STRESS DIFFERENTIALLY MODULATES BEHAVIORAL RESPONSES TO THE CANNABINOID AGONIST, WIN 55,212-2, IN ADULT RATS

FABRÍCIO LUIZ ASSINI1; REINALDO NAOTO TAKAHASHI2

2. Doutorado em Psicologia pela La Trobe University, Austrália. Professor Titular da Universidade Federal de Santa Catarina. Departamento de Farmacologia, Campus Trindade, 88049-900, Florianópolis, SC, Brazil. (48) 331 9764. takahashi@farmaco.ufsc.br

ABSTRACT

There has been a growing interest in the role of the endogenous cannabinoid system in the regulation of emotional states. The behavioral interaction between stress and cannabinoids seems to have different patterns depending on the stress carried out, i.e.: acute, repeated or variable stress. In this study, we evaluated and compared the alterations induced by two types of stress, the neonatal isolation and restraint stress. The neonatal isolation procedure was performed for 7 days – post-natal day 2 to 9 - when each pup was placed in an individual round plastic container, with no bedding, remaining for 1 h at 30°C. On the other hand, in the restraint stress procedure, 80 days old rats were immobilized in a wire chamber for 2 h. The administration of the CB1 receptor agonist WIN 55,212-2 (0.25; 0.75 and 1.25 mg/kg i.p.) was used to evaluate the effects of these two stress-procedures over cannabinoid responses of adult rats tested in the elevated plus-maze. The main finding was that only in restraint stressed animals the administration of the CB1 receptor agonist – WIN 55,212-2 - was able to interact with stress to alter the behavior of adult rats. Hence, this effect indicates that stress differentially modulates behavioral responses to this cannabinoid agonist in the elevated plus maze. The present study suggests that future research evaluating the potential use of cannabis derivatives in anti-anxiety therapies should consider the distinct interaction between different types of stress and the effects of cannabinoids.


INTRODUCTION

Cannabis use is strongly promoted by unfavorable social conditions1. Furthermore, several factors have influence over cannabis consumption, such as environmental context, basal levels of stress and anxiety2. On the other hand, it is believed that cannabinoid receptor might represent a good pharmacological target for stress-related disorders3.

Dysfunctional responding to stress is a component of some neuropsychiatric disorders, including panic disorders, post-traumatic stress disorders and drug abuse. Several studies suggests complex interactions between cannabinoid signaling and anxiety-like behavior, on one hand relatively low doses produce anxiolytic-like effects in animals depending on behavioral model4, specific test conditions5 and genetic strain6 on the other hand higher doses produces an anxiogenic profile observed in models including elevated plus-maze, light-dark exploration and social interaction task7-8.

The behavioral interaction between stress and cannabinoids has been better evaluated over the last years and seems to have different patterns in acute, repeated or variable stress9. The activation of CB1 receptor...
seems to facilitate the extinction of aversive memories\textsuperscript{10-12} and the administration of the natural cannabinoid constituent, cannabidiol, has been associated with anti-aversive behaviors\textsuperscript{13-14}. In the field of anxiety, earlier studies have shown that prior exposure of adult rats to stress sensitizes them to the anxiogenic effects of cannabinoid agonists\textsuperscript{15} and that the anxiety-like effect of cannabinoid gene disruption is context/stress dependent\textsuperscript{16}.

Different authors have shown that when anxiety, measured in the elevated plus-maze, is potentiated by prior stressor exposure it may be an interesting measure of enhanced anxiety state involved in many psychopathologies\textsuperscript{17}. This anxiogenic-like behavior of adult rats has been described 24 hours after stress\textsuperscript{18} or even, in a more persistent effect, as a consequence of neonatal isolation stress\textsuperscript{19}. It is worth noting that although both types of stress augments fear-related behavior in the EPM, the mechanisms linked to these alterations probably are different.

Therefore, it is tempting to suggest that appropriate modulation of cannabinoid system has therapeutic potential in the treatment of anxiety-related neuropsychiatric disorders. Hence the purpose of the present study was to evaluate the effects of two stress-procedures over cannabinoid responses of adult rats tested in the elevated plus-maze.

