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Research Note

Intrathecal galanin at low doses increases spinal reflex excitability in rats more to thermal than mechanical stimuli

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Summary. The neuropeptide galanin (GAL) was injected intrathecally (i.t.) in decerebrate, spinalized, unanesthetized rats and its effect on the nocifensive flexor reflex was examined. The reflex, which was evoked by intense mechanical or thermal stimulation of the foot, was recorded from the ipsilateral hamstring muscles. I.t. GAL increased reflex excitability significantly more to thermal than to mechanical stimuli. It is suggested that GAL, which is present in sensory fibers that innervate the skin, is released by the central terminals of cutaneous afferents that are much more sensitive to thermal than to mechanical stimuli.

Key words: Galanin – Spinal cord – Flexor reflex – Intrathecal – Cutaneous afferents – Thermoreceptors – Rat

Introduction

A new peptide, galanin (GAL), consisting of a 29 amino acid chain, has been recently isolated from porcine intestine (Tatemoto et al. 1983). GAL is widely distributed in the central nervous system (Rökaeus et al. 1984) and has been shown to occur in a population of small dorsal root ganglion cells (Ch'ng et al. 1985; Skofitsch and Jacobowitz 1986; Ju et al. 1987). GAL-immunoreactive (GAL-I) fibers are found primarily in laminae I-II of the dorsal horn, Lissauer's tract, the dorsolateral funiculus and the area around the central canal (Ch'ng et al. 1985). GAL-I fibers have also been identified in human digital skin (Johansson et al. 1988), and GAL-like immunoreactivity (LI) in afferent terminals is depleted by dorsal root rhizotomy or neonatal capsaicin (Ch'ng et al. 1985; Skofitsch and Jacobowitz 1986). Peripheral nerve section causes an increase in GAL-LI in dorsal root ganglion cells (Hökfelt et al. 1987). Taken together, these data may indicate that GAL, like a number of other neuropeptides, such as substance P (SP), somatostatin (SOM), and vasoactive intestinal polypeptide (VIP), could have a role in neurotransmission involving peptidergic afferents, some of which innervate the skin (Hökfelt et al. 1975; Björklund et al. 1986; Johansson and Vaalasti 1987) and are primarily unmyelinated (Hökfelt et al. 1980).

Unmyelinated cutaneous afferents have a variety of functions, including nociception, thermoception and mechanoreception (Burgess and Perl 1973; Hensel 1973). It is therefore possible that GAL may have a role in some of these sensory functions. This questions was studied by observing the effect of intrathecal (i.t.) GAL on spinal cord excitability in decerebrate, spinalized, unanesthetized rats. Spinal cord excitability was tested by evoking a nocifensive flexor reflex to intense cutaneous thermal or mechanical stimuli and the changes in the intensity of the reflex after i.t. GAL were quantified.

Methods

The magnitude of the hamstring flexor reflex in response to activation of high threshold afferents in the foot was examined in 14 decerebrate, spinalized, unanesthetized Sprague-Dawley rats (240–270 g) by recording the electromyogram (e.m.g.) from the posterior femoris/semitendinosus muscles. The animals were briefly anesthetized with Brietal (Lilly, 65 mg/kg i.p.) and a tracheal cannula was inserted. The rats were decerebrated by aspiration of the forebrain and midbrain and were ventilated. The spinal cord was exposed by a laminectomy at thoracic level and sectioned at T_{8-9}. An i.t. catheter (P.E. 10) was implanted caudally to the transection with its tip at L_{4-5}. The flexor reflex was evoked by mechanical or thermal stimulation of a hind foot. The mechanical stimulus was a standard 120 g, 0.5 s pinch to the middle 3 toes. The thermal stimulus was a copper rod, 3 mm tip diameter, heated to 80° C, applied for 0.5 s to glabrous skin on the lateral side of the foot. The intense thermal stimulus was needed to evoke a brisk,

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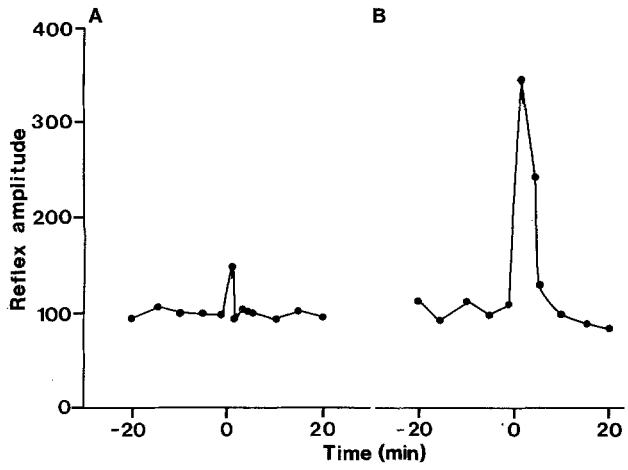

