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The Effects of Bombesin on the Afferent Sensory Transmission in the Spinal Trigeminal Nucleus of Anesthetized Rats

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=ABSTRACT=

The present study was carried out to determine the effects of intracisternal administration of three doses of bombesin (0.001, 0.01 and 0.1 μg) on afferent somatosensory transmission in single neurons of the spinal trigeminal nucleus of anesthetized rats. Lower doses (0.001 μg) of bombesin did not change the afferent sensory transmission. Medium doses (0.01 μg) of bombesin significantly ($p < 0.01$) facilitated afferent sensory transmission in the 6 to 30 min post-drug period, but higher doses (0.1 μg) inhibited responsiveness of spinal trigeminal neurons in the 16 to 35 min post-drug period. The results indicate that endogenous bombesin-like peptide present in the spinal trigeminal nucleus may participate in the processing of the somatosensory information arising from the face.

Key Words: Spinal Trigeminal Nucleus, Bombesin, Afferent Transmission

INTRODUCTION

Bombesin, isolated from amphibian skin, has a number of biologically significant actions when injected into the mammalian central nervous system (Anastasi et al, 1971; Panula, 1986; Spindel, 1986). Intracerebroventricular (ICV) administration of bombesin results in increased grooming and hyperactivity (Brown et al, 1977; Gibbs et al, 1981). Several studies have also suggested that bombesin may play a role in sensory transmission. Intrathecal injections of bombesin caused a biting and scratching behaviour response, mimicking a sensory stimulation (O'Donohue et al, 1984) and iontophoretic administration of bombesin onto the dorsal horn neurones in the cat selectively depresses nociceptive neurons (De-Koninck & Henry, 1989).

Although bombesin-like immunoreactivity and bombesin receptor have been localized within neuronal cell bodies and/or terminals in the spinal trigeminal nucleus (Moody et al, 1978; 1980; Panula et al, 1988; Roth et al, 1982; Ladenheim et al, 1992), the actions of bombesin on somatosensory processing in the neurons of the spinal trigeminal nucleus have not been elucidated. Therefore, the present study was carried out to determine the effects of intracisternal (IC) administration of bombesin on afferent somatosensory transmission in single neurons of the spinal trigeminal nucleus of anesthetized rats.

MATERIALS AND METHODS

Sprague Dawley rats (200-300 g, n=22) were anesthetized with urethane (1g/kg body weight, IP) and mounted in a stereotaxic frame with the head held in a downwardly flexed position. This allowed the dorsal surface of the spinal trigeminal nucleus to be exposed by removing the atlanto-occipital membrane, dorsum of the atlas and part of the occipital bone. In some cases the caudal-most part of the cerebellum was aspirated and the head of the rat was tilted forward to provide access to the rostral part of the nuclei. After removal of the dura the blood vessel pattern on the brain surface was drawn for use as reference coordinates in later experiments. Small supplemental doses of urethane were administered when needed. Body temperature was monitored and maintained at 36℃~37℃ with a heating pad and heating lamp.

For unit recording, a tungsten microelectrode (12 MΩ at 1 KHz, 125 μm diameter, epoxy-insulated, 8 degree tapered, A-M Systems, USA) was driven into the nucleus with a microdrive. Single neurons were recorded, amplified, filtered, threshold discriminated, and digitized for storage and display using a CED 1401 and an IBM 486 computer. Single neurons were identified initially by the presence of spontaneous activity, or by responsiveness to peripheral stimulation. During peripheral stimulation experiments, waveforms of the responses were observed carefully on the oscilloscope screen to ensure they were single neurons and not field potentials.

Cutaneous receptive fields (mainly from whiskers) were identified by listening to the recorded signal through an audio speaker while using a fine tipped probe to tap the face lightly, until the zone responding most intensely and reliably was defined. Bipolar concentric stimulating electrodes (50 μm tip, 100 μm o.d., 0.5 mm tip separation; David Kopf, Tujunga, CA) were inserted under the center of the receptive fields, which was fixed firmly to prevent any movement. Responses of individual cells to electrical stimulation of indwelling electrodes (single 0.1 ms pulses, 1.0 Hz) were characterized by generation of post-stimulus time histograms (5 min intervals). The stimulating current necessary to produce a minimal response in the spinal trigeminal units were generally in the range of 50 to 500 μA.

