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Post-ischemic treatment with cannabidiol prevents electroencephalographic flattening, hyperlocomotion and neuronal injury in gerbils

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Abstract

The potential activity of cannabidiol, a non-psychoactive constituent of marijuana, in preventing damage caused by cerebral ischemia was studied. Cannabidiol (1.25–20 mg/kg) was given 5 min after 10 min bilateral carotid occlusion in freely-moving awake gerbils. Seven days after ischemia, it antagonized the electroencephalographic flattening of total spectral power, with a dose-dependent bell-shaped curve; the neuroprotective effect was greatest with 5 mg/kg. One day after ischemia cannabidiol completely antagonized ischemia-induced hyperlocomotion, at all doses. Rectal temperature did not change during the first hour after occlusion. Histological examination showed complete survival of CA1 neurons in cannabidiol-treated gerbils. These findings suggest a potential therapeutic role of cannabidiol in cerebral ischemia, though the clear mechanism of action remains to be elucidated.

Keywords: Global ischemia; Neuroprotection; Motor activity; Temperature; Natural cannabinoid

Cannabidiol, a major non-psychoactive constituent of marijuana, has potential therapeutic effects and low toxicity. A recent overview [13] on some pharmacological aspects reported protection against neurotoxicity mediated by NMDA, AMPA and kainate type receptors in vitro in rat cortical neuron cultures exposed to toxic levels of glutamate. At submicromolar concentrations, cannabidiol blocked cell death through its antioxidant activity [8]. In vivo, cannabidiol administered orally or i.p. showed potent anti-arthritic [11], anxiolytic and anti-emetic activity [13]. Exogenous and endogenous cannabinoids may help to protect against the effects of cerebral focal and global ischemia in rodents [4,14].

The aim of the present work was to investigate for the first time the effect of post-ischemic treatment with cannabidiol on transient global cerebral ischemia in the gerbil using a range of doses similar to those active against arthritis in mice [11]. To obtain a quantitative evaluation of the ischemic damage, we evaluated different parameters known to be hardly influenced by cerebral ischemia over 7 days [16]; these were total and relative electroencephalographic (EEG) spectral power, spontaneous motor activity and neuronal damage.

Male Mongolian gerbils (*Meriones unguiculatus*) (Charles River, Calco, Como, Italy) weighing 60–80 g were housed in standard laboratory conditions. EEG, body temperature and locomotor activity were recorded for each animal. All procedures were approved under Italian Governmental decree No. 94/2000-A.

Different groups of animals received vehicle or cannabidiol (1.25, 2.5, 5, 10 and 20 mg/kg, i.p.), kindly supplied by GW Pharmaceuticals (UK), 5 min after recirculation. The drug was dissolved in an appropriate vehicle (cremophor/ethanol/saline, 1:1:18) and injected i.p. in a volume of 5 ml/kg. Sham-operated animals received the same volume of vehicle. Gerbils were anesthetized with chloral hydrate (400 mg/kg, i.p., Sigma, St. Louis, MO) and four electrodes were surgically implanted as reported elsewhere [18]. Then,

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1 week later, the animals were acclimatized in a sound-attenuated Faraday chamber for 1 h a day for 3 days. Spectral powers between 0 and 25 Hz (0.2–4.0 Hz δ, 4.2–8.0 Hz θ, 8.2–13 Hz α, 13.2–25 Hz β) were recorded using a resolution of 0.2 Hz. The EEG recordings lasted 1 h and were made 1 h before, and then 1 h, 1, 3 and 7 days after ischemia induced by 10 min carotid occlusion. The ischemia was verified only qualitatively on paper by the complete flattening of the electroencephalogram. Each 1 h spectral power was calculated as the mean of six 1 min recordings taken at 10 min intervals. After basal EEG recording, each gerbil was again lightly anesthetized and body temperature was kept at 37 °C throughout surgery with a heating lamp. Both common carotids were exposed for 10 min as previously described [4]. Rectal temperature was measured with a rectal thermistore probe three times (every 10 min) before induction of ischemia (basal) and again 30, 60, 90 and 120 min after recirculation, as previously described [18].

Spontaneous motor activity was checked in an activity cage (Ugo Basile, Varese, Italy), as previously described [4]. Cumulative horizontal counts were recorded for 30 min, 1, 3, and 7 days after ischemia.

