

044P THE ACTIONS OF SIX CANNABINOID AGONISTS IN MOUSE VAS DEFERENS PREPARATIONS UNDER TWO DIFFERENT STIMULATION CONDITIONS

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Constant current electrical field stimulation (CCFS) causes contractions of mouse isolated vas deferens (MVD) preparations. Stimulation of pre-synaptic cannabinoid CB₁ receptors results in a reduction in neurotransmitter release and as a consequence, a reduction in twitch height (Pertwee *et al*, 1995). In earlier studies, optimal stimulation parameters were determined as: constant current of 400mA, trains of 4 pulses at 5 Hz and pulse duration of 2ms at 10sec intervals between trains (K. McMillan, BPS Poster P66, Sept 2003). Using trains of 5 pulses at 15Hz, a marked decrease in potency was observed using the CB₁ agonists WIN 55, 212-2 and ACEA. The aim of this study was to evaluate the potency and efficacy of four additional cannabinoid agonists [ACPA, CP 55,940, methanandamide (AM356), and noladin ether] under two different stimulation conditions.

ICR mice (25-30g) were killed by exposure to CO₂. Each vas deferens was removed. Each end was secured with 2/0 siliconised braided sutures and placed in a 10ml bath filled with modified Mg²⁺-free Krebs Henseleit buffer at 37°C bubbled with 95% O₂ and 5% CO₂. The sutures were connected to a tissue holder and a Grass isometric force transducer type FT03. The force transducers were connected to a Grass Model C7 cabinet amplifier coupled to an Astromed K2G recorder. Two platinum electrodes were placed parallel to the preparations in the baths for CCFS, using a D330 MultiStim stimulator (Digitimer Ltd, UK).

Each vas deferens was subjected to CCFS for 45 min prior to determining cumulative concentration response curves.

Each compound was tested over the concentration range: 10⁻¹⁰ – 10⁻⁵M; 0.5 log unit increments; 3 min intervals; 50µl injections. All compounds were obtained from Tocris, UK. The results are summarised in Table 1.

Table 1: Comparison of the potency and efficacy of six cannabinoid agonists in the MVD, under two different stimulation conditions.

Agonist	5Hz, 4 pulses		15Hz, 5 pulses	
	pEC ₅₀	% inhibition	pEC ₅₀	% inhibition
WIN 55,212-2	9.75±0.31	100±0.0	7.84±0.17*	100±0.0
CP 55,940	9.55±0.27	100±0.0	8.94±0.27	100±0.0
ACEA	7.35±0.28	92.4±3.1	6.34±0.29*	69.3±5.7*
ACPA	9.19±0.14	100±0.0	7.44±0.31*	97.0±1.6
AM 356	8.27±0.13	100±0.0	7.60±0.20*	99.8±0.2
Noladin ether	7.94±0.43	95.8±1.4	6.03±0.32*	91.1±5.4

All data given are the mean ± s.e.m. *n*=4 for each compound.

* significantly different (*p*<0.05) unpaired t-test.

This study demonstrates that small changes in stimulation parameters can have a dramatic effect on the potency of cannabinoid agonists.

CP 55,940 causes inhibition which is not dependent on these stimulation conditions, suggesting that other non-cannabinoid properties might be present.

Pertwee R.G. *et al.*, (1995) *Eur. J. Pharmacol.*, 284, 241-247.