**MATERIALS AND METHODS**

**ANIMALS:** For the neonatal isolation experiment, Wistar rats were mated in the Psychopharmacology Laboratory of the Federal University of Santa Catarina, and the male offspring of these matings served as experimental subjects. Pregnant females were housed individually in plastic cages (13 x 16 inch, 208 sq inch) with free access to rat chow and tap water. Litters born before 5 p.m. on a given day were denoted as born on that day (Day 0). The litters were culled to 9-12 on postnatal day (PND) 2 with the goal of balancing the number of pups among dams. Each litter was randomly assigned to either the neonatal isolation (ISO) or nonhandled (NH) condition. Litters were weighed on PND 9 and 21 and than left undisturbed until the 10th week of life, when behavioral tests were carried out. In the restraint stress experiment, 10 week-old male rats raised in the Psychopharmacology Laboratory and housed in groups of 5 animals in 13 x 16 inch plastic cages were used. The rats were maintained under controlled temperature (23 ± 2 °C) and a 12-h light:12-h dark cycle (lights on at 7:00 a.m.). All the procedures described here were performed in accordance with the guidelines on animal care of the UFSC Committee for Ethics in the Use of Animals, which follows the “Principles of laboratory animal care” from the NIH.

**PROCEDURES:** The neonatal isolation procedure was performed for 7 days (PND 2 to 9). Each pup designated for isolation was placed in an individual round plastic container (9 cm diameter, 7 cm high), with no bedding, where it remained for 1 h at 30°C. Plastic containers were kept 20 cm apart to minimize olfactory cues from siblings. Following the isolation period, pups were returned to their home cages. Isolations were carried out
between 12 noon and 5 p.m. each day. NH control litters were left undisturbed throughout the neonatal isolation stress period, except for weekly cage cleaning and weighing on PND 9. All pups were weaned on PND 21 and housed with 5-6 per cage. In the restraint stress procedure, 10 week-old rats were immobilized in a wire chamber (5.5 cm diameter) for 2 h, and 4 hours later they were tested in the EPM test.

**DRUGS:** (R)-(+)-[2,3-dihydro-5-methyl-3-[4-(morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoazxin-6-yl]-[1--naphthalenylme]-thanone (WIN 55,212-2) (Tocris Cookson Ltd, Bristol, UK) was dissolved in saline plus 10% dimethylsulfoxide (DMSO). The drug vehicle was used as control solution. All solutions were administered by intraperitoneal (i.p.) route in a volume of 1 ml/kg. The EPM test was carried out 20 minutes after WIN (0.25, 0.75, 1.25 mg/kg) or vehicle injections.

**ELEVATED PLUS-MAZE:** The elevated plus-maze (EPM) apparatus was made of wood covered by a layer of black Formica with four elevated arms (52 cm above the floor), 50 cm long and 10 cm wide. The arms were arranged in the form of a cross, with two opposite arms being enclosed by 40-cm high walls and two being open. A central platform (10 x 13.5 cm) gave access to any of the four arms. The open arms were surrounded by a raised ledge (1-mm thick and 5-mm high) to avoid rats falling off the arms. The experimental room was dimly illuminated by a house light (9 lux). Each rat was placed on to the central platform facing a closed arm and the number of entries and the time spent (with all four paws) inside each type of arm, together with the percentage of open-arm entries in relation to the total number of arm entries was recorded for 5 min.

**STATISTICAL ANALYSIS:** The results were analyzed by two-way ANOVA with stress and treatment as factors. Post-hoc comparisons were performed using the LSD test, when appropriate. Differences were considered significant when p<0.05.

