Introduction

Calcium movement is an important factor in the activation of cells responsible for inflammation.\(^1\) Calcium does this by releasing the inflammatory mediators\(^2\)-\(^4\) or by the activation of the plasma membrane or intracellular enzymes.\(^5\) It has been reported that calcium activates the nitric oxide (NO) synthase enzyme,\(^5\) phospholipase A\(_2\) and phospholipase C. This results in the activation of the release of arachidonic acid, with resultant formation of prostaglandins, leukotrienes, and thromboxanes.

One-way to test the role of calcium in inflammation would be by preventing voltage-dependent calcium influx into cells using calcium channel blockers (CCB), e.g. verapamil and nifedipine. CCBs have been shown to possess non-cardiovascular effects. These drugs have an effect on smooth muscles and secretory cells in the gastrointestinal tract and kidney;\(^4\) they also prevent the action of neutrophils and lymphocytes \textit{in vitro} and increase the production and secretion of IL-6 and IL-8, \textit{in vitro}.\(^1\)

We have shown in our previous study, that these two CCBs inhibit the carrageenan-induced paw edema.\(^6\) It has been demonstrated that some of the CCBs can stimulate the hypothalamus-pituitary-adrenal (HPA) axis by acting on pituitary and hypothalamic levels.\(^7\) The present study was aimed to investigate the possible involvement of the HPA axis in the antiinflammatory action of CCBs.

Material and Methods

Animals

Male albino rats (200-250 g) allocated to 26 groups (eight in each) were used. The animals were allowed free access to food and water.

Drugs and solutions

Verapamil, nifedipine or ibuprofen (Rose Daro, Iran) were administered intraperitoneally (i.p.) just before the injection of 0.1 ml of 0.5% carrageenan (Sigma. Co. UK) or saline (0.1 ml) into the subplantar tissue of the hind paw.\(^7\) Nifedipine and ibuprofen were dissolved in ethanol and verapamil in saline. Verapamil and nifedipine were given in one low and one high...
dose (25 and 400 µg/kg) while ibuprofen was 12 mg/kg.6

Adrenalectomy

Bilateral adrenalectomy was performed through a dorsal incision under thiopental (40 mg/kg) anesthesia. After surgery, the rats were returned to their cages with free access to food and normal saline10 (instead of water). A control group was sham-operated with free access to food and water. One week was allowed for recovery from operative procedure.

Injection of anticitocorticotropin-releasing hormone (CRH)

A polyethylene cannula was placed in the right lateral ventricle under thiopental anesthesia for intracerebroventricular (i.c.v.) administration of corticotropin-releasing hormone receptor antagonist, α-helical corticotropin releasing factor (CRF) (AntiCRF; 9-41, Sigma Co. UK). AntiCRF was dissolved in sterile pyrogen-free water and injected at the dose of 20 µg/rat in volume of 10 µl (i.c.v.); the control animals were treated with 10 µl of sterile pyrogen-free water.11

Induction and measurement of the inflammation

Inflammatory edema was induced by subcutaneous injection of 0.1 ml of 0.5% carrageenan solution in the hind paw. The hind paw volume was measured by a plethysmometer, 4 h after carrageenan injection9 and the algebraic difference between the treated and untreated hind paw volumes was taken as the edema volume.

Another method, involving the spectrophotometry technique,12 was also used to measure the inflammation. Here Evans blue dye (Sigma, Co., UK) was injected (20 µg/kg, i.v.) to evaluate the rate of albumin leakage as an indicator of inflammation.

Statistical analysis

Data were expressed as mean±SEM. The results were analyzed by analysis of variance (ANOVA) followed by Tukey’s ‘t’ test or the Student's 't' test. The results were also consistent with the reports on the reduction of acute inflammatory model (skin inflammation). These effects are similar to the report of De Vries et al (1995), though they used different topical CCBs and a different inflammatory model (skin inflammation). The present results are also consistent with the reports on the reduction of acute pancreatitis.2

The possible mechanisms involved in the antiinflammatory activity of CCBs may be through (1) a reduction of the Ca2+ concentration in blood, causing a decrease in the vessel resistance, and consequent reduction of hydrostatic pressure in the capillaries, (2) inhibition of the release of pro-inflamma-

