PAIN IN A PARKINSON`S DISEASE RODENT ANIMAL MODEL INDUCED WITH 6-HYDROXYDOPAMINE

EVALUAREA DURERII LA UN MODEL ANIMAL DE BOALĂ PARKINSON INDUS CU 6-HIDROXIDOPAMINĂ

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Cuvinte cheie: durerea, boala Parkinson, model animal, 6-OHDA.

Abstract

Pain phenomenon, the unpleasant sensory and emotional event, appears to evidently intrude in Parkinson disease (PD), a disease formally considered to be restricted only to motor deficits. Although over a half of persons with PD suffer from pain manifestations, there are very few reports targeting this issue. Considering the cases when motor symptoms of PD are eclipsed by severe pain disclosure, there is an obvious need of clarifying the intricate implications of pain in PD context. Because there are few studies researching the link between pain and PD in clinical context, but as well in animal models we chose to explore the effects of pain stimuli on a rodent model of PD. Materials and methods: We experimentally induced a PD model in Wistar rats (n=12) by injecting in the substantia nigra, a brain area known to be involved in PD occurrence, one dose of a 6-hydroxidopamine (6-OHDA) solution (8µm 6-OHDA base and 4µm physiological saline), utilizing neurosurgery, while their control peers received same dose of saline solution. Two weeks after the intervention the animals were subjected to the hot-plate test, a behavioral task for acquiring pain sensitivity. Results: There was noticed a statistical significant ($F(1,10) = 5.67$, $p=0.038$) sensibility of the 6-OHDA rats to thermal pain stimuli (8.2 s ± 0.8 s in 6-OHDA group) as compared to their peers (13.8 s ± 1.6 s in controls). Conclusions: The involvement of pain in PD animal models is demonstrated raising questions of how it influences PD evolution. Moreover, this result increases awareness of deficient diagnostic methods of pain in PD and as a consequence, poor treatment of pain manifestations.

Rezumat

Durerea este un fenomen senzorial și emoțional neplăcut ce pare să facă parte din simptomatologia bolii Parkinson (PD), o afecțiune considerată anterior a fi caracterizată doar de deficitele motorii specifice. Deși mai mult de jumătate din persoanele diagnosticate cu boala Parkinson suferă din cauza durerii, doar foarte puține studii menționează acest aspect. Având în vedere cazurile în care s-au raportat mascarea simptomatologiei motorii de către manifestările dureroase severe prezente în acest context patologic, rezidă necesitatea evidentă de clarificare a acestor situații. Considerând numărul redus al studiilor care cercetează legătura dintre durere și PD atât în context clinic cât și în cazul modelelor animale, am optat pentru explorarea efectelor stimulilor dureroși asupra unui model animal de PD întrebuințând rozătoare. Materiale și metodă: Am indus experimental un model animal de PD la șobolani din rasa Wistar (n=12) prin injectarea acestora într-o regiune specifica a creierul cunoscută pentru implicațiile în patologia parkinsoniană, substantia nigra, a unei doze de soluție de 6-hidroxidopamină (6-OHDA; 8µm 6-OHDA bază și 4µm ser fiziologic) întrebuințând neurochirurgia, cât timp lotului de control i-a fost administrată soluție

salină. La două săptămâni după intervenție animalele au fost testate cu ajutorul testului plăcii încinse, o metodă comportamentală de evaluare a percepției durerii. Rezultate: În urma testelor comportamentale pentru durere s-au notat diferențe statistic semnificative între lotul de control (13.8 s ± 1.6 s controli) și lotul cu animale de testat (8.2 s ± 0.8 s grupul cu 6-OHDA), observându-se o sensibilitate crescută față de durerea termică la șobolanii injectați cu 6-OHDA.Concluzii: Prezența durerii la animalele cu PD indus ridică semne de întrebare cu privire la modul în care acest simptom influențează evoluția afecțiunii în sine. În plus, acest rezultat ar trebui să intensifice conștintizarea lipsei metodelor de diagnosticare a durererii în PD care în consecință conduc către un tratament necoresăpunzător al durerii.

1. Introduction

Parkinson disease (PD) was mostly known for its motor dysfunctions that occur because of the bilateral destruction of dopamine neurons located in *substantia nigra*, causing serious movement deficiencies (Vlamings et al., 2007).

