

GABA B MEDIATED ANALGESIA IN TONIC PAIN IN MONKEYS

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(Received on January 20, 1993)

Abstract : The present study was designed to characterise the analgesia produced by the GABA B agonist baclofen in tonic pain in monkeys. The effect of various doses of baclofen was studied on formalin induced pain. Baclofen was injected intraperitoneally 30 min prior to formalin injection in five doses of 2, 4, 6, 8, and 10 mg/kg and the pain was quantified for a period of one hour. Baclofen produced dose related analgesia, 6 mg/kg having maximal effect. The antinociceptive effect of baclofen could be attributed to the effect of baclofen on GABA B receptors producing presynaptic inhibition of primary nociceptive afferents in the dorsal horn of the spinal cord.

Key words : GABA baclofen formalin pain analgesia

INTRODUCTION

A number of studies have focussed on the involvement of peptidergic and aminergic neuronal circuits in the transmission and control of nociceptive messages. The role of GABA, a major inhibitory neurotransmitter in the mammalian central nervous system in analgesic mechanisms has recently emerged as an area of considerable interest (1). The analgesic action of GABA has been shown to be insensitive to opioids and this opens up the possibility of developing novel non-opioid analgesics. Baclofen, a GABA B agonist whose major clinical use is as an antispastic agent (2) reduces pain associated with spasticity (3) and is useful in the treatment of trigeminal neuralgia (4). The dose required to produce analgesia is quite different from that for improving motor performance (1). The systemic administration of baclofen produces analgesia in tail flick, hot plate, writhing, arthritis pain and shock titration tests (5, 6, 7, 8, 9, 10).

Presynaptic control of certain classes of primary afferent fibres is mediated by spinal GABAergic neurons. GABA and GABA receptors are concentrated in the superficial layers of the dorsal horn (11, 12). Stimulation of nociceptive primary afferents evoked a GABA mediated inhibition of nociceptive response of spinal neurons in cat (13).

Subcutaneous injection of dilute formalin induces

a reproducible pattern of pain-related behaviour as well as prolonged activation of C-fibers innervating the injection site as well as nociceptive neurons at the spinal cord level (14, 15). An important feature of the formalin test is that animal shows two phases of nociceptive behaviour. The first phase starts immediately after formalin injection and lasts for 3-5 min. The late phase starts after 5 min & lasts for about 40-50 min. These two phases are affected differently by different drugs and are mediated by separate neural pathways. The early phase is attributed to peripheral nociceptive stimulation and the late tonic phase to the inflammatory response (16, 17). Further, it has been suggested that the late phase of formalin test is also dependent upon plasticity in the central nervous system which occurs during the transient early phase.

Although the role of GABA has been suggested in several pain tests (mostly phasic), its involvement in the tonic pain induced during the formalin test has not been studied so far. The present study was undertaken to examine the possible involvement of GABA B receptors in tonic pain by using GABA B agonist baclofen.

METHODS

Experiments were conducted on adult male rhesus monkeys weighing between 3.5-4.5 kg. They were housed in individual barred iron cages and

provided food twice a day and water *ad libitum*. Experiments were conducted in a separate room and the monkey was conditioned to the experimental cage for 2-3 hours each day for 4-5 days prior to the beginning of the experiment. For control pain rating, 150 μ l of 10% formalin was injected subcutaneously into the palmar surface of the hand just proximal to the base of the fingers and the resultant pain was scored as described earlier (18).

Three control recordings were obtained for each monkey. An interval of at least a week was allowed before the same monkey was studied again. The hands were alternated, so that at least a fortnight elapsed before a monkey was injected formalin on a previously injected hand. Care was taken that a different site was chosen on each occasion, the previously used sites being slightly indurated. After obtaining 3 control pain ratings in 4 monkeys baclofen was injected i.p. 30 min prior to formalin injection. Five doses of baclofen were used in each monkey (2, 4, 6, 8, and 12 mg/kg) in a

random sequence.

Statistical analysis of results was done using non parametric tests. The change in pain rating with time was analysed using Kruskal-Wallis test. Pain rating with various doses of baclofen was compared with basal rating using Friedman test.

RESULTS

Pain ratings obtained with various doses of baclofen are given in Table I and depicted graphically in Fig 1. The average pain rating over 5-min blocks is represented against time. Following formalin injection pain was produced instantaneously (categories 2 and 3) and it reached its peak within 5 min. This is the early component of pain. After 5 min the pain intensity declined gradually, approaching the baseline level of zero pain, 55 min after formalin injection in control and as early as 35 min with 4, 6 and 12 mg/kg baclofen. The persistent pain following the peak represents the late component of pain.

