

PHARMACOLOGIC TREATMENT OF HYPERALGESIA EXPERIMENTALLY INDUCED BY NUCLEUS PULPOSUS

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ABSTRACT

Objective: To evaluate the effect of anti-inflammatory drugs (dexamethasone, indomethacin, atenolol and indomethacin plus atenolol) and analgesic drugs (morphine) on hyperalgesia experimentally induced by the nucleus pulposus (NP) in contact with the L5 dorsal root ganglion (DRG). **Methods:** Thirty male Wistar rats of weights ranging from 220 to 250 g were used in the study. Hyperalgesia was induced by means of a fragment of NP removed from the sacrococcygeal region that was placed in contact with the L5 dorsal root ganglion. The 30 animals were divided into experimental groups according to the drug used. The drugs were administered for two weeks after the surgical procedure to induce hyperalgesia. Mechanical and thermal hyperalgesia was evaluated using the paw pressure test, von Frey

electronic test and Hargreaves test, over a seven-week period. **Results:** The greatest reduction of hyperalgesia was observed in the group of animals treated with morphine, followed by dexamethasone, indomethacin and atenolol. Reductions in hyperalgesia were observed after drug administration ceased, except for the group of animals treated with morphine, in which there was an increase in hyperalgesia after discontinuation of the treatment. **Conclusion:** Hyperalgesia induced by NP contact with the DRG can be reduced through administration of anti-inflammatory and analgesic drugs, but a greater reduction was observed with the administration of dexamethasone.

Keywords - Spine; Intervertebral Disc; Low Back Pain; Hyperalgesia; Intervertebral Disc Displacement; Wistar Rats

INTRODUCTION

The physiopathology of lumbar disc hernias is related to mechanical compression of the nerve root and changes induced by contact between the nucleus pulposus and nerve elements^(1,2). Experimental studies have demonstrated that in the absence of mechanical compression on the nerve root, contact with the nucleus

pulposus induces functional and structural alterations to the nerve root^(3,4).

The mechanism through which the inflammatory process is induced by the nucleus pulposus remains unknown. Direct chemical irritation and the secondary immunological reaction of the adjacent tissues to the presence of the nucleus pulposus have been considered to be likely mechanisms⁽⁵⁾.

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Sensitization of the sensory nerve fibers, among which the nociceptive fibers, is the predominant phenomenon and is the common denominator for the inflammatory pain that induces the appearance of hyperalgesia, through peripheral or medullary mechanisms⁽⁶⁾. Prostaglandins and sympathomimetic amines participate in the inflammatory process, and anti-inflammatory drugs and analgesics interfere in this inflammatory process⁽⁶⁾. The aim of the present study was to evaluate the effect of different drugs (dexamethasone, indomethacin, atenolol and morphine) on hyperalgesia induced by the nucleus pulposus on the nerve root ganglion in the absence of mechanical compression.

MATERIAL AND METHODS

The protocol for this study was approved by the Ethics Committee for Animal Experimentation of the Ribeirão Preto School of Medicine, and the study was conducted in accordance with the international ethical standards for using laboratory animals. Thirty male Wistar rats weighing between 220 g and 250 g were used. Before the experiments were carried out, the animals were kept in the local vivarium for two days, to acclimatize. They were kept in cages of dimensions 40 x 60 x 20 cm, with a maximum of six rats per cage, under controlled conditions of temperature (22 to 25°C) and light-dark cycling (12 x 12 h), with free access to food and water, before being subjected to the tests. The experimental model used in the study consisted of a surgical procedure for removing the nucleus pulposus from the sacrococcygeal region and placing it on the L5 dorsal root ganglion (DRG)⁽⁷⁾. After the surgical procedure, the animals were divided into experimental groups with five animals in each group. The groups were determined according to the drug administered (dexamethasone, indomethacin, atenolol, indomethacin plus atenolol, morphine or saline solution).

Surgical procedure

Surgical protocol – The animals were anesthetized with an intraperitoneal injection of 10% ketamine (0.1 ml/100g), 2% xylazine (0.07 ml/100g) and 5% fentanyl (0.001 ml/100g).

The nucleus pulposus (NP) was removed from the sacrococcygeal region (base of the rat's tail) by means of a medial incision at the transition between the fourth sacral vertebra and the first coccygeal vertebra. The intervertebral disc was exposed bilaterally and the NP was

removed by means of a transverse incision in the fibrous annulus. The NP was collected (Figure 1), weighed on a precision balance (mean weight of 4 to 5 µg) and placed on the L5 DRG, which was exposed by means of partial laminectomy. The surgery to expose the L5 DRG and place the NP fragment was carried out with the aid of a lens of 10x magnification. After the procedure, the surgical wound was closed as a single layer involving the muscle fascia and skin.

