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Increased pain perception and attenuated opioid antinociception in paradoxical sleep-deprived rats are associated with reduced tyrosine hydroxylase staining in the periaqueductal gray matter and are reversed by L-DOPA

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ABSTRACT

Paradoxical sleep deprivation (PSD) increases pain sensitivity and reduces morphine antinociception. Because dopaminergic neurons in the periaqueductal gray matter (PAG) participate in pain modulation and opioid-induced antinociception, we evaluated the effects of PSD on thermal pain sensitivity, morphine- and L-DOPA-induced antinociception and dopaminergic functionality in the PAG by assessing tyrosine hydroxylase (TH) immunoreactivity. Rats that were subjected to 96 h of PSD received vehicle, morphine (2.5, 5 or 10 mg/kg), L-DOPA (50 or 100 mg/kg) or L-DOPA (50 mg/kg) + morphine (2.5 and 5 mg/kg) and were tested with a 46 °C hot plate 1 h after. The paw withdrawal latency responses to the hot plate were decreased in PSD rats and were modified by the highest dose of morphine, L-DOPA and L-DOPA + morphine. Analgesic effects were observed in control groups for all of the morphine doses as well as 100 mg/kg of L-DOPA and L-DOPA (50 mg/kg) + morphine (5 mg/kg). The number of cell bodies that were immunopositive for TH in the PAG was reduced in PSD rats. In conclusion, increased thermal sensitivity was reversed by L-DOPA and could be caused by a reduction TH levels in the PAG. Our data also suggest a relationship between central dopaminergic networks and opiate-induced analgesia in rats.

1. Introduction

Pain and sleep disturbances are closely correlated and previous studies have demonstrated a bidirectional relationship between them. Sleep disturbances are common in patients who experience acute and chronic pain (Morin et al., 1998; Ohayon, 2005), and a number of studies have focused on the effects of sleep loss in the context of pain perception (Carli et al., 1987; Damasceno et al., 2009; Landis et al., 1998; Lautenbacher et al., 2006; May et al., 2005; Nascimento et al., 2007; Onen et al., 2001). Most sleep deprivation occurs during the paradoxical-sleep/rapid-eye-movement (PS/REM) phase, which occurs during the second half of the night. It is during this phase that most restorative processes within the central nervous system (CNS) and other parts of the body occur, and this is the reason for the substantial number of studies that have focused on paradoxical sleep deprivation (PSD).

Previous experiments have shown that pain sensitivity to noxious stimuli is increased following PSD in healthy human subjects (Kundermann et al., 2004; Moldofsky et al., 1975; Moldofsky and Scarisbrick, 1976; Roehrs et al., 2006) and experimental animals (Hicks et al., 1979; May et al., 2005; Nascimento et al., 2007; Onen et al., 2000, 2001). Onen et al. (2001) showed that 72 h of PSD lead to a significant increase in behavioral pain responses in rats when challenged with mechanical, thermal and electrical stimuli. Experimental animals that underwent 96 h of PSD showed a decrease in paw-withdrawal responses to a noxious thermal stimulus (50 °C), and this response persisted even after 24 h of sleep recovery (Nascimento et al., 2007). These effects of PSD on nociception appear to be dependent on the intensity of the noxious thermal stimuli and on the age of the animal (May et al., 2005).

The mechanisms that govern the ability of PSD to change nociceptive responses are still poorly understood. Many structures in the brainstem that control sleep are also involved in the modulation of pain processing (McCarley 2004; Pertovaara and Almeida, 2006). Therefore, PSD may promote biochemical changes in the regions involved in the modulation and control of pain. Several studies have demonstrated that PSD can reduce the antinociceptive effect of morphine (Ukponmwan et al., 1984), which suggests that normal PS is important for the antinociceptive activity of endogenous and exogenous opiates.

The periaqueductal gray (PAG) plays an important role in nociceptive modulation. Studies have correlated the antinociceptive

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effects of morphine to the ventrolateral PAG (vPAG) and other midbrain areas (Manning et al., 1994; Pert and Yaksh 1975; Yaksh et al., 1976). Alterations in neural transmission within the PAG modify the antinociceptive effects of morphine (Lane et al., 2005), and electrophysiological experiments suggest that opioids may activate the PAG output neurons that project to the rostroventral medulla and the spinal cord (Reichling and Basbaum, 1990).

There is a network of dopaminergic neurons within the vPAG. This network is a part of the dorsocaudal A10 group of Hökfelt (Hökfelt et al., 1976) and is composed of both small and large neurons (Hasue and Shammah-Lagnado, 2002). Dopamine (DA) neurons of the PAG region that project locally or to different brain structures may communicate with neural areas that are involved in the affective aspects of pain (Hasue and Shammah-Lagnado, 2002; Ottersen, 1981).

