\)C for 20 min. After trypsinization, 1 ml of Dulbeco’s modified Eagle’s medium (DMEM, Gbco) supplemented with 10% fetal bovine serum was added to the tubes. The dissociated cells were collected with filters, and were washed with Hanks and then...
centrifuged at 1500 r/min for 5 min. Supernatants were decanted and the cells resuspended in a volume of DMEM to give a concentration of approximately $2 \times 10^9$ cells/L. The trypan blue exclusion showed more than 90% cellular viability, which was not altered when samples were tested at random for trypan blue staining at the end of an experiment. The cell suspensions with Fura 2 AM at a final concentration of 5μmol/L were kept in a humidified incubator with 95% air and 5% CO$_2$ at 37 °C for 50 min. The fluorescence spectrophotometer (RF25000, Japan) was used for fluorescence determinations. Basal and drug-stimulated peak values were obtained, 0.01% Triton X-100 was added to determine the maximum fluorescence, and the minimum fluorescence was calculated using 4 mmol/L egtazic acid.

116 Experimental protocols

11611 Determination of the effect of DEX on egtazic acid-induced fever in the rabbit. New Zealand white rabbits were divided into 3 groups, ACSF + NS, EGTA + NS and EGTA + DEX groups. 20 min after animals were infused intracerebroventricularly (icv) with 150μl of ACSF or egtazic acid (EGTA), NS (1 ml/kg) or DEX (5 g/L, 1 ml/kg) was injected via marginal vein.

11612 Examination of the effect of DEX on [Ca$^{2+}$] in dissociated hypothalamus cell.

11613 Determination of the effect of actinomycin D on DEX antipyresis. 15 min after the separate groups of animals were infused (icv) 150μl of ACSF or egtazic acid, rabbits were injected (icv) with 100μl of actinomycin D or ACSF, and DEX (5 mg/kg) or NS was given intravenously (iv) 5 min after treatment with actinomycin D or ACSF.

117 Procedures for prevention of contamination

The laboratory and all materials were routinely sterilized using the method previously described[11,12], experiments were performed under aseptic conditions.

118 Data analysis For statistical analysis of body temperature response, the febrile response was represented by the mean thermal response curve and the 6 h thermal response index (TRI$_6$). All values were expressed as $\bar{x} \pm s$. Statistical differences were determined by an unpaired student’s t test and differences were considered to be significant if $P < 0.05$.

2 RESULTS

As can be seen in Fig11 and Table 1, the ranges of colon temperature changes in the ACSF + NS group were less than 0.13 °C Infusion (icv) of egtazic acid caused a rapid rise in colon temperature compared with the ACSF group ($P < 0.001$). Rabbits treated with DEX (5 mg/kg, iv) 20 min after infusion of egtazic acid developed fever, which was significantly milder compared with rabbits treated with an equal volume of NS (iv).

Table 1 Thermal response index between 0 and 360 min (TRI$_6$) after infusion (icv) shown in Fig11

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TRI$_6$/ °C h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF + NS</td>
<td>5</td>
<td>0.143 ±0.039</td>
</tr>
<tr>
<td>EGTA + NS</td>
<td>8</td>
<td>0.9154 ±0.1175</td>
</tr>
<tr>
<td>EGTA + DEX</td>
<td>9</td>
<td>0.7152 ±0.1153</td>
</tr>
</tbody>
</table>

$P < 0.001$ vs ACSF + NS group. $P < 0.01$ vs EGTA + NS group. ($\bar{x} \pm s$)
Resting $[Ca^{2+}]_i$, in these dissociated hypothalamus cells was $14011 \pm 1410 \text{ nmol (} n = 6 \text{)}$, and 50 mmol KCl stimulated a rapid increase from $14011 \pm 1410 \text{ nmol}$ to $21010 \pm 1410 \text{ nmol}$. As shown in Fig.12, DEX did not induce any increase in $[Ca^{2+}]_i$ in dissociated hypothalamus cells.

No significant differences were found between $\Delta T_R$ of rabbits infused with actinomycin D and those with ACSF. Rabbits in EGTA + ACSF group developed fever, and actinomycin D (icv, 15 min after infusion of egtazic acid) did not markedly affect egtazic acid-induced fever (Table 2).