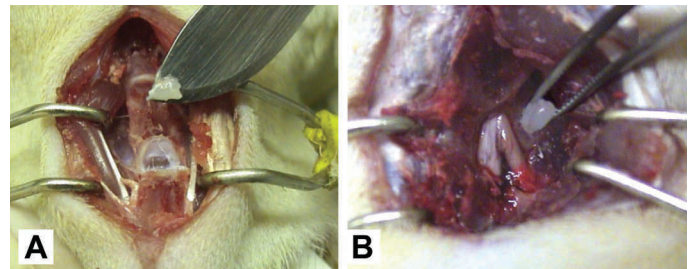


Figure 1 – Exposure of an intervertebral disc in the sacrococcygeal region (A) and deposition of the NP fragment on the L5 dorsal root ganglion (B).

Experimental groups

The animals were divided into six experimental groups with five animals in each group, according to the drug administered. The drugs were administered for a 15-day period after the surgical procedure that placed the NP in contact with the L5 DRG. With the exception of morphine, which was administered 30 minutes before carrying out the tests to evaluate the hyperalgesia, the other drugs were administered every day over this two-week period after the operation. Morphine was administered on the third day, first week and second week after the operation.

- Group I – dexamethasone – 0.5 mg/kg – subcutaneously.
- Group II – indomethacin – 2 mg/kg – subcutaneously.
- Group III – atenolol – 1 mg/kg – intraperitoneally.
- Group IV – indomethacin (2 mg/kg subcutaneously) + atenolol (1 mg/kg intraperitoneally)
- Group V – morphine – 4 mg/kg – intraperitoneally.
- Group VI – saline solution – 3 ml – intraperitoneally.

Evaluation of the hyperalgesia

The hyperalgesia was evaluated in order to determine the threshold of tolerance for mechanical stimuli (constant paw pressure test and von Frey electronic test)^(8,9) and thermal stimuli (Hargreaves test)⁽¹⁰⁾ before the surgical procedure and after drug administration. The tests were carried out on the third day after the operation and weekly thereafter until the seventh week

after the operation. They were performed by an independent examiner who was unaware of which experimental group each animal belonged to. The tests to evaluate hyperalgesia were performed during the period when drugs were administered (two weeks) and then for a further five weeks after drug discontinuation, i.e. for a total period of seven weeks.

Constant paw pressure test – The latency of the nociceptive response was determined by means of the test described by Randall and Selitto⁽⁸⁾. In this method, a constant pressure of 20 mmHg (measured using a sphygmomanometer) is applied by means of a syringe propelled by air compression, to an area of 15 mm² on the dorsal surface of the hind paws of the rats. The pressure is withdrawn when the animal presents a “typical reaction” that is interpreted as nociceptive. This “typical reaction” is signaled by brief apnea and concomitant retraction of the front paws. The duration of the latency (latency time) is determined for each animal, from the time of application of the pressure to the appearance of the typical reaction. The intensity of the hyperalgesia is quantified through the reduction in time difference, calculated by subtracting the latency time in seconds, before and after the treatment that was used.

Increasing pressure test on the rat paws (von Frey electronic test) – The mechanical hyperalgesia was evaluated by means of increasing pressure test on the rat paws, which is known as the von Frey electronic test (tenociception)⁽⁹⁾. The method consisted of using an electronic anesthesia meter (model 1601C, Life Science Instruments[®]) that had a force transducer connected to a digital force counter expressed in grams (g). The precision of the apparatus was 0.1 g and it was calibrated to register a maximum force of 150 g, while maintaining a precision of 0.1 g up to a force of 80 g. The contact between the force transducer and the paw was made by means of a disposable polypropylene pointer of 0.5 mm in diameter that was adapted to the transducer. The animals were placed in acrylic boxes measuring 12 x 20 x 17 cm, in which the floor consisted of a mesh of size 5 mm², made of non-malleable wire of 1 mm in thickness, and they were left there for 15 minutes before the experiment, to adapt to the environment. Mirrors positioned 25 cm below the experimentation boxes made it easier to see the soles of the animals' paws. The investigator applied a linearly increasing force to the center of the soles of the rats' paws through the mesh spaces, until the animal produced a response of withdrawing and

shaking the paw that had been stimulated. The stimuli were repeated up to six times, generally until the animal presented three similar measurements with a clear response of leg shaking after withdrawing the paw.

