M. ZUBRZYCKA1*, A. JANECKA2

EFFECT OF GALANIN ON SUBSTANCE P- AND VASOACTIVE INTESTINAL POLYPEPTIDE-INDUCED NOCICEPTIVE TRIGEMINO-HYPOGLOSSAL REFLEX IN RATS

1Department of Experimental Physiology, Medical University, Lodz, Poland
2Laboratory of Biomolecular Chemistry, Medical University, Lodz, Poland

Substance P (SP), vasoactive intestinal polypeptide (VIP) and galanin (GAL), present in primary sensory neurons, are involved in transmission of nociceptive signaling from the peripheral to central nervous system. In this study we investigated the effect of GAL on SP-induced or VIP-induced evoked tongue jerks (ETJ) in response to noxious tooth pulp stimulation during perfusion of the cerebral ventricles with SP or VIP solutions. The experiments were carried out on rats under chloralose anesthesia. It was shown that both, SP and VIP, perfused through the cerebral ventricles enhanced the ETJ amplitude as compared with control, but the effect produced by SP was stronger. The intracerebroventricular perfusion of GAL 5 minutes before SP caused a dose-dependent inhibition of SP-induced ETJ, whereas GAL perfused through the cerebral ventricles 5 minutes before VIP did not reduce the excitatory effect of VIP on ETJ. These results indicate that the antinociceptive effect of GAL perfused through the cerebral ventricles, tested on the trigemino-hypoglossal reflex in rats, is specifically mediated by the SP-ergic system.

Key words: antinociception, perfusion of cerebral ventricles, evoked tongue jerks

INTRODUCTION

Substance P (SP), vasoactive intestinal polypeptide (VIP) and galanin (GAL), present in the primary sensory neurons, are involved in transmission of nociceptive signals from the peripheral to central nervous system (1, 2).

GAL is a 29/30-amino-acid neuropeptide (3), that is present in sensory and spinal dorsal horn neurons (4-7). GAL modulates synaptic transmission at both, pre- and postsynaptic level (8, 9). It has been proposed that GAL produces a
biphasic, dose-dependent effect on nociception through activation of anti-nociceptive (inhibitory) GALR1 receptors or pro-nociceptive (excitatory) GALR2 receptors (5, 10). There are also multiple lines of evidence suggesting that under normal conditions, the effect of exogenous GAL is predominantly inhibitory and due to blocking the excitatory effect of SP (8 - 10). Numerous studies have been conducted to elucidate the mechanism of antinociceptive effect of GAL in the brain and spinal cord after its intrathecal (i.t.) (8, 11), intracerebroventricular (i.c.v.) (12) or intra-PAG (13) administration. The exact mechanism underlying the antinociceptive effect of GAL via GALR1 is unclear, but some recent data indicate that GALR1 is preferentially expressed in a subpopulation of glutamatergic interneurons (14). In contrast, GALR1 was not found in GABAergic interneurons and did not co-localize with its endogenous ligand (was not present in the local GAL neurons). GAL released from both, sensory and interneuron nerve endings, could then act on GALR1-containing glutamatergic interneurons, resulting in attenuation of glutamatergic activation of pain projection neurons and antinociception. On the other hand, data published by Hua et al. (8) indicate that antinociception of i.t. administered GAL is due to its inhibitory action on sites both, presynaptic and postsynaptic to the primary afferents, which prevent development of spinal sensitization and hyperalgesia. Inhibition of release of SP from small afferents (via GALR1), and blockade of spinal neuron activation-induced prostaglandin (PGF\(_2\)) production (via GALR1/2) are two potential mechanisms.

In our previous papers we have used the nociceptive trigemino-hypoglossal reflex of tongue jerks, caused by tooth pulp stimulation, to study the role of different neuropeptides in pain transmission in the orofacial area in rats. In the present study we tried to determine the interactions of GAL with SP and VIP in the same experimental model.

MATERIALS AND METHODS

Experimental animals and anaesthesia

The experimental protocol in the present study was approved by the Local Ethical Committee for Animal Research and it complies with the European Community guidelines for the use of experimental animals. Male Long-Evans rats weighing 340-360 g were used for the experiments. The animals were kept under standard conditions: temperature 22°C, a 12 h light-dark cycle, and allowed tap water and rodent chow ad libitum. The rats were anaesthetised with a single intraperitoneal injection of chloralose solution in a dose of 150 mg/kg body weight. For each experiment n = 10 animals were used.

