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Moxidectin Interference on Motor Activity of Rats

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ABSTRACT

The present study investigated the effects of the moxidectin (MXD) in some parameters of rat motor function and neurochemical. The general activity in the open field and the motor coordination in the wooden beam were employed to evaluate the MXD effects. The results showed that, in the open field, even at high doses (2.0 and 20.0 mg/kg), the MXD did not alter the locomotion and the rearing frequencies. However, MXD was able to impair the motor coordination of the animals at wooden beam. Neurochemical studies of striatal GABA and dopamine neurotransmitters showed a reduced levels of dopamine and its metabolite, homovanillic acid, without interference on striatal GABA levels. Since GABAergic receptor stimulation had an inhibitory effect on dopaminergic striatal system, the decreased motor coordination could be attributed to an action of MXD on dopamine system via GABA activation.

Key words: Moxidectin, GABA, motor coordination, rat, open field, wooden beam

INTRODUCTION

Moxidectin (MXD) is a milbemycin compound produced by a combination of fermentation and chemicals synthesis. MXD is obtained by chemical modification of nemadectin, the natural compound is produced when *Streptomyces cianogriseus* is ground under controlled culture conditions (Takigushi et al., 1980). The milbemycins were discovered in 1973, as acaricidal and insecticidal compounds for crop protection by Sankyo scientists and their name reflected this (*milbe* mite + *myc* fungus + *in* pharmaceutical product) (Campbell, 1989; Steel, 1993; Shoop et al.,1995; Lanusse et al. 1997). The mylbemicins anthelmintics have a broad-spectrum activity against internal and external parasites in animals. MDX is a milbemycin endectocide compound active at extremely low dosages against a wide variety of nematode and arthropod parasites of domestic animals (Campbell, 1989; Lifschitz et al., 2002; Njue et al., 2004).

Milbemicyn and avermectins (AVM) are highly lipophilic substances that dissolve in most organic solvents, and MXD solubility in water is 4.3mg/L. Their high lipophilicity accounts for a wide tissue distribution and long residence in plasma, and allows these substances to cross the cellular barriers (Lanusse et al., 1997). After treatment, this drug is found mainly in liver and fat (Molento et al., 2004).

MXD peak plasma concentration (C_{max}) were achieved 8h post-treatment (subcutaneous administration of 200μg/kg to cattle), and the

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concentration of MXD in fat after 28 days of treatment in cattle has been shown to be ninety-fold higher than that detected in plasma. A great proportion of MXD parent drug would accumulate in fat, such phenomenon explaining the long plasma residence time obtained for this drug, after an early peak concentration followed by a fast decline (distribution phase) in the plasma concentration profile between the C_{max} and 10 days post-treatment (Lanusse et al., 1997). When administered orally to ovine and rats, this peak will be of 10 hours (Joint FAO/WHO, 1995).

MXD showed moderate toxicity when administered orally to rats and mice (50% lethal dose (LD 50) 50-100 mg/kg (Joint FAO/WHO, 1995). When MXD is administered to the animals in high doses, the adverse effects observed are characterized by neurotoxicity signs as depression, ataxia, tremors, anxiety, vision difficulty, coma and death.

Although the antiparasitic activity of the milbemycins has been described since more than two decades, its mechanism of action is still not totally elucidated. The main difficulty is due to controversial studies performed in different biological systems with different methodologies, which provides inconclusive data. In this respect, studies about the AVM's mechanism of action are more abundant than those of the milbemycins. The molecular structures of the two groups of so-called 'endectocide' compounds are superimposable (Shoop et al., 1995). They share some structural and physicochemical properties, and their broad-spectrum antiparasitic activity against nematodes and arthropods at extremely low dosage rates (Steel, 1993) is based on a common mode of action (Shoop et al., 1995; Lanusse et al., 1997; Fisher and Mrozik, 1989; Turner and Schaeffer; 1989; Arena et al., 1991, 1992, and 1995; Roder, 1998; Spinosa et al., 1999 and 2002; Forrester et al., 2002).