Hargreaves thermal hyperalgesia test (plantar test) – Thermal hyperalgesia was evaluated by means of the plantar test of Hargreaves *et al*⁽¹⁰⁾. This test consists of heating up the plantar region of the rat's hind paw, using a directed infrared light source, until the animal presented the behavior of withdrawal of the paw or until the heating of the paw reached a predetermined time limit (cutoff time of 12 seconds).

The animals were placed in individual acrylic compartments and were positioned over a surface of special glass that allowed homogenous passage of light and heat, for five minutes, so that they would adapt to the environment. After this period, an infrared light source was placed under each of the animal's hind paws and was switched on together with an electronic timer. When the animal withdrew the paw, the light source and timer stopped automatically.

Three measurements were made, at intervals of five to ten minutes. The latency measurements on paw withdrawal were then expressed as percentage assessments relative to the control paws (contralateral paws) at each observation time. In situations in which the animal did not present a reaction, a maximum cutoff time for the experiment of 12 seconds was stipulated, in order to preserve the animal's physical integrity.

Statistical analysis – The results were presented as the mean value for each experimental group and its standard deviation. The statistical analysis was performed by means of the one-way ANOVA test, followed by the Bonferroni test, and the significance level was set at $p < 0.05$.

RESULTS

It was shown that administration of the anti-inflammatory drugs had the capacity to reduce the mechanical and thermal hyperalgesia induced through contact between the nucleus pulposus and the dorsal root ganglion (Figures 2, 3, 4, 5 and 6). Among the drugs used in this study, dexamethasone was the one that presented the largest reduction in hyperalgesia, and this effect increased after its administration was discontinued. Atenolol and indomethacin also reduced the intensity of hyperalgesia, but to a lesser extent than shown by dexamethasone. Combined use of atenolol and indo-

methacin presented a greater reduction of hyperalgesia than did the separate administration of these drugs.

