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Cardiorespiratory action of opioid/tachykinin agonist peptide hybrid in anaesthetized rats: Transduction pathways

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ABSTRACT

AWL3106 composed of opioid (dermorphin) and tachykinin (substance P_{7-11}) pharmacophores is a new compound with high analgesic potency and markedly reduced ability to induce tolerance and dependence.

The present study aimed to determine the respiratory and cardiovascular responses evoked by this peptide in urethane-chloralose anaesthetized, spontaneously breathing rats in the presence or absence of vagal connection.

Intravenous injection of AWL3106 at a dose of 0.3 μmol/kg in intact rats resulted in apnoea lasting 5.1 ± 0.7 s. Breathing that followed was of diminished frequency (F) and augmented tidal volume (V_T) with no significant impact on minute ventilation. AWL3106-challenge induced biphasic fall in arterial blood pressure with no effect on heart rate. Midcervical and supranodosal sectioning the vagal nerves prevented the occurrence of the apnoea and abrogated the post-AWL3106 reduction in F but failed to eliminate the increase in V_T. Hypotensive response appeared to be less profound following supranodose vagotomy. NaloxoneHCl abolished solely the occurrence of apnoea. However additional blockade of tachykinin NK_1 receptors with SR140333 was required to abolish V_T increase, deceleration of breathing and to markedly suppress AWL3106-induced hypotension.

The present study shows that extravagally controlled stimulation of V_T maintains fairly regular ventilation by levelling the bradypnoeic effects. Although the peptide showed no cardiac effects, hypotension occurring beyond the vagal loop may limit future therapeutic benefits of this chimeric compound.

1. Introduction

Respiratory depression is the most threatening side effect of the prolonged opioid analgesia. Therefore the search for finding new compounds of high analgesic potency with reduced side-effects of opioids is still in progress. AWL3106 is a new analgesic peptide, where the opioid pharmacophore – dermorphin is hybridized with synergistically acting C-terminal pentapeptide of substance P (SP_{7-11}). This novel peptide compound given intrathecally showed an analgesic ceiling at nearly 100% of MPE (Lipkowski et al., 2013).

Of the two constituent pharmacophores: dermorphin, a heptapeptide isolated from amphibian skin, shows very high affinity and selectivity for μ opioid receptors, and an extremely potent antinociceptive effect (Mizoguchi et al., 2011). Earlier studies on respiratory effects of dermorphin reported diverse results, since they were carried out in conscious rats and the peptide was applied to the brain ventricles (Feuerstein and Faden, 1983; Paakkari et al., 1990). However, with the use of anaesthesia: an apnoea, depression of respiratory rate and of cardiovascular variables were described (Portolano et al., 1991), similar to subsequently reported effects of an intravenous administration of dermorphin, which comprised the increase in tidal volume as well (Wojciechowski et al., 2007).

The second pharmacophore, substance P_{7-11}, constitutes C-terminal pentapeptide of substance P – an undecapeptide distributed in the central and peripheral neural network, a natural endogenous ligand of tachykinin NK_1 receptors. Of its multiple functions, substance P serves as pain transmitter, neuromodulator in baro- and chemotransduction and an excitatory mediator of respiratory drive (Helke and Seagard, 2004; Montandon et al., 2011; Ostuka and Yoshioka, 1993). Furthermore, it shows impact on blood pressure and heart rate, operating both peripherally and in the central nervous system (Walsh and Williams, 2006). At the spinal level substance P acts in concert with endogenous opioid systems to regulate analgesic responses to nociceptive stimuli (Foran et al., 2000).

Systemic administration of C terminal sequence of the parent peptide – SP_{7-11} in anaesthetized rats decreased tidal volume ensued by a transient increment and slowed the respiratory rate, concomitant with reduction in arterial blood pressure and heart rate (Wojciechowski

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et al., 2016).

μ-Opioid and tachykinin NK_1 receptors are involved in cholinergic transmission in the airways, showing inhibitory and facilitatory effects, respectively (Barnes, 1992). Coincident activation of both opioid receptors and SP-immunoreactive neurons within the intrapulmonary airways, located in vagal nodose ganglia (Zhuo et al., 1997), should modify the respiratory pattern. Chimeric peptide, AWL3106, expresses high affinity for both types of receptors (Lipkowski et al., 2013). The present experiments aimed to determine the respiratory and cardiovascular motor output responses evoked by activation of opioid and SP_{7-11} pathways of this compound, not as yet described.

2. Materials and methods

2.1. Animals and surgical procedures

All animal procedures were performed in accordance with EU Directive 2010/63/EU for animal experiments and approved by the IV Local Commission for the Care and Use of Laboratory Animals.

Fifty six adult male Wistar rats were anaesthetized with an intraperitoneal injection of 750 mg/kg urethane and 150 mg/kg of α-chloralose (Sigma-Aldrich, Poznań, Poland). Supplementary doses of anaesthesia were administered i.p. as assessed by response(s) to nociceptive stimuli.

Animals placed in the supine position breathed spontaneously room air through the tracheal cannula. The right femoral artery and vein were catheterised for recording of cardiovascular parameters and drug administration, respectively. Cervical or supranodose vagi were bluntly dissected and prepared for vagotomies in two sets of experiments. In either of them, section was performed before measuring the respiratory variables and administration of AWL3106. Rectal temperature was maintained close to 37–38 °C by a heating pad. At the conclusion of each experiment, the animal was killed by anaesthetic overdose.

