Involvement of Potassium Channels in the Release of Glucocorticoids

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The present investigation was undertaken to study the effect of various potassium channel modulators on release of glucocorticoids in dogs. Single dose intravenous administration of pinacidil and cromakalim produced significant fall in serum cortisol levels. KRN2391 and nicorandil did not produce any significant change in serum cortisol levels in dogs. Intravenous administration of synthetic adrenocorticotropic hormone (ACTH) produced a significant rise in cortisol levels. Pinacidil, cromakalim and KRN2391 prevented ACTH-induced rise in cortisol levels in dogs. Gilbenclamide neither significantly altered cortisol levels nor produced any change in ACTH-induced rise in cortisol levels. In conclusion, our data provide an evidence for the involvement of potassium channels in the release of glucocorticoids. It is possible that differential effects of potassium channel openers on glucocorticoid levels is due to diverse subtypes of potassium channels.

Secretion of hormones from endocrine gland is regulated by calcium-dependent mechanisms. Intracellular Ca\textsuperscript{2+} concentration is the key internal regulator in the secretion of insulin\textsuperscript{1} and also for other hormones. Calcium channel blockers can reduce endocrine hormone release\textsuperscript{2}. Verapamil, an agent that inhibits Ca\textsuperscript{2+} current, inhibited the K\textsuperscript{+} and ouabain-induced release of adrenocorticotropic hormone (ACTH), growth hormone (GH) and thyroid stimulating hormone (TSH) but had no effect on the release of these hormones by secretagogues in incubated rat pituitary tissues\textsuperscript{3}. Hyperpolarization induced by potassium channel openers (PCOs) prevents Ca\textsuperscript{2+} entry through voltage operated channels and reduces agonist-induced accumulation of inositol-1,4,5-trisphosphate (IP\textsubscript{3}) and consequently Ca\textsuperscript{2+} mobilization from intracellular stores. PCOs can thus act as indirect Ca\textsuperscript{2+} entry blockers and be expected to have a pharmacological profile similar to that of the Ca\textsuperscript{2+} antagonists\textsuperscript{4}.

Soro et al.\textsuperscript{4} reported that in a large group of patients with essential hypertension, the excretion rates of each of major metabolite of corticosterone are higher than in normal control subjects. Whitworth et al.\textsuperscript{5} suggested that relative or local cortisol excess might be responsible for hypertension. In the light of these observations, the objective of the present investigation was to study the effect of short term treatment of potassium channel modulators on glucocorticoids in dogs.

MATERIALS AND METHODS

Mongrel dogs (12-20 kg) of either sex were used for the study. The animals were housed at ambient temperature (21±1\degree) and relative humidity (55±5\%) with fixed 12 h light/dark cycles and free access to food and water. At least 7 d were allowed for adaptation before the animal were used in the experiments. Necessary approval was obtained from the departmental ethics committee on animal experimentation. The mongrel dogs were procured from the refuge department, Ahmedabad Municipal Corporation, Ahmedabad.

Dogs were divided into six groups of five animals each. Group I serving as control received saline. Group I to V were treated with pinacidil, cromakalim, KRN2391 or nicorandil (40 \mu g/kg, i.v.) respectively. Group VI was treated with gilibenclamide (2 mg/kg, i.v.). Dogs were anaesthetized with chloralose (60 mg/kg, i.v.) and 30 min after post anaesthesia, the drugs were administered through saphenous vein.
Blood samples were collected at 0h i.e. before treatment and 1, 2, 3 and 6 h after drug treatment from saphenous vein. Serum was separated and analysed for cortisol levels using the DSL-2000 cortisol radioimmunoassay kit.

In another set of experiments, dogs were anaesthetised with chloralose (60 mg/kg, i.v.) and 30 min later drugs were administered as mentioned above. Fifteen minutes after drug administration, synthetic ACTH (Synacthen, 2 μg/kg, i.v.) was administered and blood samples were collected at 0, 5, 10, 15, 20 and 30 min. Serum was separated and serum cortisol levels were estimated using the DSL-2000 cortisol radioimmunoassay kit. The dogs were rehabilitated after the experiments were completed and returned to the Refuge Department, AMC, Ahmedabad.

Statistical analysis:

Results were analyzed using analysis of variance followed by Tukey's Test. An alpha level of 5% was taken as the level of statistical significance.

RESULTS

Single intravenous dose of pinacidil, cromakalim, produced a significant fall in serum cortisol levels observed upto 6 h, whereas, glibenclamide did not produce any significant change in serum cortisol levels when compared with control (Table 1).

Intravenous administration of single dose of synthetic ACTH (2 μg/kg) in dogs produced a significant rise in serum cortisol levels (Table 2). ACTH-induced rise in serum cortisol levels was significantly prevented by single intravenous dose of pinacidil, cromakalim and KRN2391. Whereas, nicorandil and glibenclamide did not produce any change in ACTH-induced rise in serum cortisol levels (Table 2).

