Calcium channel blockers reduce inflammatory edema in the rat: Involvement of the hypothalamus-pituitary-adrenal axis

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ABSTRACT

Objective: To evaluate the role of calcium channel blockers and their mechanisms of action on acute inflammation of rat paw.

Material and Methods: The study was conducted using carrageenan-induced rat paw inflammation model. Two different doses of nifedipine and verapamil (25 and 400 µg/kg, i.p.) were used. Edema was assessed by calculating the volume changes and by extravasation of Evans blue dye.

Results: Nifedipine reduced edema dose-dependently, whereas verapamil was effective only at low dose. Adrenalectomy prevented the effect of nifedipine and verapamil. With low dose of nifedipine 66% of antiinflammatory effect was observed. Pretreatment with \( \alpha \)-helical corticotropin releasing factor (CRF 9-41), a corticotropin-releasing hormone (CRH) receptor antagonist, had the same effect as that of adrenalectomy for either doses of verapamil, but only the effect of low-dose nifedipine was prevented completely.

Conclusion: Our data suggest that verapamil and nifedipine exerts a potent antiinflammatory action possibly through pituitary adrenocortical activation.

KEY WORDS: Acute inflammation, adrenalectomy, carrageenan

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 \( \text{in vitro} \) and increase the production and secretion of IL-6 and IL-8, \( \text{in vitro} \). \(^1\)

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. \(^7-8\) 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. \(^9\) 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.

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 saline (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 anticomitin-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.

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 injection and the algebraic difference between the treated and untreated hind paw volumes was taken as the edema volume.

Another method, involving the spectrophotometry technique, 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 test or the Student’s ‘t’ test. The results were considered statistically significant if P<0.05.

Results

Carrageenan injection increased the hind paw volume compared to normal group (0.47±0.04 Vs 0.03±0.01) and both CCBs caused a significant reduction in inflammatory edema (P<0.01). The effect of nifedipine was dose-dependent, nifedipine (400 μg/kg) and verapamil (25 μg/kg) had the same effect as ibuprofen (12 mg/kg) (Table 1).

As shown in Table 1, adrenalectomy blocked the antiedema effect of verapamil in both doses and of nifedipine (only 400 μg/kg), but the administration of a dose of 25 μg/kg nifedipine and 12 mg/kg ibuprofen caused significant inhibition of inflammation (P<0.01 and P<0.001 respectively). Table 1 also shows that pretreatment with CRH receptor antagonist blocked the antiedema effect of verapamil but not of nifedipine (400 μg/kg), and ibuprofen (12 mg/kg), i.e. they reduced the hind paw edema significantly (P<0.05 and P<0.01 respectively) in spite of pretreatment with CRH receptor antagonist. Measurement of Evans blue in the hind paw tissue showed that in an intact animal the dye in nifedipine (400 μg/kg) and ibuprofen (12 mg/kg) groups was significantly lower in content than in the carrageenan (untreated) groups (P<0.001) (Table 2).

In the adrenalectomized (ADX) animals, only with 25 μg/kg nifedipine, the dye content was significantly decreased compared to the carrageenan group (P<0.05) (Table 2).

In the group which received CRH receptor antagonist plus nifedipine (400 μg/kg), the content of Evans blue dye was significantly decreased compared to the carrageenan group (P<0.05) (Table 2).

Discussion

The results of the present study indicate that CCBs (verapamil and nifedipine), that selectively block L-type calcium channel, exerted a potent antiedema effect in rat paw. 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.

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>Nifedipine</th>
<th>Ibuprofen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Vehicle</td>
<td>25 μg/kg</td>
<td>400 μg/kg</td>
</tr>
<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>
</tr>
<tr>
<td>ADX</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>
</tr>
<tr>
<td>AntiCRF</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>
</tr>
</tbody>
</table>

*P<0.01, **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<0.001 significant difference between ibuprofen with both doses of verapamil and 400 μg/kg of nifedipine. CRH antagonist-administered rats: **P<0.05 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 means±SEM (n=8 per group).
tory mediators, leading to inhibition of the activity of PLA₂ and/or PLC, the enzymes responsible for the synthesis of eicosanoids and leukotrienes, and stabilization of the cell membrane integrity (by inhibiting Ca²⁺ influx), thus preventing tissue injury and inflammation. To find out the possible mechanism(s) of the antiinflammatory activity of CCBs, we studied the effect of verapamil and nifedipine in adrenalectomized, and CRH receptor antagonist pretreated rats. The observations from both experiments showed that CCBs inhibit inflammation. Both CCBs can activate the HPA axis. The activation of the HPA axis by verapamil can explain the antiinflammatory effect of this drug, but the inhibition of the HPA axis only tended to antagonize the effect of nifedipine. We cannot explain the discrepancy of results between nifedipine and verapamil. So we suggest an involvement of the hypothamic-pituitary-adrenal (HPA) axis in the action of these drugs.

It is conceivable that since these CCBs have diverse molecular structures, they have different modes and sites of action in the calcium system. The difference between the effects of these CCBs has also been reported by other researchers who stated that verapamil has predominant effect on the heart and nifedipine on the vessels. It has also been demonstrated that there is a significant suppressive effect on skin sensitivity by these CCBs. The dose-dependent suppressive effect was seen with nifedipine but not with verapamil. It has been reported that nicotine enhances the nifedipine-induced analgesia. Rodler et al have reported that verapamil can increase the inflammatory cytokines like IL-6 in a high dose but not in a low dose.

Ibuprofen (positive control for antiinflammatory study) inhibited the increase of paw volume by up to 83%, and the antiinflammatory effect of verapamil (25 µg/kg) and nifedipine (400 µg/kg), was similar to ibuprofen. Reduction of the efficacy of ibuprofen on reducing the paw volume in adrenalectomized and antiCRF treated rats, suggest participation of the adrenal gland and CRF receptors in the antiinflammatory activity of ibuprofen.

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 interleukins or CCBs (verapamil and nifedipine), like other antiinflammatory drugs, may effect on protein and fluid leakage via different mechanisms involving arachidonic acid metabolites.

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 antiinflammatory 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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**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 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 Vehicle 25 µg/kg</th>
<th>400 µg/kg</th>
<th>Nifedipine Vehicle 25 µg/kg</th>
<th>400 µg/kg</th>
<th>Ibuprofen (12 mg/kg)</th>
</tr>
</thead>
<tbody>
<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>
<td>7.2±0.4</td>
<td>5.5±0.07</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>
<td>6.5±1.01</td>
<td>3.5±0.12</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>
<td>5.7±4.0</td>
<td>6.1±0.5</td>
</tr>
</tbody>
</table>

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

**Notes:**

- P<0.001 and P<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.

- P<0.05 significant difference between 400 µg/kg of nifedipine with carrageenan in ADX rats.

- P<0.05 significant difference between 400 µg/kg of nifedipine with carrageenan in CRH antagonist administered rats.

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