

Effects of Anesthesia and Nociceptive Stimulation in an Experimental Model of Brachial Plexus Avulsion

Š. VACULÍN, R. ROKYTA

Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University, Prague, Czech Republic

Received January 6, 2003

Accepted April 25, 2003

Summary

Unilateral dorsal rhizotomy of brachial plexus nerves (C5-Th1) performed under general anesthesia is known to induce self-mutilation in rats. The aim of this study was to determine the effect of different anesthetic agents, and of pre-rhizotomy nociceptive stimulation on the appearance of self-mutilation. Self-mutilation appeared in 78 % of animals after rhizotomy had been performed under pentobarbital anesthesia. When ketamine was used as the general anesthetic, self-mutilation was almost suppressed (13 %) and consisted of superficial erosions. Mechanical nociceptive stimulation, when applied just before the induction of ketamine anesthesia and subsequent rhizotomy, provoked self-mutilation in 91 % of rats. Furthermore, a serious type of self-mutilation consisting of total amputation of the distal part of the forepaw was present in 28 % of all self-mutilating animals after previous nociceptive stimulation. In terms of self-mutilation, these results suggest 1) the crucial role of anesthesia, especially that which involved NMDA receptors (ketamine), and 2) the need of an additional factor to chronic deafferentation, formed either by activity of nociceptive pathways just before rhizotomy (nociceptive stimulation preceding ketamine anesthesia) or by injury discharges (pentobarbital anesthesia).

Key words

Rhizotomy • Deafferentation • Autotomy • Neuropathic Pain • Prior Nociception

Introduction

An extensive dorsal rhizotomy at the cervicothoracic level (C5-Th1) in the rat is used as a model of central pain (Basbaum 1974) which often develops after brachial plexus avulsion in man (Nashold and Ovelment-Lewitt 1991, Mailis 1996, Berman *et al.* 1998, Ali *et al.* 2002) and children (Rossitch *et al.* 1992a, Al-Qattan 1999). Physiological changes following rhizotomy in rats implicate development of a chronic pain

syndrome in the ipsilateral limb. The changes include a delayed and transient increase of mechanical nociceptive thresholds of unoperated limbs (Kayser *et al.* 1990), changes in opioid and neurokinin-1 receptor distribution in the spinal cord (Goff *et al.* 1998, Hohmann *et al.* 1999), changes of patterns of neuronal activity in the spinal cord, lateral and medial thalamus and the cerebral cortex (Albe-Fessard and Rampin 1991, Vaculín *et al.* 2000) as well as the onset of self-mutilation (Basbaum 1974, Albe-Fessard *et al.* 1979).

The dorsal rhizotomy model differs from other chronic pain models due to the localization of the nerve lesion, which is proximal to the neuronal body. It prevents any further afferent discharges that occur in other models – sciatic nerve transection/ligation (Wall *et al.* 1979, Bennett and Xie 1988, Kim and Chung 1992, Lee *et al.* 2000) – in which lesions are localized in the distal part of the axon (related to the neuronal body). In these models the injured part of the nerve is believed to produce abnormal afferent activity which might be responsible for dorsal horn hypersensitivity and hyperexcitability. On the other hand, in the rhizotomy model the connection between the first (spinal ganglion) and the second (dorsal horn) neurons is totally interrupted and the corresponding spinal cord neurons are without any peripheral inputs. Consequently, their abnormal firing and subsequent pain are expected to be of central origin.

The onset of self-mutilation in rats is of vital interest in the present study. The self-mutilating behavior consists of scratching, chewing and biting the extremity and is considered to be a correlate to chronic pain (Albe-Fessard *et al.* 1979, Mailis 1996, Kaupila 1998). The development of these symptoms is not regular and is modulated by various factors: the number of roots sectioned and prior chemical pain (Basbaum 1974, Dennis and Melzack 1979), administration of “analgesic” antibiotics (Albe-Fessard and Suaudeau 1992) or antiepileptics (Duckrow and Taub 1977), and by the sex and type of lesion (Rossitch *et al.* 1992b). Influences of seasonal effects (Albe-Fessard *et al.* 1987) and genetical basis (Devor and Raber 1990, Shir *et al.* 2001) for conversion of nerve injury into a chronic pain condition have been demonstrated. The suppression of self-mutilation by postoperative administration of NMDA receptor antagonist MK-801 has been published (Tseng 1998, Wong *et al.* 1998).

