Presynaptic dopaminergic agonists increased gripping-generated immobility episodes in the myelin-mutant taipep rat

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A B S T R A C T

The taipep rat is a myelin mutant whose immobility episodes (IEs) can be caused by gripping them by the tail. Electroencephalographic recordings during IEs show a rapid-eye movement sleep-like pattern, similar to narcolepsy-cataplexy in canines. Systemic administration of α2-adrenoceptor agonists and the α1 antagonist increase gripping-generated IEs. Furthermore, adrenergic α2 antagonists decrease them. Serotonin receptors are also involved in the regulation of IEs, because the 5-HT1A, 5-HT1B serotonin-receptor agonists decrease the IE frequency, as do the postsynaptic-serotonergic 5-HT2A,2C agonists. The rats were maintained under standard conditions with a 12:12 h light:dark cycle, lights on at 0700, with free access to rodent pellets and tap water. Drugs were freshly prepared using sterile water and administered intraperitoneally at 0800 with the observation lasting 90 min. The IEs were caused by gripping the rat’s tail every 5 min. Systemic injection of (-)-quinpirole, R(+)-7-hydroxy-2-(dipropylamino)tetratin (7-OH-DPAT), or trans-(±)-3,4,4a,10b-Tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol (±) PD 128,907 increased both the frequency and mean duration of the IEs. The IEs produced by (-)-quinpirole were blocked by the previous administration of (-)-sulpiride and those by 7-OH-DPAT were blocked by tiapride. Systemic injection of sulpiride reduced gripping-generated IEs, but not the changes with either tiapride or U-99194, two other antagonists. In canine narcolepsy, systemic administration of D2-dopaminergic agonists increases the frequency of cataplexies and decreased them by using (−)-sulpiride similar to the pharmacological profile in taipep cataplexies. Because of this evidence, we proposed taipep rats as an adequate model of this sleep illness and for the evaluation of anticataplexic drugs.

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In our work, we evaluated the effects of systemic injection of D2/D3-agonists and antagonists on gripping-generated IEs in *taiep* rats and we also evaluated the behavioral changes caused by these drugs. The *taiep* rats were supplied by our animal-house facilities. Animals were under 12:12 light:dark cycle (lights on at 0700), with controlled temperature 21 ± 2 °C, relative humidity between 30% and 45%, and with free access to balanced rodent pellets (Zeigler, USA) and tap water. Rats were tested at 0800, when the susceptibility peak of gripping-generated IEs occurred [4].

Tests were done in acrylic cages (22 × 26 × 35 cm). The IEs were caused by gripping the base of the rat's tail for 10 s every 5 min, the most potent way to generate immobilities [4]. When immobility was not produced, the animal was put into the observation box again. If an IE was produced, the duration and latency to the first immobility was recorded by two-trained observers, one of them blind to the treatment and the dosage of the drug used.

Before each gripping-generated IE, we measured the behavioral state of the rats with a qualitative scale as (0)-indicating somnolence characterized by closed eyes, no movement, and regular breathing; (1)-quiet rat, awake but without movement; (2)-low motor activity characterized by sniffing, horizontal head movements, or whisker activity; (3)-regular motor activity showing allogrooming, scratching, or exploration; and (4)-high motor activity with displacement and other motor activities such as rearing or jumping, which are not common in the mutant [8,9].

The procedures described have been done in compliance with the laws and codes approved in the seventh title of the regulations of the general law of health regarding Health Research of the Mexican government (NOM-062-ZOO-1999) and in accordance with the National Institute of Health guidelines revised [3]. All experimental procedures were approved by the University's Animal Care and Use Committee.

The agonists used were (-)-quinpirole hydrochloride; R(+)-7-Hydroxy-2-((dipropylamino)tetratin hydrobromide (7-OH-DPAT); or trans-(±)-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]benzopyrano[4,3-b]1,4-oxazin-9-ol hydrochloride ([(±) PD 128,907] and the antagonists were (-)-sulpiride, tiapride hydrochloride, and 5,6-dimethoxy-2-((di-n-propylamo)lin)indan maleate (U-99194). All were purchased from Sigma–Aldrich (St. Louis, Mo, USA).

