Seizure liability assessments using hippocampal brain slice: comparison of multiple preclinical species

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ABSTRACT

Introduction: Hippocampal brain slices can be used as an in vitro assay to characterize seizure liabilities early in drug development. Traditionally, this assay has relied upon the rat hippocampal slice which sometime possesses limited translational value. Species differences are often noted during in vivo seizure liability assessments and drug safety testing may benefit from early ranking of the various animal models. Methodology: Transverse hippocampal brain slices were isolated from animal models (e.g., rat, minipig, dog, nonhuman primate) and population spikes (PSs) were evoked through Schaffer collateral pathway stimulation via a concentric bipolar stimulating electrode and recorded using conventional in vitro electrophysiological techniques via an extracellular electrode placed into the CA1 cell body layer. Results: All preclinical animal hippocampal slices displayed a concentration-dependent increase in PS area and number in the presence of the pro-convulsant Pentylenetetrazol (PTZ; 0.1-10 mM) - in good agreement with previously published in vivo and in vitro PTZ data. However, nuanced and distinct differences were observed in certain species with respect to their sensitivity to PTZ. For instance, PS area reached significance at lower thresholds in nonhuman primates compared to rats (1 mM vs. 10 mM) whereas rats demonstrated greater evoked PS numbers when compared to monkeys (2.3 vs 1.8). Discussion: Our results suggest the nuanced differences between hippocampal slices isolated from different preclinical animal models should influence the design of non-clinical seizure liability studies and their associated data interpretation. Hippocampal brain slices from multiple preclinical animal models may benefit from early ranking of the various animal models.

MATERIALS AND METHODS

Coronal hippocampal brain slices (400-500 µm) from Sprague-Dawley rats (n = 4; 2-3 months), cynomolgus monkeys (n = 5; 3-5 years), beagle dogs (n = 3; 6-14 months) and minipigs (6-9 months) were isolated and allowed to recover at room temperature for at least 1 h prior to use. Extracellular recording microelectrodes (1.3 MΩ, filled with 3 M NaCl) were placed into the CA1 cell body layer and a concentric bipolar stimulating electrode was positioned in the CA2 stratum radiatum. PSs were evoked through stimulation of the Schaffer collateral pathway at 30 s intervals using constant current pulses (0.1 ms duration) of varying voltages (e.g., 0-70 V). Voltages were adjusted to ~50-80% the maximum amplitude evoked (or to generate PSs with an amplitude of 1 mV if possible). Slices with PS amplitudes less than 0.2 mV were discarded (mean PS amplitude: 0.97±0.2 mV, n = 21). All experiments were performed between 30-36 °C. PS responses were quantified as pS areas (the area above and below the 0 mV line).

RESULTS

Table 1. PTZ Dose-Dependent Changes in Population Spike Area and Number

<table>
<thead>
<tr>
<th>Animal</th>
<th>First Myoclonic Jerk</th>
<th>First Tonic Convulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>103 ± 15</td>
<td>153 ± 25</td>
</tr>
<tr>
<td>Monkey</td>
<td>40.5 ± 14.2</td>
<td>581 ± 29.7</td>
</tr>
<tr>
<td>Rat</td>
<td>40.5 ± 14.2</td>
<td>404 ± 31.7</td>
</tr>
<tr>
<td>Minipig</td>
<td>24.9 ± 11.8</td>
<td>692 ± 11.4</td>
</tr>
</tbody>
</table>

All data is represented as Average ± SD

DISCUSSION AND CONCLUSION

All preclinical animal species tested showed some level of PTZ-associated seizure like activity in the hippocampal brain slice assay. Interestingly, each animal possessed unique nuances to their sensitivity to PTZ which strongly correlates with in vivo data. For instance, dogs appear to be quite sensitive to PTZ-associated changes in PS morphology and frequency. Conversely, rat tissue exhibited greater concentration-dependent changes in PS morphology (e.g. generation of multiple population spikes) whereas NHPs (and to a lesser extend minipigs) tissue displayed greater PS area changes to higher PTZ concentrations. These data highlight important differences to consider between preclinical species as well as the complex nature of induced seizure like activity; important issues in safety pharmacology. Taken together, our results suggest that hippocampal brain slices can be considered as an early screening tool to compare species susceptibility to drug-induced seizure.