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Research report

The effect of δ-9-tetrahydrocannabinol on forebrain ischemia in rat

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Abstract

The purpose of the study was to evaluate the effect of δ-9-tetrahydrocannabinol (THC), the major psychoactive constituent of marijuana, on ischemic neuronal injury. A 12-min ischemic insult was induced by a reduction in systolic blood pressure to a mean of 50 mm Hg, followed by bilateral carotid artery occlusion at a middle ear temperature of 37.5°C. THC at either a low (0.1 mg/kg; $n = 8$) or high (10 mg/kg; $n = 8$) dose was injected i.p. every 12 h for 7 days prior to ischemia. Non-treated ischemic ($n = 8$) animals formed the control group. The animals were sacrificed 3 weeks post-ischemia for quantitative histopathology. THC at either dose did not significantly reduce ischemic neuronal damage in the hippocampus. The high dose THC-treated group showed significantly less neocortical injury, compared to either the control or low-dose THC groups ($p < 0.05$). The striatum was markedly protected by both low and high dose THC ($p < 0.001$). This regionally specific protection implies that either the hippocampus undergoes suprathreshold ischemic injury or that mechanisms of ischemic injury vary in different brain regions.

Keywords: Forebrain ischemia; δ-9-tetrahydrocannabinol; Striatum; Hippocampus

1. Introduction

Marijuana, the New World colloquialism for *Cannabis sativa*, has been inhaled or ingested for millennia in Asia. δ-9-THC is its major psychoactive constituent, and has euphoriant properties. These effects were exploited by Syrian chiefs, inciting professional agents to murder their adversaries. They were known as the Hashshasin, or ''hashish-eaters,'' giving rise of course, to the term assassin [3]. Marijuana was brought to the attention of the Western World by the 12th century crusaders, and subsequently either vilified as an instrument of insanity or championed as a panacea. It is of considerable interest to stroke biologists for a variety of reasons. Cannabinoid receptors densely decorate brain regions selectively vulnerable to ischemia. Activation of these receptors reduces influx of calcium [11], diminishes cAMP levels [5], and enhances cerebral blood flow [9]. These permissive effects provide the ability to modulate multiple pathways in the ischemic cascade. It is with these factors in mind that we undertook to evaluate the effect of chronic δ-9-THC administration on ischemic brain injury using a rat model of forebrain ischemia.

2. Materials and methods

All male 24 Sprague–Dawley rats used in this study were treated in accordance with guidelines of the Canadian Council on Animal Care and local animal care committees approved all protocols. They were divided into three groups: vehicle-treated ($n = 8$); low-dose ($n = 8$) and high-dose THC ($n = 8$). δ-9-Tetrahydrocannabinol was dissolved in a solution of polyvinyl pyrolidone (PVP; Sigma) prepared in ethanol. This solution was evaporated at 60°C and dried, concentrated THC-PVP was suspended in saline at concentrations of 1 mg/ml and 10 mg/ml. A 30% PVP solution was used as the control vehicle. In the THC groups, animals were randomly assigned to either a 0.1 mg/kg or a 10-mg/kg dose. All drugs (including the

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vehicle) were administered by daily intraperitoneal (i.p.) injection for seven days pre-ischemia.

Each rat was fasted for 12 h, pre-treated with 0.5 mg/kg of atropine, and then anesthetized with 50-mg/kg pentobarbital. Forebrain ischemia was induced through bilateral carotid artery occlusion coincident with a reduction in systemic blood pressure to a range of 45–50 mmHg through aspiration of blood into a heparinized syringe. After 12 min, blood flow through the carotid arteries was restored and the aspirated blood reinfused. Brain temperature was monitored before, during, and after the ischemic insult using a tempanic membrane probe and was maintained at 37.5 ± 0.1°C using a thermal regulated servo-controlled heated water blanket and/or an overhead heating lamp. We have previously shown that in this model, middle ear temperatures correlate with brain temperature [15]. Blood gas analyses, glucose levels and hematocrit determinations were obtained prior to and following the ischemic insult.

