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Research report

Levodopa analgesia in experimental neuropathic pain

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ABSTRACT

Levodopa has been shown to produce analgesia in various clinical and experimental settings, but its use for chronic pain treatment has not been established. We have undertaken a study of the antiallodynic actions of levodopa in a rat model of painful mononeuropathy. When administered systemically, levodopa produced a decrease in tactile and cold allodynia lasting at least 3 h. Direct intrathecal (i.t.) levodopa injection at lumbar levels produced a similar, though shorter, antiallodynic effect. This effect was blocked by the D2-type receptor antagonist sulpiride, which supports the involvement of the spinal dopaminergic system in the analgesic action of levodopa on neuropathic pain. These results provide experimental support on the antiallodynic effect of levodopa in neuropathic pain and suggest that at least part of the analgesic action takes place in the spinal cord and involves dopaminergic D2-type receptors.

1. Introduction

Neuropathic pain is a chronic pathological condition of varied etiology for which there is no single treatment at present that works in all conditions. Antidepressants, anticonvulsants, opioids, NMDA antagonists, cannabinoids and topical drugs are used with varied effectiveness to treat pain [16,44]. Antidepressants, either tricyclic or serotonin–noradrenaline reuptake inhibitors, are often considered as first choice treatment for several conditions of neuropathic pain. Their mechanism does not appear to rely on their mood-enhancing properties but rather on the increase of monoaminergic neurotransmission, which has a modulatory action on nociceptive circuits [28,30,36,45–47]. Spinal projections of both brainstem serotonergic and noradrenergic neurons mediate antinociception [4,18,45,46]. Dopaminergic terminals in spinal cord have also been shown to modulate nociceptive transmission [3,17,19,23,31,42] and to enhance antinociception mediated by other monoamines [31].

Levodopa is the precursor of dopamine. It is endogenously synthesized by the enzyme tyrosine hydroxylase in catecholaminergic neurons, where it is subsequently transformed into dopamine by the L-amino acid decarboxylase. Exogenously administered levodopa increases the dopaminergic activity and has been used in dopamine-deficiency pathologies like Parkinson's disease [26] and restless legs syndrome (RLS) [10]. Central pain and/or increased pain sensitivity often accompany these pathologies and levodopa treatment relieves this symptom in a way that appears to be independent of movement disorders [7,20,37,41]. Levodopa has also been clinically tested as a non-traditional analgesic drug in different chronic pain conditions, like bone metastasis pain [13,32] and neuropathic pain [15,24] although at present it is not a treatment of choice for these pathologies. In animal experiments of acute pain, levodopa has been shown to be antinociceptive [34,39], acting via the dopaminergic systems in brain and/or spinal cord, but no study has been reported on the analgesic activity of levodopa in chronic pain, so clinical tests cannot be backed by experimental data.

The aim of the present study was to test the analgesic properties of levodopa in a rat model of neuropathic pain.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 250–300 g at the beginning of the experiments were used for the present study. The experimental procedures followed the ethical guidelines of the IASP for the investigations of experimental pain in conscious animals [49] and were approved by the Ramon y Cajal Hospital Animal Welfare Ethic Committee. The rats were bred in the Ramón y Cajal Hospital stabulary premises and fresh male breeders from Charles River (France) were introduced to the colony every 4–5 months. The animals were housed in groups of 3 with food and water *ad lib* and maintained at 24 °C room temperature under a 12 h light/12 h dark schedule. The behavioral tests were started at the 3rd hour of the light period.

2.2. Drugs and treatments

For intraperitoneal (i.p.) injections, levodopa was prepared in 0.2 M HCl and buffered at pH 6.8 with 7% $NaHCO_3$, to a final concentration of 10 mg/ml. DOPA-

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decarboxylase inhibitor carbidopa was injected previously, 50 mg/kg, i.p., to prevent peripheral formation of catecholamines. Fifteen minutes later, levodopa was injected i.p. at 200 mg/kg. Pain tests were performed at 20, 90, 180 min and at 24 h post-injection.