Dopaminergic neuron depletion in the vPAG with 6-hydroxydopamine (6-OHDA) decreases opioid-induced antinociception in rats (Flores et al., 2004), and a recent study suggests that DA may have a direct antinociceptive effect in addition to the modulation of the antinociceptive effects of morphine (Meyer et al., 2009). Both D1 and D2 dopamine receptors are expressed by PAG neurons. D1 receptors are associated with morphine analgesia (Flores et al., 2004), and D2 receptors are associated with dopamine analgesia (Meyer et al., 2009).

Previous studies have demonstrated that tyrosine hydroxylase (TH), the enzyme that catalyzes the rate-limiting step of catecholamine biosynthesis, is sensitive to periods of PSD (Damasceno et al., 2008), which makes TH an interesting marker that may be used to understand the correlation between the dopaminergic system and PSD.

Therefore, we hypothesized that the increase in pain sensitivity that is observed after periods of PSD might be related to a reduction in dopaminergic functionality in the PAG. In this study, we assessed the effect of PSD on thermal pain sensitivity, morphine- and L-DOPA (a DA precursor)-induced antinociception and dopaminergic functionality in the PAG by the measurement of TH immunoreactivity.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (250–300 g) were used. The rats were housed in cages with free access to food and water in a room under controlled light/dark cycle conditions (12 h light/12 h dark; lights on at 6:00 a.m.) and ambient temperature (23 ± 1 °C). All experimental protocols complied with the ethical guidelines for investigations of experimental pain in conscious animals and were previously approved by The Animal Studies Ethical Committee of the State University of Rio de Janeiro (CEUA/032/2010).

2.2. Paradoxical sleep-deprivation procedure

The method used to induce PSD was the “inverted flower pot” technique (Jouvet et al., 1964) for 96 h. In this technique, rats were placed individually in tanks on circular platforms (6 cm in diameter) that were surrounded by water. When the animals entered the paradoxical phase of sleep, they lost skeletal muscle tone, fell into the water and were awakened. Food and water were easily accessible. The control groups were maintained in cages in the same room for the duration of the study.

2.3. Hot-plate test

Thermal hyperalgesia responses were assessed using the hot-plate test (Lejca Scientific Instruments, Barcelona, Spain) at a temperature of 46 °C. The experiments were videotaped and evaluated by 2 independent observers who were blinded to the experimental conditions. The results represent the mean latency of the removal of 1 of the hind paws from the hot plate apparatus (i.e., paw withdrawal latency). To avoid paw damage, a cutoff of 90 s was established.

2.4. Experimental design

Immediately after 96 h of PSD, rats in both the control and PSD groups were tested on the hot plate to evaluate thermal sensitivity. Vehicle (physiological saline), morphine (2.5, 5 and 10 mg/kg, Cristália — Brazil) or L-DOPA (50 and 100 mg/kg, Sigma-Aldrich — USA) was intraperitoneally administered to both control and PSD rats (n = 8 per group) 1 h before the hot-plate test. In another experiment, L-DOPA (50 mg/kg) was administrated 30 min before treatment with morphine (2.5 and 5 mg/kg). In all experiments, L-DOPA was combined with benserazide, a peripheral decarboxylase inhibitor, at a dose ratio of 4:1 dissolved in 0.2 M HCl (in physiological saline), and the solution was adjusted to a pH of 6.8 (for details, see Paalzow, 1992).

2.5. Tissue preparation and TH immunohistochemistry

Immediately after 96 h of PSD, rats (n = 7 per group) from the control and PSD groups were anesthetized with thiopental (70 mg/kg) and transcardially perfused with 0.9% NaCl, which was followed sequentially by 4% paraformaldehyde in phosphate buffer and 4% paraformaldehyde with 10% sucrose. After perfusion, the brains were removed and immersed overnight in 20% sucrose in phosphate buffer at 4 °C for cryoprotection prior to sectioning. After each brain was frozen, 30-μm coronal sections were obtained and collected on gelatinized slides.

Eight 30-μm sections, each separated by 90 μm, were collected and processed for immunohistochemistry. The first section was collected at approximately −7.64 mm from the bregma, and the last section was collected −8.72 mm from the bregma (vPAG) (Paxinos and Watson, 1998).