For histological determination, 7 days after the ischemic insult, three gerbils from the sham, vehicle and cannabidiol (5 mg/kg) groups were anesthetized and transcardially perfused with 4% formaldehyde. Brains were removed and paraffin-embedded. Five serial 5 μm coronal sections were cut at the level of the hippocampus and stained with cresyl violet. Neurons with a normal appearance in the pyramidal cell layer of the CA1 sector were counted blind (from coded slides) in each section for each group.

Since ischemia-induced hyperlocomotion is maximal on day 1 after occlusion [4] and the EEG power decrease may be related to pronounced damage of neurons on day 7 [16], only findings obtained respectively 1 and 7 days after ischemia for spontaneous motor activity and EEG are shown.

In sham-operated gerbils the spectral power activity remained unchanged (Fig. 1). Vehicle-treated ischemic gerbils showed a decrease of 88% from the pre-ischemic value (Fig. 1A). When the mean total spectral power was plotted against the log of the cannabidiol doses, a parabola was obtained ($r^2 = 0.99$, $P < 0.001$), the maximally effective dose being 5 mg/kg. Significant changes were found in the mean relative spectral power distribution in vehicle- and cannabidiol-treated ischemic groups (Fig. 1B, D). The δ frequency increased in vehicle- and cannabidiol-treated groups at 10 and 20 mg/kg, with complete recovery at 1.25, 2.5 and 5 mg/kg. The θ frequency did not change in any of the groups but complete recovery of β activity was seen in the cannabidiol 1.25, 2.5 and 5 mg/kg groups. The ischemia-induced α decrease was not affected by cannabidiol. Body temperature did not significantly change in any group (data not shown).

There were marked differences between treatments in

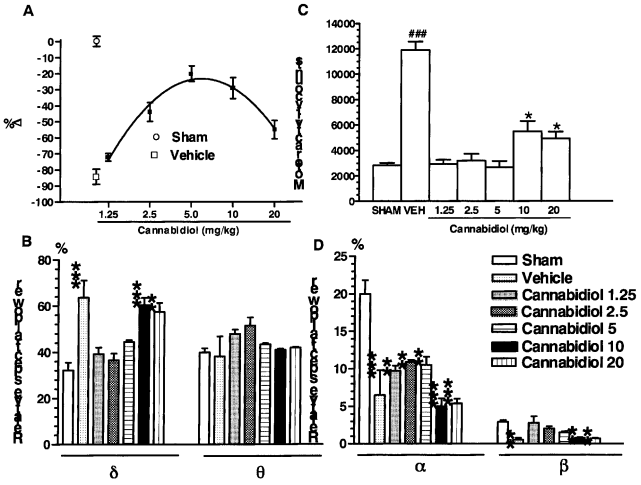

Fig. 1. (A) Parabolic regression curve of total spectral power (mean ± SEM), evaluated as the difference (Δ%) from the pre-ischemic value. (B,D) Cortically derived relative spectral power distribution in freely-moving awake gerbils 7 days after ischemia with increasing doses (log) of cannabidiol. (C) Effect of cannabidiol on spontaneous motor activity (mean ± SEM) 1 day after ischemia evaluated for 30 min as the number of horizontal counts. The compound was given i.p. 5 min after 10 min bilateral carotid occlusion. $N = 8$ for each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham-operated gerbils; $^{\#\#\#}P < 0.001$ vs. all remaining groups (C) (ANOVA, Tukey's test).

spontaneous motor activity ($P < 0.00001$) 1 day after ischemia (Fig. 1C). Vehicle-treated gerbils had a significant increase of mean horizontal counts while sham-operated animals did not. Cannabidiol at all tested doses antagonized the ischemia-induced hypermotility evaluated 1 day after ischemia, even if at the highest doses (10 and 20 mg/kg) the antagonism was not full.

Histological examination of the hippocampus showed a mean loss of 41% of neuronal cells in vehicle-treated gerbils, but complete recovery in the animals given cannabidiol 5 mg/kg (Table 1).

The present study demonstrates the neuroprotective effect of a non-psychoactive constituent of marijuana, cannabidiol, against ischemic damage, 5 mg/kg being the most effective dose. These findings agree with previous results in a rat stroke model in which i.v. cannabidiol before the onset of ischemia reduced infarct size by 60% [8].