Rats were subjected to three different doses (0.001, 0.01 and 0.1 μg, IC, in 10 μl volume of saline containing 0.2% bovine serum albumin) of bombesin (Sigma, Co). After an initial 15 min recording period, bombesin was administered and recording was continued up to 40 min post-injection. Post-stimulus time histograms were constructed for quantitative measurements of intensity or latency of unit responses. Histograms were displayed on a graphics computer terminal. A cursor routine on the computer was used to determine the single unit firing rates (in spikes/sec) during control and experimental epochs (6.7 ± 0.7 -20.3 ± 1.3 msec poststimulus). The following formula was used to calculate the firing rate in an epoch: (No of spikes/No. of sweeps) $\times$(1000/No. of msec in epoch). Evoked unit responses (EURs: EUR = ER-SD) were expressed as the evoked unit discharge rate (ER) measured during experimental epoch, minus the spontaneous discharge (SD). Spontaneous discharge rates, in spikes/sec, were measured by making similar calculations on the 150 and 200 msec poststimulus epoch, in which time the histograms flattened. These EURs were calculated from histograms generated during the pre-drug 15 min (EURcont) and post-drug period (EURdrug), and were compared with each other. The bombesin-induced modulation of sensory transmission was expressed in terms of the percent change from the averaged EUR value calculated during pre-drug control period ($100 \times$(EURdrug-EURcont)/EURcont). When three EUR values during a 15 min pre-drug control period showed more than 5% of variation from the

averaged EUR value, then the effects of drug were not considered as data.

RESULTS

Quantitative determination of the effect of bombesin on afferent sensory transmission to the spinal trigeminal neurons was carried out by generating post-stimulus time histograms of single unit responses to the subcutaneous electrical stimulation of the center of a receptive field located in the whisker area. Figure 1 shows results of three experiments where we tested the effects of three doses (0.001, 0.01 and 0.1 μg) of bombesin (IC) on the transmission of the somatosensory evoked unit responses recorded in three spinal trigeminal