Chemicals

The artificial cerebrospinal fluid (aCSF) was prepared according to Daniel and Lederis (15) and contained: 120 mM NaCl; 26 mM NaHCO\(_3\); 4.8 mM KCl; 2.8 mM CaCl\(_2\); 1.3 mM MgSO\(_4\); 1.2 mM KHPO\(_4\) and 10 mM glucose. GAL and VIP were purchased from Tocris, Bristol, UK and SP was
obtained from Peninsula Lab., San Diego, USA. Solutions of these peptides for intracerebroventricular (i.c.v.) perfusions were prepared in aCSF.

**Perfusion of cerebral ventricles in rats**

Perfusion of cerebral ventricles was performed as described previously (16). The rat’s head was fixed by introduction of ear bars into the external auditory meati and fixing the maxilla with jaw clamps in a stereotaxic instrument specially adapted for perfusion of the cerebral ventricles (Fig. 1). The skin of the animal’s head was incised in the midline and the skull bones were exposed. On the basis of co-ordinates given by De Groot’s stereotaxic atlas, the sites for drilling holes in the skull bones were determined: The system of cerebral ventricles was perfused by inserting stainless steel cannulae into both lateral ventricles and to the cerebellomedullary cistern. The container with perfusion fluid was positioned 20 cm above the animal’s head. The outflow cannula, inserted into the cerebellomedullary cistern, was connected to a polyethylene tube ca 100 cm long which provided the outflow for the perfusion fluid. The flow rate at the end of the tubing in the course of perfusion was 0.5 - 0.7 ml/10 min. After control perfusion with aCSF the cerebral ventricles were perfused with peptide solutions.

**Tooth pulp stimulation**

After placing the animal’s head in a stereotaxic instrument, the tips of both lower incisors were cut off with a dental separator and stainless steel wire electrodes were inserted into the pulp and

![Fig. 1. Position of a rat skull in a stereotaxic instrument adapted for perfusion of cerebral ventricles. A- inflow cannulae for lateral ventricles, B – outflow cannula for cerebellomedullar cistern.](image-url)
fixed with dental cement. The pulp bipolar stimulation was delivered 6 times per min, with a train of four electrical impulses, of 200 Hz frequency, 3 ms single impulse duration with 2 ms intervals and 4 - 5 V amplitude, using a programmed stimulator. Trains of 4 impulses were delivered to the pulp at 10 s intervals. A Grass stimulator, model S4K, connected with a gating circuit, was used. The amplitudes of electrical impulses stimulating the incisor pulp were adjusted individually for each animal. At the beginning of each experiment the intensity of stimulus inducing maximal evoked tongue jerks (ETJ) was determined. Then, the amplitude of impulses was reduced to obtain the amplitude of ETJ equal to the half of the maximal value.

Recording tongue jerks

The tip of the animal’s tongue was attached with a silk thread to an isotonic rotating tensometric transducer. The amplitude of tongue jerks was recorded on a paper using a Line Recorder TZ-4620 (Laboratorni Pристoje Praha, Czech Republic) (Fig. 2).

The tongue was stretched with the same force, ca. 5.8 G throughout the experiment, the amplification of the recorder also remained unchanged. For each animal during the first 10 min of the control perfusion with aCSF the amplitude of tongue jerks evoked by tooth pulp stimulation was recorded. The mean amplitude of ETJ was regarded as an indicator of magnitude of the trigemino-hypoglossal reflex.

Statistical analysis

Statistical analyses were performed using Prism 4.0 (GraphPad Software Inc.). The data are expressed as means ± SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) followed by a post-hoc multiple comparison Student Newman-Keuls test. The combination experiments were analyzed using two-way analysis of variance (ANOVA) and a post-hoc multiple comparison Student Newman-Keuls test was used for multiple comparisons between groups. A probability level P<0.05 or lower was considered as statistically significant.