Immunohistochemistry was conducted according to the avidin–biotin-peroxidase protocol (ABC Elite kit; Vector). The sections were incubated in phosphate-buffered saline/0.2% Triton X-100 (PBS-T) with 10% normal goat serum (Vector) for 2 h to block nonspecific binding. Sections were incubated for 72 h at 4 °C with a rat anti-tyrosine-hydroxylase polyclonal antibody (AB 151, Chemicon, 1:5,000) in PBS-T, and after washing in PBS, they were incubated for 18 h with biotinylated goat anti-rabbit-IgG (Santa Cruz) diluted 1:200. Additional washes in PBS were performed, and the sections were incubated for 2 h with the ABC kit (1:50).

Finally, the sections were reacted with the SG chromogen and a hydrogen peroxide solution (Vector SG kit) and analyzed for the number of cells that expressed TH (i.e., those with a blue SG label in the soma and dendrites). Positive cells distributed throughout the vPAG were counted in each coronal section. The microscopic counting analysis was performed by an investigator who was blinded to the experimental conditions. Data represent the average cell count from 8 sections.

Control sections were incubated with normal goat serum with the omission of the primary antibody and immunoreactivity detection. All sections were examined under light microscopy using an Olympus BX 40 microscope. Images were captured with a cooled charged–coupled device camera (Sony DXC 151A).

2.6. Statistics

The results are presented as a mean ± S.E.M., and all data analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc., USA). One-way analysis of variance (ANOVA) was used for comparisons of data between the doses followed by the Newman–Keuls test for post-hoc comparisons. Differences between the control and PSD

groups for each dose and differences in immunohistochemical data were determined using Student's unpaired two-tailed *t*-test. Results were considered to be statistically significant when $p \leq 0.05$.

3. Results

3.1. Nociceptive behavior

As shown in Fig. 1, PSD for 96 h significantly decreased the paw withdrawal latency in rats that received vehicle (−75.89%, $p \leq 0.05$). As expected, acute treatment with 2.5, 5 and 10 mg/kg morphine increased paw withdrawal latency in non-sleep-deprived rats (54.45%, 75.37% and 63.89%, respectively) (Fig. 1A). In sleep-deprived rats, acute treatment with 2.5 or 5 mg/kg morphine did not increase paw withdrawal latency; however, a significant effect was observed with the highest dose (10 mg/kg) ($p \leq 0.05$).