MXD and ivermectin are believed to act by binding to glutamate- and gamma-aminobutyric acid (GABA)-gated chloride channels, resulting in somatic and pharyngeal muscle paralysis of the parasite, respectively (Duce and Scott, 1985; Dawson et al., 2000; Feng et al., 2002; Lifschitz et al, 2002; Molento et al.,2004; Njue et al, 2004). In the insects and arthropods, the AVMs interfere at the transmission among nervous and muscular cells, because the GABAergic receptors are located at the neuromuscular junction. These inhibitory receptors are found only in invertebrates (insects, crustaceans and nematodes) and belong to the superfamily of ligand-gated ion channels (Arena, 1994; Cleland, 1996; Cully et al., 1996; Njue et al., 2004). AVMs also interact with GABA receptors in brain of vertebrates (mammals), but its affinity for the invertebrate animals receptor is approximately 100 times greater (Schaeffer and Haines, 1989).

Thus, most of the studies accomplished on the mechanism of action of the macrocyclic lactones in mammals involve the GABA neurotransmitter, since this neurotransmitter has an important role in the regulation of the motor activity (Agmo and Giordano, 1985; Tegnér et al., 1993; Kriem et al., 1998). The goal of the current study was to evaluate the possible effects of the MXD in the general activity of rats observed in the open field and in their motor coordination at the wooden beam. Also, neurochemical analysis of striatal GABA and dopamine neurotransmitters were performed.

MATERIAL AND METHODS

Animals

Male Wistar rats, weighing 180-370 g, which come from local colony, were used. Seven days before the experiments, the animals were housed, in number of five, in plastic cages with metallic cover (40 x 50 x 20cm) at a controlled room temperature (22 ± 2ºC), with a 12h light-dark cycle (the lights came on at 07:00). Water and food were available *ad libitum*. The animals were randomly divided into control and experimental groups. Behavioral tests on control and experimental rats were intermixed in order to minimizing circadian changes. All the observations were made between 08:00 and 18:00. The animals used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA.

Drugs

Moxidectin (Cydectin - Fort Dodge Animal Health Ltd., Campinas) was diluted in sweet almond oil (Industrial Leclerc Ltd., São Paulo), obtaining the concentrations of 0.2, 2.0 and 20 mg/ml, just before subcutaneous (SC) injections of 1.0 ml/kg from one of these preparations.

The lowest MXD dose employed was 0.2 mg/kg, i.e., a therapeutic dose widely used clinically in

different animal species; the remaining doses, i.e., 2.0 and 20.0 mg/kg were increased in a logarithmic scale.

Open Field

Sixty four male rats were divided into four groups: three experimental (n = 16 for each group; 0.2, 2.0 or 20.0mg/kg of MXD) and one control (n = 16; 1.0 ml/kg sweet almond oil), and tested in an open field. The open field apparatus was a round wooden arena, 970 mm in diameter and metallic wall, 280 mm of height, painted white, with the floor divided into 25 areas almost equal, delineated in black. During the experiment, three 40W white bulbs hanging 720 mm from the floor provided continuous illumination of the arena. Hand-operated counters were employed to score locomotion (number of floor areas entered) and rearing frequencies (number of times that an animal stood on its hind legs) and a chronometer was used to measure the duration of immobility (total time without spontaneous movements). For open field observations, each rat was individually placed in the center of arena and its behavioral parameters were recorded over 3 min at 24 and 72 h after the treatment. The rats returned to their home cage immediately after the observations. The animals of the control and experimental groups were observed at random. The open field apparatus was washed with a 5% ethanol solution before introduction of each animal in the arena, in order to eliminate any possible influences of smell left by previous subjects.