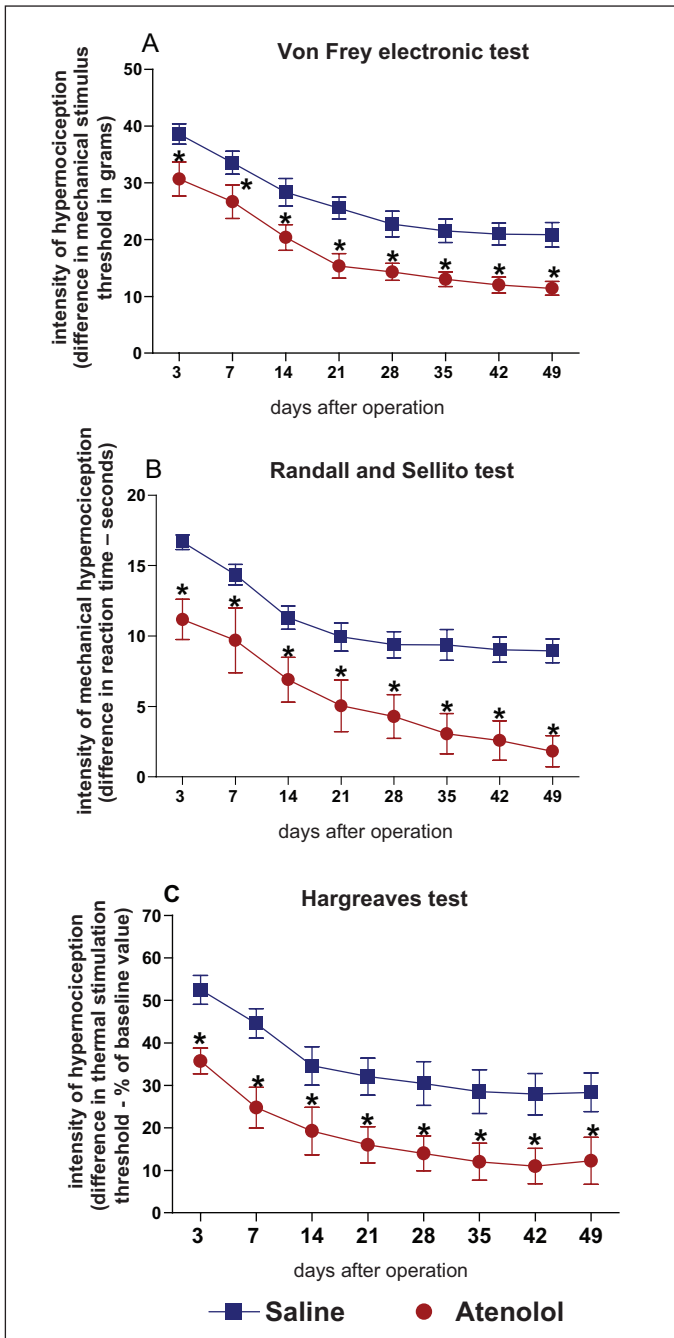


Figure 2 – Intensity of hyperalgesia in animals subjected to surgery to induce lumbar disc hernia that was treated postoperatively with atenolol. After the operation, the animals were treated with atenolol (1.0 mg/kg) for the first seven days and were followed up for 49 days, with weekly measurements. The intensity of hyperalgesia was evaluated through three different behavioral tests: increasing pressure test on the rat paws (von Frey electronic test) (A); constant pressure test on rat paws (Randall and Sellito, modified) (B); and Hargreaves thermal test (C). The measurements represent the mean ± SEM of six animals per group. *p < 0.05, compared with the control group that was treated with saline solution (MANOVA, followed by Student’s t test with Bonferroni inequality).

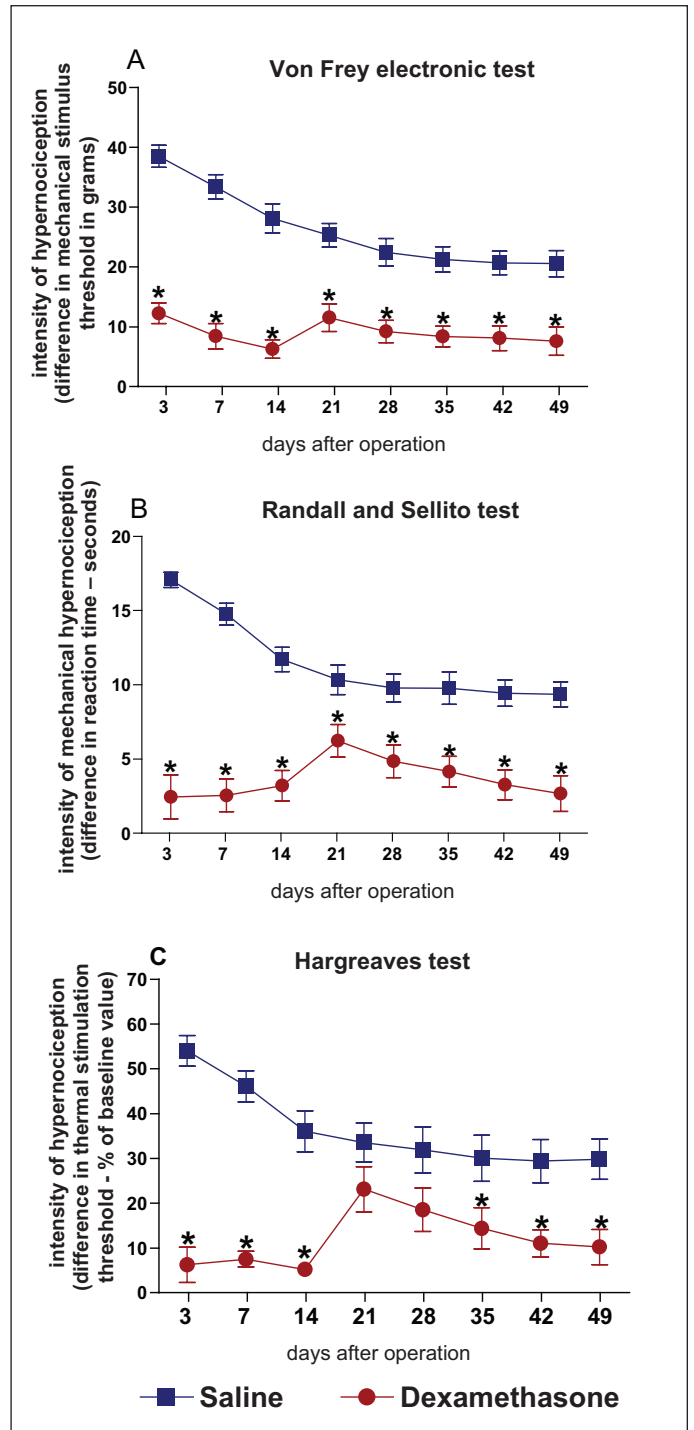


Figure 3 – Intensity of hyperalgesia in animals subjected to surgery to induce lumbar disc hernia and postoperative treatment with dexamethasone. After the operation, the animals were treated with dexamethasone (0.5 mg/kg) for the first seven days and were followed up for 49 days, with weekly measurements. The intensity of hyperalgesia was evaluated through three different behavioral tests: increasing pressure test on the rat paws (von Frey electronic test) (A); constant pressure test on rat paws (Randall and Sellito, modified) (B); and Hargreaves thermal test (C). The measurements represent the mean ± SEM of six animals per group. *p < 0.05, compared with the control group that was treated with saline solution (MANOVA, followed by Student’s t test with Bonferroni inequality).