For intrathecal (i.t.) injections, levodopa was prepared as a 40 mM stock solution in 0.02 M HCl and diluted 1:16 in Hank's balanced salt solution (HBSS) to deliver 50 nmoles of levodopa in 20 μL. Supplements of 0.02 M HCl were added when preparing lower doses or vehicle, to equal chloride and pH of injections. (S)-(−)-sulpiride was prepared 40 mM in 0.02 M HCl and diluted in HBSS with supplements of HC when required, as mentioned above. All drugs were from Sigma–Aldrich.

2.3. Animal surgery

2.3.1. Rat model of neuropathic pain

A peripheral mononeuropathy was produced in young adult rats by the chronic constriction injury (CCI) method developed by Bennett and Xie [5] with little variations. Briefly, the left sciatic nerve was exposed by blunt dissection through biceps femoris and 4 loose ligatures, 1 mm apart, were done with 6/0 silk. The ligatures barely compressed the nerve so that blood circulation was maintained. The muscles were repositioned with one resorbable stitch and the skin was closed with staples that were withdrawn 7 days later. In our experience, using this procedure, the animals typically show signs of neuropathic pain by the first week after CCI and maintain stabilized signs for more than 3 weeks thereafter. These signs include tactile and cold allodynia and heat hyperalgesia. Pain tests started 14 days after CCI (pre-injection values) and treatments (drugs or vehicle) were administered the next day.

2.3.2. Direct lumbar puncture

Intrathecal injections were performed by a direct lumbar puncture method that has been described in detail elsewhere [12]. Briefly, the animal was anaesthetized with isoflurane (4% for induction until the puncture and 2.5% for the rest of the procedure, in 100% O_2) and placed on an elevated surface with hind limbs hanging out to widen the intervertebral space between L3 and L4 vertebrae. The back was shaved, a small incision was made in the skin and the fascia overlying L3–L4 was blunt dissected to clear the intervertebral entry site. A 25G × 1inch neonatal lumbar puncture needle (Becton–Dickinson, ref. 405243) was advanced into the subarachnoidal space and CSF was allowed to flow into the needle cup. After 35–40 μL of CSF had flown to the needle cup, this liquid was withdrawn and replaced by 20 μL of the treatment to be delivered subarachnoidally. A 1 ml syringe was then fitted to the needle (vaseline was applied around the open end of the syringe to easy this step) and the treatment was slowly pushed into the intrathecal space, under visual control, by a small bolus of air. The needle was then immediately withdrawn and the time was recorded. The skin incision was closed with staples and the animal was transferred to the test cage, where it woke up and moved normally in less than 10 min.

2.4. Pain tests

Rats were tested for both tactile and cold allodynia. Basal measurements were performed before any surgery, so as to discard animals that showed to be hyper reactive to the tests. Post-CCI measurements were performed 14 days after surgery and only rats showing either a tactile withdrawal response below 4 g or a withdrawal response time to cold above 4 s in the CCI limb were eligible for experiments. For behavioral tests, the animals were housed in transparent square cages (17 cm × 22 cm × 14 cm) with a grid bottom made of 7 mm wire mesh. The rats were allowed to accommodate to the cage for at least 10 min. The stimuli were applied from the bottom and the response was observed directly or with the help of a mirror.

Tactile allodynia was assessed by estimating the threshold for leg withdrawal in response to the mid-plantar application of von Frey nylon calibrated monofilaments as proposed by Chaplan et al. [8]. The sequence of filament strengths was 0.4, 0.6, 1, 2, 4, 6, 8 and 15 g. The withdrawal threshold was calculated by the "up and down" method described by Dixon [14], starting with filament strength of 2 g and changing to a higher strength if there was no reaction during a 6 s application or to a lower strength if the rat had lifted the paw. The test was stopped when five filaments had been tested after the first change of direction or if the test limits (0.4 or 15 g) had been reached. The test was recorded as a series of "O" (when there was no response) or "X" (when there was a lifting response) and that record was compared with the Dixon's table to obtain a value (D_{50}) that could be translated into the estimated withdrawal threshold, following the formula

$$\text{Threshold (g)} = \frac{10^{(Xf+K\delta)}}{10000}$$

where Xf is the value (in log units) of the final von Frey filament used; K is Dixon's tabular value for pattern of positive/negative responses; and δ is the mean difference (log units) between stimuli (here 0.224).