Fig. 1A, B. Percent increase of the magnitude of the flexor reflex over control response levels (expressed as 100%) after i.t. injection of 10 ng/μl GAL in the same experiment. In **A** the reflex was evoked by a 120 g, 0.5 s pinch to the middle three toes. In **B** the reflex was evoked by touching a copper rod (3 mm diameter, 80° C) for 0.5 s to glabrous skin on the lateral aspect of the foot. GAL was injected at 0 min. The total time between the injections in **A** and **B** was 50 min

stable flexor reflex and was applied at various sites demarcated on the skin in order to reduce tissue damage and changes in receptor sensitivity.

Reflexes were recorded as e.m.g. activity via stainless-steel needle electrodes inserted in the ipsilateral biceps femoris/semitendinosus muscles. The number of action potentials elicited during the reflex was integrated (1.5 s for mechanical, 2.5 s for thermal stimuli). Heart rate and rectal temperature were monitored and maintained within normal limits. GAL (Bachem, Bubendorf, Switzerland) was dissolved in normal saline and injected i.t. in a 10 μl volume, followed by a 10 μl saline flush of the catheter. The correct location of the catheter tip was confirmed after every experiment.

Results

The four concentrations of GAL used, 1 ng/μl, 10 ng/μl, 100 ng/μl and 1 μg/μl, were given once in ascending order in some experiments. In other experiments the effect of the same dose of GAL on both thermal and mechanical stimulus-evoked reflexes was tested (Fig. 1). At all doses GAL caused an increase in spinal cord excitability over control levels. When the reflex was evoked by mechanical stimuli, the response was more briefly facilitated than when the reflex was evoked by the thermal stimuli (Fig. 2A). The difference between the duration of the facilitation was statistically significant for the 10 ng/μl and 1 μg/μl doses (Mann-Whitney U-test).

The size of the facilitation over control response levels was also examined. The peak response increase, such as that illustrated at 1 min post-