Although motor impairments including tremor, rigidity, postural instability or bradykinesia (Blaszczyk, 1998) are well defined symptoms of PD, current research highlights also other aspects of this disease that have been omitted in the past.

In this way, cognitive deficits covering manifestations from mild memory impairments to disturbances of intellectual nature and even dementia are encountered (Avila et al., 2009; Georgiev et al., 2010; Kramberger et al., 2010; Possin, Filoteo, Song, & Salmon, 2008).

Other aspects of the disease that have been uncovered to be part of the symptomatology include perturbed bladder function, bowel function and pain (Boivie, 2009).

Interestingly enough, although Parkinson himself described pain as specific trait of PD (Parkinson, 2002), its mechanisms are still lacking understanding, because of this it is underdiagnosed and as a consequence undertreated.

In the reduced amount of literature following pain in the context of PD, it was estimated that it develops in 40% of diagnosed patients with PD (Ford, 2010).

Moreover, another study conducted later on in a large group of patients (179 patients with PD) declared an 83% of pain suffering patients (Beiske, Loge, Ronningen, & Svensson, 2009).

What it is even more striking is that pain does not correlate with age, severity or duration of the illness (Beiske et al., 2009), transforming the matter of PD pain occurrence in a veritable enigma. Equally interesting are the reports affirming that pain manifestations in PD can develop to be so severe to surpass motor deficits of this disorder (Ford, 2010).

Under these circumstances, pain becomes a fascinating phenomenon to be studied in the context of PD and therefore this work concentrated in studying it.

In the attempt of creating similar conditions to the human disease it was developed an animal model of PD, largely utilized today, by injecting 6-hydroxidopamine (6-OHDA) right into the brain area blamed for the origin of neuron degeneration (substantia nigra).

The chemical substance causes neurodegeneration in a selective manner targeting only dopamine nerve terminals (Berretta et al., 2005; da Conceicao, Ngo-Abdalla, Houzel, & Rehen, 2010; Iancu, Mohapel, Brundin, & Paul, 2005).

Consequently, employing the rodent model of 6-OHDA and a control group, we studied their behavioral reactions to thermal induced pain stimuli through hot-plate test.

2. Materials and Methods

Animals

A number of 12 experimentally male Wistar rats with weights ranging from 200g to 250 g were introduced into the experiment process.

The animals were hosted in a in a room with controlled temperature (22℃), precise light-dark cycle (12h cycle, commencing at 8:00h) and housed in groups of two or three animals per cage, with unrestricted access to food and water.

The study was approved by the Local Ethic Board and managed in accordance to Helsinki Declaration. Also, all the animals were handled in conformity with animal bioethics guidelines from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania,

and all performed procedures respecting the European Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery

All the performed surgical procedures were achieved under anesthesia and carried out in aseptic conditions.

The rats were fastened in the stereotaxic device with the nose aligned 11° below the horizontal plane. In order to prevent debilitating outcomes of bilateral lesions, only right-unilateral lesions were performed.

The lesion of the dopaminergic neurons located in substantia nigra were induced using 6-OHDA (Sigma) as following: free-base 6-OHDA (8µg) dissolved in 4 µl physiological saline incorporating 0.1% ascorbic acid.

This solution was administered through Hamilton syringe over 4.50 min and the syringe was left in place 5 min after the injection, being afterwards slowly removed.

The control group was also subjected to surgery and injected with saline in the same brain area. The coordinated employed were the following: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos, 2006).

Pain testing started in the fifteenth day after surgery and included hot-plate behavioral task.

Hot-Plate Test

This investigation tool of painful stimuli consists of a device designed by Ugo Basile through which pain sensitivity is quantified.

The heated surface of the apparatus, maintained at a temperature of 55℃ using a thermostat, is delimited by a plastic cylinder which keeps the rat from escaping.

The necessary time for a reaction to stimuli, in this case thermal stimuli, defines the latency time. In the current experiment it was monitored the latency time until two types of behaviors occurred: licking the paw and jumping (Arcan et al., 2013).

Contralateral rotation behavior

To verify if the 6-OHDA induced lesions in the substantia nigra were effective we utilized the rotational behavior, a characteristic test for evaluating the dopaminergic neurons denervation (Hudson et al., 1993).

The test consists in administering to rats with unilateral lesions dopamine releasing drug in the unaffected side, which causes the rat to rotate towards it.

