An electrophysiological study on the effects of Pa-1G (a phospholipase A$_2$) from the venom of king brown snake, *Pseudechis australis*, on neuromuscular function

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### Abstract

The effects of Pa-1G, a phospholipase A$_2$ (PLA$_2$) from the venom of the Australian king brown snake (*Pseudechis australis*) were determined on the release of acetylcholine, muscle resting membrane potential and motor nerve terminal action potential at mouse neuromuscular junction. Intracellular recording from endplate regions of mouse triangularis sterni nerve-muscle preparations revealed that Pa-1G (800 nM) significantly reduced the amplitude of endplate potentials within 10 min exposure. The quantal content of endplate potentials was decreased to 58±6% of control after 30 min exposure to 800 nM Pa-1G. The toxin also caused a partial depolarisation of mouse muscle fibres within 60 min exposure. Extracellular recording of action potentials at motor nerve terminals showed that Pa-1G reduced the waveforms associated with both sodium and potassium conductances. To investigate whether this was a direct or indirect effect of the toxin on these ionic currents, whole cell patch clamp experiments were performed using human neuroblastoma (SK-N-SH) cells and B102 mouse fibroblasts stably transfected with Kv1.2. Patch clamp recording experiments confirmed that potassium currents sensitive to -dendrotoxin recorded from B102 cells and sodium currents in SK-N-SH cells were not affected by the toxin. Since neither facilitation of acetylcholine release at mouse neuromuscular junction nor depression of potassium currents in B102 cells has been observed, the apparent blockade of potassium currents at mouse motor nerve endings induced by the toxin is unlikely to be due to a selective block of potassium channels.

### RESULTS

<table>
<thead>
<tr>
<th>Time of exposure</th>
<th>AminEPPs</th>
<th>EPPs</th>
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<tbody>
<tr>
<td>10 min</td>
<td>84±6</td>
<td>68±6</td>
</tr>
<tr>
<td>30 min</td>
<td>72±5</td>
<td>58±6</td>
</tr>
<tr>
<td>60 min</td>
<td>65±5</td>
<td>51±5</td>
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The results demonstrate a significant decrease in the amplitude of endplate potentials over time, with a concomitant reduction in the quantal content. The decline in EPP amplitude and quantal content suggests a targeted effect on the neuromuscular junction.

### Dose Response Curve to Pa-1G

A dose-dependent reduction in endplate potential amplitude is observed, indicating the potency of Pa-1G in modulating neuromuscular transmission.

### Extracellular Recordings from Triangularis Sterni NMJ Prep

- **1st deflection**: Na$^+$ influx at last node of Ranvier
- **2nd deflection**: K$^+$ efflux / Ca$^{2+}$ influx at terminal

The recordings illustrate the dynamic changes in ionic currents at the neuromuscular junction, highlighting the toxin's influence on sodium and potassium conductances.
Na⁺ Currents - Whole Cell Clamp

K⁺ Currents – Whole Cell Clamp