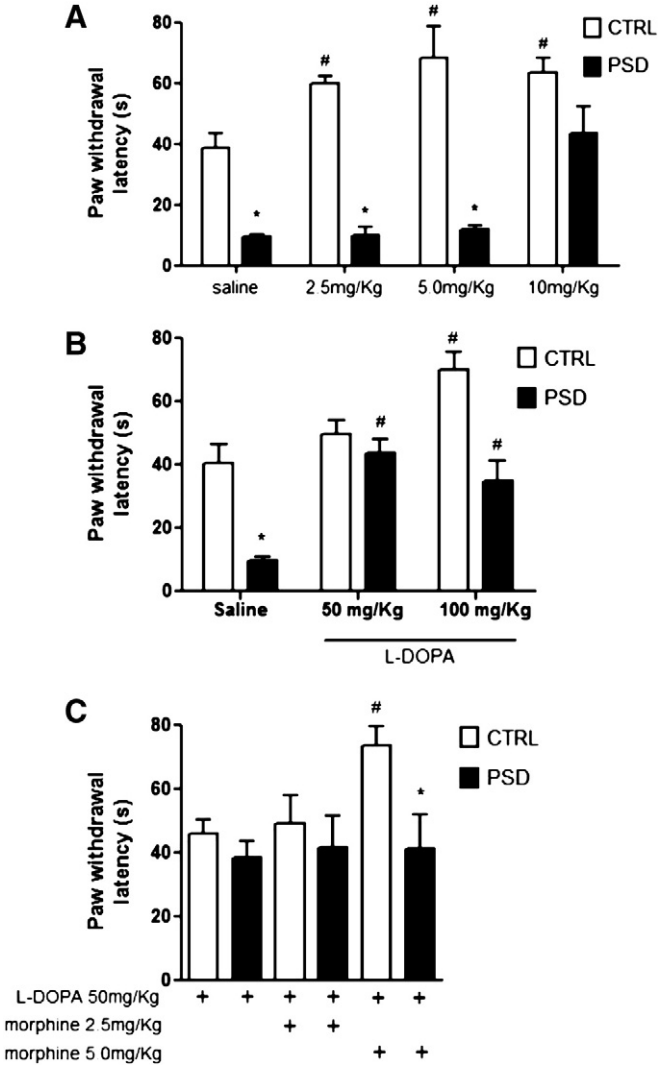

Fig. 1. Effects of acute treatment with morphine, L-DOPA and L-DOPA plus morphine on hind paw withdrawal latencies in control and paradoxical sleep deprived rats submitted to a hot-plate test (46 °C) after 96 h of paradoxical sleep deprivation. (A) Morphine was administered intraperitoneally in 3 different doses (2.5, 5 or 10 mg/kg), 60 min prior to the test. (B) The dopamine precursor, L-DOPA, and the DOPA decarboxylase inhibitor, benserazide (at a 4:1 ratio) were administered intraperitoneally in 2 different doses (50 or 100 mg/kg) 60 min prior to the test. (C) L-DOPA (50 mg/kg) and morphine (2.5 or 5 mg/kg) were administrated 90 and 60 min prior to the test, respectively. Each column represents the mean ± SEM of eight rats. * p-value ≤ 0.05 differs from the respective control group (unpaired *t*-test), # p-value ≤ 0.05 differs from the respective saline group (one-way ANOVA followed by Newman–Keuls test).

The treatments with L-DOPA at 50 and 100 mg/kg increased paw withdrawal latency in sleep-deprived rats (+346.61% and +256.65%, respectively; $p \leq 0.05$) (Fig. 1B). In control rats, this effect was observed at the highest dose of L-DOPA (+76%, $p \leq 0.05$) and after L-DOPA 50 mg/kg plus morphine 5 mg/kg treatment (+49,28%, $p \leq 0.05$) (Fig. 1B and C). However, the combination of L-DOPA plus morphine in all of the doses evaluated did not increase the L-DOPA analgesic effect in sleep-deprived rats (Fig. 1C).

3.2. Tyrosine hydroxylase immunohistochemistry

In control rats, the distribution of cells expressing TH was similar to previous reports (Flores et al., 2004). Immunostaining was present throughout the cell body and was predominant in the eminence of the main neurite. Moreover, TH-positive neurons were observed throughout the vPAG and consisted of large cells located in the lateral area and smaller neurons surrounding the aqueduct of Sylvius (Fig. 2). In this experiment, only the large neurons were counted. Sleep-deprived rats exhibited a significant reduction (−31.5%) in the number of TH-immunoreactive cells in the PAG compared to controls (Figs. 2 and 3).

4. Discussion

Our results clearly demonstrate that 96 h of PSD significantly increased the pain sensitivity of animals subjected to a noxious thermal stimulus (46 °C). We also evaluated the effects of acute morphine treatment on PSD-induced hyperalgesia. Morphine reversed this hyperalgesia but only at the highest of the 3 doses administered. In addition, we tested the role of dopamine in this reversal by the administration of L-DOPA (with and without morphine) and through the quantification of the number of TH-immunoreactive cellular bodies in the vPAG of sleep-deprived and control rats. L-DOPA alone prevented PSD-induced hyperalgesia. The immunohistochemical analysis revealed a reduction in the number of TH-immunoreactive cell bodies in sleep-deprived rats.

Our results are consistent with the results obtained by Nascimento et al. (2007), who reported that 96 h of PSD reduced the latency of paw withdrawal in animals subjected to a thermal stimulus at 50 °C. Onen et al. (2001) also showed that PSD for 72 h reduced the pain threshold for thermal stimuli as determined by the tail flick test. Based on the subsequent type of nociceptive fiber activation, PSD appears to increase the pain sensitivity of rats in a manner that is independent of the noxious temperature (Damasceno et al., 2009). However, in our study, the noxious temperature was set at 46 °C, which predominantly activates C nociceptive fibers (May et al., 2005) that express μ-opioid receptors. We used this experimental paradigm to observe morphine antinociception.

Previous studies have investigated opioid systems in PSD-induced hyperalgesia in rats. Ukponmwan et al. (1984, 1986) have shown that the analgesic effects of phosphoramidon and morphine are abolished after 96 h of PSD. Nascimento et al. (2007) also demonstrated that PSD may reduce the antinociceptive effect of morphine because the acute administration of 2.5 and 5 mg/kg morphine did not alter the paw withdrawal response of rats challenged with a noxious temperature of 50 °C. These data suggest that PS is important for the antinociceptive activity of opiates. Similarly, in our study, we concluded that PSD reduced the antinociceptive effects of morphine at the same doses in rats tested at 46 °C on a hot plate. We only observed an increase in the hind paw withdrawal latency at the highest dose of morphine, 10 mg/kg. Morphine treatment with 10 mg/kg, but not 3 mg/kg decreases locomotor activity (Smith et al., 2009). Therefore, it is important to note that the reduction in hind paw response at 10 mg/kg may be due to a secondary reduction in locomotor activity rather than an analgesic effect.