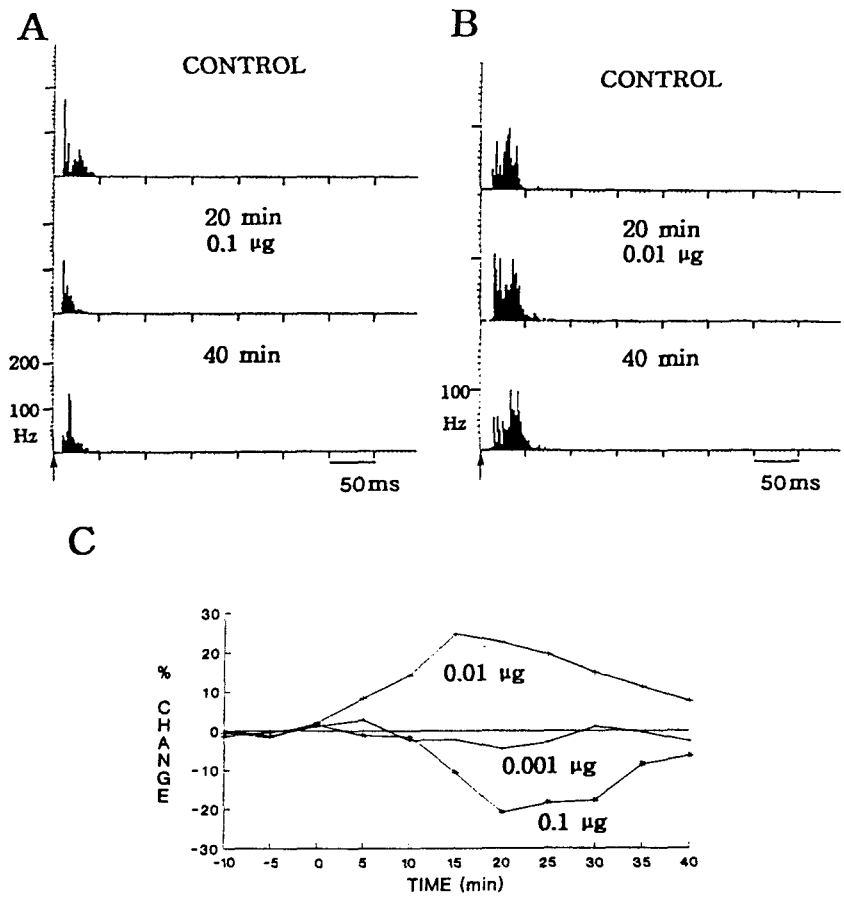

Fig. 1. Effects of bombesin on sensory responses of three neurons recorded from the spinal trigeminal nucleus. A,B: Post-stimulus time histograms obtained from two spinal trigeminal neurons. Evoked unit responses were triggered by subcutaneous electrical stimulation under the whisker pad during the pre-drug control period and during 20 and 40 min post-drug periods (0.1 μg in A, 0.01 μg of bombesin in B). Small y-axis ticks= 10 spikes/s, small x-axis ticks 5 ms, bins= 1 ms. An arrow indicates the time when the peripheral stimulation was delivered. C: Percentage change of sensory response of three spinal trigeminal neurons during pre-drug 15 min and during post-drug 40 min. Data from the administration of the medium and the high doses of bombesin were obtained from the analysis of post-stimulus time histograms generated from the cells of B and A, respectively.

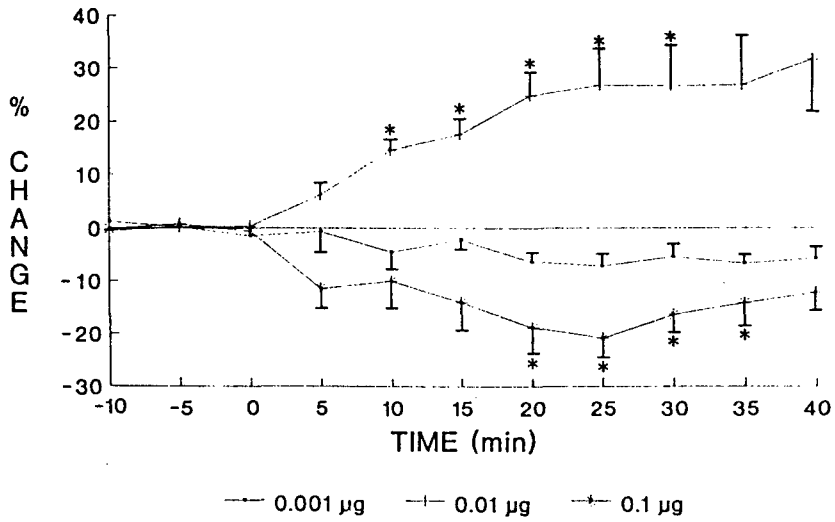

*Fig. 2. Comparison of the time courses of average change in sensory responsiveness induced by three different doses (0.001, 0.01 and 0.1 μg, IC) of bombesin during the 40 min post-drug period. Note the stability of the afferent sensory transmission during the 15 min pre-drug control period. Number of spinal trigeminal neurons tested: low doses (n=7), medium doses (n=7) and high doses (n=7). The evoked unit responses (EURdrug) obtained during the post-drug periods were compared with the values of the evoked unit response (EURcontrol) calculated during pre-drug control period. *= p <0.01, Newman-Keuls Test.*

neurons. The stability of the afferent sensory transmission was ensured by monitoring evoked unit responses (EURs) of the three poststimulus time histograms (5 min duration for each histogram) generated during a 15 min control period before the bombesin administration (Fig. 1C). Histograms (Fig. 1A, B) generated at 20 min after administration of bombesin showed that the EURs were inhibited (−20.8%) and facilitated (+22.8%) by high (0.1 μg) and medium (0.01 μg) doses, respectively. These effects were greatly diminished after 40 min of drug administration. Although histograms were not shown, low doses (0.001 μg) of bombesin did not exert any modulatory influence on the sensory transmission in a spinal trigeminal neuron (Fig. 1C).

