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Preliminary data regarding some pain manifestations in a MPTP- induced rat model of Parkinson's disease

Date preliminare privind unele manifestări ale durerii într-un model de șobolan MPTP-indus a bolii Parkinson

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Cuvinte cheie: *durere*, *boala Parkinson*, *1-metil-4-fenil-1,2,3,6-tetrahidropiridină*, *șobolan*

Abstract

Parkinson's disease (PD) is less known as a disease causing pain syndromes, although pain is found in 40-80 % of PD patients, as described by the very few reports in this area of research. Moreover, in some PD patients, pain is so severe and intractable that it overshadows the motor symptoms of the disorder. Still, pain in PD frequently goes under acknowledged and undertreated. Also, the studies regarding pain perception in the existing animal models of PD are very few. We experimentally induced the PD model in rats by injecting subcutaneously one dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 20 mg/kg, while the control group received saline. The behavioral testing for pain included the hot-plate task and was performed 7 days after MPTP injection. In this way, our rat model resulted from the acute treatment with a low dose of MPTP, exhibited an increased sensitivity to pain perception, as demonstrated by the significant decrease in the values of the latency time in hot-plate for rats treated with MPTP, as compared to the controls. In this way, further studies in this area of research seem warranted.

Rezumat

Boala Parkinson (BP) este mai puțin cunoscută ca o boală care generează durere, deși durerea este prezentă la 40-80% dintre pacienții cu BP, așa după cum este descris de foarte puține rapoarte în această ramură a cercetării. Mai mult decât atât, la unii pacienți cu BP, durerea este atât de severă și de puțin tratabilă încât maschează simptomele motorii ale bolii. În continuare, durerile în BP rămân în mod frecvent neînțelese și netratabile. De asemenea, studiile privind percepția durerii la modele existente de animale cu BP sunt foarte puține. Noi am indus experimental un model BP la șobolani prin injectarea subcutanată a unei doze de 1-metil-4-fenil-1,2,3,6-tetrahidropiridină (MFTP), 20 mg/kg, în timp ce grupul de control a fost tratat cu soluție de clorură de sodiu 0,9%. Testările comportamentale privind durerea au inclus testul plăcii încinse (hot-plate) și a au fost realizate la 7 zile după administrarea MFTP. În acest fel, modelul nostru de șobolan rezultat din tratamentul acut cu o doză scăzută de MFTP, a prezentat o creștere a sensibilității la percepția dureroasă, așa după cum a fost demonstrat de o scădere semnificativă a valorilor timpului de latență pe placa încinsă pentru șobolanii tratați cu MFTP, comparativ cu grupul de control. Astfel, par a fi justificate o serie de studii suplimentare în acest domeniu de cercetare.

1. Introduction

In addition to the well defined motor deficits, which are including tremor, rigidity, postural instability or bradykinesia (Błaszczyk, 1998), there are also other non-motor symptoms that affect the Parkinson's disease

(PD) patients, such as disturbances in memory, bladder function, bowel function and pain (Boivie, 2009).

Although pain was described as a component of the disease even by Parkinson itself (Parkinson, 1817) the pain component in PD is poorly understood and the number of studies regarding this subject is still limited.

In this way, if in 1998 Ford estimated in a comprehensive review that pain occurs in approximately 40% of patients with PD (Ford, 1998), in 2009 a complex study on 179 PD patients published by Beiske *et al.* stated that pain was reported in 83 % of patients (Beiske *et al.*, 2009).

Moreover, in Beiske *et al.* study, pain was not associated with age, disease duration or severity of the disease (Beiske et al., 2009), which makes the interaction between pain manifestation and PD pathology even more complicated to interpret.

Generally, five types of painful sensations are associated with PD patients: musculoskeletal, radicular-neuropathic pain, dystonic pain, central neuropathic pain and akathisia (Ford, 1998).

Also, there are reports stating that pain in PD can become sometimes severe enough to overshadow even the motor symptoms of the disorder (Ford, 1998).

In this context, the objective of the present study was to determine some pain manifestations, as studied in the specific hot-plate behavioral task, in an 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced rat model of Parkinson's disease, as compared to a saline-treated control group.

Materials and methods

Animals

Adult male Wistar (n=10) rats, weighing 200–250 g and about 20 weeks of age, were housed in groups of three or four animals per cage and kept in a room with controlled temperature (22 ℃) and a 12:12 h light / dark cycle (starting at 08:00 h), with food and water ad libitum.

Rats were allowed to adapt to the experimental room conditions for one week before the beginning of the behavioral experiment (hot-plate task).

The animals were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

We experimentally induced the PD model in rats by injecting subcutaneously one dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 20 mg/kg, while the control group received saline (Le Bars *et al.*, 2001).

The behavioral testing for pain included the hot-plate task and was performed 7 days after MPTP injection.