TABLE I : Pain rating during 5 min blocks (avg \pm std.) of 4 monkeys.

| Time | Basal | 2 mg/kg | 4 mg/kg | 6 mg/kg | 8 mg/kg | 12 mg/kg |
|------|-----------------|-----------------|----------------------|-----------------------|-----------------------|-----------------------|
| 5 | 1.93 \pm 0.17 | 1.97 \pm 0.12 | 1.94 \pm 0.35 | 1.87 \pm 0.41 | 1.90 \pm 0.31 | 2.08 \pm 0.38 ** |
| 10 | 1.28 \pm 0.12 | 1.01 \pm 0.20 | 0.81 \pm 0.13 | 0.86 \pm 0.32 | 0.74 \pm 0.39 ** | 0.62 \pm 0.12 |
| 15 | 0.95 \pm 0.24 | 0.62 \pm 0.27 | 0.41 \pm 0.33 | 0.48 \pm 0.35 | 0.28 \pm 0.23 | 0.37 \pm 0.19 |
| 20 | 0.92 \pm 0.14 | 0.54 \pm 0.41 | 0.44 \pm 0.21 | 0.17 \pm 0.20 ** | 0.48 \pm 0.38 | 0.51 \pm 0.30 |
| 25 | 0.54 \pm 0.13 | 0.31 \pm 0.37 | 0.09 \pm 0.04 | 0.09 \pm 0.09 * | 0.16 \pm 0.22 | 0.12 \pm 0.10 |
| 30 | 0.46 \pm 0.17 | 0.26 \pm 0.29 | 0.09 \pm 0.04 * | 0.01 \pm 0.01 * | 0.12 \pm 0.11 | 0.04 \pm 0.04 * |
| 35 | 0.14 \pm 0.04 | 0.17 \pm 0.02 | 0 | 0 | 0.02 \pm 0.03 | 0 |
| 40 | 0.14 \pm 0.07 | 0.08 \pm 0.08 | 0 | 0 | 0 | 0 |
| 45 | 0.04 \pm 0.05 | 0.03 \pm 0.03 | 0 | 0 | 0 | 0 |
| 50 | 0.01 \pm 0.01 | 0 | 0 | 0 | 0 | 0 |
| 55 | 0 | 0 | 0 | 0 | 0 | 0 |
| 60 | 0 | 0 | 0 | 0 | 0 | 0 |
| AVG | 0.54 \pm 0.07 | 0.41 \pm 0.15 | 0.31 \pm 0.09 | 0.29 \pm 0.11** | 0.31 \pm 0.13 | 0.31 \pm 0.08 |