Fig. 2. Original recordings of evoked tongue jerks (ETJ) induced by incisor pulp stimulation in rat during perfusion of cerebral ventricles: 10 min control perfusion with aCSF, mean amplitude 24 mm (A) and 10 min perfusion with 100 nM GAL, mean amplitude 14 mm (B).
RESULTS

In the first experiment we measured the effect produced by i.c.v. administration of SP (100 nM) alone and GAL (100 nM) alone, expressed as the amplitude of the evoked retransory movement of the tongue after electrical tooth pulp stimulation (ETJ). The amplitude of the movements was 24 mm for control and this value was regarded as 100%. The obtained values were 148.00 ± 11.09 (35.5 mm) and 55.16 ± 6.12% (13.2 mm), respectively. Then GAL, in three different concentrations (50, 100 and 200 nM) was perfused 5 min before SP (100 nM). GAL perfused 5 min before SP produced a dose-dependent inhibition of the excitatory effect induced by SP, and the obtained amplitudes of ETJ were 127.4 ± 10.23 (30.5 mm), 76.87 ± 9.29 (18.4 mm) and 41.20 ± 6.73% (9.8 mm), respectively, (Fig. 3).

In the similar experiment, when VIP (100 nM) and GAL (100 nM) were perfused separately the obtained ETJ values were 131.72 ± 9.71 (30.2 mm) and 63.14 ± 8.65% (14.5 mm), respectively. GAL (50, 100 and 200 nM) perfused 5 min before VIP (100 nM) did not cause inhibition of excitatory effect induced by VIP and the obtained values were 137.66 ± 11.89 (31.6 mm), 130.19 ± 16.95

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Fig. 3. Effects of perfusion of cerebral ventricles in rats with aCSF and with the following peptides: substance P (SP) (100 nM), galanin (GAL) (100 nM), GAL (50, 100, 200 nM), perfused 5 min before SP (100 nM) on evoked tongue jerks (ETJ) induced by tooth pulp stimulation. The data represent mean ± SEM of 10 rats per group. Amplitude of ETJ after i.c.v. perfusion with aCSF was considered a 100% response. The statistical significance of differences between groups was assessed with two-way ANOVA and a post-hoc multiple comparison Student Newman-Keuls test. A probability level of 0.05 was regarded as statistically significant.
DISCUSSION

The trigemino-hypoglossal reflex induced by tooth pulp stimulation is an important measure of nociception. In this study we have investigated the effect of three neuropeptides (SP, VIP, and GAL) on the transmission of impulses in the brain stem between the sensory nuclei of the trigeminal nerve and the motor nuclei of the hypoglossal nerve, after stimulation of nerve terminals in the tooth pulp in rats. We have shown that both, SP and VIP, perfused through the cerebral ventricles enhanced the nociceptive effect induced by tooth pulp stimulation, measured as the amplitude of ETJ. The effect produced by GAL was antinociceptive. Further we have demonstrated that the i.c.v. perfusion of GAL prior to SP resulted in a dose-dependent inhibition of SP-induced ETJ, which indicated the possibility of post-synaptic antagonistic effect of GAL on the
excitatory effect of SP. GAL perfused prior to VIP did not reduce the excitatory effect of VIP on ETJ. Similar results were obtained by Xu et al. (11) in the nociceptive flexor reflex on decerebrated rats. The facilitatory effect of SP given intrathecally was significantly inhibited after pretreatment with intrathecal GAL, but GAL did not reduce the effect produced by VIP (11).

It is possible that some dorsal horn neurons respond to both SP and GAL, whereby GAL could hyperpolarize the cell membrane (17) and thus reduce the depolarizing effect of SP (18). GAL present in the dorsal horn may originate from primary afferents, local dorsal horn neurons and descending bulbospinal systems. Probably one of these systems may antagonize SP-induced depolarization. This effect may be due to modulation of SP-ergic transmission by GAL, both, at the pre-synaptic level, by inhibition of SP release in midbrain structures and at the post-synaptic level, by blocking the SP-ergic receptors.

It is not clear if there are any interactions between GAL and VIP in the orofacial area. The obtained results do not indicate that such interactions exist. In spite of numerous studies involving GAL, its role in the nervous system is to be fully elucidated. GAL may have multiple functions and may interact with other neurotransmitters and modulators in ways which have not yet been identified.

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Author’s address: Dr Maria Zubrzycka, Department of Experimental and Clinical Physiology, Medical University of Lodz, 90-215 Lodz, ul. Mazowiecka 6/8, Poland. Fax: (4842) 6784277; e-mail: ajanecka@zdn.am.lodz.pl