**RESULTS**

Figure 1 shows the effects of neonatal isolation on the behavior of rats, treated with WIN or saline vehicle, in the EPM test. Two-way ANOVA showed a significant effect of treatment on the % of time spent on the open arms [F(3,63)= 3.64 p<0.01], as well as on the % of open-arms entries [F(3,63)= 4.68 p<0.01], but not on closed-arm entries. There were no effects of stress or the interactions between stress and treatment on the EPM measures. Post-hoc comparisons indicated that WIN (0.75 mg/kg) reduced the % of time and the % of entries in the open arms in NH rats (p<0.05). In the ISO group the dose of 1.25 mg/kg reduced both the % of time and the % of entries in the open arms (p<0.05), whereas the dose of 0.75 mg/kg significantly reduced the % of time in the open arms (p<0.05).

Figure 1 shows the effects of neonatal isolation on the behavior of rats, treated with WIN or saline vehicle, in the EPM test. Two-way ANOVA showed a significant effect of treatment on the % of time spent on the open arms [F(3,63)= 3.64 p<0.01], as well as on the % of open-arms entries [F(3,63)= 4.68 p<0.01], but not on closed-arm entries. There were no effects of stress or the interactions between stress and treatment on the EPM measures. Post-hoc comparisons indicated that WIN (0.75 mg/kg) reduced the % of time and the % of entries in the open arms in NH rats (p<0.05). In the ISO group the dose of 1.25 mg/kg reduced both the % of time and the % of entries in the open arms (p<0.05), whereas the dose of 0.75 mg/kg significantly reduced the % of time in the open arms (p<0.05).
arms \[F(3,63)= 3.64 p<0.01\], as well as on the
% of open-arms entries \[F(3,63)= 4.68 p<0.01\],
but not on closed-arm entries. There were no
effects of stress or the interactions between
stress and treatment on the EPM measures.
Post-hoc comparisons indicated that WIN
\((0.75 \text{ mg/kg})\) reduced the % of time and the %
of entries in the open arms in NH rats \((p<0.05)\).
In the ISO group the dose of 1.25 mg/kg
reduced both the % of time and the % of
entries in the open arms \((p<0.05)\), whereas the
dose of 0.75 mg/kg significantly reduced the %
of time in the open arms \((p<0.05)\).

DISCUSSION

In the present study we investigated
the effects of the cannabinoid agonist WIN
55,212-2 on the behavior of adult rats in the
elevated plus-maze following neonatal
isolation or restraint stress. Our data
demonstrate that the anxiolitic-like effect of the
cannabinoid agonist Win 55,212-2 was
dependent on the type of stress used, once
this treatment has consistently reversed only
the restraint-induced anxiogenic-like effect in
the EPM.

As mentioned in Introduction, anxiety-
like behaviors in the EPM can be potentiated
by prior exposure to an inescapable stressor, a
situation that has been called fear-potentiated
plus-maze\(^{17}\). Following this, previous studies
investigating the effects of acute restraint
stress on EPM behavior reported an increase
in anxiety-like responses\(^{20-21}\). In agreement
with these findings, in the present study we
showed that 2 h of restraint stress induced
anxiety-like behavior in the EPM when
measured 4 h later. This behavioral effect of
restraint was reversed by a low dose of WIN
55,212-2 administered 20 minutes before
testing, thus suggesting that the activation of
the cannabinoid system counteracts the effects
of stress in the EPM. In line with these results,
other authors have been suggesting that WIN
55,212-2 in a similar concentration than our, is
able to counteracts restraint-induced gastric
ulcers\(^{22}\) and that the activation of CB1
receptors by endocannabinoids opposes the
behavioral and neuronal responses to aversive
stimuli\(^{23}\). On the other hand, the fact that Hill
and Gorzalka\(^{15}\) reported anxiogenic-like
responses after a low dose of the cannabinoid
agonist HU-210 in chronically stressed rats
might suggest that acute and chronic stress
activate different physiological substrates that
interact in opposite ways with the cannabinoid
system.

Unexpectedly, our hypothesis that
neonatal isolation could affect cannabinoid
modulation of anxiety expressed in the
elevated plus maze was not confirmed. It is
known that this protocol induces persistent
alterations in the HPA-axis\(^{24}\) and in the
basolateral amygdala\(^{25}\) of adult rats and that
these areas\(^{26,27}\) are involved in behavioral
effects induced by cannabinoids.