The effect of pre-deafferentation pain is known from both clinical and experimental studies. The phantom limb pain is more likely to develop in patients who had suffered pain in the limb before the amputation (Appenzeller and Bicknell 1969). The level and type of pain before the amputation correlate positively with phantom limb pain in man (Jeanmonod *et al.* 1993). Dennis and Melzack (1979) showed the influence of prior chemically induced pain on the onset of chewing behavior in rats with sectioned dorsal roots.

The aim of this study was to compare the effect of different anesthesia during the operation and to evaluate the influence of acute mechanical nociception closely preceding the rhizotomy on the incidence of self-mutilation in rats.

Methods

Wistar male rats were used in this study. The rats were randomly divided into three groups: the first and the second group (Groups I and II) received ketamine anesthesia (intramuscular injection of ketamine 100 mg/kg), the third group (Group III) received pentobarbital anesthesia (intraperitoneal injection of pentobarbital 40 mg/kg). Xylazine (16 mg/kg i.m.) were added in all groups. When necessary the anesthesia was supported by the injection of one quarter of the first dose in all groups. No other anesthetics or analgesics were used.

The clamping of a forelimb induced pre-rhizotomy nociceptive stimulation in Group I. Metatarsus of the forelimb was clamped for 1-3 min using a crocodile clip (toothed jaws, force 4.8 N) just before the induction of anesthesia. In Groups II and III, the crocodile clip was used in the same way, but after the induction of anesthesia to cause similar skin micro-traumatization without any unpleasant sensation.

After this procedure, dorsal rhizotomy was performed as follows. Under general anesthesia, incisions of skin and muscular layers were performed between base of the skull and the second thoracic vertebra. Vertebral arcs were opened, Th2 vertebra was identified by its prominent process, and hemilaminectomy was performed between C4 and Th1. The dura mater was opened and dorsal roots were identified by their position with respect to the vertebra. Evulsion of dorsal roots C5-Th1 was carefully performed to avoid bleeding. The distribution of blood vessels, however, caused occasional bleeding during the rhizotomy. The animals in which the spinal cord had been damaged were excluded from the study. Finally, the muscles and the skin were sutured in layers. After the surgery, animals were caged separately with free access to water and food and were observed daily for a maximum of up to 80 days. After the onset of self-mutilation and depending on its gravity, the rats were either excluded from the group within 2-7 days or underwent subsequent experimentation.

Two statistical tests were used. The 2 x 2 Tables Fischer's Exact Test was used to evaluate levels of statistical significance of the number of self-mutilating animals and to establish the distribution of self-mutilation types between individual groups on a daily basis. The Sign Test was used for the evaluation of significance of the number of self-mutilating animals and of the distribution of self-mutilation types in each group.

The experiments were approved by the Committee for Animal Care and Use of the Third Faculty of Medicine, Charles University, Prague, and conducted in compliance with the guidelines of the Ethics Committee of the International Association for the Study of Pain (Zimmermann 1983).

Results

Following the surveillance of all rats from different groups freely moving in their cages only occasional evidence of changes in behavior due to deafferentation could be seen. Programmed behavior (grooming) was identical with that of intact animals. During locomotion only occasional misplacement of the deafferentated limb was noticeable. Sitting on their hind legs, the rats kept the deafferentated forelimb in extension, but when the forelimb was to be used again, almost no visible differences could be noticed.

To evaluate the sensitivity of the deafferentated forelimb, light pinching with surgical forceps was applied to both forelimbs. Touching the intact extremity became impossible while pinching of the deafferentated paw did not evoke any response.

Only a qualitative pattern of self-mutilation was used to evaluate the abnormal behavior in rats. Two distinct types of self-mutilation were observed. Type A consisted of superficial wounds mostly on the dorsal surface of the forearm. The majority of scratching appeared in a partially deafferentated area corresponding to the distribution and to the overlapping of neighboring receptive fields in rats. This type of self-mutilation was noted in all groups.