Severe interference with cerebral blood flow has a pronounced effect on electrical activity, which is reflected in

Table 1
Neuronal cell count 7 days after reperfusion in the CA_1 region of the hippocampus of sham-operated or ischemic gerbils treated with vehicle or cannabidiol (5 mg/kg, i.p.) 5 min after 10 min bilateral carotid occlusion

Treatment	Neuronal cells[a] (mean ± SEM)
Sham-operated	319.40 ± 15.63
Vehicle + ischemia	187.00 ± 29.10***
Cannabidiol + ischemia	299.30 ± 7.41

[a] Mean of five hippocampal sections from the same coronal plane for each animal. $^{***}P < 0.001$ vs. sham-operated and cannabidiol groups (Tukey's test). $N = 3$ for each group.

EEG flattening. Since the decrease in EEG power 7 days after ischemia may be related to severe neuronal damage (delayed death), these cells being replaced by astrocytes in the hippocampal sub-field [16], the lasting protection in cannabidiol-treated ischemic gerbils indicates that the compound may contrast the cascade of pathological events that lead to neuronal death. The hippocampus is a preferred area for global ischemia-induced damage and the CA1 sector is the most selectively vulnerable to the reduced cerebral blood flow [19]. The survival of CA1 neurons 7 days after ischemia may account for the protection against EEG flattening in cannabidiol-treated gerbils.

The neuroprotective effect obtained with cannabidiol is in accordance with that exerted by other psychoactive CB1 synthetic cannabinoid agonists like CP 55,940, given 5 min after recirculation using the same ischemia model [4], and WIN 55,212-2, tested in a rat model of focal ischemia [14]. However, the latter was administered 30 or 40 min before carotid occlusion.

The dose-dependent bell-shaped curve is not surprising for cannabinoids since a similar pattern has been seen for the anti-inflammatory effect of cannabidiol [11] in a murine collagen-induced arthritis model and for the reinforcing properties of CP 55,940 using an i.c.v. self-administration paradigm in rats [3].

Cannabidiol also played an important role in protecting against ischemia-induced motor hyperactivity. This paradoxical behavior may indicate a reduction of the ischemic animal's ability to form spatial maps [15] because of the loss of CA_1 neurons. These findings agree with those in ischemic gerbils given CP 55,940 in the same test [4].

The mechanism of cannabidiol's neuroprotection has still to be clarified. During an ischemic episode, large amounts of the excitatory neurotransmitter glutamate are released, leading to neuronal death. Since cannabidiol prevented neuronal death in rat cortical neuron cultures exposed to toxic levels of glutamate, through antioxidant activity [13], a similar mechanism can be suggested for our animals.

Cannabidiol has been reported to inhibit anandamide amidase [20] and transporter activity [2,17]. Since anandamide belongs to a group of N-acyl-ethanolamines which are formed in large amounts in injured neurons [9], a neuroprotective effect through an increase of anandamide by cannabidiol can be suggested. Furthermore, cannabidiol was recently found to be a full, though weak, agonist of human vanilloid receptor type 1 (VR_1) [2], which has been found in the brain [5,13], particularly in the hippocampus where it is functionally active [1] and in the locus coeruleus where it stimulates glutamate release [12]. The ability of cannabidiol to desensitize VR_1 to the action of capsaicin [2] opens up the possibility of its having anti-inflammatory action, partly by desensitizing sensory nociceptors. An interesting, though speculative, explanation is that cannabidiol might act as a partial agonist/antagonist for VR_1 stimulation. This would account for the bell-shaped curve obtained by fitting the mean total spectral power with increasing doses. At lower doses cannabidiol might antagonize or desensitize VR_1 while at higher doses it could act as an antagonist.

Cannabidiol was active against ischemia-induced hyperlocomotion at all doses tested. One explanation is that stimulation of VR_1 receptors by the highest doses may have a locomotor inhibitory effect, as shown in rats and mice [6,7]. Cannabinoids promote hypothermia, which is neuroprotective in some settings. However, any such effect can be excluded in our experiments since body temperature was held constant during the early stages and the compound had no hypothermic effect [10].

Cannabidiol has been found to block clinical symptoms of arthritis in a murine collagen-induced arthritis model [11]. Since cytotoxic and vasogenic edema appears during and after bilateral carotid occlusion in gerbils [19], an anti-inflammatory action of cannabidiol on cerebral ischemia cannot be ruled out.

In summary, cannabidiol given after ischemic insult in gerbils antagonized the ischemia-induced EEG flattening and hyperlocomotion by promoting the survival of CA_1 neurons. Further studies are obviously needed to elucidate the mechanism of action, but these findings, together with the well-documented low toxicity, suggest a therapeutic role as an anti-ischemic drug for this naturally occurring non-psychoactive ingredient of marijuana.

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