Although 63 spinal trigeminal units were isolated and tested to see the effects of the bombesin on the afferent sensory transmission, results from only 21 units were considered as data. Since the other 42 spinal trigeminal units exhibited more than 5% variation of EUR values during the 15 min pre-drug control period, results from these neurons were disregarded. Figure 2 shows the overall effects of the bombesin on the afferent sensory transmission through 21 spinal trigeminal neurons during the 40 min post-drug period. Although low doses (0.001 μg) of bombesin did not change the afferent sensory transmission, medium doses (0.01 μg) facilitated sensory transmission. This facilitatory effects were statistically significant in the 6 to 30 min post-drug period (Newman-Keuls Test, $p < 0.01$). The facilitatory effects observed in the 31 to 40 min post-drug period exhibited large variations as indicated by the values of the standard of error of means. This variation was due to three neruons which did not show recovery up to 60 min post-drug period. In contrast to the medium doses, high doses (0.1 μg) of bombesin exerted inhibitory influences

on the afferent sensory transmission through the spinal trigeminal neurons. This inhibition was statistically siginificant in the 16 to 35 min post-drug period (Newman-Keuls Test, $p<0.01$). The absolute magnitude of the modulation appeared to be greater in facilitation than in inhibition.

DISCUSSION

This study has demonstrated that bombesin may influence afferent somatosensory transmission in the spinal trigeminal neurons of anesthetized rats. The major effects of bombesin on the sensory responses evoked by subcutaneous electrical stimulation to the center of the receptive field located under the whiskers were facilitatory for medium doses (0.01 μg), and inhibitory for high doses (0.1 μg). Low doses (0.001 μg), however, did not exert any influences. Bombesin-induced sensory modulatory effects appeared to be dose-dependent and were maximal during 20 to 30 min after drug administration.

Although this study did not test the effects of bombesin on the transmission of afferent impulses evoked by natural, cutaneous stimulation of the receptive field, the significance of the bombesin-induced modulatory effects on the afferent transmission through the spinal trigeminal neuron should not be disregarded. Previously, we have successfully tested movement-induced afferent modulation in the primary somatosensory cortex and the ventroposterior thalamus of awake rats which were implanted with stimulating wire electrodes, to deliver ascending afferent impulses under the thenar eminence of the forepaw (Chapin and Woodward, 1982; Shin & Chapin, 1990; Shin et al, 1992; 1993; 1994).

Bombesin-induced facilitation of the afferent sensory transmission has not been reported in any major sensory systems of intact animals. However, previous in-vitro studies on other brain loci such as hippocampus, and ventral root fibers of the spinal cord have reported bombesin-induced reversible excitatory effects on spontaneous activity, lasting 5 to 25 min (Suzue et al, 1981; Dreifuss & Raggenbass, 1986). In this study, spontaneous activities of the spinal trigeminal neurons did not appear to be influenced by bombesin. Since spontaneous activities were treated as background noise, they were subtracted from the unit responses evoked by peripheral stimulation of the receptive field. Although bombesin-induced facilitation of sensory responses in four spinal trigeminal neurons were diminished around 30 to 40 min post-drug period, the augmentation of the afferent sensory transmission in three other neurons appeared to be longer lasting since it did not recover until 60 min after drug administration. Similar bombesin-induced long lasting (up to 120 min) sensory stimulatory effects have been reported by observing the bite/scratch behaviour response elicited after intraspinal injection of bombesin (Bishop et al, 1986).

Previously, bombesin-induced inhibition was also observed in small number of neurons (3 %) of rat hypothalamic arcuate slice preparation (Lin & Pan, 1993), and 26 of 78 nociceptive cells of the superficial dorsal horn of cat spinal cord were inhibited by the iontophoretic application of bombesin (De Koninck & Henry, 1989). In this study, inhibition of afferent sensory transmission was observed after administration of high doses of bombesin. Since the intensity of the peripheral stimulation to the center of the receptive field was not strong enough to activate nociceptive transmission, the inhibitory influence of the bombesin could not be tested on the nociceptive spinal trigeminal neurons. This inhibition of afferent sensory transmission by high doses of bombesin may not be considered to be a physiological phenomenon occurring in normal animals, since the concentration of bombesin may be too high.

In conclusion, the result of this study provides evidence that bombesin/gastrin-releasing peptide and its receptor (Ladenheim et al, 1992) present in the spinal trigeminal nucleus

may participate in the processing of afferent sensory information arising from the whisker, which is one of the most important tactile organs in the rat.

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