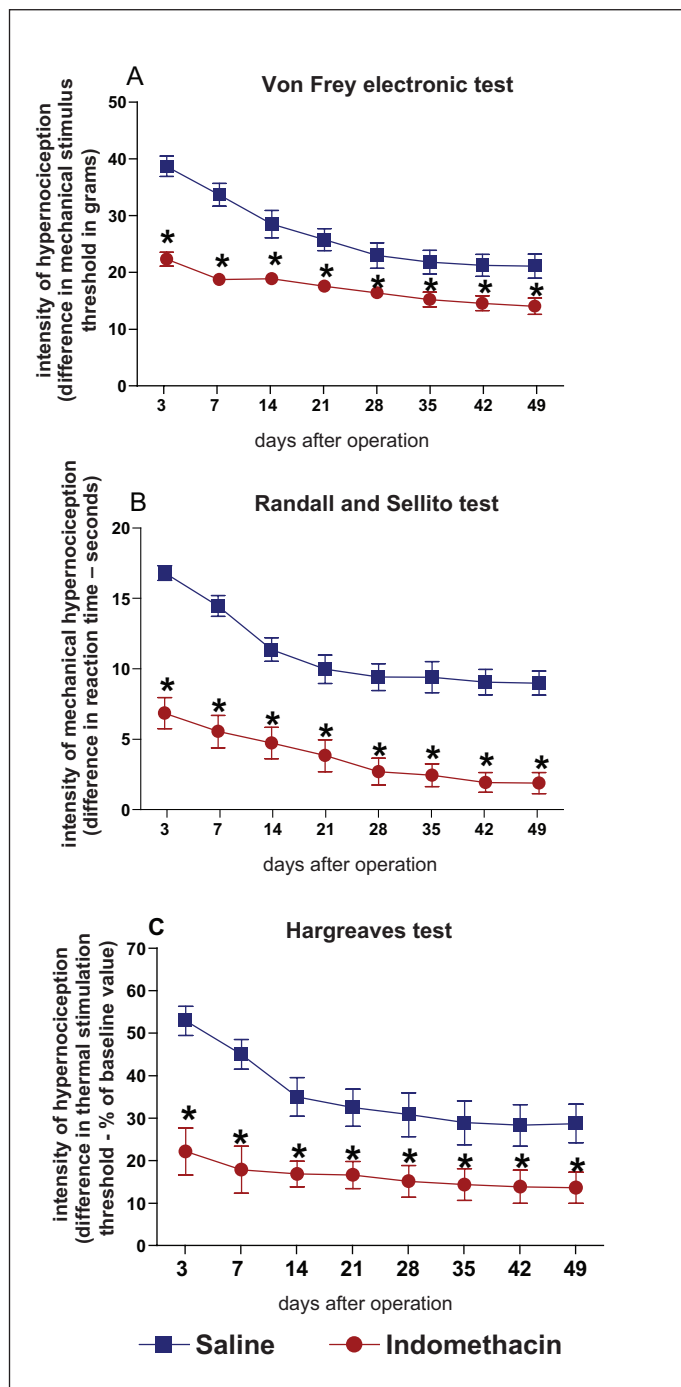


Figure 4 – Intensity of hyperalgesia in animals subjected to surgery to induce lumbar disc hernia that was treated postoperatively with indomethacin. After the operation, the animals were treated with indomethacin (2.0 mg/kg) for the first seven days and were followed up for 49 days, with weekly measurements. The intensity of hyperalgesia was evaluated through three different behavioral tests: increasing pressure test on the rat paws (von Frey electronic test) (A); constant pressure test on rat paws (Randall and Sellito, modified) (B); and Hargreaves thermal test (C). The measurements represent the mean ± SEM of six animals per group. *p < 0.05, compared with the control group that was treated with saline solution (MANOVA, followed by Student’s t test with Bonferroni inequality).