The mechanism by which these PSD-induced changes occur during the nociceptive responses is unclear, but several lines of evidence suggest that these alterations may result from changes in different

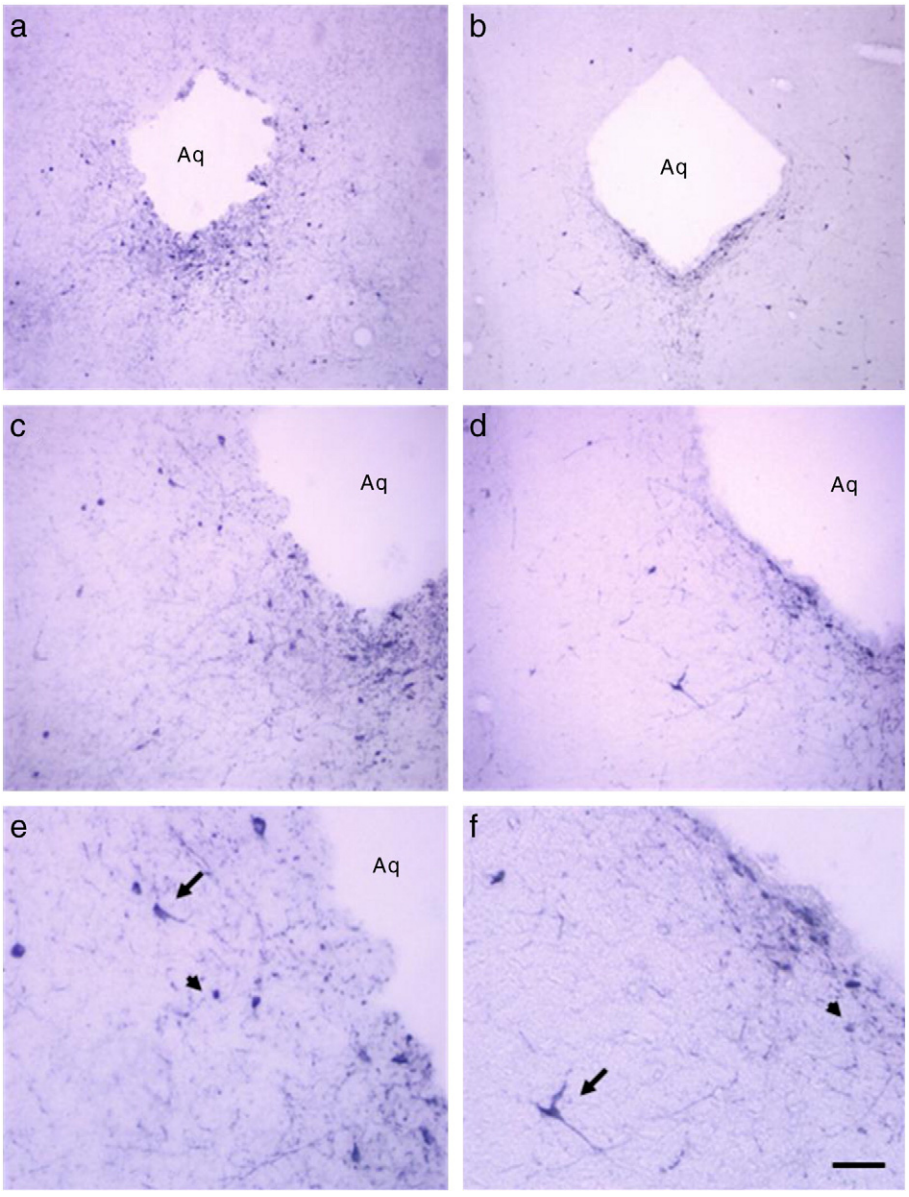

Fig. 2. Photomicrographs of periaqueductal gray matter immunostained with an antibody to tyrosine hydroxylase. (a, c, e) Coronal sections from control rats showing dense tyrosine hydroxylase labeling in the periaqueductal gray matter. (b, d, f) Coronal sections from paradoxical sleep-deprived rats showing a reduction in tyrosine hydroxylase immunostaining in the periaqueductal gray matter. The black arrows indicate large cells, and the black arrowheads indicate small cells. Aq, aqueduct of Sylvius. Scale bars represent 50 μm (a, b), 100 μm (c, d) and 25 μm (e, f).