The second type of peripheral lesions was characterized by total self-amputation of the distal portion of the deafferentated forelimb (Type B). This type of lesion was observed only in Group III and I, i.e. when the animals were operated under pentobarbital or under ketamine to which previous nociceptive stimulation had

been applied. The rate at which the distal parts of the forelimb were removed was very high (even within a single day) following the onset, and ceased when approximately first third of the forearm had been amputated. The distribution of the two types of lesion in the different operated groups is shown in Figure 1.

The appearance of self-mutilation in different groups was compared. The self-mutilation appeared in the majority of animals which had been operated under ketamine preceded by the nociceptive stimulation (Group I) and those operated under pentobarbital anesthesia (Group III). In this Group III, 7 out of 9 animals showed self-mutilation. However, this dependence was assessed as non-significant ($p=0.18$), probably because of the size of the sample. In Group I (animals anesthetized under ketamine preceded by nociceptive stimulation) 11 out of 12 (91.6 %) developed self-mutilation indicating a significant dependence of the self-mutilation on the clamping of the forelimb just before the rhizotomy performed under ketamine anesthesia ($p=0.006$).

On the contrary, in the animals operated under ketamine without preceding nociceptive stimulation (Group II) only two rats out of 15 (13.3 %) developed self-mutilation. The day of onset was 54 and 68 days, respectively. In both rats included in Group II the self-mutilation was classified as Type B (superficial wounds).

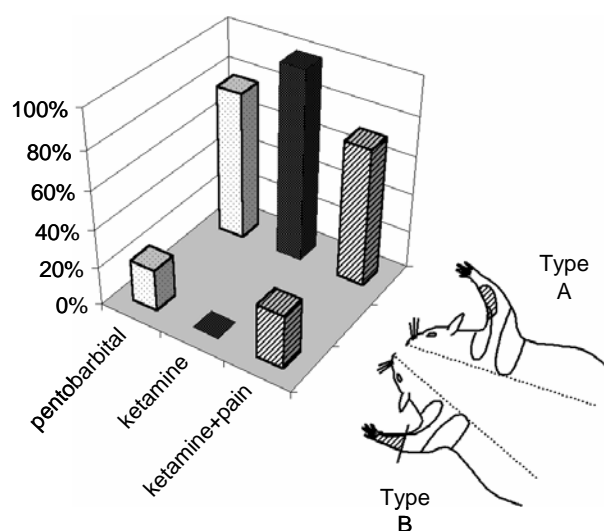


Fig. 1. The distribution of self-mutilation types among the groups. Only self-mutilating animals were included, the bars illustrate the type of the self-mutilation within the groups in percentage. Distributions were not statistically significant; note an absence of the Type B (self-amputation of distal portion of the deafferentated forelimb) in ketamine group II. Superficial wounds (Type A) were noted in all groups.

