Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol17Issue1abst006P.pdf

Leelamine, a low affinity CB_1 receptor ligand, has cannabinoid-like behavioural effects in rats

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Introduction: Leelamine ([(1R,4aS,10aR)-1,4a-Dimethyl-7-propan-2-yl-2,3,4,9,10,10a-hexahydrophenanthren-1-yl]methanamine) is a diterpene amine found in the pine tree[1,2]. In recent years, leelamine has been investigated for its anticancer activity in melanoma[3]. However, it was first discovered as a low-affinity CB_1 receptor (CB1R) ligand[4]. The aim of the present study was to further assess the cannabinoid-like pharmacological properties of leelamine in behavioural and radioligand binding studies.

Method: Male Sprague-Dawley rats (250-300g) were injected intraperitoneally with 25mg/kg leelamine or vehicle (1:1:18, ethanol:cremophor:saline) at time-point 0. At time-points (minutes) -15, 15, 30, 60 and 120 animals were tested in the hot-plate test (55°C±1) and their core body temperature was measured with a rectal probe. To measure catalepsy, a bar test was performed at time-point 15. Locomotor activity was assessed using a photo beam break system during the intervals between behavioural tests and injection. Binding experiments with [^{3}H]leelamine and its displacement by either unlabelled leelamine or the cannabinoid receptor agonist CP55,940 were conducted using whole brain membranes from CB1R knockout mice. 10-12 µg of protein were incubated with 1200-1600cpm (80-105pM) of [^{3}H]leelamine for 30 minutes. Bound and free radioligand were separated by centrifugation, and quantified using scintillation counting.

Table 1: Results are express as Veh (mean±SEM) vs leelamine (mean±SEM). Number of animals treated with Veh-Leelamine is shown in parentheses. Student's t-test * P<0.05 veh vs leelamine.

	Time-points (minutes, relative to leelamine or vehicle injection)				
Measurements	-15	15	30	60	120
Latency to first hind paw lick (s)	17.60±1.63 vs 19.72±1.47 (20-21)	**21.49±1.57 vs 31.21±1.88 (20-20) ***	**17.37±1.67 vs 28.28±2.39 (21-21) ***	**16.28±1.82 vs 23.76±2.41 (14-14) ***	13.64±2.08 vs 20.62±3.46 (7-7)
Core body temperature, change from baseline (°C)		**1.11±0.21 vs 0.143±0.19 (20-21) ***	**0.85±0.19 vs -0.26±0.22 (20-22) ***	**0.63±0.34 vs -1.49±0.41 (14-15) ***	**0.83±0.36 vs -2.14±0.47 (7-8) ***

Results: Acute administration of leelamine induced a significant antinociceptive effect, increasing the latency to hind paw lick in the hot-plate test during the first 60 minutes after leelamine administration (Table 1). Throughout the trial duration (120 minutes), leelamine-treated rats had significantly lower core body temperature (Table 1) and locomotor activity effect (Figure 1), compared with vehicle-treated controls. However, no significant effects were found in the bar test (1.63±0.21s vs 2.06±0.33s; N=18-22). In brain membranes from CB1R knockout mice, [^{3}H]leelamine exhibited specific binding and homologous displacement by unlabelled leelamine, but not by CP55,940.

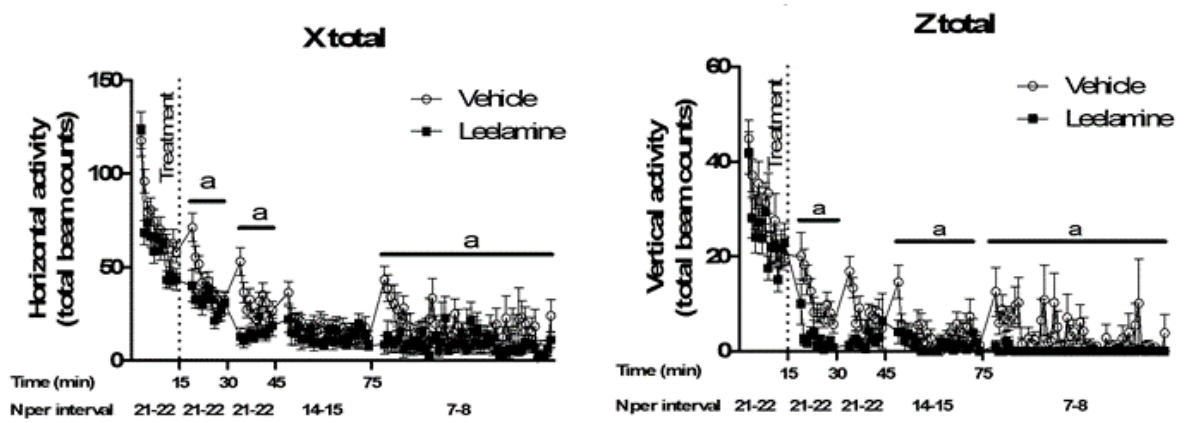

Figure 1: Effects of leelamine administration on horizontal activity (left panel) and vertical activity (right panel). Graphs represent total beam counts every minute. Repeated measures ANOVA were performed on data within each of the intervals, a: effect of leelamine treatment.

Conclusions: The low affinity CB1R ligand leelamine exhibits cannabimimetic behavioural effects, but also binds to a site distinct from CB1R. Further research is needed to determine the identity of the non-CB1R binding site and whether it is involved in the behavioural effects of leelamine.

Acknowledgements: Funding from the Wellcome Trust, the Irish Research Council and the National University of Ireland Galway is gratefully acknowledged.

References:

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