

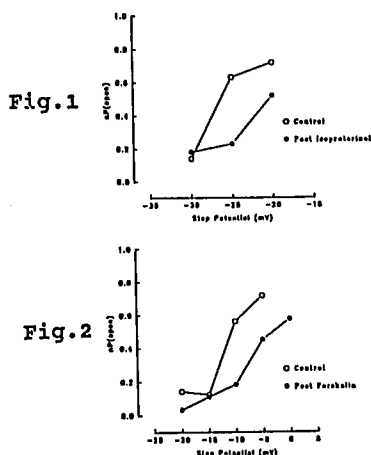
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**B-AGONISTS DECREASE THE OPEN-STATE PROBABILITY OF VOLTAGE DEPENDENT CALCIUM CHANNELS IN AIRWAY SMOOTH MUSCLE.** M. Tomasic, J.P. Boyle, and M.I. Kotlikoff. Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania.

Unitary conductance (20 to 35 pS) single channel Ca currents were recorded from cultured human and from fresh enzymatically dissociated canine and porcine airway smooth muscle cells using the on-cell patch clamp technique with the microelectrode solution containing 80 mM BaCl<sub>2</sub>, 10 mM HEPES--pH 7.4 (Ba(OH)<sub>2</sub>); and the bath solution containing 120 mM NaCl, 20 mM CsCl, 5mM EGTA, and 10 mM HEPES--pH 7.4 (NaOH). In some experiments, both microelectrode and bath solutions contained the dihydropyridine agonist, Bay K 8644 (500 nM), to increase the resting open-state probability of the voltage dependent calcium channels (VDCC).

Membrane patches were stepped to various depolarizing potentials from a holding potential of -70 mV. Open-state probabilities were determined from amplitude histograms obtained from 25 to 30 traces at each depolarizing step-potential. After control recordings were made, isoproterenol was added to the recording bath (100  $\mu$ M final concentration). Fig 1 shows the rightward shift in the voltage-dependent open-state probability curve after the addition of isoproterenol. In other experiments, forskolin (a membrane permeant adenylate cyclase activator) was added to the bath to a final concentration of 1 to 10  $\mu$ M. Fig 2 shows a similar right shift of the open-state probability after the addition of 1  $\mu$ M forskolin. Bath addition of dibutyryl-cyclic AMP also reduced the open-state probability of the VDCC from control levels (data not shown).

These results indicate that  $\beta$ -agonists reduce the open-state probability of VDCC probably through activation of the adenylate cyclase pathway.



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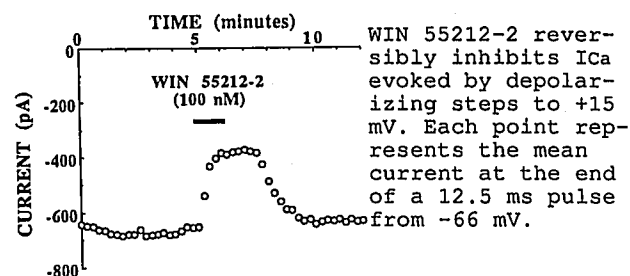
**TITLE:** CANNABINOIDS INHIBIT A HIGH-THRESHOLD CALCIUM CURRENT  
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Medicinal properties of *Cannabis sativa* and its major biologically active constituent,  $\Delta^9$ -tetrahydrocannabinol (THC), have been known for years.<sup>1</sup> THC has been used as an antiemetic, and analogs show promise as analgesics.<sup>2</sup> Recently a high-affinity THC receptor has been identified that has a CNS distribution consistent with the behavioral effects of THC.<sup>3</sup> The cellular mechanisms of action of THC are uncertain. One action of THC is to inhibit adenylyl cyclase (AC).<sup>4</sup> As neurotransmitters that inhibit AC often affect ionic channels by other pathways, we have investigated a link between cannabinoid receptors and calcium channels.

Calcium currents (I<sub>Ca</sub>) were recorded from NG108-15 neuroblastoma x glioma cells using the perforated patch modification of the whole-cell patch clamp technique. Tetrodotoxin and ion substitution were used to suppress sodium and potassium currents, respectively. The cannabanomimetic alkylaminoindole, WIN 55212-2, potently, stereospecifically and reversibly inhibited a component of the high-threshold voltage-sensitive I<sub>Ca</sub>. Maximal inhibition of I<sub>Ca</sub> was 40% at 100 nM WIN 55212-2. For several reasons the inhibition is not likely to be due to a decrease in cAMP level. Half-maximal inhibition of I<sub>Ca</sub> was seen at 10 nM, whereas half maximal inhibition of AC (in a neuroblastoma line) is observed at 300 nM WIN 55212-2.<sup>5</sup> Furthermore, prior incubation with 8-(chlorophenylthio)cAMP (100  $\mu$ M) and/or isobutylmethylxanthine (1 mM) did not prevent I<sub>Ca</sub> inhibition by WIN 55212-2.

Given the central role of calcium channels in neurotransmitter release and in patterned firing of action potentials, it is possible that inhibition of these channels contributes to the profound behavioral changes produced by THC.

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<sup>1</sup>Cannabinoids as Therapeutic agents, CRC Press, Boca Raton, FL, 1-20, 1986.  
<sup>2</sup>ibid, 105-120.  
<sup>3</sup>J Neurosci, 11:563-583, 1991.  
<sup>4</sup>Molec Pharmacol, 27:429-436, 1985.  
<sup>5</sup>D Haycock, personal communication.