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Reduced Formalin Nociceptive Responses in a Rat Model of Post - Surgical Pain

[1]A.S. Oyadeyi, [1]A.O. Afolabi, [1]F.O. Ajao and [2]G.F. Ibironke

[1]Department of Physiology, Ladoke Akintola University of Technology,
P.M.B. 4000, Ogbomoso, Oyo State, Nigeria
[2] Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Abstract: The responses to inflammatory pain were assessed in a rat model of post surgical pain using the formalin test. There were significantly reduced nociceptive responses in the second phase of the formalin test in both ipsilateral and contralateral limbs. The results either reflect reduced activity in mechanically insensitive afferents, Aδ and C fibers that mediate the second phase response, or a diffuse noxious inhibitory controls mechanism (DNIC).

Key words: Pain · surgery · incision · formalin · DNIC

INTRODUCTION

One out of every two patients suffers intense or very intense pain during the first few days post surgery [1], clinical and preclinical models of post-surgical pain are therefore becoming increasingly important for investigating the patho physiological mechanisms of this condition [2-5]. Recently, a new animal model of postoperative pain has been introduced [6], where the quantifiable mechanical allodynia and non-evoked pain behaviour produced parallel the postoperative course of patients.

It is now known that there are two types of postoperative pain. Pain during rest (Rest pain) and pain during function (Incident pain) [7]. Four recent studies have investigated the action of cyclooxygenase (COX) inhibitors on post surgical pain, three of these [3, 8] demonstrated that post-surgical intrathecal administration of indomethacin or a selective COX- 2 inhibitor (JTE 522) 5 minutes following plantar incision can reduce tactile allodynia.

From the fore-going, it appears there is considerable information about the physiological and pharmacological processes that underlie post surgical pain while there is a relative paucity of experiments that have examined if enhanced responses during chemogenic or inflammatory conditions can also develop post surgically. Indeed, LaBuda and co [9] had earlier hypothesized the possibility of inflammatory conditions been a significant clinical problem in conditions of nerve damage and given the similarity between nerve damage and surgery, this possibility also exists post surgically. One common experimental condition to explore inflammatory pain responses is the formalin test [10]. The test involves a subcutaneous injection of dilute formaldehyde into the plantar surface of the hind paw that causes a characteristic pattern of behavioural responses such as elevation and licking of the injected paw. There is an initial period of responding during the first 5-10 min of the test that is followed by a 5-10 min period of diminished responses that gradually return and continue for an additional 20-40 min. The second period of responding is thought to reflect an inflammatory process [11], signaled by peripheral mechanically insensitive afferents (MIAs) and C - fibres [12].

Combining the methods of paw incision to model post surgical pain and the formalin test to model inflammatory pain, the purpose of the present experiment was to determine if an increase in inflammatory responses developed following the onset of a post surgical pain condition.

MATERIALS AND METHODS

Animals: Adult (150-200 g) male Sprague - Dawley rats were used. They were housed in the faculty animal house with free access to food and water.

Surgery: Surgery was performed as previously described [6], with minor modifications. Briefly, rats were anaesthetized with sodium pentobarbital (40 mg kg^{-1} i.p

Corresponding Author: Mr. A.S. Oyadeyi, Department of Physiology, Ladoke Akintola University of Technology, P.M.B. 4000, Ogbomoso, Oyo State, Nigeria

supplemented a s necessary) and the plantar surface of the left hind limb was prepared in a sterile manner. A 1 cm longitudinal incision was made through the skin and fascia, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The plantaris muscle was elevated and incised longitudinally. Following haemostasis with gentle pressure, the skin was apposed with two single interrupted sutures while the wound was cleaned with iodine solution.

Formalin test: Two days after surgery, rats received 0.2 ml of 1% formalin on the dorsal surface of the ipsilateral (n=11) or contralateral (n=11) or right hind paw of intact control animals (n=13). Time spent biting or licking the injected paw was recorded between 0-5 min (first phase) and 20-40 min (second phase).

Statistical analysis: Comparisons were done using the t-test while statistical significance was accepted at $p<0.05$.

RESULTS

In the experimental group of animals, there was significant decrease ($p<0.05$) in the second phase response of the ipsilateral side compared to the contralateral side. Similarly there was significantly decreased second phase responses in the ipsilateral side of experimental animals compared to the ipsilateral side of intact control animals. Although the first phase responses were also decreased when compared with the ipsilateral side of experimental and intact control animals these did not attain statistical significance (Fig. 1).