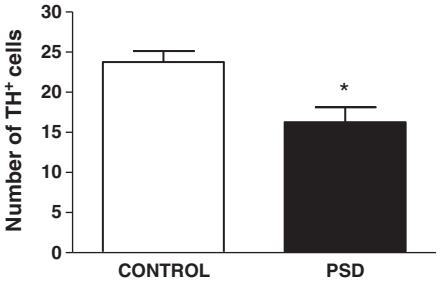

Fig. 3. Number of large tyrosine hydroxylase immunoreactive cells counted in the ventrolateral periaqueductal gray matter of the control (n = 7) and paradoxical sleep-deprived (n = 7) groups. Data are presented as mean ± SEM. *p value ≤ 0.05 differs from the control group (unpaired *t*-test).

neurotransmitter systems and their receptors. Several studies have suggested that animals subjected to PSD exhibit a low level of activity at the receptors for endogenous opioid peptides (Ukponmwan et al., 1986). In fact, these receptors are reduced in animals deprived of PS (Fadda et al., 1991). PSD also promotes the inhibition of opioid protein synthesis (Shapiro and Girdwood, 1981) and modifies μ-opioid receptor binding in limbic areas of the brain (Fadda et al., 1991). However, Nascimento et al. (2007) reported no differences in μ-opioid receptor binding in brain regions that are involved in pain control, which suggests that other neurotransmitter systems may be involved in PSD-induced changes in the response to morphine.

DA is a neurotransmitter that is associated with several of the behavioral changes observed after PSD. An increase in receptor sensitivity (see for a review: Tufik et al., 2009) and DA release after 24 h of total sleep deprivation has been described (Volkow et al., 2008); however, no significant alterations of dopamine transporters have been observed (Martins et al., 2010).

Several studies have demonstrated that DA plays a central role in nociception within supraspinal regions and modulates the antinociceptive effect of morphine on the PAG (Paalzow, 1992; Wood, 2008). L-DOPA has been used to treat pain associated with diabetic neuropathy, bone metastasis and herpes zoster (Nixon, 1975; Ertas et al., 1998; Kernbaum and Hauchecome, 1981), and the analgesic effect occurs after conversion to dopamine, which acts on D2 receptors (Shimizu et al., 2004).

In this study, L-DOPA was administered with benserazide to prevent its peripheral decarboxylation, and reversed the hyperalgesia induced by PSD; an analgesic effect of L-DOPA in control rats was observed at the highest dose administered. The combination of L-DOPA 50 mg/kg and morphine did not increase the analgesic effects of L-DOPA because the paw withdrawal latencies of control and sleep-deprived rats treated with morphine were in the same range regardless of whether morphine administration was accompanied by L-DOPA (Fig. 1A and C). However, the paw withdrawal latencies in the control group were similar for the highest doses of morphine (Fig. 1A) and L-DOPA (Fig. 1B). These data indicate that central dopamine had a direct effect on PSD-induced hyperalgesia and suggest a relationship between the central dopaminergic network and opiate-induced analgesia in rats. In fact, the vPAG features dopaminergic neurons that are associated with this opiate response. Depletion of these neurons using 6-hydroxydopamine (6-OHDA) or through intracerebral injections of D1 DA receptor antagonists into the vPAG reduces the analgesic action of morphine (Flores et al., 2004). Recently, another group has provided evidence for the role of DA in the modulation of nociception in the PAG. Meyer et al. (2009) have shown that DA inhibits the GABAergic system in the vPAG in a manner similar to opioids.

In this study, we observed and quantified the labeling of dopaminergic neurons in the vPAG of sleep-deprived and control rats using TH immunoreactivity. TH activity is regulated by feedback inhibition and phosphorylation, and TH also undergoes transcriptional and translational regulation that is induced by changes in the physiological state (for a review, see Kumer and Vrana, 1996). Immunohistochemical analysis revealed a reduction in the number of cell bodies labeled with TH in PSD animals. Flores et al. (2004) showed that the dopaminergic circuitry in the PAG is composed of 2 different types of neurons (large and small) and reported that large neurons participate in supraspinal nociceptive responses. Large neurons, which also exhibit a multipolar morphology and abundant arborization, are often located adjacent to the PAG. Small cells exhibit a rounded morphology and are more frequently located in the vicinity of the surface of the aqueduct of Sylvius. In our study, we observed the 2 dopaminergic cell types described previously in the literature. Sleep-deprived rats showed small dopaminergic neurons in regions closer to the aqueduct of Sylvius and a reduction in the number of large dopaminergic neurons.

Meyer et al. (2009) has suggested that there may be synaptic connections between dopaminergic and GABAergic neurons in the vPAG, which implicates the participation of dopaminergic neurons in analgesia. Considering the involvement of vPAG dopaminergic neurons in the response to opioid drugs and our observations of the antinociceptive effect of L-DOPA and the TH labeling in sleep-deprived rats, we suggest that there is a relationship between the behavioral data and the observed alterations in TH immunohistochemistry. The reducing in TH staining in sleep-deprived rats indicated a reduction in dopaminergic activity in the vPAG. This event might be associated with a decrease in endogenous analgesic mechanisms, which resulted in an increased sensitivity to painful stimuli and with the changes observed in the antinociceptive responses that were induced by morphine.