Cold allodynia was assessed by the duration of the paw lifting response during the first minute after spraying 0.5 ml acetone to the foot with the help of a syringe and a blunt needle. Acetone evaporation produces an innocuous cooling of the paw that is felt as painful only in a neuropathic limb so the animal lifts that leg one or more times as a withdrawal response to pain. The total time spent by the rat with the limb raised from the floor during the first minute can be thus considered as a measurement of perceived pain.

2.5. Rotarod tests for motor coordination

The integrity of sensory-motor capacities of animals treated with systemic levodopa was tested with the Rotarod treadmill (Panlab). Rats were first trained to walk on the rotating drum for 3 consecutive days with 3 sessions per day of 3 min at constant speeds of 5, 10 and 14 RPM (1 min for each speed). On the third day, the last session was replaced by a session of accelerating speed like the one used for testing. Testing consisted in recording the latency to fall from the rotating drum accelerating from 4 to 40 RPM in one minute (3 repetitions, 6 min apart). Rats were tested before and starting 20, 90, 180 min and 24 h after i.p. injection of levodopa + carbidopa (200 mg/kg + 50 mg/kg, as described above) or the equivalent amount of vehicle. As a positive control of the sensitivity of the Rotarod test to show impaired sensory-motor coordination, additional rats receiving i.p. injections of ethanol (2 g/kg of a 20% ethanol dilution in saline) were tested.

2.6. Statistical analyses

GraphPad Prism 4 software was used for data handling and statistical analyses. Normal distribution of 14 days post-CCI responses to tactile and cold stimuli was first checked. Pooled data from 106 animals failed to pass the D'Agostino and Pearson omnibus normality test for cold responses ($p < 0.05$ for left limbs, $p < 0.001$ for right limbs) as well as for tactile responses ($p < 0.001$ for both limbs). Furthermore, collected data had upper cut-off. Consequently, non-parametric statistics were chosen for data analysis. Central values were represented by medians and data dispersions were represented by interquartile ranges. Differences within an experimental group were checked by Friedman's one-way analysis by ranks of paired data, followed *post hoc* by the Wilcoxon matched-pair test with Bonferroni's correction, consisting in multiplying the obtained p by the number of analyzed points. Differences between experimental groups were checked by Kruskal-Wallis test of unpaired data, followed by Dunn's multiple comparisons post-test.

3. Results

3.1. Systemic levodopa

Levodopa + carbidopa (200 mg + 50 mg/kg) or vehicle were injected i.p. in rats with CCI. As shown in Fig. 1(A), a drastic decrease of both cold and tactile allodynic responses was observed 20 min after levodopa treatment (Friedman test $p < 0.001$ for cold and $p < 0.01$ for tactile responses). The analgesic effects of systemic levodopa on cold allodynia lasted more than 3 h and were more pronounced than the analgesic effects on tactile allodynia. The unoperated limb did not show any signs of allodynia after CCI of the contralateral sciatic nerve and the treatment did not produce measurable changes in this response. However, the present methodology does not allow us to detect if the treatment produces an increase in the nociceptive threshold in the unoperated limb.

Systemic levodopa, at the dose injected in the present study, did not impair the performance of normal rats in the Rotarod treadmill (Fig. 1(B)), a test which is commonly used to evaluate motor coordination (animals treated with 2 g/kg ethanol, used as a positive control drug producing altered sensory-motor coordination, clearly showed impaired Rotarod performance lasting more than 90 min). At the doses here used, systemic levodopa produced short-lasting (<15 min) ataxia and piloerection in the rats, followed by increased locomotion, stereotyped movements (licking, rearing) and aggressive/defensive behaviors when housed with other animals between tests. These signs disappeared before 3 h after injection. The maintenance of the antiallodynic activity of levodopa for more than 3 h suggests that this effect was unrelated to sedation, ataxia or altered locomotion.

3.2. Intrathecal levodopa

When levodopa was intrathecally injected at lumbar levels in rats with CCI, analgesic effects on cold and tactile allodynias, reproducing those observed after systemic levodopa, were obtained (Fig. 2). The antiallodynic effects of i.t. levodopa were