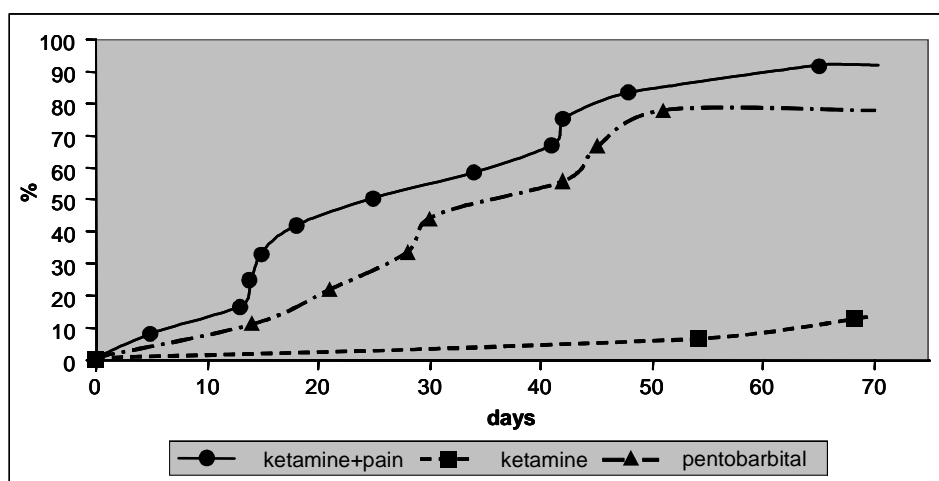


Fig. 2. The number of animals, which developed self-mutilation, during the period after deafferentation. The difference in self-mutilation onset between Group II (animal anesthetized by ketamine) and Group I (animals subjected to nociceptive stimulation prior to ketamine anesthesia) were found significant ($p < 0.05$) from day 15, between Groups II and III (animals operated under pentobarbital anesthesia) from day 28. No significant difference in the self-mutilation onset was found between Groups III and I.

Discussion

There are conflicting views about the correlation between self-mutilation and pain sensation in animal models (Rodin and Kruger 1984, Coderre *et al.* 1984). However, the evidence rather inclines to the hypothesis that self-mutilation is a behavioral correlate of pain sensation (Kauppila 1998). Two types of self-mutilation have been described in papers of Albe-Fessard *et al.* (1979) and corroborated in the course of our observations as well as a tendency to extension of the deafferented limb (Vejsada and Hník 1983). It is evident that rats exhibiting amputation suffered from unpleasant sensations more frequently. On the other hand, it is still not clear whether this sensation is actually painful or not.

Many authors are in favor of the notion that NMDA receptors are involved in the development of neuropathic pain (Dougherty and Willis 1991, Mayer *et al.* 1999, Petersen-Zeitz and Basbaum 1999). In a brachial avulsion model, the effect of anesthesia employed has not yet been described, however, non-competitive NMDA antagonist (MK 801) was found to suppress autotomy when administered immediately after rhizotomy (Tseng 1998) or continually for 7 days (Wong *et al.* 1998). Since ketamine is a non-competitive antagonist of NMDA receptor and pentobarbital spares NMDA receptor intact, our results support these findings, while accenting the importance of the sensation felt at the moment of deafferentation. Blumenkopf and Lipman (1991) have shown that chronic local anesthesia of the sciatic nerve does not cause self-mutilation. This fact corresponds well with our results. It seems to be evident

that other factor(s) are required for the development of neuropathic pain additionally to chronic deafferentation.

Rygh *et al.* (1999) have demonstrated that acute severe noxious stimulation causes a long-term increase of the excitability of wide-dynamic-range neurons of spinal cord and have suggested that it may be important for the development of chronic pain disorders. Seltzer *et al.* (1991) showed the importance of injury discharges in a sciatic nerve transection model. When a nerve is being severed, damaged sensory fibers emit a barrage of impulses that last for many seconds, or even minutes. We suppose that rhizotomy alone might cause depolarization of the distal portion of the proximal axon (related to the neuronal body) and thus evoke extensive neuromediator release on terminal synapses in the dorsal horn. In such a case, the dorsal horn neuron sensitization might occur *via* NMDA receptors. When NMDA receptor antagonist is used for anesthesia, sensitization and subsequent behavioral correlates do not develop. On the other hand, application of pre-anesthesia pain might mimic the effect of injury discharges (extensive neuromediator release) and thus trigger an intracellular cascade resulting in dorsal horn cell hyperexcitability and sensitization.

Acknowledgements

This study was supported by Research Goal VZJ13/98:11120005 and Centre for Neuropsychiatric Studies, LN 00B122. These results were presented in preliminary form at the Meetings of the Czech and Slovak Physiological Societies in Hradec Králové, 2000 (Vaculín and Rokyta 2000) and Plzeň, 2003 (Vaculín *et al.* 2003).