5. Conclusions

Our data suggest that PSD could interfere with dopaminergic transmission in the PAG as indicated by the observed reduction in TH staining and the L-DOPA-induced decrease in PSD-hyperalgesia. Moreover, the changes induced in the dopaminergic system may be associated with an increase in the sensitivity to noxious stimuli and changes in the antinociceptive response induced by morphine.

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References

Carli G, Montesano A, Rapezzi S, Paluffi G. Differential effects of persistent nociceptive stimulation on sleep stages. Behav Brain Res 1987;26:89–98.
Ertas M, Sagduyu A, Arac N, Uludag B, Ertekin C. Use of levodopa to relieve pain from painful symmetrical diabetic polyneuropathy. Pain 1998;75:257–9.
Damasceno F, Skinner GO, Cordeiro JF, Ferraz MR, Almeida OM. Sleep deprivation affects sexual behavior and tyrosine hydroxylase (TH) levels in sexually experienced male rats. Physiol Behav 2008;94:405–11.
Damasceno F, Skinner GO, Gomes A, Araujo PC, Almeida OM. Systemic amitriptyline administration does not prevent the increased thermal response induced by paradoxical sleep deprivation. Pharmacol Biochem Behav 2009;94:51–5.
Fadda P, Tortorella A, Fratta W. Sleep deprivation decreases mu and delta opioid receptor binding in the rat limbic system. Neurosci Lett 1991;129:315–7.
Flores JA, El Banoua F, Galan-Rodriguez B, Fernandez-Espejo E. Opiate antinociception is attenuated following lesion of large dopamine neurons of the periaqueductal gray: critical role for D1 (not D2) dopamine receptors. Pain 2004;110:205–14.
Hasue RH, Shammah-Lagnado SJ. Origin of the dopaminergic innervation of the central extended amygdala and accumbens shell: a combined retrograde tracing and immunohistochemical study in the rat. J Comp Neurol 2002;454:15–33.
Hicks RA, Coleman DD, Ferrante F, Sahatjian M, Hawkins J. Pain thresholds in rats during recovery from REM sleep deprivation. Percept Mot Skills 1979;48:687–90.
Hökfelt J, Johansson O, Fuxe K, Goldstein M, Pardk D. Immunohistochemical studies on the localization and distribution of monoamine neuron systems in the rat brain. I. Tyrosine hydroxylase in the mes- and diencephalon. Med Biol 1976;54:427–53.
Jouvet D, Vimont P, Delorme F, Jouvet M. Study of selective deprivation of the paradoxal sleep phase in the cat. C R Seances Soc Biol Fil 1964;158:756–9.
Kernbaum S, Hauchecome J. Administration of levodopa for relief of herpes zoster pain. J Am Med Assoc 1981;246:132–4.
Kumer SC, Vrana KE. Intricate regulation of tyrosine hydroxylase activity and gene expression. J Neurochem 1996;67:443–62.
Kundermann B, Spernal J, Huber MT, Krieg JC, Lautenbacher S. Sleep deprivation affects thermal pain thresholds but not somatosensory thresholds in healthy volunteers. Psychosom Med 2004;66:932–7.
Landis CA, Robinson CR, Levine JD. Sleep fragmentation in the arthritic rat. Pain 1998;34:93–9.
Lane DA, Patel PA, Morgan MM. Evidence for an intrinsic mechanism of antinociceptive tolerance within the ventrolateral periaqueductal gray of rats. Neuroscience 2005;135:227–34.
Lautenbacher S, Kundermann B, Krieg JC. Sleep deprivation and pain perception. Sleep Med Rev 2006;10:357–69.
Manning BH, Morgan MJ, Franklin KB. Morphine analgesia in the formalin test: evidence for forebrain and midbrain sites of action. Neuroscience 1994;63:289–94.
Martins RCS, Andersen ML, Garbuio SA, Bittencourt LR, Guindalini C, Shih MC, Hoexter MQ, Bressan RA, Castiglioni MLV, Tufik S. Dopamine transporter regulation during four nights of rem sleep deprivation followed by recovery – an in vivo molecular imaging study in humans. Sleep 2010;33:243–51.
May ME, Harvey MT, Valdovinos MG, Kline RHt, Wiley RG, Kennedy CH. Nociceptor and age specific effects of REM sleep deprivation induced hyperalgesia. Behav Brain Res 2005;159:89–94.
McCarley RW. Mechanisms and models of REM sleep control. Arch Ital Biol 2004;142:429–67.
Meyer PJ, Morgan MM, Kozell LB, Ingram SL. Contribution of dopamine receptors to periaqueductal gray-mediated antinociception. Psychopharmacology 2009;204:531–40.
Moldofsky H, Scarisbrick P, England R, Smythe H. Musculosketal symptoms and non-REM sleep disturbance in patients with "fibrositis syndrome" and healthy subjects. Psychosom Med 1975;37:341–51.
Moldofsky H, Scarisbrick P. Induction of neurasthenic musculoskeletal pain syndrome by selective sleep stage deprivation. Psychosom Med 1976;38:35–44.
Morin CM, Gibson D, Wade J. Self-reported sleep and mood disturbance in chronic pain patients. Clin J Pain 1998;14:311–4.
Nascimento DC, Andersen ML, Hipolide DC, Nobrega JN, Tufik S. Pain hypersensitivity induced by paradoxical sleep deprivation is not due to altered binding to brain mu-opioid receptors. Behav Brain Res 2007;178:216–20.
Nixon DW. Letter: use of L-DOPA to relieve pain from bone metastases. N Engl J Med 1975;292:647.
Ohayon MM. Relationship between chronic painful physical condition and insomnia. J Psychiatr Res 2005;39:151–9.