More importantly, the effects of
neonatal isolation over anxiety behavior
evaluated in the elevated-plus maze during
adulthood are not conclusive. While some
authors have shown an anxiogenic-like
response\(^{19,28}\), others have suggested lack of
effects\(^{29}\). Hence, one possible explanation for
the absence of effect of WIN 55,212-2 in the
neonatal isolated animals is that the neonatal
isolation procedure was not stressful enough
to induce anxiogenic-like behavior in the EPM, as was the restraint-stress. Indeed McIntosh et al. \(^{30}\) have proposed that short and long term periods of maternal separation stress differentially affect anxiety in adult rats. Pups briefly separated (10-15 min) from the dam on a daily basis for the first 2 weeks of life, show reduced HPA responsiveness to stressors and less anxiety in tests such as the EPM. In contrast, longer periods (2 h or more) of daily maternal separation are reported to increase HPA responsiveness to stressors and overt anxiogenic-like responses in the EPM\(^{31}\). Hence, the lack of effect of neonatal isolation in the present study was not entirely a surprise, since we isolated pups for 1 hour from PND 2-9, which could have been more than sufficient to induce anxiolytic-like effects and, on the other hand, might not have been intense enough to induce anxiogenic-like effects.

Taken together, we show that the behavioral outcome of WIN 55,212-2 in the EPM was dependent on the basal level of stress because only when stress was robust enough to produce an anxiogenic-like response in control animals we observed a positive interaction between cannabinoid receptor activation and the expression of anxiety in the EPM. Indeed, this context-dependent interaction has been shown by others authors, Haller et al. \(^{16}\) have shown that a high light context was anxiogenic to CB1 receptor knock-out mice evaluated in the EPM. Moreover, the cannabinoid receptor antagonists, SR 141716A\(^{32}\) and AM251\(^{33}\), induced marked changes during second exposure to the maze. As mice show heightened fear in the second exposition to the EPM\(^{34-35}\) and we have observed a positive interaction of cannabinoid receptor activation when control animals exhibited an anxiogenic-like behavior we may suggest that the modulation of eCB system in the EPM is better associated with a stressful condition.

It must be conceded that in the present study we have not tried a pharmacological blockade of neither CB1 nor CB2 receptors and this might be one of the limitations of our work. However, given that numerous studies have previously shown that WIN possesses affinity at CB1 and CB2 receptors, the results we obtained could be acceptable without the above control. Certainly, further studies should evaluate the participation of CB1\(^{36}\), CB2\(^{37}\) and non-CB1/ non-CB2 receptors\(^{38}\) under stressfull conditions.

In conclusion, we have provided the first demonstration that acute restraint stress in adult rats is more effective than neonatal isolation for the evaluation of stress and cannabinoid interaction-induced anxiety in the EPM. Additional studies are necessary to better understand these responses. The anxiolytic-like effect of WIN 55,212-2 in restraint stressed-animals reported here confirms a potential use of these drugs in stress induced-anxiety pathologies.

REFERÊNCIAS

3 Carrier EJ, Patel S, Hillard CJ. Endocannabinoids in neuroimmunology and
26 Pistis M, Perra S, Pillolla G, Melis M, Gessa GL, Muntoni AL. Cannabinoids modulate neuronal firing in the rat basolateral amygdala: evidence for CB1- and non-CB1-mediated...
ANEXOS

Fig. 1: Effects of neonatal isolation stress on WIN 55,212-2 effects in non-handled (left) and isolated (right) animals evaluated in the elevated plus-maze. Data are presented as mean values and SEM of 8-9 animals. * p<0.05 compared to the respective control group (LSD post-hoc test).

Fig. 2: Effects of restraint stress on WIN 55,212-2 effects in naïve (left) and stressed (right) animals evaluated in the elevated plus-maze. Data are presented as mean values and SEM of 8-10 animals. * p<0.05 compared to the respective control group. # p<0.05 compared to the naive control group (LSD post-hoc test).