Three weeks post-ischemia, each rat was perfusion-fixed with 1 liter of 10% buffered formaldehyde (pH 7.25). The brains were fixed for two weeks prior to sectioning. The brains were cut coronally into 1.5-mm slices, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Serial sections (5 μm) were cut and stained with hematoxylin and eosin. All sections were examined to determine the quantitative and topographic extent of brain damage. For quantification of ischemic neuronal injury, standardized sections of the cerebral cortex, hippocampus and striatum were used [7,14]. The hippocampal CA1 and CA2/3 sectors were scrutinized on a microscopic grid and percent ischemic neuronal injury was defined by direct visual counting of all neurons. Similarly in the dorsolateral striatum, neuronal injury was also determined as a percentage of dead neurons within three 25 μm^2 areas. In the neocortex, the total number of necrotic neurons was counted.

All results are expressed as means ± S.E.M. Statistical significance between groups was determined by using ANOVA, with Scheffè's test for multiple comparison between groups. Significance was assumed at $p < 0.05$.

Table 1
Physiologic parameters[a]

Physiologic parameter	Experimental Group[c]		
	Control	High-dose THC	Low-dose THC
Weight (g)			
Initial	277 ± 8	281 ± 8	294 ± 9
Pre-ischemia	285 ± 8	248 ± 7	293 ± 9
Pre-perfusion Fixation	371 ± 11[b]	376 ± 7[b]	409 ± 10[b]
Blood glucose (mM)			
Pre-ischemia	4.8 ± 0.4	4.4 ± 0.2	4.2 ± 0.2
Post-ischemia	4.6 ± 0.3	4.7 ± 0.3	4.3 ± 0.3
Hematocrit			
Pre-ischemia	48 ± 2	45.0 ± 0.7	50 ± 1
Post-ischemia	45 ± 2	44.0 ± 0.7	48.0 ± 0.8
pH			
Pre-ischemia	7.32 ± 0.01	7.321 ± 0.008	7.333 ± 0.009
Post-ischemia	7.31 ± 0.01	7.28 ± 0.01	7.30 ± 0.01
P_aCO_2			
Pre-ischemia	39.5 ± 0.8	38.6 ± 0.8	38 ± 1
Post-ischemia	40 ± 1	41.3 ± 0.8	39 ± 1
P_aO_2			
Pre-ischemia	137 ± 4	141 ± 5	149 ± 2
Post-ischemia	136 ± 5	138 ± 4	144 ± 2
Blood pressure (mmHg)			
Pre-ischemia	132 ± 5	138 ± 5	131 ± 4
During ischemia	46.8 ± 0.6	47.1 ± 0.7	46.9 ± 0.4
Post-ischemia	115 ± 6	122 ± 5	118 ± 6

[a] Values are expressed as the Mean ± S.E.M.
[b] Denotes a statistically significant difference between pre-perfusion fixation and initial body weights.
[c] Initial Weight refers to body weight at time of randomization to either control or THC treatment groups. Pre-ischemia weight is the body weight just prior to the ischemic insult and pre-perfusion fixation is the body weight just prior to perfusion fixation.

3. Results

Physiological variables are presented in Table 1. High-dose THC rats showed weight loss prior to stroke although this failed to reach significance. Both of the THC treated groups had significant weight gain following ischemia. There were no other significant differences between the groups.

Qualitative observations confirmed that animals had bilateral injury confined to selectively vulnerable brain regions. Striatal injury (Figs. 1 and 2) was confined to the dorsolateral sector and in some control animals this had progressed to cavitation, usually associated with ex vacuo ventricular dilatation. In the neocortex, injury was limited to the middle layers. Hippocampal damage was greater in the CA1 than CA2/3 sector.

Quantitative histological results are shown in Figs. 3 and 4. Analysis demonstrated a lack of THC protection in both the CA 1 and CA 2/3 sectors. CA1 sector injury was 0.88 ± 0.05 in control animals compared to 0.8 ± 0.1 and 0.6 ± 0.2 in the high and low dose THC groups, respectively. CA2/3 sectors demonstrated a trend to protection in the high and low-dose treated groups, but this failed to reach significance. Neocortical examination revealed significantly fewer necrotic neurons in the high-dose group only ($p < 0.05$), which experienced a cell death count of only 20 ± 13 in the standardized section. In the control