References

- ALBE-FESSARD D, RAMPIN O: Neurophysiological studies in rats deafferented by dorsal root sections. In: *Deafferentation Pain Syndromes: Pathophysiology and Treatment*. BS NASHOLD Jr, J OVELMEN-LEWITT (eds), Raven Press, New York, 1991, pp 125-139.
- ALBE-FESSARD D, SUAUDEAU C: Action of antibiotics in two animal models of pain. In: *Advances in Pain Research and Therapy*. F SICUTERI, L TERENIUS, L VECCHIET, M MAGGI, M NICOLODI, M ALESSANDI (eds), Raven Press, New York 1992, pp 55-62.
- ALBE-FESSARD D, NASHOLD BS Jr, LOMBARD MC, YAMAGUCHI Y, BOUREAU F: Rat after dorsal rhizotomy, a possible model for chronic pain. In: *Advances in Pain Research and Therapy*. JJ BONICA (ed), Raven Press, New York, 1979, 761-766.
- ALBE-FESSARD D, RAMPIN O, SANDERSON P: Comparison of forelimb deafferentation syndrome in man and animals. In *Trends in Cluster Headache*. F SICUTERI, L VECCHIET, M FANCIULLACCI (eds), Elsevier, Amsterdam, 1987, pp 29-40.
- ALI Z, MEYER RA, BELZBERG AJ: Neuropathic pain after C7 spinal nerve transection in man. *Pain* **96**: 41-47, 2002.
- AL-QATTAN MM: Self-mutilation in children with obstetric brachial plexus palsy. *J Hand Surg* **24**: 5477-5499, 1999.
- APPENZELLER O, BICKNELL JM: Effects of nervous system lesions on phantom experience in amputees. *Neurology* **19**: 141-146, 1969.
- BASBAUM AI: Effect of central lesions on disorders produced by multiple rhizotomy in rats. *Exp Neurol* **42**: 490-501, 1974.
- BENNETT GJ, XIE YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**: 87-107, 1988.
- BERMAN JS, BIRCH R, ANAND P: Pain following human brachial plexus injury with spinal cord root avulsion and the effect of surgery. *Pain* **75**: 199-207, 1998.
- BLUMENKOPF B, LIPMAN JJ: Studies in autotomy: its pathophysiology and usefulness as a model of chronic pain. *Pain* **45**: 203-209, 1991.
- CODERRE TJ, GRIMES RW, MELZACK R: Deafferentation in animals as a model for the study of pain: an alternative hypothesis. *Brain Res* **319**: 213-228, 1984.
- DENNIS GD, MELZACK R: Self-mutilation after dorsal rhizotomy in rats: effects of prior pain and pattern of root lesions. *Exp Neurol* **65**: 412-421, 1979.
- DEVOR M, RABER P: Heritability of symptoms in an experimental model of neuropathic pain. *Pain* **42**: 51-67, 1990.
- DOUGHERTY PM, WILLIS WD: Enhancement of spinothalamic neuron responses to chemical and mechanical stimuli following combined microiontophoretic application of N-methyl-D-aspartic acid and substance P. *Pain* **47**: 85-93, 1991.
- DUCKROW RB, TAUB A: The effect of diphenylhydantoin on self-mutilation in rats produced by unilateral multiple dorsal rhizotomy. *Exp Neurol* **54**: 33-41, 1977.
- GOFF JR, BURKEY AR, GOFF DJ, JASMIN L: Reorganization of the spinal dorsal horn in models of chronic pain: correlation with behaviour. *Neuroscience* **82**: 559-574, 1998.
- HOHMANN AG, BRILEY EM, HERKENHAM M: Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. *Brain Res* **822**: 17-25, 1999.
- JEANMONOD D, MAGNIN M, MOREL A: Thalamus and neurogenic pain, physiological, anatomical and clinical data. *Neuroreport* **4**: 475-478, 1993.
- KAUPPILA T: Correlation between autotomy-behavior and current theories of neuropathic pain. *Neurosci Biobehav Rev* **23**: 111-129, 1998.
- KAYSER V, BASBAUM AI, GUILBAUD G: Deafferentation in the rat increases mechanical nociceptive threshold in the innervated limbs. *Brain Res* **508**: 329-332, 1990.
- KIM SH, CHUNG JM: An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in rat. *Pain* **50**: 355-363, 1992.

