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ORIGINAL ARTICLE

Attenuation of neuropathic pain by sodium butyrate in an experimental model of chronic constriction injury in rats

Ankesh Kukkar, Nirmal Singh, Amteshwar Singh Jaggi*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, India

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Background/Purpose: The present study was designed to investigate the potential of sodium butyrate, a histone deacetylase (HDAC) inhibitor, in chronic constriction injury (CCI)-induced neuropathic pain in rats.
Methods: Neuropathic pain was induced by placing four loose ligatures around the sciatic nerve. Acetone drop, Von frey hair, pin prick and hot plate tests were performed to assess cold allodynia, mechanical allodynia, and mechanical and heat hyperalgesia, respectively. The level of tumor necrosis factor (TNF)-α was measured in the sciatic nerve as an inflammatory marker.
Results: CCI was associated with the development of cold allodynia, mechanical allodynia, and mechanical and heat hyperalgesia, along with an increase in TNF-α level. Administration of sodium butyrate (200 and 400 mg/kg, oral) for 14 days in CCI-subjected rats significantly attenuated behavior related to injury-induced pain and the increase in TNF-α level.
Conclusion: It may be concluded that the anti-inflammatory actions mediated by sodium butyrate are responsible for its beneficial effects in neuropathic pain in rats.

Introduction

Neuropathic pain has been described as the "most terrible of all tortures which a nerve wound may inflict" and arises as a consequence of nerve injury either of the peripheral or central nervous system. Following peripheral nerve injury, a cascade of events in the primary afferents leads to peripheral sensitization, resulting in spontaneous nociceptor activity, decreased threshold, and increased response to suprathreshold stimuli.[1] Despite the recent advances in identification of peripheral and central sensitization mechanisms related to nervous system injury, the effective treatment of patients suffering from neuropathic pain remains a clinical challenge. Although numerous treatment

* Corresponding author. Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147002, Punjab, India.
E-mail address: amteshwarjaggi@yahoo.co.in (A.S. Jaggi).

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options are available for relieving neuropathic pain, there is no consensus on the most appropriate treatment.[2] Consequently, there is still a considerable need to explore novel treatment modalities for neuropathic pain management.

HDACs (also called lysine deacetylases) are a class of enzymes that regulate the gene expression by removing the acetyl group from ε-N-acetyl lysine amino acid present on histone proteins.[3,4] Several studies have suggested the upregulation of HDAC in different inflammatory diseases, including polycythemia vera, essential thrombocythemia, rheumatoid arthritis, neuroblastoma, and pancreatic cancer.[5–7] Accordingly, HDAC inhibitors are proposed to have significant therapeutic potential as anti-inflammatory and immunosuppressive drugs.[8] Preclinical studies have shown that HDAC inhibitors produce beneficial effects in various pathological conditions such as rheumatoid arthritis,[9] Rubinstein—Taybi syndrome, Rett syndrome, Friedreich's ataxia, Huntington's disease and multiple sclerosis,[10] and acute central nervous system injury including ischemic and hemorrhagic stroke.[11]

It has been suggested that increased expression of HDAC within the superficial dorsal horn is key to maintenance of persistent pain.[12] The pharmacological inhibition of class IIa HDAC in the spinal cord has been shown to attenuate inflammatory complete-Freund's-adjuvant-induced thermal hyperalgesia in rats.[13] Sodium valproate has been shown to produce beneficial effects in different neuropathic pain conditions, including those due to chemotherapeutic agents, and its pain-attenuating effects have been at least partly attributed to HDAC inhibition.[14] Sodium butyrate is a noncompetitive inhibitor of HDAC[15] and it selectively inhibits subtypes I and IIa.[16] The present study was designed to investigate the neuropathic-pain-attenuating potential of sodium butyrate in chronic constriction injury (CCI)-induced neuropathic pain in rats.

Materials and methods

Experimental animals

Sprague—Drawly rats of either sex weighing 200—250 g (procured from Punjab University, Chandigarh, India) were used in the present study. They were housed in the animal house with free access to water and a standard laboratory chow diet (Kisan Feeds, Mumbai, India). The rats were exposed to normal cycle of 12 hours light and 12 hours dark. The experimental protocol was duly approved by the Institutional Animals Ethics Committee (IAEC) and care of animals was carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. — 107/1999/CPCSEA).

Drugs and reagents

Sodium butyrate was obtained from Sigma—Aldrich (St Louis, MO, USA). Bovine serum albumin and Folin—Cicalteu phenol reagent were obtained from S.D. Fine (Mumbai, India). All the chemicals used in the present study were of analytical grade. The tumor necrosis factor (TNF)-α assay kit was procured from RayBiotech (Norcross, GA, USA).

Induction of neuropathy by CCI

Peripheral neuropathy was induced by CCI as described by Bennett and Xie with slight modification,[17] using silk 4-0 sutures instead of chromic gut sutures, because it has been documented that the latter initiate inflammatory reactions in the sciatic nerve.[18] The rats were deeply anesthetized with chloral hydrate (350 mg/kg, intraperitoneal). The hair on the lower back and thighs of the rats was shaved, and the skin was sterilized with 0.5% chlorhexidine. The skin of the lateral surface of the left thigh was incised and a cut made directly through the biceps femoris muscle to expose the sciatic nerve. Once exposed, the sciatic nerve was ligated with silk 4-0 thread at four sites with a 1-mm gap. The ligatures were loosely tied until a short flick of the ipsilateral hind limb was observed. The muscle and skin were closed in two layers with the use of thread, and topical antibiotics were applied. All surgical procedures were carried out under normal sterile conditions. As a result of the distinct development of postural defects in the paws of CCI control animals, the behavioral studies were not blinded for comparison between the normal controls, sham controls, and CCI control groups. However, for all other groups the behavioral tests were blinded.

Behavioral examination

Paw cold allodynia (acetone drop test)

Cold allodynia was assessed by spraying 100 μL of acetone onto the surface of the rat paw (placed over a wire mesh), without touching the skin. The response of the rat to acetone was noted for 20 seconds and was graded on a 4-point scale as defined by Flatters and Bennett[19] (0, no response; 1, quick withdrawal, flick or stamp of the paw; 2, prolonged withdrawal or repeated flicking; and 3, repeated flicking of the paw with licking of the paw). Acetone was applied three times to the hind paw, with a gap of 5 minutes between the acetone applications, and the individual scores noted at 20-second intervals were added to obtain a single score over a cumulative period of 1 minute. The minimum score was 0 and the maximum possible score was 9.

Mechanical hyperalgesia (pin prick test)

Mechanical hyperalgesia was assessed by the pinprick test, as described by Erichsen and Blackburn-Munro.[20] The surface of the injured hind paw was touched with the point of a bent gauge needle (at 90° to the syringe) at intensity sufficient to produce a reflex withdrawal response. The paw withdrawal duration was recorded in seconds and the normal quick reflex withdrawal response was given the value of 0.5 seconds.

Paw heat hyperalgesia (hot plate test)

The thermal nociceptive threshold, as an index of thermal hyperalgesia, was assessed by the Eddy's hot plate, maintained at a temperature of 52.5 ± 1.0°C. The rat was placed on the hot plate and withdrawal latency, with respect to licking of the hind paw, was recorded in seconds. The cut-off time of 15 seconds was maintained.[21]