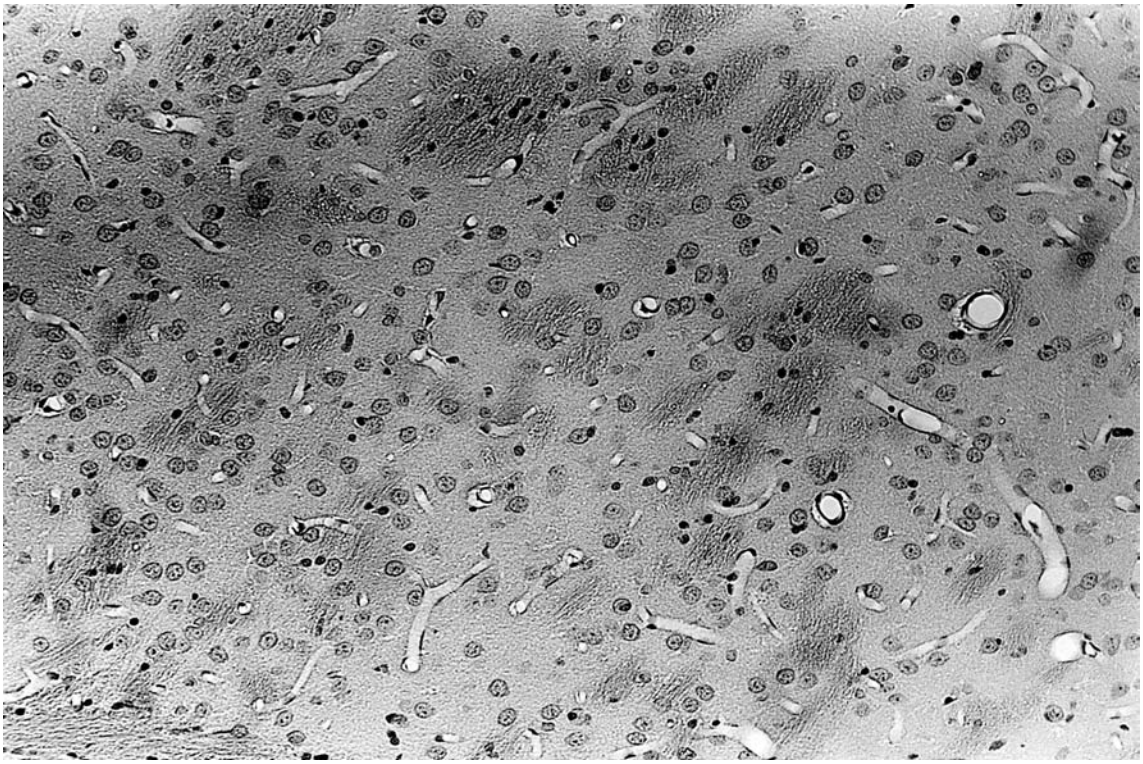

Fig. 1. Photomicrograph of dorsolateral striatum (×120) showing normal morphology.

group, the total number of injured neurons was 99 ± 44 and 80 ± 45 in the low dose THC group. The striatum demonstrated substantial amelioration of ischemic injury in both treated groups: high dose 0.03 ± 0.02; low dose