Hot-plate test

The investigation of pain sensibility was performed using a hot-plate (Ugo Basile).

A plastic cylinder is used to confine the rat to the heated surface of the plate which is maintained at 55 ˚C using a thermostate.

The reaction time (the latency time) to two different types of behavior was monitored: licking the paw and jumping (Arcan *et al.*, 2013).

Statistical analysis

Results were expressed as mean ± S.E.M.

The results were analyzed statistically by one way Anova. $p<0.05$ was taken as the criterion for significance (Georgescu and Dascalu, 2003).

Results and discussions

In this way, our rat PD model resulted from the acute treatment with a low dose of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, exhibited an increased sensitivity to pain perception, as demonstrated by the significant decrease in the values of the latency time in hot-plate for rats treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ($F(1,8) = 5$, $p = 0.041$), as compared to the controls (Fig. 1.).

As previously mentioned, the latency time is expressed in seconds and is referring to the

reaction time to two different types of behavior: licking the paw and jumping (11.5 s ± 1.5 s in controls vs. 6.7 s ± 0.6 s in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine group).

As we previously mentioned, lately there is a significant increase in the interest directed towards pain manifestations in PD, especially since it seems that pain may even precede the first signs of Parkinsonism, as regional pain can appear before the motor disturbances or before a certain increase in muscle tone (Ford, 1998; Boivie, 2009).

Additionally, the abovementioned study of Beiske *et al.* reported that only 34% of the patients were on analgesic medication.

Therefore, pain seems to be a significant clinical problem in PD and moreover, it is mainly left untreated.

There is also an increase attention regarding the connections between pain and oxidative stress.

In this way, while lately oxidative stress has been demonstrated to be implicated in many degenerative neurological and psychiatric conditions, including PD (Hritcu *et al.*, 2008), Alzheimer's disease (Padurariu *et al.*, 2010) schizophrenia (Ciobica *et al.*, 2011) or depression (Stefanescu şi Ciobică, 2012), it is now considered that, oxidative stress can contribute also to persistent pain (Khalil *et al.*, 1999; Kim *et al.*, 2004; Schwartz *et al.*, 2008).

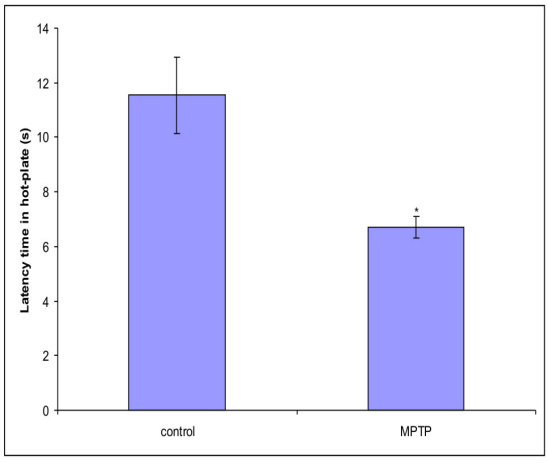

Fig. 1. Latency time, as expressed in seconds, in hot-plate behavioral task. The values are mean ± SEM (n=7 for control group, n=3 for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine group).*p = 0,041 vs. control group.

In this way, removal of excessive reactive oxygen species by free radical scavengers, such as phenyl N-tert-butylnitrone (PBN) and 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), produced significant analgesic effects in both neuropathic pain (Tal, 1996; Kim *et al.*, 2004; Lee *et al.*, 2007) and inflammatory pain (Hacimuftuoglu *et al.*, 2006).

Furthermore, the number of neurons showing mitochondrial ROS production was significantly increased in the lumbar spinal dorsal horn in spinal nerve ligated neuropathic rats (Park *et al.*, 2006).

Moreover, increased levels of extracellular hydrogen peroxide were also observed in the spinal trigeminal nucleus after formalin injection into the lip of the rat, and this increase coincided with pain behaviors (Viggiano *et al.*, 2005).

Additionally, it was demonstrated that superoxide dismutase, which converts free-radical superoxide to hydrogen peroxide, was very effective in reducing inflammation indicators and hyperalgesia after carrageenan injection into the rat paw (Wang *et al.*, 2004).

However, while is becoming clear that ROS are involved in persistent pain, the mechanisms by which they contribute to pain are still not clear.

In this way, since there are several reports indicating the toxicity of MPTP administration in rats (Lee *et al.*, 1992), and even articles citing some oxidative stress-induced effects of MPTP administration (Ali *et al.*, 1994), we could speculate for a possible relevance of oxidative stress in this MPTP-induced model and pain manifestations, as studied in hot plate behavioral task.

However, further studies are necessary in order to confirm these aspects, especially by using an increased number of animals.

Conclusions

Our data are suggesting, for the first time in our best of knowledge, an increased sensitivity to pain in a MPTP-induced rat model of PD.

In this way, further studies in this area of research seem warranted.

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