Wooden Beam

The motor coordination was evaluated through a new model, the wooden beam. This model was adapted from the one described by Jeffery and Blakemore (1997). The apparatus was a wooden beam, 18 mm in width and thickness and 2 m in length, with a 100 mm^2 and 18 mm in thickness platform in each extremity. The beam had a 200 mm height feet, at platforms and at center, painted white with two black vertical marks delimiting 1m in the central portion (Fig. 1). During the experiment, this beam was leaning on a balcony of the observation room. Thirty rats were used in the wooden beam test, divided in three experimental groups (n=10 for each group; 0.2, 2.0 or 20.0 mg/kg of MXD). Initially, each rat was trained to walk on the beam, in 5 min daily sessions, in the following way: on the first day, positive reinforcement (small portion of condensed milk) was put on both platforms and, following, the rat was introduced to get used to the environment and to the reinforcement. Next day, the animal was put at beam, close to the platform with the reinforcement, with the head turned back to this. On the following days, the rat was put at beam, however, at more and more distant from the platform with reinforcement, until the animal crossed the beam, reaching the opposite platform and returned to the initial platform, always receiving the reinforcement at the end of each crossing. The training period (7 to 10 days) was considered complete when each rat could reliably cross the beam without stalling (four crossings), few footstep errors were made at this stage. The animals that were not capable to walk in the beam in 10 days were eliminated from the experiment. Once trained, the rats were submitted to three observation sessions (24 h before and 24, 72 h after the treatment). During each evaluation, a score (Table 1) was attributed for each step with the pelvic member, turned for the observer, when the rat walked in the central portion of beam (1m among the vertical marks) at each cross. At the end of each session, the scores obtained by each animal in the four crossings were added. Before each animal was tested, the wooden beam was cleaned with a cloth moistened in water and after that all the animals of each cage, with 5% ethanol solution.

Table 1 - Scores for the evaluation of the rat behavior at wooden beam (Jeffery and Blakemore, 1997).

Score	Foot Position
0	Normal: foot positioned on top of beam, no slippage
1	Minor error: foot slip so that part of the foot was visible below the lower surface of beam
2	Major error: whole foot slipped below the lower surface of the beam

Determination of striatal neurotransmitters and metabolite levels

Eighteen male rats were divided into two groups: the experimental (n = 9) received 0.2 mg/kg of MOX) and the control (n = 9) 1.0 ml/kg sweet almond oil). Rats treated with as without MOX were decapitated 72 h after the treatments. Brains were dissected on dry ice and prepared as described previously (Felicio et al., 1996). Briefly, the striatum was weighted and stored -70 °C. During the weeks following the sample collection, perchloric acid was added to the tissues, which were then homogenized by sonication one week before the neurochemical evaluations. Dopamine (DA) and its metabolites, homovanillic acid (HVA) and 3,4 dihydroxyphenylacetic acid (DOPAC) were measured by HPLC (Shimadzu, model 6A) with a C-1 column (Shimpak-ODS), an electrochemical detector (Shimadzu, model 6A), a sample injector (valve for 20 µl), and an integrator (Shimadzu, model 6^A Chromatopac). GABA was measured by HPLC (HP, model 1100) with a Beckman 5 µ Ultrasphere ODS-PTH column, a sample injector (valve for 1.0 ml). Each sample was run for 25 min to DA and its metabolites and 28 min to GABA. The limit of detection was 2 pg for DA, DOPAC and 20 pg for HVA and GABA.

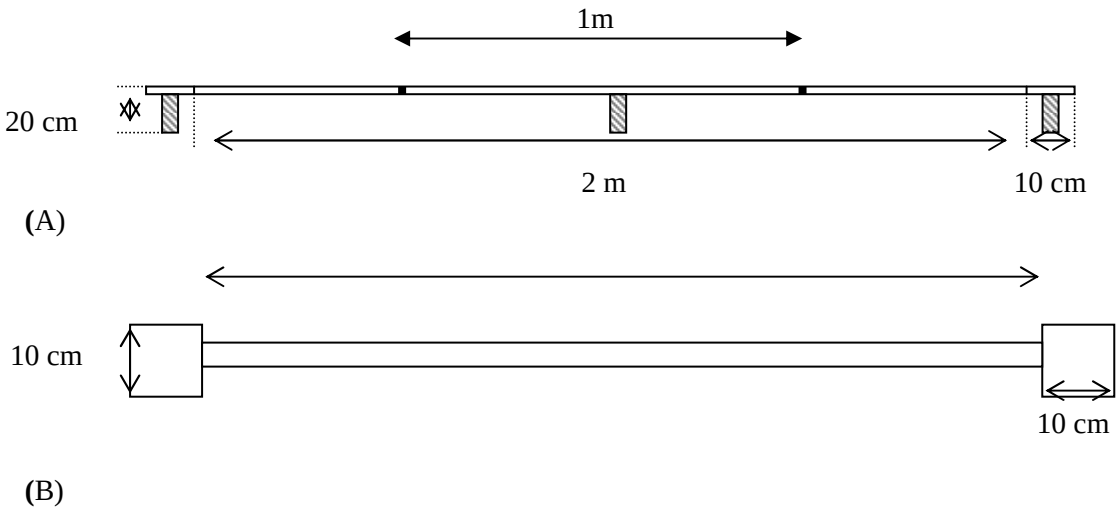

Figure1 - Scheme of wooden beam (thickness = 18 mm). (A) Lateral view and (B) superior view.