- LEE BH, WON R, BAIK EJ, LEE SH, MOON CH: An animal model of neuropathic pain employing injury to the sciatic nerve branches. *Neuroreport* **11**: 657-661, 2000.
- MAILIS A: Compulsive targeted self-injurious behaviour in humans with neuropathic pain: a counterpart of animal autotomy? Four case reports and literature review. *Pain* **64**: 569-578, 1996.
- MAYER DJ, MAO J, HOLT J, PRICE DD: Cellular mechanisms of neuropathic pain, morphine tolerance, and their interactions. *Proc Natl Acad Sci USA* **96**: 7731-7736, 1999.
- NASHOLD BS Jr, OVELMEN-LEWITT J: *Deafferentation Pain Syndromes: Pathophysiology and Treatment*. Raven Press, New York, 1991.
- PETERSEN-ZEITZ KR, BASBAUM AI: Second messengers, the substantia gelatinosa and injury-induced persistent pain. *Pain Suppl* **6**: S5-S12, 1999.
- RODIN BE, KRUGER L: Deafferentation in animals as a model for the study of pain: an alternative hypothesis. *Brain Res* **319**: 213-228, 1984.
- ROSSITCH E Jr, OAKES WJ, OVELMEN-LEVITT J, NASHOLD BS Jr: Self-mutilation following brachial plexus injury sustained at birth. *Pain* **50**: 209-211, 1992a.
- ROSSITCH E Jr, LYERLY MA, OVELMEN-LEVITT J, YOUNG JN, NASHOLD BS Jr: Deafferentation syndrome in the rat: effects of sex, age, and lesion type. *Surg Neurol* **37**: 89-93, 1992b.
- RYGH LJ, SVENDSEN F, HOLE K, TJØLSEN A: Natural noxious stimulation can induce long-term increase of spinal nociceptive responses. *Pain* **82**: 305-310, 1999.
- SELTZER Z, COHN S, GINZBURG R, BEILIN B: Modulation of neuropathic pain behavior in rats by spinal disinhibition and NMDA receptor blockade of injury discharge. *Pain* **45**: 69-75, 1991.
- SHIR Y, ZELTSER R, VATINE JJ, CARMİ G, BELFER I, ZANGEN A, OVERSTREET D, RABER P, SELTZER Z: Correlation of intact sensibility and neuropathic pain-related behaviors in eight inbred and outbred rat strains and selection lines. *Pain* **90**: 75-82, 2001.
- TSENG S-H: Suppression of autotomy by N-methyl-D-aspartate receptor antagonist (MK-801) in the rat. *Neurosci Lett* **240**: 17-20, 1998.
- VACULÍN Š, ROKYTA R: Actual knowledge about experimental model of brachial plexus avulsion. *Physiol Res* **49**: P25, 2000.
- VACULÍN Š, FRANĚK M, ROKYTA R: Dorsal rhizotomy changes the spontaneous neuronal activity of nuclei in the medial thalamus. *Physiol Res* **49**: 279-283, 2000.
- VACULÍN Š, FRANĚK M, ROKYTA R: Brachial plexus injury in young rats. *Physiol Res* **52**: 44P, 2003.
- VEJSADA R, HNÍK P: Motor impairment after unilateral hind limb deafferentation in young rats. *Physiol Bohemoslov* **32**: 289-294, 1983.
- WALL PD, DEVOR M, INBAL R, SCADDING JW, SCHONFELD D, SELTZER Z, TOMKIEWICZ MM: Autotomy following peripheral nerve lesions: experimental anaesthesia dolorosa. *Pain* **7**: 103-111, 1979.
- WONG CS, CHERN CH, TUNG CS: Intrathecal administration of excitatory amino acid receptor antagonists or nitric oxide synthase inhibitor reduced autotomy behavior in rats. *Anesth Analg* **87**: 605-608, 1998.
- ZIMMERMANN M: Ethical guidelines for investigation of experimental pain in conscious animals. *Pain* **16**: 109-110, 1983.

Reprint requests

Š. Vaculín, Department of Normal, Pathological and Clinical Physiology, Third Faculty of Medicine, Charles University, Ke Karlovu 4, 120 00 Prague 2, Czech Republic. E-mail: svaculin@lf3.cuni.cz