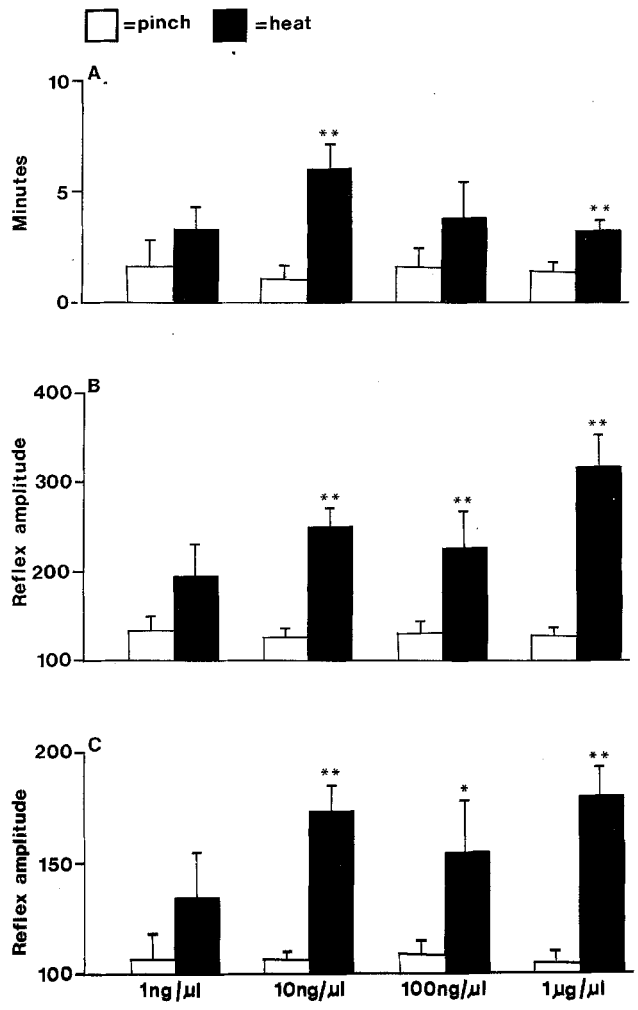

Fig. 2A–C. Comparison of the mean facilitatory effect over control response levels of 4 doses of i.t. GAL (1 ng/μl, 10 ng/μl, 100 ng/μl and 1 μg/μl) on the flexor reflex. In all experiments the reflex was evoked by mechanical or thermal stimuli. The duration (**A**), magnitude of peak facilitation over control level (**B**) and average increase in the response over control levels during the first 5 min postinjection (**C**) are presented. In **B** and **C** control level = 100%. Variability is presented as standard error of the mean. Differences between the groups were calculated with the Mann-Whitney U-test. N = 5–7 in each group. * = $p < 0.05$; ** = $p < 0.01$

injection in Fig. 1A and B, was calculated. At all doses, except for 1 ng/μl, the peak increase in response magnitude was significantly greater with thermal than mechanical stimuli (Fig. 2B). The magnitude of the average response increase over control levels during the first 5 min post-injection was also calculated in order to compare the present data which previous studies (Wiesenfeld-Hallin 1986, 1987a). A significantly larger increase was obtained with all doses, except for the lowest dose, of GAL to thermal than mechanical stimuli (Fig. 2C).

Discussion

In previous studies using similar techniques, i.t. SP, SOM and VIP caused increased reflex excitability (Wiesenfeld-Hallin 1986, 1987a). SP facilitated the flexor reflex with both mechanical and thermal stimuli, whereas SOM and VIP primarily with thermal stimuli. It was concluded that SP is released by polymodal nociceptors that respond to noxious mechanical and thermal stimuli, whereas SOM and VIP by receptors sensitive to cutaneous thermal, but not mechanical stimuli. These conclusions are supported by reports of the spinal release of some of these peptides after mechanical (SP) and thermal (SO and SOM) skin stimuli (Kuraishi et al. 1985; Duggan et al. 1987). No such data are available for VIP or GAL. The present results also indicate that GAL may be released by a class of receptors that respond intensely to thermal and weakly to mechanical stimuli. Cutaneous thermoreceptors make up a small population of skin afferents (Burgess and Perl 1973; Hensel 1973) and thermoreceptive unmyelinated afferents responding vigorously to cooling or warming and poorly to mechanical stimuli have been identified in the rat (Iggo 1969; Lynn and Carpenter 1982; Fleischer et al. 1983). It is not possible to determine with this experimental model whether the observed effects are due to pre- or postsynaptic mechanisms. However, it was previously discussed (Wiesenfeld-Hallin 1986) that these modality-specific results may be due to an interaction between neuropeptides and other transmitter(s) released by primary afferents.

Since the differences between the effects of i.t. GAL with mechanical or thermal stimulation on spinal excitability only involve changes in the type of sensory input, it may be assumed that the observed effect is due to a sensory function of GAL. However, other functions of GAL in the spinal cord cannot be excluded, since GAL is not only present in primary afferents, but also in interneurons in the dorsal horn (Rökaeus et al. 1984; Ch'ng et al. 1985) and even in some motoneurons (Ch'ng et al. 1985). The monosynaptic reflex is not affected by the doses of i.t. GAL used in the present study and therefore motoneuron involvement in the polysynaptic flexor reflex facilitation is unlikely (Wiesenfeld-Hallin et al. 1988).

The doses of GAL used in this study always caused an increase in spinal excitability, which has not been previously described. At a higher dose (10 μg/μl) i.t. GAL causes a moderate depression of the reflex lasting about 30 min (Wiesenfeld-Hallin et al. 1988). In addition it has been shown that high doses of GAL (3 and 10 μg) cause a reversible increase in the latency of responses in the tail flick and hot plate tests in the mouse, with no signs of motor impairment (Post et al. 1988). One possibility is that low doses of GAL activate spinal mechanisms that respond to afferent inputs and cause reflex facilitation. This effect is too brief to be detected by behavioral tests. Higher doses activate intraspinal mechanisms that use GAL as a neurotransmitter/neuromodulator which has a long lasting inhibitory effect. Dose-dependent changes in spinal excitability have, for example, been demonstrated for i.t. noradrenaline (Wiesenfeld-Hallin 1987b). Further studies are needed to clarify these and other possible roles for GAL in sensory and spinal cord function.

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