Table 1

Comparison of the effect of ibuprofen with different doses of verapamil and nifedipine on the volume of hind paw (ml) induced by carrageenan in intact, adrenalectomized and CRH antagonist-treated group of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Carrageenan</th>
<th>Sham</th>
<th>Verapamil</th>
<th>Vehicle</th>
<th>25 µg/kg</th>
<th>400 µg/kg</th>
<th>Nifedipine</th>
<th>25 µg/kg</th>
<th>400 µg/kg</th>
<th>Ibuprofen (12 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03±0.01</td>
<td>0.47±0.04</td>
<td>-</td>
<td>0.48±0.08</td>
<td>0.095±0.02</td>
<td>0.27±0.06</td>
<td>0.5±0.1</td>
<td>0.17±0.02</td>
<td>0.08±0.02</td>
<td>0.09±0.018</td>
<td>0.36±0.045</td>
</tr>
<tr>
<td>ADX</td>
<td>-</td>
<td>0.7±0.07</td>
<td>0.48±0.05</td>
<td>0.63±0.05</td>
<td>0.69±0.06</td>
<td>0.63±0.06</td>
<td>0.64±0.07</td>
<td>0.39±0.04</td>
<td>0.65±0.06</td>
<td>0.28±0.05</td>
<td>0.36±0.045</td>
</tr>
<tr>
<td>AntiCRF</td>
<td>-</td>
<td>0.86±0.05</td>
<td>0.54±0.06</td>
<td>0.7±0.1</td>
<td>0.78±0.05</td>
<td>0.81±0.07</td>
<td>0.75±0.06</td>
<td>0.7±0.06</td>
<td>0.54±0.08</td>
<td>0.36±0.045</td>
<td></td>
</tr>
</tbody>
</table>

*p<0.01, *p<0.001 and *p<0.001 in control (intact) rats: significant difference between 25 µg/kg of nifedipine with 25 µg/kg verapamil, 400 µg/kg nifedipine with 400 µg/kg verapamil, and 12 mg/kg ibuprofen with 25 µg/kg of nifedipine and 400 µg/kg verapamil respectively. ADX rats: *p<0.05 significant difference between 25 µg/kg of nifedipine with carrageenan, 25 µg/kg verapamil, sham; 400 µg/kg nifedipine and verapamil, *p<0.01 significant difference between ibuprofen with both doses of verapamil and nifedipine, CRH antagonist-administered rats; *p<0.001 significant difference between 400 µg/kg of nifedipine with carrageenan, sham, either doses of verapamil and 25 µg/kg of nifedipine. *p<0.001 significant difference between ibuprofen with carrageenan, sham, both doses of verapamil and 25 µg/kg of nifedipine. Data represents mean±SEM (n=8 per group).
Table 2
Comparison of the effect of ibuprofen with different doses of verapamil and nifedipine on the content of Evans blue dye (µg/100 mg tissue) induced by carrageenin in intact, adrenalectomized and CRH antagonist-treated group of rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>Carrageenan</th>
<th>Sham</th>
<th>Verapamil</th>
<th>Nifedipine</th>
<th>Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>25 µg/kg</td>
<td>400 µg/kg</td>
<td>Vehicle</td>
<td>25 µg/kg</td>
<td>400 µg/kg</td>
</tr>
<tr>
<td>Control</td>
<td>3.3±0.6</td>
<td>7.3±0.23</td>
<td>-</td>
<td>7.6±0.3</td>
<td>6.9±0.4</td>
<td>8±0.06</td>
</tr>
<tr>
<td>ADX</td>
<td>-</td>
<td>5.9±0.74</td>
<td>6.4±1</td>
<td>6.2±0.7</td>
<td>4.4±0.8</td>
<td>4.6±0.28</td>
</tr>
<tr>
<td>AntiCRF</td>
<td>-</td>
<td>5.24±0.58</td>
<td>6.1±0.7</td>
<td>6.5±0.5</td>
<td>5.7±0.5</td>
<td>4.6±0.5</td>
</tr>
</tbody>
</table>

abP<0.001 and abP<0.01 in control (intact) rats: significant difference between 400 µg/kg of nifedipine with 25 µg/kg nifedipine, both doses of verapamil and carrageenan, and ibuprofen with both doses of verapamil, 25 µg/kg nifedipine and carrageenan respectively. cP<0.05 significant difference between 25 µg/kg of nifedipine with carrageenan in ADX rats. dP<0.05 significant difference between 400 µg/kg of nifedipine with carrageenan in CRH antagonist administered rats.

Data represents means±SEM (n=8 per group).

Antiedema action of calcium channel blockers

The Evans blue content of the inflamed paw decreased to 58% by ibuprofen. This was significantly more compared to both the doses of verapamil and 25 µg/kg of nifedipine. Adrenalectomy and antiCRF treatment of rats resulted in the elimination of the inhibitory effect of ibuprofen on the dye extravasation. It can be claimed that ibuprofen needs an intact HPA for its action. The effects of CCBs on Evans blue content showed that only nifedipine is effective, whereas the water content in control, ADX and antiCRF groups decreased, by 52%, 41% and 37% by nifedipine respectively. These findings suggest that verapamil may increase vascular permeability to proteins through secretion of interleukins13 or CCBs (verapamil and nifedipine), like other antiinflammatory drugs, may effect on protein and fluid leakage via different mechanisms involving arachidonic acid metabolites.18

The present study has shown that nifedipine and verapamil reduced acute carrageenan-induced paw edema in the rats. It is probable that the HPA axis mediates the antiinflammatory effects of verapamil. However, it is likely that the antiedema effect of nifedipine may involve both peripheral and HPA mechanisms. Further study to determine the relevance of this finding in humans should be undertaken.

Acknowledgements

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References

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