*P<0.05; **P<0.01

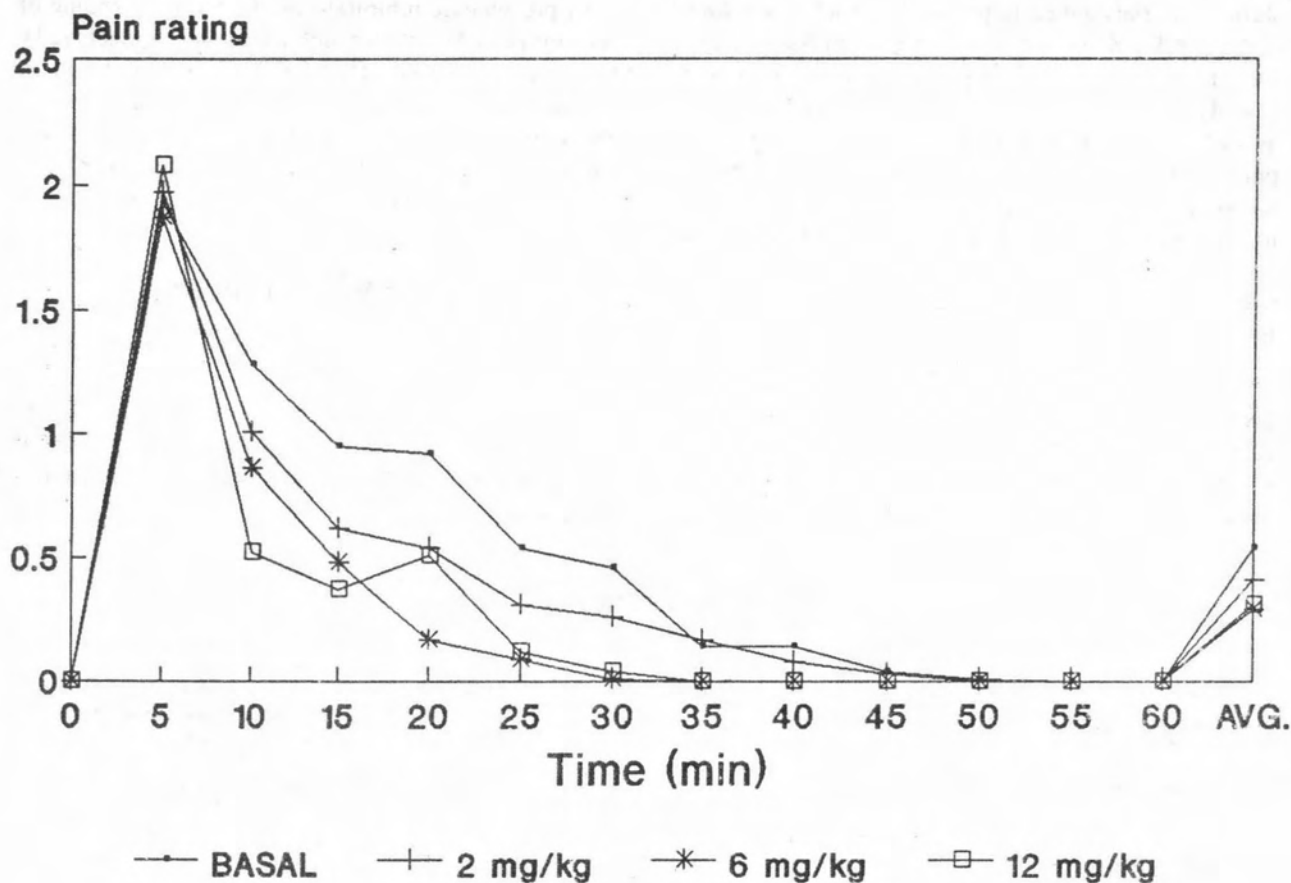


Fig. 1 : Basal pain rating curve along with pain rating obtained with 2, 6 and 12 mg/kg baclofen is represented during one hour period. Effect of 4 and 8 mg/kg baclofen are not depicted here for clarity.

Different doses of baclofen produced analgesia to varying extent. Pain scores were not altered during the early component of pain. During the late component, however, various degrees of analgesia were produced. Initially, i.e., during 10 and 15 min blocks analgesia was most significant with 12 and 8 mg/kg baclofen (0.62 ± 0.12 and 0.28 ± 0.23 , $P < 0.05$ and $P < 0.01$ respectively as compared to control). From 20 min onwards 6 mg/kg baclofen was more effective in producing analgesia. Pain rating at 20 min block decreased from 0.92 ± 0.14 to 0.17 ± 0.02 ($P < 0.01$). During 25 min block pain rating reduced from 0.54 ± 0.13 to 0.09 ± 0.09 which was statistically significant ($P < 0.05$). This decrease continued during the 30 min block when the pain rating was 0.01 ± 0.01

as compared to the control value of 0.46 ± 0.17 ($P < 0.05$). The maximum decrease in average pain rating for one hour period was obtained with 6 mg/kg baclofen (from 0.54 ± 0.07 to 0.29 ± 0.11 , $P < 0.01$)

DISCUSSION

The effect of baclofen, a GABA B agonist was studied on tonic pain in monkeys. Baclofen was found to produce a dose dependent alleviation of pain. There was no effect of baclofen on the initial pain rating which is attributed to the direct stimulation of peripheral nociceptors. This phase of pain is directly due to activation of C fibres (14, 15). During the late phase of pain, which is in part dependent upon

plasticity in the central nervous system which occurs during the transient early phase (19), baclofen induced a dose related analgesia (6 and 8 mg/kg baclofen producing maximal analgesia). Sustained neural activity in the spinal cord induced by the first phase, as well as local inflammatory changes during second phase are necessary for the full manifestation of the second phase (5). Inflammation alone seems insufficient to elicit the behaviour as seen in the second phase, as stimuli which induce an even greater degree of inflammation, such as yeast and carrageenan, produce little pain-like behaviour in rats (21).

The dorsal horn of the spinal cord is said to contain higher concentration of GABA B receptors than A receptors (22). A high concentration of GABA B receptors sensitive to baclofen has been reported in nociceptive primary afferent endings in the superficial

dorsal horn (12). These GABAergic interneurons mostly exert presynaptic inhibition on the terminal ending of nociceptive afferents through axoaxonic contacts (23). The antinociceptive effect of baclofen produced in formalin pain could thus be attributed to the effect of baclofen on GABA B receptors on primary nociceptive afferents at the spinal level in a dose related manner producing presynaptic inhibition of primary nociceptive afferents.

ACKNOWLEDGEMENTS

This research work was supported by the research grant from Department of Science and Technology, India (Grant SP/so/B43/89). The authors are grateful to Mr. Rajinder Kumar and Mrs. Chander Kanta for their technical and secretarial assistance respectively.

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