Statistical Analysis

The Bartlett test was used to evaluate the data homocedasticity. Analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test, was used to analyze the data from the open field test. The Friedman test for repeated measures, followed by the Dunn test for multiple comparisons was employed to the data from wooden beam test. The neurochemical data were analyzed by Mann-Whitney-U-test. In these experiments, $p<0.05$ was the criterion for statistical significance.

RESULTS

Fig. 2 shows the effects of the administration of the MXD in the general activity of rats observed in the open field. Although $GABA_B$ receptor agonist (baclofen) inhibited dose-dependently the locomotor activity (Agmo and Giordano, 1985; Nissbrandt and Engberg, 1996; Paredes et al., 1997), in the current experiment, the ANOVA did not show significant differences in the locomotion frequencies ($F24=0.90$; $F72=0.29$; $df=3/63$, $p>0.05$) and rearing frequencies ($F24=0.94$; $F72=1.79$; $df=3/63$, $p>0.05$) among control and experimental groups. On the other hand, significant differences among the groups were observed in the immobility duration when the rats were observed after 72 h ($F24=1.79$; $F72=5.23$; $df=3/63$, $p<0.05$); the Tukey-Kramer test showed reduction in the duration of immobility of the animals that received 0.2 or 20 mg/kg of MXD, at the 72 h post-treatment, when compared to the animals of the control group.

Although MXD did not show significant effects on the locomotion frequencies, this was able to impair motor coordination of rats evaluated at the wooden beam (Fig. 3). Significant differences were observed in the sum of the scores of the rats that received 0.2 mg/kg ($Fr=16.632$, $p<0.05$), 2.0 mg/kg ($Fr=16.800$, $p<0.05$) and 20 mg/kg ($Fr=15.842$, $p<0.05$) of MXD. The Dunn test of multiple comparisons showed that there was an increase in the sum of the scores after 24 and 72 h of the administration of the different doses of the drug, when compared to the sum of the scores obtained 24 h before the injection. At this time (base line), after the training, almost all rats were able to cross the beam with no error.

Table 2 shows the effects of MXD on striatal GABA, dopamine and its metabolite levels. MXD treatment reduced the striatal dopamine and HVA levels. No differences were observed between MXD and controls in striatal GABAergic and DOPAC levels as well as in HVA/DA and DOPAC/DA ratios.

DISCUSSION

The behavioral observations were accomplished 24 and 72 h after the administration of MXD, since post SC administration its C_{max} were achieved 8 h post-treatment in cattle and had a half-life of 80 h (Lanusse et al, 1997; Joint FAO/WHO, 1995). MXD showed a long plasma residence time after an early peak concentration, followed by a fast decline (distribution phase) in the plasma concentration profile between the C_{max} and 10 days post-treatment (Lanusse et al, 1997).

In the present work, the possible effects of the MXD in the general activity of the rats observed in the open field were studied. General activity is an index for evaluating behavioral changes induced in animals, not only by physiological and genetic manipulation, but also by toxicological ones. Among the techniques used to assess general activity, open field techniques stand out, making it possible to measure various behavioral parameters, among which are those related to emotional, exploratory and motor behavior.

