

043P EFFECT OF ELECTRICAL FIELD STIMULATION PARAMETERS ON THE POTENCY OF TWO CANNABINOID AGONISTS IN THE MOUSE VAS DEFERENS

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Electrical field stimulation of the mouse isolated vas deferens is a well-established assay for the study of cannabinoid agonists (Pertwee *et al.*, 1995). Stimulation of pre-synaptic CB₁ receptors results in a reduction in neurotransmitter release and subsequent contractions. The aim of this study was to investigate the effect of number of pulses per train and frequency on the potency of two chemically different CB₁ agonists (Arachidonyl-2'-chloroethylamide (ACEA) and WIN 55,212-2) in the mouse vas deferens.

ICR mice (25-30g) were killed by exposure to CO₂. Each vas deferens was removed, cleaned, secured with sutures and placed in a 10ml tissue bath containing Mg²⁺-free modified Krebs-Henseleit buffer at 37°C and bubbled with 95% O₂ and 5% CO₂. The sutures were connected to a tissue holder and a Grass isometric force transducer which was then connected to an amplifier and recorder. Each vas deferens was subjected to constant current electrical field stimulation via two platinum electrodes, using a D330 MultiStim stimulator. Stimulation parameters were: constant current of 400mA, pulses of 2msec duration and 0.1min intervals between trains. After 45 min, a cumulative concentration response curve (CCRC) was started (Range: 10⁻¹⁰ – 10⁻⁵M; 0.5 log unit increments; 3 min intervals).

Four different number of pulses per train and four different frequencies (Hz) were used in these experiments (Table 1).

Table 1 Mean pEC₅₀ values for ACEA and WIN 55,212-2 in the MVD.

| (Hz) | 5 | 10 | 15 | 20 |
|--------------|--------------|-------------|-------------|-------------|
| pulses/train | ACEA | | | |
| 3 | 6.88 ± 0.40 | 6.96 ± 0.18 | 6.12 ± 0.15 | 6.79 ± 0.20 |
| 4 | 7.16 ± 0.28 | 6.30 ± 0.39 | 6.78 ± 0.07 | 6.43 ± 0.10 |
| 5 | 6.83 ± 0.49 | 6.62 ± 0.10 | 6.40 ± 0.28 | 6.54 ± 0.11 |
| 6 | 6.97 ± 0.10 | 7.00 ± 0.07 | 6.79 ± 0.04 | 6.70 ± 0.21 |
| pulses/train | WIN 55,212-2 | | | |
| 3 | 9.08 ± 0.10 | 9.37 ± 0.14 | 9.42 ± 0.42 | 8.25 ± 0.08 |
| 4 | 9.75 ± 0.30 | 8.75 ± 0.22 | 8.63 ± 0.25 | 8.18 ± 0.17 |
| 5 | 9.95 ± 0.10 | 8.31 ± 0.30 | 7.68 ± 0.20 | 8.31 ± 0.11 |
| 6 | 8.65 ± 0.12 | 8.48 ± 0.13 | 8.66 ± 0.14 | 8.57 ± 0.10 |

Data are expressed as Mean and SEM, n = 4 – 6 / group.

These results demonstrate that small changes in stimulation parameters cause changes in the sensitivity of the tissue to the agonists. This indicates that comparison of results from different laboratories is difficult when different stimulation conditions are used.

Pertwee R.G. *et al.*, (1995) *Eur. J. Pharmacol.*, 248: 241-247.