As demonstrated in Table 3, DEX (iv) markedly attenuated egtazic acid-induced fever. Actinomycin D (3 nmol, icv) administered 5 min before treatment with DEX completely abolished antipyretic action of DEX, actinomycin D took effect 225 min after icv administration. There was no significant difference between $\Delta T_R$ of EGTA + ACSF + NS group and that of EGTA + AM + DEX group.
Table 2. TR\(_i\) between 0 and 360 min of rabbits after infusion (icv) of ACSF, ACSF + AM, EGTA + ACSF and EGTA + AM respectively

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TR(_i)/ °C h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF</td>
<td>4</td>
<td>0158 ±0122</td>
</tr>
<tr>
<td>ACSF + AM</td>
<td>4</td>
<td>0176 ±0115(^3)</td>
</tr>
<tr>
<td>EGTA + ACSF</td>
<td>7</td>
<td>9105 ±1117</td>
</tr>
<tr>
<td>EGTA + AM</td>
<td>6</td>
<td>8154 ±0193(^3)</td>
</tr>
</tbody>
</table>

\(^3 P > 0105\) vs ACSF group.  \(^33 P > 0105\) vs EGTA + ACSF group.  \(( \bar{x} ± s )\).

Table 3. TR\(_i\) between 0 and 360 min of rabbits after infusion (icv) in EGTA + ACSF + NS, EGTA + AM + DEX and EGTA + ACSF + DEX groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TR(_i)/ °C h</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGTA + ACSF + NS</td>
<td>7</td>
<td>9184 ±1165(^3)</td>
</tr>
<tr>
<td>EGTA + AM + DEX</td>
<td>6</td>
<td>10127 ±2184(^3)</td>
</tr>
<tr>
<td>EGTA + ACSF + DEX</td>
<td>8</td>
<td>7131 ±1149</td>
</tr>
</tbody>
</table>

\(^3 P < 0105\) vs EGTA + ACSF + DEX group.  \(( \bar{x} ± s )\).

3 DISCUSSION

The hypothesis that the antipyretic effect of glucocorticoids results from inhibition of cytokine, PG and CRH synthesis can not explain an antipyretic action even after delayed treatment with glucocorticoids. Coelho et al. speculated that very rapid actions of glucocorticoids are probably the result not of the changes in the synthesis of protein, but rather of other mechanisms such as the proposed direct influence of glucocorticoids with membrane phospholipid, or changes in transmembrane ion currents\(^[3]\).

In the present work, it was first demonstrated that DEX markedly inhibited the febrile response induced by administration (icv) of egtazic acid. Although some studies suggested that the effect of Ca\(^{2+}\) ions on thermoregulation appears to be due to a nonselective depression of neuronal activity\(^[10]\), the results in our earlier study show that egtazic acid-induced decrease in concentration of cellular Ca\(^{2+}\) in the hypothalamus and the subsequent increase in hypothalamus cAMP content, which in turn alters the "setpoint", may be an important link in the pathogenesis of egtazic acid-induced fever\(^[12]\). Previous studies have shown that glucocorticoid binding specifically in the anterior hypothalamus acts to downregulate or inhibit endotoxin-induced fever\(^[14,15]\). Therefore, inhibition of egtazic acid-induced fever by DEX may be related to a direct action between DEX and neurons within the thermoregulatory center.

An increasing amount of evidence shows that some of the effects of steroids can not be explained by a genomic mode of action on the target cells\(^[16,17]\). Shao2Ying HUA and Yi2Zhang CHEN reported that glucocorticoid can hyperpolarize the membrane potential of guinea pig ganglion neurons through its neuronal membrane receptor in vitro with a latency of less than 2 min, and the steroid-induced hyperpolarization was accompanied by a change in the input resistance of the cell, indicating an involvement of some kinds of ion channels in the action of glucocorticoid\(^[17]\). However, the results presented in Fig12 demonstrate DEX did not influence \([ Ca^{2+} ]\), in the dissociated hypothalamus cells. Thus, the possibility that the suppression of egtazic acid-induced fever by DEX is ascribed to the changes in transmembrane calcium ion currents may be excluded. On the other hand, the antipyretic action of...
DEX on egtazic acid-induced fever took place 220 min after administration of DEX (Fig 11), thus it appears that the antipyretic action of DEX may be related to some genomic mechanisms. Furthermore, pretreatment (icv) with actinomycin D, which interferes with gene transcription, completely abolished the antipyretic action of DEX on egtazic acid-induced fever, and neither normal body temperature nor egtazic acid-induced fever were influenced by administration (icv) of actinomycin D. These results indicate that DEX inhibits centrally egtazic acid-induced fever via activating certain gene expressions.

In conclusion, the present results support the hypothesis that the antipyretic action of glucocorticoids on egtazic acid-induced fever is the result of its direct effect on the function of neurons within the brain via activating certain gene expressions, but may be independent of the changes in transmembrane calcium ion currents in hypothalamus neurons. Therefore, together with the data of the previous studies, it may be likely inferred that the antipyretic action of glucocorticoids on pyrogen-induced fever results from its direct action on the thermoregulatory center via gene transcription in addition to the inhibition of cytokine, PG and CRH synthesis.

REFERENCES


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Fura22

(dexamethasone, DEX)

(22

μmol/L)  

([Ca

2+

]i)

(016 nmol)  

(22

μmol/L)  

(60–120 μmol/L)  

([Ca

2+

]i)


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