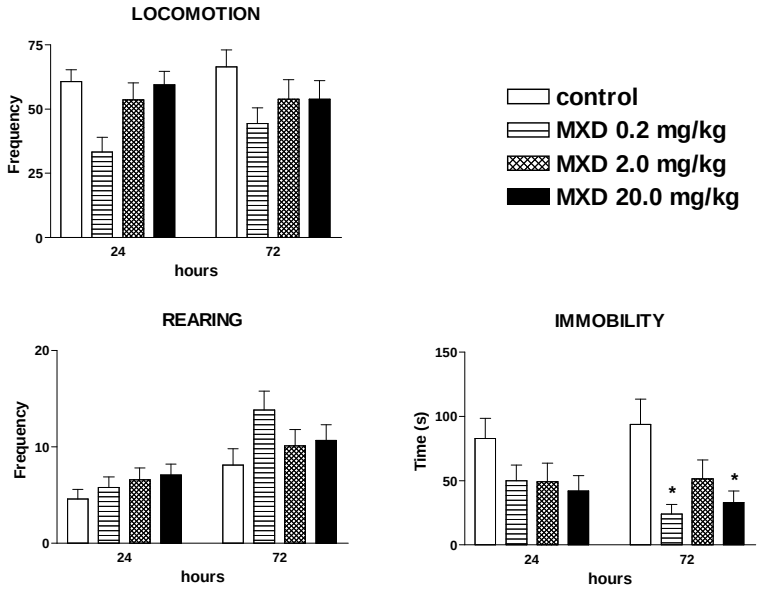

Figure 2 - The effects of moxidectin (MXD) on general activity of rats observed in open field. Data are means ± standard errors of mean. *$p<0.05$, ANOVA and Tukey-Kramer (in relation to control group).

Table 2 - The effects of moxidectin (MXD) on striatal GABA, dopamine and its metabolite levels (ng/g tissue). Animals were treated with 0.2 mg/kg MXD, 72 h. before the assay. Data are means ± standard errors of mean. N = 9 per group.

	Groups	
	Control	MXD (0.2 mg/kg)
GABA	905.50 ± 163.13	778.45 ± 146.55
DA	3,511.90 ± 638.91	1,537.30 ± 397.69*
HVA	141.24 ± 24.02	73.20 ± 17.41*
DOPAC	204.66 ± 39.08	119.04 ± 28.39
HVA/DA	0.040 ± 0.006	0.058 ± 0.007
DOPAC/DA	0.006 ± 0.008	0.008 ± 0.010

*p<0.05, in relation to control group. Mann-Whitney U-test.

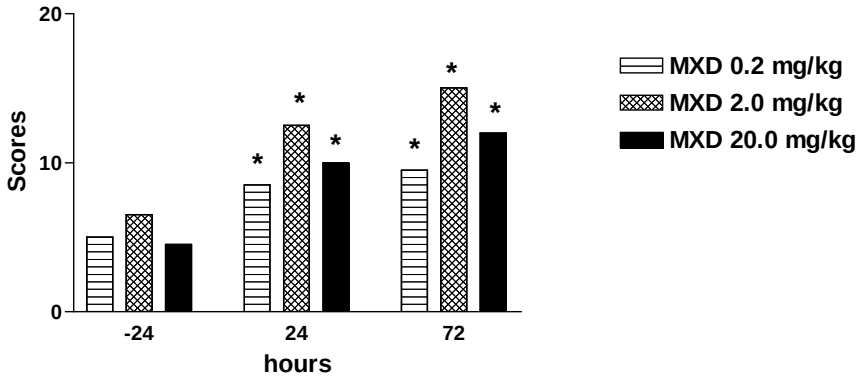

Figure 3 - The effects of moxidectin (MXD) on motor coordination of rats evaluated at wooden beam. Data are medians of the sum of errors for the four crossings obtained 24 h before (-24) and 24 and 72 h after the treatment. *p<0.05, Friedman test and Dunn test (in relation to -24 h group).

The first exposure of the animal to the device has a sharper emotional component than the remaining agents of exposure (Moniz et al., 1994; Batatinha et al., 1995; Massoco et al., 1995). This method provides a direct behavioral observation and is very much used in psycho-pharmacology studies of animals, because it is simple and is available for a fast and easy measure of the several behaviors that can be clearly defined. The general activity measured in the open field can be useful to define the doses, administration ways and the latency to the effect of a chemical substance to be studied.