2.2. Apparatus and measurements

The tracheal cannula was connected to a pneumotachograph head, linked to a Research Pneumotach System (RSS 100 HR, Hans Rudolph inc., Shawnee, KS, USA) and a computerized recording system (Windows software version 3.07.02, KORR Medical Technologies Inc., Salt Lake City, UT, USA) for measuring and recording ventilatory parameters: tidal volume (V_T), respiratory frequency (f), minute ventilation (V_E), inspiratory (T_I) and expiratory (T_E) times. Arterial blood pressure and heart rate were measured with a BP-2 blood pressure monitor (Columbus Instruments, Columbus, OH, USA). The costal electromyogram of the diaphragm was recorded with bipolar electrodes connected to NL 104 amplifier (Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The recordings were registered with an Omnilight 8M 36 apparatus (Honeywell, Tokyo, Japan).

2.3. Drugs

Hybrid peptide AWL3106, the opioid and tachykinin receptor agonist was synthetized in the Department of Neuropeptides (Mossakowski Medical Research Centre, Warsaw, Poland) by classic solid phase peptide synthesis using Boc-protected amino acids. Briefly, the sequences of both pharmacophores were performed by stepwise elongation. The mixture was then added with HOSu, and after some time a deprotection was performed using TFA/H20 while the further purification was accomplished by RP-HPLC. The structure of tested dermorphin/SP_{7-11} hybrid is presented in Fig. 1.

The drugs were prepared freshly from powder before each experiment. AWL3106, SR140333 were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, Poznań, Poland) and subsequently diluted in 0.9% NaCl to the final concentration of DMSO not exceeding 6%.

The dose of AWL3106 (0.3 μmol/kg), derived from preliminary experiments summarized in Fig. 2, was injected in a volume of 1 ml/kg into the femoral vein. Each drug bolus was immediately flushed with an 0.2 ml aliquot of saline.

Naloxone hydrochloride (Tocris Bioscience, Bristol UK) – an opioid receptor antagonist, was dissolved in physiological saline and injected intravenously at a dose of 2 mg/kg 2 min before AWL3106 challenge.

To antagonize the activity of tachykinin NK_1 receptors 2.0 μmol/kg of SR140333 (Tocris Bioscience, Bristol UK) was used. The blocker was dissolved in DMSO and diluted with saline so that the final concentration of DMSO did not exceed 6%, such solution was administered intravenously 10 min before AWL3106 injection.

2.4. Treatment schedule and groups

The cardio-respiratory responses to intravenous bolus of AWL3106 were tested in the following experimental designs: (i) intact rats: doses 0.15 and 0.6 μmol/kg (n = 14), 0.3 μmol/kg (n = 9), (ii) subjected to midcervical (n = 9) or (iii) supranodosal (n = 6) vagotomy, (iv) after blockade of opioid receptors with naloxone hydrochloride (n = 9), (v) following simultaneous blockade of opioid and tachykinin NK_1 receptors with naloxone hydrochloride and SR140333 (n = 9), either of which in the intact animals.

Each individual value of V_T, F, V_E were determined by averaging the variables measured for five respiratory cycles. The ventilatory parameters were derived from the integrated pneumotachograph system and assessed as indicated in the tables.

2.5. Data analysis

All experimental data were analyzed by two-way analysis of variance (ANOVA) following repeated measurements, with a surgical treatment (intact/vagotomized animals) or a receptor blockade and an effect of injected chimera or its components at different time points (pre-challenge and defined time points post challenge) as a differentiation factors. Tukey-test was used as a post-hoc. In all cases $P < 0.05$ was considered significant. Data are presented as means ± S.E.M.

3. Results

The dose of AWL3106 was derived from preliminary experiments. Fig. 2 shows maximal effect of different doses of AWL3106 on tidal volume (Fig. 2A) and frequency of breathing (Fig. 2B) in intact rats. The most vivid impact on an increase in V_T and reduction in respiratory rate resulted from the dose of 0.3 μmol/kg of AWL3106. This dose was applied in the following experiments.

Fig. 3 shows representative recordings of cardio-respiratory patterns induced by AWL3106 challenge in intact (A) and midcervically vagotomized rats (B), following blockade of opioid receptors with naloxone hydrochloride (C), and at simultaneous treatment of opioid and tachykinin NK_1 receptors with naloxone hydrochloride and SR140333, respectively (D).

3.1. Cardio-respiratory pattern triggered by an intravenous injection of AWL3106 in anaesthetized rats. Contribution of vagal pathways

Intravenous injection of 0.3 μmol/kg of AWL3106 in the neurally intact rats evoked an apnoea of mean duration of 5.1 ± 0.7 s (Fig. 3A). Respiration that followed was of reduced frequency (F) and increased tidal volume (V_T), without significant depressive impact on minute ventilation (V_E) (Table 1).

Bilateral midcervical vagotomy (AWL3106 CV) as well as section of supranodose vagi (AWL3106 NV), by itself, significantly uplifted baseline values of tidal volume and lowered the respiratory rate (Table 1). Both neurotomies prevented the occurrence of apnoea and abrogated the post-AWL3106 reduction in respiratory rate, however failed to eliminate completely the increase in V_T induced by this