**TABLE 1: EFFECT OF POTASSIUM CHANNEL MODULATORS ON CORTISOL LEVELS IN DOGS.**

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.91±0.34</td>
<td>2.40±0.40</td>
<td>2.68±0.36</td>
<td>2.69±0.17</td>
<td>2.53±0.41</td>
</tr>
<tr>
<td>Pinacidil</td>
<td>2.07±0.14</td>
<td>0.54±0.20*</td>
<td>0.85±0.20*</td>
<td>1.05±0.39*</td>
<td>1.77±0.28*</td>
</tr>
<tr>
<td>Cromakalim</td>
<td>2.19±0.29</td>
<td>1.26±0.23*</td>
<td>1.51±0.17*</td>
<td>1.93±0.20*</td>
<td>1.43±0.20*</td>
</tr>
<tr>
<td>KRN2391</td>
<td>2.15±0.21</td>
<td>1.38±0.35*</td>
<td>1.90±0.20*</td>
<td>2.43±0.42</td>
<td>2.31±0.28</td>
</tr>
<tr>
<td>Nicorandil</td>
<td>2.01±0.13</td>
<td>1.59±0.14*</td>
<td>2.07±0.21</td>
<td>2.33±0.30</td>
<td>2.10±0.36</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>1.72±0.38</td>
<td>1.91±0.05</td>
<td>1.93±0.14</td>
<td>2.30±0.21</td>
<td>2.12±0.14</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M., (n=5), * Significantly different from control (p<0.05).

DISCUSSION

In most secretory organs, the secretory products are stored in granules or vesicular structures, and release of the secretory products is thought to occur by exocytosis. Calcium-dependent mechanism is responsible for the release of their respective secretory products. ACTH-stimulated secretion of glucocorticoids seems to depend on a rise of intracellular Ca²⁺. The major site of action of calcium is within the mitochondria wherein the precursor of glucocorticoids is synthesised. Hyperpolarization induced by PCOs may inhibit the ability of intracellular stores to refill after Ca²⁺ release has occurred in excitable and non-excitable cells.

In the present study, administration of single dose of pinacidil and cromakalim, significantly decreased serum cortisol levels that could be observed upto 6 h (Table 1). The decrease in blood glucocorticoid levels may be due to hyperpolarization of adrenocortical cells resulting in decreased intracellular Ca²⁺ concentration and thereby decreased glucocorticoid release.

During stimulation of the adrenal cortex by pituitary corticotrophin (ACTH), the steroids are synthesised de novo and then diffuse out to the exterior. In the present study, administration of ACTH produced a significant rise in serum cortisol levels in dogs. Except nicorandil, preadministration of all other PCOs significantly prevented this rise in serum cortisol levels induced by ACTH in dogs. Studies on quartered glands have shown that calcium is an important factor in increased steroid production in response to ACTH. Janus et al. have reported that in the absence of calcium, ACTH increases steroidogenesis within the gland, but there is little or no increase in corticosterone output. Furthermore, excess of potassium, which depolarises cortical cells, causes a small, transient increase in steroid out put. In the present
### TABLE 2: EFFECT OF POTASSIUM CHANNEL MODULATORS ON ACTH-INDUCED RISE IN CORTISOL LEVELS IN DOGS.

<table>
<thead>
<tr>
<th>SERUM CORTISOL (µg/ml)</th>
<th>0 min</th>
<th>5 min</th>
<th>10 min</th>
<th>15 min</th>
<th>20 min</th>
<th>30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>2.70±0.18</td>
<td>2.28±0.14</td>
<td>2.10±0.70</td>
<td>1.83±0.16</td>
<td>2.23±0.19</td>
<td>1.93±0.16</td>
</tr>
<tr>
<td><strong>ACTH-control</strong></td>
<td>1.24±0.23</td>
<td>9.23±0.98*</td>
<td>8.60±0.80*</td>
<td>8.05±1.12*</td>
<td>8.25±0.90*</td>
<td>7.67±0.75*</td>
</tr>
<tr>
<td><strong>Pinacidil</strong></td>
<td>0.90±0.19</td>
<td>1.73±0.31**</td>
<td>1.87±0.28**</td>
<td>2.90±0.47**</td>
<td>3.99±0.72**</td>
<td>2.77±0.36**</td>
</tr>
<tr>
<td><strong>Cromakalim</strong></td>
<td>1.15±0.23</td>
<td>4.58±0.95**</td>
<td>6.22±0.68**</td>
<td>6.00±0.64**</td>
<td>5.75±0.62**</td>
<td>5.80±0.99**</td>
</tr>
<tr>
<td><strong>KRN2391</strong></td>
<td>2.18±0.24</td>
<td>3.14±0.85**</td>
<td>3.38±0.29**</td>
<td>4.20±0.56**</td>
<td>6.06±0.89**</td>
<td>6.13±0.43**</td>
</tr>
<tr>
<td><strong>Nicorandil</strong></td>
<td>2.40±0.32</td>
<td>7.46±0.99*</td>
<td>7.43±0.82*</td>
<td>8.70±0.90*</td>
<td>7.80±0.86*</td>
<td>7.40±0.68*</td>
</tr>
<tr>
<td><strong>Glibenclamide</strong></td>
<td>1.33±0.31</td>
<td>7.30±0.94*</td>
<td>8.20±0.92*</td>
<td>7.50±0.63*</td>
<td>6.28±0.85*</td>
<td>6.95±0.75*</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M., (n=5). * Significantly different from control at p<0.05. **Significantly different from ACTH-control p<0.05.

In conclusion, our data suggest that potassium channels are involved in the release of glucocorticoids. Since potassium channel modulators have different effect on glucocorticoid levels, it seems that there is involvement of different types of potassium channels in the action of these modulators. It is possible that same type of \( K_{ATP} \) channel from different tissues display varying sensitivities to PCOs. Our studies also suggest that pinacidil and cromakalim can be considered as useful drugs in hypertension associated with increased glucocorticoid levels.

**ACKNOWLEDGEMENTS**

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**REFERENCES**