Locomotion by the rat in an open field is inversely associated with anxiety ratings, depending upon whether the locomotion appears purposeful and whether the animal exhibits other exploratory behaviors such as rearing or approaching the center of the open field, in contrast to exhibiting anxiety-related behaviors such as hesitant locomotion, freezing, shivering, and defecation (Dishman et al., 1996). As the GABAergic drugs are ansiolitcs, the rats of this study that received 0.2 or 20 mg/kg of MXD and were observed 72 h after the injections, showed significant reduction of immobility when compared with the rats of control group, and a loss of anxiety was noticed. The lack of effect of 2.0 mg/kg of MXD may be due to biphasic action of GABAergic drugs (Lloyd and Morselli, 1987; Car and Wisniewski, 1998; Silva et al., 2005).

In this experiment, just a few of significant alterations were detected after the drug administration. This indicated that, even in high doses, MXD did not modify the rat's exploration behaviors in the open field, showing just a weak motor inhibition effect. On the other hand, MDX was able to impair the motor coordination of the animals observed at the wooden beam.

The animal's immobility in the open field and the impairment of motor coordination presented an

inversed-U-shape profile 72 h after the treatments, and not in the classical dose-response curve. It is possible that both facts expressed the motor impairment induced by MXD. Additionally, even in a therapeutic dose widely used clinically in different animal species, the motor incoordination was observed.

It is well established that most neurons located within the striatum are GABAergic. The firing pattern of nigral dopaminergic neurons would be modulated differentially by disinhibition of $GABA_A$ inputs arising from substantia nigra pars reticulata and disinhibition of pallidonigral GABAergic inputs mediated by $GABA_B$ receptors (Tepper et al., 1995). Tegnér et al. (1993) observed that in the lamprey brain stem-spinal cord, a model system to study how the neuronal networks controlling motor behavior in vertebrates, both receptors, $GABA_A$ and $GABA_B$, showed a modulator role, i.e, they acted together in the coordination of the locomotion. Kriem et al. (1998) found that GABAergic transmission in the substantia nigra pars reticulata (SNR), and not in the substantia nigra pars compacta (SNC), would play a crucial role in the control of motor activity of rats exposed to high pressure, and the $GABA_A$ and $GABA_B$ receptors, in SNR, had an opposite role in the regulation of the movements. It has been shown that both the receptors, $GABA_A$ and $GABA_B$, have a modulator role in the control of locomotion, and they have an opposite roles in the regulation of this behavior (Tegnér et al., 1993; Kriem et al., 1998). It is also known that the stimulation of $GABA_B$ receptor reduces the locomotor activity and $GABA_A$ receptor is not involved in the locomotion-reducing effect (Agmo and Giordano, 1985; Paredes et al., 1997). The effective doses of some GABAergic drugs that impair motor execution, reduce ambulatory activity and frequently also motor coordination, as evaluated by open field activity and a treadmill test (Dishman et al., 1996). The present results showing a significant decrease on striatal dopaminergic system activity suggested that MXD should activate the GABAergic system, resulting in a reduced motor coordination in consequence of inhibition of striatal dopamine release. In fact, not only the dopamine levels were decreased but also its metabolite levels, the HVA.

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RESUMO

A moxidectina (MXD) é uma droga antiparasitária amplamente empregada em animais domésticos; seu mecanismo de ação, em mamíferos, envolve o neurotransmissor ácido gama-aminobutírico (GABA). Esse neurotransmissor tem papel importante na função motora. Assim, no presente trabalho estudaram-se os efeitos da MXD em alguns parâmetros comportamentais ligados a função motora de ratos e também em sistemas de neurotransmissão central. A atividade geral no campo aberto e a coordenação motora na trave elevada foram empregadas para avaliar os efeitos de diferentes doses de MXD. Os resultados mostraram que: no campo aberto, mesmo as doses maiores (2.0 e 20.0 mg/kg) de MXD não alteraram as freqüências de locomoção e levantar. Por outro lado, a MXD foi capaz de prejudicar a coordenação motora dos animais avaliada na trave elevada. Estudos neuroquímicos dos níveis estriatais de GABA e dopamina mostraram redução dos níveis de dopamina e seu metabólito, ácido homavanílico, sem interferência nos níveis de GABA estriatal. Considerando que a estimulação de receptores GABAérgicos tem efeito inibidor sobre o sistema dopaminérgico estriatal, nós atribuímos a redução na coordenação motora a ação da MXD sobre o sistema dopaminérgico via ativação do GABA.

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