The drugs were freshly dissolved in sterile water, expressed as a free drug, and the volume adjusted to 1 mL/kg of the rat's weight. Animals received an ip injection of sterile water as a control. All the drugs were used in an increasing dosage scheme every 48 h to allow a washout period between sessions. For the experiments using a Latin-square design, the antagonist drugs were injected 15 min before the corresponding agonist.

The analyses of the data were done with the Friedman ANOVA (χ² test), followed by a Dunn test or a Tukey test for the comparisons among all treatments, with P < 0.05 considered statistically significant [24] and using Sigma Stat v. 3.5 software.

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>IEs (mean ± SE)</th>
<th>IEs (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS + SS</td>
<td>2.3 ± 0.8</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>SS + AGO</td>
<td>13.8 ± 1.2a</td>
<td>13.8 ± 1.5c</td>
</tr>
<tr>
<td>ANTAG + SS</td>
<td>2.5 ± 0.7</td>
<td>2.2 ± 1.9</td>
</tr>
<tr>
<td>ANTAG + AGO</td>
<td>7.0 ± 1.3c</td>
<td>7.2 ± 2.0b</td>
</tr>
</tbody>
</table>

**SS** = sterile water; **AGO** = (−)-sulpiride or (−)-quinpirole; **ANTAG** = tiapride or 7-OH-DPAT. Antagonists were injected 15 min before agonists; both by ip route.

* a SS + AGO being different from all other treatments. ANOVA (F(2,15), P < 0.001) followed by Tukey test P < 0.05.

b Different from SS + AGO group (P > 0.05).

SEM is no longer used, SE is.

In the table adjust the numbers so that the ± sign is in the columns under one another in the third box down, the two results should be on the same level horizontally. These changes make the table results easier to read and compare the numbers.

to 11 ± 2 min, 12 ± 2 min, and 10 ± 2 min (χ² = 14.5, df = 4, P = 0.006; followed by the Dunn test, P < 0.05).

PD 128,907, a specific D₃ agonist, produced a significant increase in gripping-generated IEs with the 400 and 800 μg/kg doses (χ² = 15, df = 4, P < 0.005; followed by the Dunn test, P < 0.05; see Fig. 1C) and also increased the mean duration of the IEs at these doses (χ² = 17.1, df = 4, P < 0.002; followed by the Dunn test, P < 0.05). This agonist also significantly increased the latency with the 400 μg/kg dose from 20.8 ± 5.2 min to 67.0 ± 6.9 min (χ² = 13.2, df = 4, P < 0.01; followed by the Dunn test, P < 0.05).

The administration of 7-OH-DPAT or PD 128,907 did not produce any change of the ongoing behavioral activity or side effects. For (−)-quinpirole the administration of higher doses (800 and 1600 μg/kg) produced regular activation and an increase of yawning frequency (data not shown), but no side effects.

The tiapride and U-99194 did not change the frequency or mean duration of the IEs, but sulpiride significantly decreased gripping-generated IEs with the 800 and 1600 μg/kg doses (χ² = 15.6, df = 4, P < 0.004; followed by the Dunn test, P < 0.05; see Fig. 2), but not their mean duration (χ² = 7.6, df = 4, P = 0.1). Using a Latin-square design, (−)-quinpirole (200 μg/kg) or 7-OH-DPAT (400 μg/kg) increased gripping-generated IEs, which were significantly decreased by the previous injection of (−)-sulpiride or tiapride (Tukey test P < 0.05, see Table 1).

Our results clearly showed that dopaminergic receptors from the D₂ family modulate gripping-generated cataplexies in *taiep* rats. The agonists clearly increased the frequency and mean duration of the IEs caused by gripping them by the tail and (−)-sulpiride significantly decreased them.