In the current experiment we chose to administer pergolide (0,3 mg/kg) (Herrera-Marschitz, Arbuthnott, & Ungerstedt, 2010), an ergoline semisynthetic derived dopaminergic agonist for both D1 and D2.

Following the observation, as it was predicted the rodents without 6-OHDA lesions presented with reduced contralateral rotation, while those with 6-OHDA lesions showed increased contralateral rotation.

Histological analysis

When the experiment was finished all the included animals were sacrificed by decapitation under light ether anesthesia.

Afterwards, their brains were removed and introduced in a solution of sucrose/formalin with a concentration of 30% and subsequently frozen and cut into coronal sections (50µm) by operating a freezing microtome, stained with cresol violet to verify the need point of the syringe.

From the gathered experimental data, solely the information from the accurately located lesions in the substantia nigra was introduced in the statistical analysis.

Statistical analysis

Animal behavior recorded utilizing hot-plate task was statistically analyzed with the help of one-way analysis of variance (ANOVA). Statistically significant was considered an F value for which $p<0.005$. All the obtained results are expressed as mean ±SEM.

3. Results

In effect, the outcomes of this experiment indicate that our rat model created by injecting 6-OHDA in the substantia nigra exhibited a significant decrease in the values of latency time, which signifies an increased sensitivity to pain perception of the rats treated with 6-OHDA ($F(1,10) = 5.67$, $P=0.038$; $^*P<0.05$) in comparison with control peers (Figure 1).

As explained before, the latency time represents the time of reaction measured in seconds between the placement of the animal on the plate and specific behavior occurrences (13.8 s ± 1.6 s in controls vs. 8.2 s ± 0.8 s in 6-OHDA group).

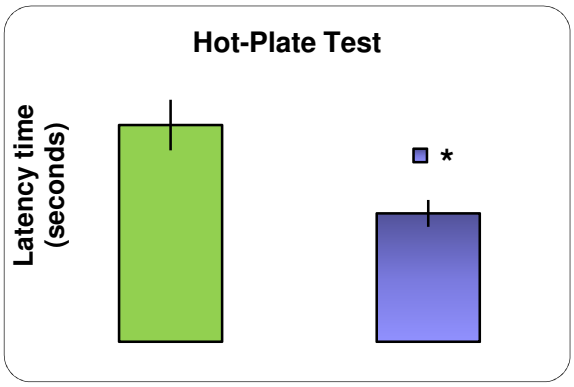

Figure 1. Latency time in hot plate behavioural task, expressed in seconds. The means are ±SEM (n=7 control group; n=5 6-OHDA group). *p=0.038 vs. control group

4. Discussion

This research investigated the influence of PD on pain perception in rodent animal models of PD induced with 6-OHDA. In this way, fourteen days after surgical procedure of intranigral 6-OHDA inoculation is performed, the process of dopamine neurons destruction is completed (Ferro et al., 2005), inducing the characteristic cognitive impairments for modeling PD.

Afterwards, thermal pain stimuli were inflicted on the rats and the latency time measurements indicated an accentuated decrease in pain resistance.

Therefore, our results showed that administration into substantia nigra of 6-OHDA produced pain sensitization. These findings are in accordance with previous data in regard to pain manifestations in the context of PD, accentuating on the need to quantify pain in patients diagnosed with PD (Ababei, 2015; Beiske et al., 2009; Ford, 2010).

While the motor problems of PD are widely known (tremor, rigidity, postural instability, slowness of movement), pain is a trait that is less explored.

Although, Parkinson himself pointed out pain as a condition that accompanies PD, few studies follow its influence and record its intensity in the context of PD. Among these studies, the data collected indicates that approximately 40% to 80% of patients with PD suffer from pain experiences (Beiske et al., 2009; Ford, 2010).

Inquiries in regard to the causative factors of pain and PD co-occurrence have been made and recent researches indicate to oxidative stress as being a common determinant in both conditions.

Thus, to this extent, recent studies indicate to free radicals as being the causative factors implicated in the pathogenesis and/or progression of PD, generating oxidative disturbances of dopaminergic neurons located in this specific brain area, substantia nigra (Bisaglia, Soriano, Arduini, Mammi, & Bubacco, 2010; Chalimoniuk, Stepien, & Strosznajder, 2004; Ciobica, Hritcu, Artenie, & Padurariu, 2008; Wypijewska et al., 2010).