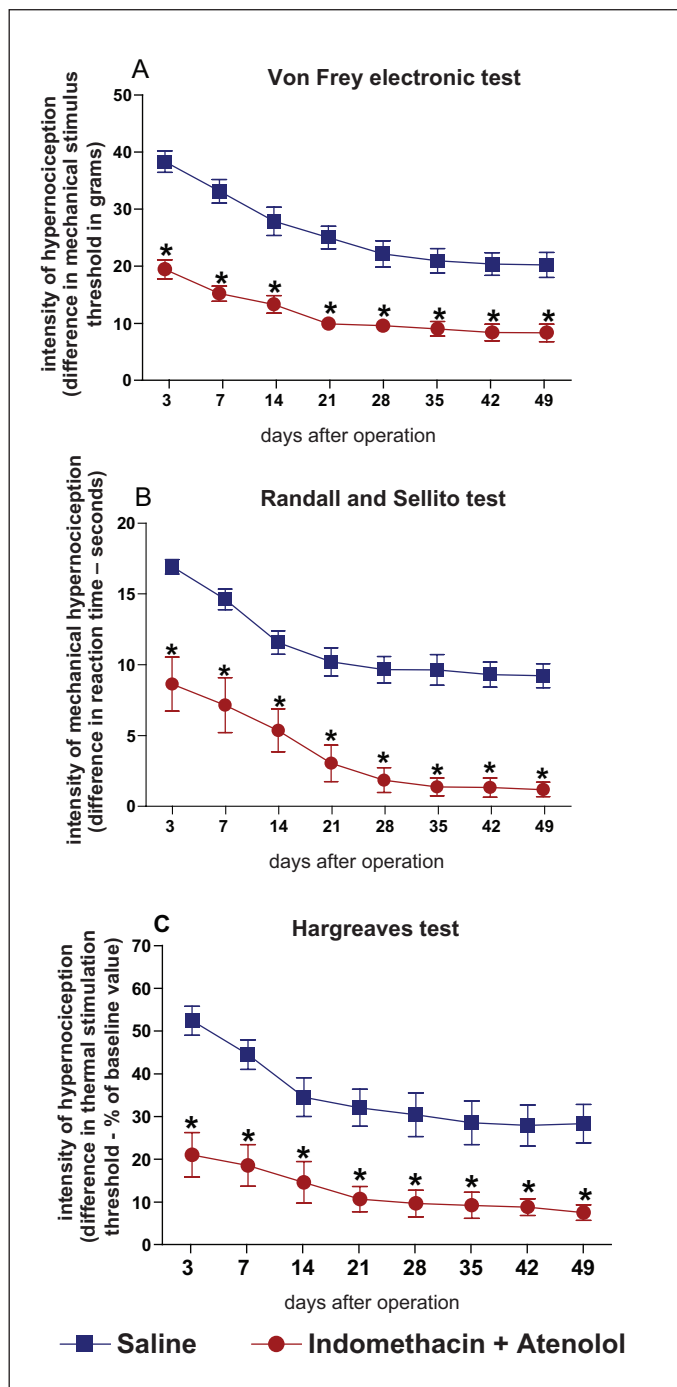


Figure 5 – Intensity of hyperalgesia in animals subjected to surgery to induce lumbar disc hernia that was treated postoperatively with indomethacin and atenolol. After the operation, the animals were treated with indomethacin (2.0 mg/kg) and atenolol (1.0 mg/kg) simultaneously for the first seven days and were followed up for 49 days, with weekly measurements. The intensity of hyperalgesia was evaluated through three different behavioral tests: increasing pressure test on the rat paws (von Frey electronic test) (A); constant pressure test on rat paws (Randall and Sellito, modified) (B); and Hargreaves thermal test (C). The measurements represent the mean ± SEM of six animals per group. *p < 0.05, compared with the control group that was treated with saline solution (MANOVA, followed by Student’s t test with Bonferroni inequality).

