NEUROACTIVE PROPERTIES OF THE MULTIFUNCTIONAL PROTOTYPE LASSBio-881: FOCUS ON THE CANNABINOID SYSTEM

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Aim: The CB1 cannabinoid receptor is the most abundant G-protein-coupled receptor in the CNS and its activation promotes analgesia and stimulates the appetite, among other actions. LASSBio-881, a compound developed at UFRJ, has antinociceptive, anti-inflammatory and antioxidant effects in vivo, and also binds to CB1 receptors (Bioorg. Med. Chem. 15:241, 2007). The compound has significant neuroprotective effects as well (Balassiano et al., SBFTE, 2009). However, the type of interaction with the CB1 receptor is unknown. We have investigated its central nervous system effects, particularly the involvement of CB1 receptors, by observing synaptic modulation in vitro and animal behavior in vivo, and characterized the mode of interaction with heterologously expressed receptors.

Methods and Results: Animal procedures were approved by the Ethics Committee on Animals Research of CCS/UFRJ, protocols DFBICB 009 and 029. LASSBio-881 and the CB1 agonist, WIN 55212-2 (WIN) were assayed in *Xenopus* oocytes expressing human CB1 and two G-protein-gated K+ channels (Kir3.1 and Kir3.4) by current recordings. Effects on miniature spontaneous inhibitory postsynaptic currents (mIPSC) were evaluated in cultured rat hippocampal neurons through whole-cell patch-clamp recordings at 0 mV. Pulses of control solution, LASSBio-881 and the CB1 antagonist AM251 were applied sequentially in each cell. To evaluate the effect on feeding behavior, mice receiving LASSBio-881 at the dose of 20 mg/kg or vehicle i.p. were observed after fasting for 24 h. Chow consumption and body weight changes were observed for 24 h. Effects on two signs that compose the cannabinoid tetrad were assayed by the catalepsy test and rectal temperature measurements.
At near-saturating concentration (20 microM) LASSBio-881 did not activate K+ currents in oocytes expressing CB1 receptors. To the contrary, the compound reduced the baseline current, which changed by -30.7±2.6% (mean±s.e.m., n=7, $P<0.001$) relative to the response to the CB1 agonist WIN (500 nM). When both were co-applied, the response was 12.6±1.6% of that induced by WIN alone (n=7, $P<0.001$). In hippocampal neurons, WIN (100 nM) increased the frequency of miniature inhibitory post-synaptic currents in some cells (n = 6). The average interval between events was 217.9 ± 54.3 ms in control conditions, 158.2 ± 22.6 ms during pulses of LASSBio-881 20 microM, and 249.9 ± 38 ms in 1 microM AM251. The effects of LASSBio-881 and AM251 on mIPSC did not reach statistical significance. The compound (50 mg/kg i.p.) had a post-fasting hypophagic effect similar to that of AM251, in mice. A single dose of LASSBio-881 one hour before reintroducing chow pellets reduced consumption by 37%. The cumulative consumption compared to the group receiving vehicle remained significantly lower after 24 h (n=8, $P<0.05$). The immobility induced by WIN was reduced by 68% with a single dose of the compound (n=12, $P<0.05$), although it could not reverse the hypothermic effects of WIN, possibly because of its own effect on the TRPV1 channel.

Conclusion: The data corroborate the hypothesis that LASSBio-881 is a cannabinoid antagonist, as one additional aspect of its multifunctional properties**.**

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