For narcoleptic dogs, it has been demonstrated that systemic administration of dopaminergic D₂- or D₃-agonists increased the frequency of cataplexies in the food-elicited cataplexy test (FECT). The systemic administration of a dopaminergic antagonist, such as raclopride or (−)-sulpiride, decreased the frequency of cataplexies in the FECT [15,18].

The side effects produced by systemic administration of dopaminergic D₂- or D₃-agonists in narcoleptic dogs strongly limited the range of doses tested, mainly by causing vomiting [15], which obviously interferes with the FECT. It is clear that systemic administration of a dopaminergic D₂- or D₃-agonists such as (−)-quinpirole, (−)-3-PPP, or 7-OH-DPAT produced an increase in cataplexies attacks [15]. The dopaminergic D₂- or D₃-antagonists raclopride, sulpiride, and haloperidol decreased the frequency and duration of cataplexies [15,18].

Acute administration of (−)-sulpiride (300 and 600 mg op) statistically reduced cataplexy without affecting the hypnogram in canine narcoleptics [18], but under chronic administration an increase of the mean duration of drowsiness and light- and deep-sleep bouts were obtained, as well as a decrease of the total time
spontaneously immobile for more than 10 days, which supports the possibility of using (−)-sulpiride in human beings [18]. In concordance, we obtained a significant reduction of gripping-generated IEs with (−)-sulpiride on taiep rats.

In narcoleptic dogs, local injection of (−)-quinpirole or 7-OH-DPAT into the sustancia nigra pars reticulate (SNr) or into the ventral tegmental area (VTA) increased the time spent in cataplexy during the perfusion time, but no changes were obtained with D1 agonists [19,21,12,18]. These pharmacological effects were correlated with the higher density of D2 receptors measured with 3H-spiperone in narcoleptic canines in the rostral caudate, nucleus accumbens, and amigdala [1]. Using autoradiography for D2 receptors, we have shown that taiep rats are not different than the control Sprague-Dawley rats, but the taiep rats had higher levels of D1 receptors in the caudate-putamen, entopeduncular nucleus, and the SNr [10]. The dopamine levels are higher in the striatum and nucleus accumbens in the taiep rats than in the Sprague-Dawley control rats [26], and an increase in the discharge rate in the SNr neurons during IEs has been reported in the mutant [25]. In future experiments we will address the effects of local perfusion on the dopaminergic nuclei in the myelin mutant.

Not only dopaminergic D2 (auto- and heteroreceptors) and D1 (mainly postsynaptic receptors) participate in the regulation of the sleep-wake cycle. As for other monoamines, dopamine plays a critical role because the dopaminergic D2- or D3-agonists increased either slow-wave sleep (SWS) or rapid-eye movement (REM) sleep [27,2]. The systemic administration of a D1-receptor agonist produced the opposite effects, an increase of wakefulness and a concomitant decrease of the SWS and REM sleep. These effects can be obtained in normal dogs and rats [27,2], therefore it is probable that the dopaminergic D2- or D3-agonists increased cataplexies and also produced an increase of both types of sleep.

There are few clinical reports that associate demyelinating diseases, mainly multiple sclerosis, with narcolepsy-cataplexy [23,20,28]. The sleep alterations such as daytime sleepiness and the sudden loss of muscle tone (cataplexy) are important symptoms in patients with myelin alterations. These clinical observations correlated with those obtained in taiep rats that suffer a progressive myelin loss and also cataplexies and sleep alterations [14,4].

In summary, central D2-D3-agonists significantly suppress cataplexy, whereas most D2- or D3-antagonists and the antagonists decreased cataplexy attacks aggravate it. This suggests an involve-
ment of presynaptic dopaminergic receptors in the regulation of canine and \textit{taiep} cataplexies. Importantly, most anticataplectic drugs used in humans take action through blocking the dopamine-transporter mechanism, supporting that dopamine plays a central role in the regulation of cataplexy and the promotion of wakefulness.

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