When compared to the contralateral side of intact control animals, there was significant ($p<0.05$) decrease in both phases of the formalin test in the contralateral side of experimental animals (Fig. 1).

DISCUSSION

The present study demonstrates that inflammatory pain responses during both phases of the formalin test were significantly reduced following post - incision injury compared to intact control animals. The reduced responding was evident as a decrease in paw licking and paw biting in the incised paw. This finding, together with the relatively small and non-statistically significant reduction of responding during the initial 5 min of the formalin test, indicates that hyperalgesia to an inflammatory condition does not develop post incision and this supports previous reports of reduced chemogenic pain following sciatic nerve damage [13, 14]. We considered the possibility that the reduced responding was a behavioural adaptation by the animal that made it reluctant to groom the incised limb. As previously done [14, 15], we applied a layer of coloured lipstick to the dorsal surface of selected rats on postoperative day 2; this was cleaned away within 15 h without any apparent reluctance to clean the incised paw.

The model of post surgical pain used here is a well characterized animal model [6]. The procedure results in ongoing and stimulus - evoked behaviours in addition to properties that are thought to reflect characteristics of clinical pain conditions, such as mechanical allodynia [6],

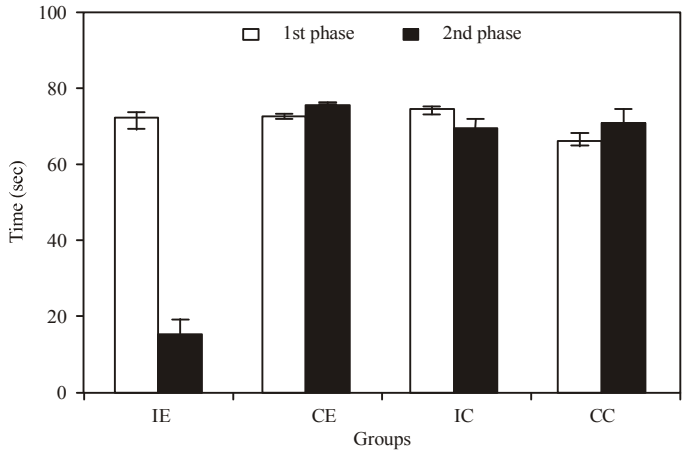

Fig. 1: Nociceptive responses in both phases of the formalin test in experimental and control animals
IE : Ipsilateral side of experimental animals, CE : Contra lateral side of experimental animals
IC : Ipsilateral side of intact control animals, CC : Contra lateral side of intact control animals

reduction of tactile allodynia by the administration of indomethacin [7] and the partial efficacy of non-steroidal anti-inflammatory drugs and gabapentin in alleviating the hyperalgesia and allodynia produced in the model [16]. This therefore lends validity to the present report.

However, we have re-examined the earlier report of Vissers and Co [13], who reported reduced nociceptive responses in a rat model of neuropathic pain, we also re-examined the data upon which our own similar subsequent report was based [14] and compared them with the previous work of LaBuda and Co [9], who reported enhanced formalin nociceptive responses in another model of neuropathic pain previous work of LaBuda and others [9]. Although we could find no other work on responses to inflammatory pain in any rat model of neuropathic or post - surgical pain, our re-examination convinced us that the reports must be interpreted with some caveats in mind. While the report of LaBuda and Co [9] is consistent with the well - established phenomenon of heightened as measured by release of inflammatory indicators, inflammatory responses, seguel to tissue or nerve injury [17], we are reluctant to accept our earlier report [14] and that of Vissers *et al*. [13] as truly reflecting attenuated inflammatory responses, in spite of tissue and nerve damage, in the rat models used. Of course this is not presuming either faulty methodology or spurious data in those studies, we only see an alternative explanation of their results.

We speculate that the reduced responses to inflammatory pain more probably reflects a diffuse noxious inhibitory controls (DNIC) mechanism. DNIC describes a situation where painful stimulation at one body site can suppress pain at more distant loci, it occurs in the presence of two concurrently applied pain stimuli [18]. The short time - lapse between surgery and pain testing in our previous report [14] and that of Vissers *et al*. [13] opened up the possibility of DNIC while the long time lapse in the work of LaBuda and Co [9] might have ruled out a DNIC mechanism.

Finally, we conclude that this report of reduced responses to inflammatory pain is consistent with earlier reports [13, 14] although the mechanisms might more accurately reflect DNIC and not necessarily reduced inflammatory responses. More research is needed to shed more light on the possibilities.

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