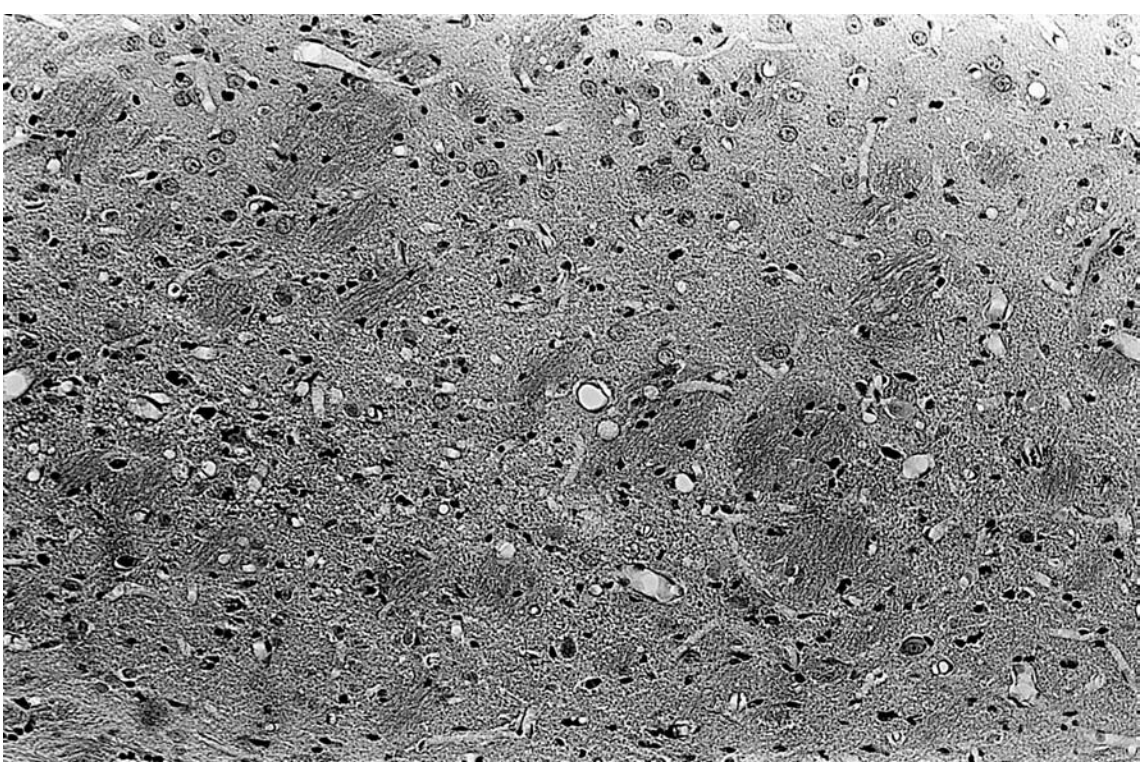

Fig. 2. Photomicrograph (×120) of dorsolateral striatum obtained 3 weeks post-ischemia from control rat. Ischemic neuronal changes consisting of retraction of cell body, eosinophilia of cytoplasm, disappearance of Nissl bodies, pyknosis, and hyperchromasia of nucleus are illustrated.

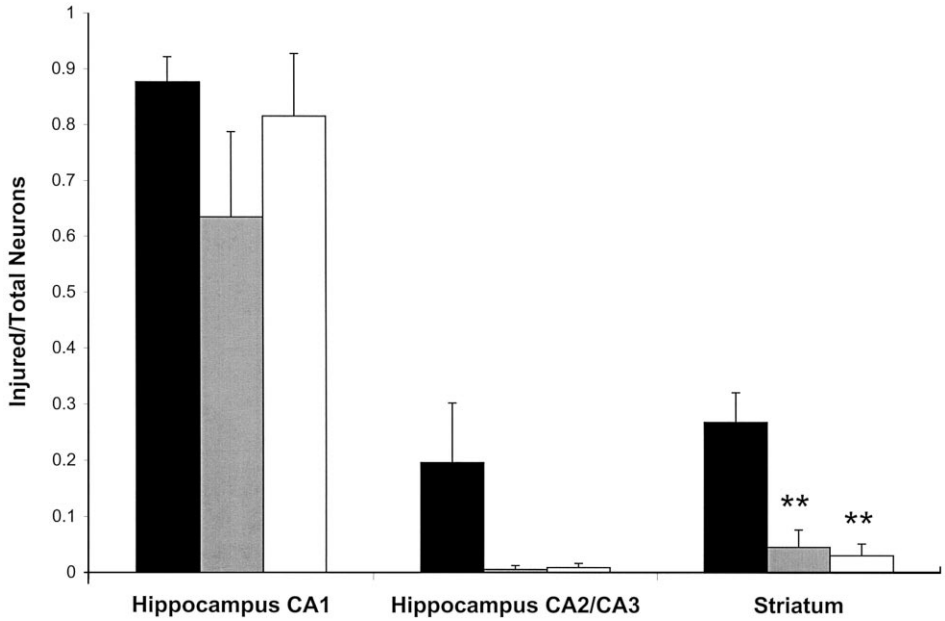

Fig. 3. Quantitative results showing the effect of high (open bars) and low (grey bars) dose THC on ischemic brain injury. Vehicle-treated control animals are shown in black. Injury is expressed as mean ± S.E.M. $^{**}P < 0.001$ compared to control.

0.05 ± 0.03; ($p < 0.001$). In contrast, injury in the control group was 0.27 ± 0.04.