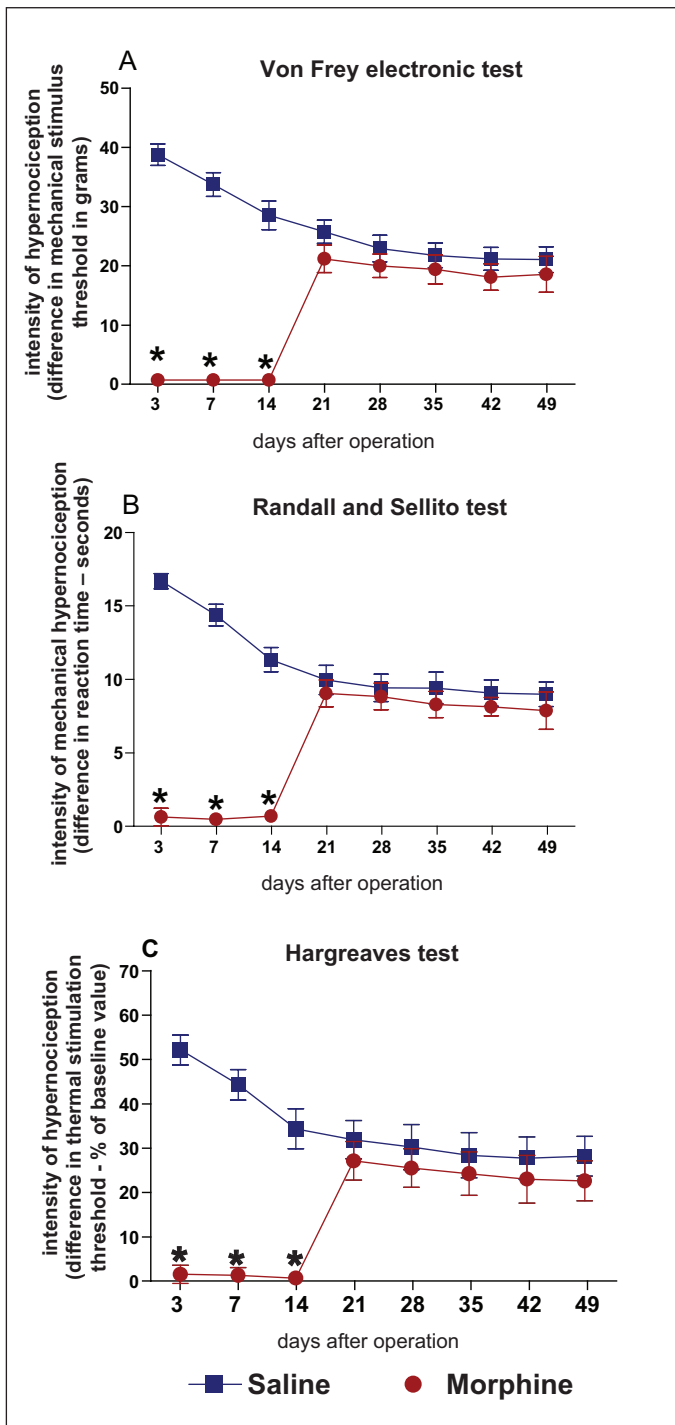


Figure 6 – Intensity of hyperalgesia in animals subjected to surgery to induce lumbar disc hernia that was treated postoperatively with morphine. After the operation, the animals were treated with morphine (4 mg/kg) for the first seven days and were followed up for 49 days, with weekly measurements. The intensity of hyperalgesia was evaluated through three different behavioral tests: increasing pressure test on the rat paws (von Frey electronic test) (A); constant pressure test on rat paws (Randall and Sellito, modified) (B); and Hargreaves thermal test (C). The measurements represent the mean \pm SEM of six animals per group. * $p < 0.05$, compared with the control group that was treated with saline solution (MANOVA, followed by Student's *t* test with Bonferroni inequality).

Administration of morphine produced the largest reduction in the intensity of hyperalgesia in the three tests performed. When its administration was discontinued, the values in the group treated with this drug did not present any difference in relation to the control group. Contrary to what was seen with the administration of the anti-inflammatory drugs, the reduction in hyperalgesia caused by morphine was not maintained after its withdrawal.

DISCUSSION

The inflammatory process induced by contact between NP and nerve structures has been widely reported⁽²⁾. Sensitization of the receptors is the common denominator in the inflammatory process involved in the mechanism for inflammatory pain. Functional regulation of the pain receptors leads to a state of hyperalgesia or allodynia, and there are two groups of mediators involved in this process, acting directly to sensitize the nociceptors: eicosanoids and sympathomimetic amines⁽⁶⁾. The capacity of these mediators to sensitize pain receptors has been demonstrated in animals and humans⁽⁶⁾.

Inflammatory mediators come from three basic sources: blood, resident cells and the nociceptor itself. These substances can produce direct activation (bradykinin, serotonin and histamine at high doses), indirect sensitization (bradykinin, histamine, substance P, TNF- α , leukotrienes and cytokines) or direct sensitization (prostaglandins, serotonin and noradrenalin) of nociceptors⁽¹¹⁻¹⁵⁾.

In an experimental model developed for this purpose, we observed the effects from contact between NP and the DRG, which defined the model that was used in the present study⁽⁷⁾. This study was designed taking into consideration the induction of hyperalgesia that occurs through the inflammatory process that results from contact between the elements of the NP and DRG.