Also, it is now admitted that oxidative stress might have a contribution to persistent pain (Khalil, Liu, & Helme, 1999; Kim et al., 2004; Schwartz, Lee, Chung, & Chung, 2008).

Our laboratory has a rich history concerning the research in PD field. Thus, data from our previous studies where it was also used the rodent model of PD through unilateral administration of 6-OHDA into substantia nigra and into the ventral tegmental area, demonstrated the negative repercussions of 6-OHDA on memory capacity along with increased oxidative stress levels, both relevant elements in the context of PD (Hritcu, Ciobica, & Artenie, 2008).

Furthermore, after observing the performance of this model we tried to test different substances that could enhance the chances of recovery or at least stop the progression of the induced PD symptomatology. Along these lines, there was tested the influence of low-dose nicotine administration during a short period of time in a 6-OHDA rat animal model and it was observed that it has beneficial outcomes on memory processes and as well in improving the brain

oxidative stress levels (Ciobica, Padurariu, & Hritcu, 2012).

Nicotine and pain bond seems to be quite strong, several reports indicating nicotine's antinociceptive actions. In a paper following the history of nicotine as a therapeutical agent it is specified its function in pain conditions (Powledge, 2004). Thereupon a clinical trial is cited where the postoperative analgesic proprieties of nicotine were tested on 20 women subjected to gynecological intervention, with the mention the both the control and nicotine group had free access to morphine. The results indicate to a high significant analgesic potential of nicotine compared to the control group, where placebo was administered (Powledge, 2004).

Another study where it was followed the influence of nicotine administration on different pain subtypes that appear after spinal cord injury in smoking and non-smoking patients proved that a complex relationship exists between these factors.

In this way, the situation presented itself as follows: nonsmoker patients accusing neuropathic and musculoskeletal symptoms presented with pain reduction after nicotine exposure, while, on the contrary, tobacco users with the same type of pain accused an increase in pain severity (Richardson, Ness, Redden, Stewart, & Richards, 2012).

For these aforementioned reasons, the need of extending studying in this matter of nicotine and pain interaction is without question necessary. In addition, knowing that dopamine agonists are used in clinical practice as a therapeutical option for PD (Brusa et al., 2005), we investigated the effects of pergolide mesilate.

As mentioned before, this is an ergoline semisynthetic product with dopaminergic agonist proprieties for both D1 and D2, suspected to possess also neuroprotective functions, ergo, the reason why we wanted to explore its proprieties. After testing it on the same 6-OHDA induced PD animal model, our findings advocate that pergolide improves special memory and also brain oxidative balance (Ciobica, Olteanu, Padurariu, & Hritcu, 2012).

Considering our afore mentioned interest towards pergolide action in the context of PD and the different influences that it exerts on various levels of PD symptomatology, we are interested in its effect on pain amendment in the context of this complex disorder that PD unfolds to be. In the context of present literature the subject of pergolide in connection to pain is poorly investigated. Among the few studies existing on the matter it appears that this dopamine agonist compound has an enhancement role in the antinociceptive therapy employing transcranial direct current cathodal stimulation (Terney et al., 2008).

It was observed that, aside the significantly reduction of experimental induced pain using cathodal transcranial direct current stimulation of the primary cortex, pergolide enhanced the antinociceptive effect by contributing to the decrementation of pain sensation and prolonging the analgesic result (Terney et al., 2008).

Having this in mind, with these potential positive results on pain when pergolide is administered, our research group is interested in testing its actions in the field of pain manifestations occurring in PD.

In consequence, considering the benefits that nicotine and pergolide bring to oxidative balance and also the improvements in memory abilities of the modeled PD, in addition they might bring a generous contribution in the fight against pain symptoms (seeming to be found in PD), seeing, as marked above, that pain is also associated with oxidative stress.

Therefore, having in mind this quite strong link between pain and PD and also a possible factor explaining to some extent their occurrence it is clearly necessary to study deeper the implications of these phenomena, considering especially the need of determining and treating pain, a real and unpleasant event that interferes with PD.

5. Conclusions

Our data indicating toward increased pain sensitivity in a 6-OHDA-induced rat model of PD is raising awareness towards pain, the poorly explored symptom of the disorder.

Therefore, it instigates for further research in this area of interest, raising questions concerning current management of therapy in patients suffering from PD.

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