4. Discussion

These results are remarkable for a variety of reasons. Particularly notable is the dramatic degree of protection afforded by THC, surpassing a p-value of 0.001 in the striatum and $p < 0.05$ in the neocortex. This agent, in fact, has proven to be more effective than any other pharmaceutical agent in our laboratory (including hypothermia) at protecting the striatum. The striatum is notoriously insensitive to standard anti-ischemia drugs. There is both an interesting and compelling co-localization of cannabinoid receptors and zones of ischemic salvage. The densest binding of receptors in the rat is the dorsolateral quadrant of the striatum [4], corresponding perfectly to the most vulnerable sector of the striatum to ischemia. Strikingly, this is the exact striatal segment afforded maximal protection by administration of THC. This finding is of potential clinical significance as cannabinoid receptor densities are uniquely conserved across species, including humans [5]. With chronic exposure to THC maximum changes in cannabinoid receptor mRNA transcripts within the hippocampus and striatum occurs at 7 days [16]. Although striatum and cortex were significantly protected by THC, this was not the case for hippocampal CA1 sector injury. This sector subtends significantly fewer cannabinoid receptors than the dentate and CA3 region [4]. However, this therapeutic escape may also reflect that the selectively vulnerable CA1 region undergoes a suprathreshold injury during ischemia. Alternately, mechanisms that contribute to irreversible brain injury may vary between brain regions. Cannabinoid receptor binding varies among different cannabinoids [5], with THC Ki perhaps being insufficient for CA1 protection. A potential error of interpretation in our results would be if THC was binding to non-cannabinoid receptors, but this has not yet been shown. Hypothermia, a known effect of THC, was not a factor in this study as rigorous temperature control was maintained throughout the experiment.

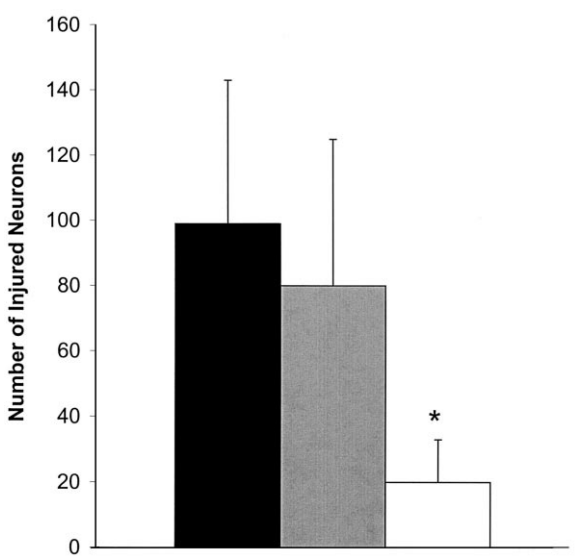

Fig. 4. Quantitative results showing the effect of high (open bars) and low (grey bars) dose THC on neocortical brain injury. Vehicle treated control animals are shown in black. Injury is expressed as mean ± S.E.M. $^{*}P < 0.05$ compared to control.

The pharmacologic effects of cannabinoid compounds provide a rational counter-mechanism to components of the ischemic cascade. They induce a reversible inhibition of cAMP production [5], thereby limiting activation of potentially harmful protein kinases. The mechanism of curbing adenylate cyclase activity is via a G protein, Gi [5]. A synthetic, non-psychotropic cannabinoid, HU-211, is a non-competitive NMDA antagonist and blocks Ca^{2+} influx [11]. It has reduced ischemic injury in both focal and forebrain ischemia models [1,2]. The fact that our paradigm demonstrated less hippocampal protection suggests that THC binds NMDA receptors less avidly than HU-211. The potential detrimental effect of increasing arachidonic acid levels after ischemia [13] was not observed in this study. Regional cerebral blood flow was not measured by us, but has been noted to increase in human subjects after marijuana smoking [9].

The implications of this study are protean. Patients who fail conventional strategies of stroke prevention may ultimately benefit from judicious use of THC, singly or in combination with other agents. Cannabinoids have already proven to be of use in spasticity [12], chronic pain states [10], and chemotherapy-induced nausea [6]. Future directions of laboratory research include induction of ischemia either global (forebrain) or the more clinically relevant focal while blocking cannabinoid receptors, as well as investigating expression of receptor genes [8] in a stroke paradigm.

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