Onen SH, Alloui A, Eschalier A, Dubray C. Vocalization thresholds related to noxious paw pressure are decreased by paradoxical sleep deprivation and increased after sleep recovery in rat. Neurosci Lett 2000;291:25–8.
Onen SH, Alloui A, Jourdan D, Eschalier A, Dubray C. Effects of rapid eye movement (REM) sleep deprivation on pain sensitivity in the rat. Brain Res 2001;900:261–7.
Ottersen OP. Afferent connections to the amygdaloid complex of the rat with some observations in the cat. III. Afferents from the lower brain stem. J Comp Neurol 1981;202:335–56.
Paalzow GHM. L-DOPA induces opposing effects on pain in intact rats: (−)-sulpiride, SCH 23390 or α-methyl-DL-p-tyrosine methylester hydrochloride reveals profound hyperalgesia in large antinoceptive doses. J Pharmacol Exp Ther 1992;263:470–9.
Paxinos G, Watson J. The rat brain in stereotaxic coordinates. 4th ed. New York: Academic Press; 1998.
Pert A, Yaksh T. Localization of the antinociceptive action of morphine in primate brain. Pharmacol Biochem Behav 1975;3:133–8.
Pertovaara A, Almeida A. Descending inhibitory systems. In: Cervero F, Jensen TS, editors. Handbook of clinical neurology. Amsterdam: Elsevier; 2006. p. 179–92.
Reichling DB, Basbaum AI. Contribution of brainstem GABAergic circuitry to descending antinociceptive controls: II. Electron microscopic immunocytochemical evidence of GABAergic control over the projection from the periaqueductal gray to the nucleus raphe magnus in the rat. J Comp Neurol 1990;302:378–93.
Roehrs T, Hyde M, Blaisdell B, Greenwald M, Roth T. Sleep loss and REM sleep loss are hyperalgesic. Sleep 2006;29:145–51.
Shapiro C, Girdwood P. Protein synthesis in rat brain during sleep. Neuropharmacology 1981;20:457–60.
Shimizu T, Iwata S, Marioca H, Masuyama T, Fukuda T, Nomoto M. Antinociceptive mechanism of L-DOPA. Pain 2004;110:246–9.
Smith MA, Greene-Naples JL, Lyle MA, Iordanou JC, Felder JN. The effects of repeated opioid administration on locomotor activity: I. Opposing actions of mu and kappa receptors. J Pharmacol Exp Ther 2009;330:468–75.
Tufik S, Andersen MA, Bittencourt LRA, De Mello MT. Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. Annals of the Brazilian Acad of Sciences 2009;81:521–38.
Ukponmwan OE, Rupreht J, Dzoljic MR. REM sleep deprivation decreases the antinociceptive property of enkephalinase-inhibition, morphine and cold-water-swim. Gen Pharmacol 1984;15:255–8.
Ukponmwan OE, Poel-Heisterkamp AL, Dzoljic MR. REM sleep deprivation antagonizes morphine-induced akinesia and catalepsy. Sleep 1986;9:415–22.
Volkow ND, Wang GJ, Telang F, et al. Sleep deprivation decreases binding of [11 C] raclopride to dopamine D2/D3 receptors in the human brain. J Neurosci 2008;28: 8454–61.
Wood PB. Role of central dopamine in pain and analgesia. Expert Rev Neurother 2008;8:781–97.
Yaksh TL, Yeung JC, Rudy TA. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: observation of differential effects within the periaqueductal gray. Brain Res 1976;114:83-103.