This study was conducted with the aim of furnishing knowledge about the hyperalgesia mechanism induced by NP, using drugs with known sites and mechanisms of action.

The treatment with indomethacin (a nonsteroidal anti-inflammatory drug) had the capacity to reduce the intensity of hyperalgesia by around 50% in relation to the control group, in the initial evaluation made on the third day. Atenolol (an inhibitor of sympathomimetic amines) showed the smallest reduction in the intensity of the acute pain. The combination between indomethacin and

atenolol showed similarities with the reduction induced by indomethacin, thus suggesting that at this stage of the process, the participation of prostaglandins should be greater than that of sympathomimetic amines, regarding the production of hyperalgesia. After the chronic condition of hyperalgesia became established (14 days) the results from administration of atenolol became similar to those from administration of indomethacin, thus suggesting that participation of sympathomimetic amines may be more important at this stage.

The neuron hypersensitivity produced during the inflammatory process had two distinct components: one that was dependent on activation of the enzymes cyclooxygenase (COX) 1 and 2, thus leading to production of eicosanoids (of which the most important would be prostaglandin); and another that was dependent on release of sympathomimetic amines such as noradrenalin^(13,16). Nonsteroidal anti-inflammatory drugs (NSAIDs) may present some limitations to their efficacy, considering their mechanism of action for inhibiting COXs^(17,18). The results suggest that in the acute phase, the prostaglandin component is of greater importance in inducing hyperalgesia. However, the results obtained through administration of the inhibitor of beta-adrenergic receptors (atenolol) indicated that at some time, this component might also participate in a more pronounced manner than seen with the prostaglandins, especially if the possibility of development of the characteristics of neuropathic pain is taken into consideration, since sympathomimetic amines may be involved in this transition. Experimental studies have suggested that this effect may be present, even if the inflammatory component is still active^(19,20). A change in the inflammatory state to a neuropathic state may occur, with the eicosanoids produced through activation of COXs as the protagonists, involving action by the sympathomimetic amines that are produced by the second arm of the cytokine cascade, thereby explaining the observation that atenolol produced effects similar to those of indomethacin after the condition became chronic, i.e. after 14 days.

Dexamethasone produced the largest reduction in hyperalgesia in the acute phase, similar to what has been observed with its clinical use. Although the mechanisms of action of corticosteroids are extremely diversified, with studies showing new forms of action for inducing analgesia and reducing the inflammatory process⁽²¹⁾, the inhibition of synthesis of prostaglandins and sympathomimetic amines may have been responsible for

the intense effect that dexamethasone had with regard to reduction of hyperalgesia. Another additional type of action may have been its effect on TNF- α , which had been shown to participate in inducing hyperalgesia in disc hernias⁽²²⁾.

Administration of morphine considerably reduced the mechanical and thermal hyperalgesia, but after its administration had been discontinued, the hyperalgesia recurred and its intensity reached levels similar those presented by the group that was treated with saline solution. This temporary effect of the opioid, which was observed only for as long as it was administered may be explained by the recent observations that only the neuron fibers relating to pain and nociception (type C fibers) presented receptors for morphine⁽⁶⁾. The real and exclusive participation of type C nociceptor fibers deserves future study, because if type C fibers are really the only ones that have receptors for morphine, at least during the acute phase, it may be that only this type of nerve fiber is responsible for the mechanical and thermal sensitivity. However, it has been suggested that changes occur in type A delta fibers and that, through the injury process, these fibers would start to innervate layers of the spinal cord in which the neurons conduct stimuli of a variety of types, such as touch^(23,24). These neurons are known as WDR (wide dynamic range); they would be activated by sensitized type A delta fibers and would contribute towards observation of allodynia. If these fibers were not affected by morphine but were participants in neuropathic processes, it could be that continuation of treatment with morphine would not have such a pronounced effect at later times during the treatment, probably because a neuropathic process would become established after the end of the acute phase.

Administration of different classes of anti-inflammatory drugs and an analgesic reduced the intensity of the hyperalgesia induced by contact between NP and L5 DRG. The action of the drugs occurred through their action on the cascade of the inflammatory process triggered by NP, and the different responses demonstrated the intensity of the participation of eicosanoids and sympathomimetic amines in this process.

CONCLUSIONS

Administration of different types of anti-inflammatory drugs reduced the intensity of the experimental hyperalgesia induced through contact between NP and L5 DRG, and dexamethasone presented the largest effect.

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