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Enhancement of wind-up by the combined administration of adenosine A_1 receptor ligands on spinalized rats with carrageenan-induced inflammation

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Abstract

The adenosine A_1 receptor agonist N6-cyclopentyladenosine (CPA) is very effective in reducing wind-up in intact but not in spinalized adult rats with carrageenan-induced inflammation, suggesting an adenosine-mediated supraspinal modulation. Since wind-up is a spinal cord mediated phenomenon but highly influenced by descending modulatory systems, especially in situations of sensitization, we assessed the possible involvement of adenosine in the modulation of wind-up. We studied the effect of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT) in the presence and in the absence of the adenosine A_1 receptor agonist CPA. The experiments were carried out in spinalized male Wistar rats under α-chloralose anaesthesia. Withdrawal reflexes, studied as single motor units, were activated by noxious mechanical and high-intensity repetitive electrical stimulation (wind-up). While CPA and CPT were not able to induce any change on wind-up when injected alone, the combination of the two drugs, in any order, lead to an important enhancement of wind-up. This enhancement not always paralleled an increase of responses to noxious mechanical stimulation, indicating that the effect is mainly located in the spinal cord. In addition, the enhancement of wind-up was not further increased by the administration of the opioid receptor antagonist naloxone. We conclude that the depression of the wind-up phenomenon observed in spinalized animals is, at least in part, dependent of adenosine systems and can be relieved by the combined administration of CPA and CPT.

Keywords: Pain; Single motor unit; Spinal cord; CPA; CPT; Naloxone

Adenosine is an effective antinociceptive agent whose actions have been located on spinal cord neurones, mainly through the A_1 receptor [21,26,30]. This receptor is highly concentrated on dorsal horn neurons [12] and is situated pre- and postsynaptically [14,20,22,29]. Adenosine A_1 receptors are also present in most areas of the brain [27] and supraspinal inhibitory activity of the adenosine A_1 system has been suggested by electroencephalography [10] and other techniques [34]. In a previous work carried in our lab [25], we observed that the adenosine A_1 receptor selective agonist N6-cyclopentyladenosine (CPA) was a very effective antinociceptive drug in adult rats with an intact spinal cord, but not in spinalized animals. This observation indicates that the main action of systemic CPA was located supraspinally in adult rats or, at least, that the antinociception induced by systemic CPA requires the presence of supraspinal modulation in preparations using the whole adult animal with carrageenan-induced inflammation. Furthermore, our experiments showed that CPA antinociceptive effect was more intense in sham-spinalized animals, indicating a surgery-mediated enhancement of descending inhibitory systems involving the adenosine A_1 receptor. A phenomenon also depicted with the administration of different opiates [18].

CPA is also very effective in reducing wind-up [25,26,32], which is a centrally mediated phenomenon that has been defined as a progressive and frequency-dependent facilitation of the responses of spinal cord neurons observed on the application of constant and high intensity repetitive electrical stimuli. It is a phenomenon that shares some common mechanisms with central sensitization and is mediated by NMDA and NK1 receptors [7,9], although other systems are involved

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in its generation or maintenance (see [19] for further discussion). Although wind-up is a spinal cord-mediated phenomenon, it is highly influenced by descending modulatory systems, especially in situations of sensitization [16,17], and the level of wind-up responses is much lower in spinalized animals than in animals with an intact spinal cord [16].

It is not clear why wind-up is lower in spinalized animals. A possible explanation is that the transection of facilitatory descending influences reduces the activity of some nociceptive neurones. But, it is also possible that an endogenous inhibitory control within the spinal cord is released as a consequence of the interruption of inhibitory descending modulation. This has been suggested with opioid systems based on the observation that the administration of the opioid receptor antagonist naloxone increases the spinal cord activity in spinalized animals [15,18]. We have checked the possibility of a similar involvement of the endogenous adenosine A_1 system in the modulation of spinal cord activity by studying in spinalized animals the effect of the adenosine A_1 receptor antagonist 8-cyclopentyl-1,3-dimethylxanthine (CPT). Due to the implication of opioids in this endogenous control and to the suggested interaction between opioids and adenosine [1,33], we have also studied the effect of the combined administration of naloxone and adenosine ligands in the same preparation.

The experiments were carried out on adult male Wistar rats weighing 250–330 g with carrageenan-induced inflammation. Preparatory surgery was performed in all cases under halothane anaesthesia (5% in oxygen for induction and 2% for maintenance). The cannulation of the trachea, two superficial branches of the jugular veins (for the administration of anaesthesia and drugs) and one carotid artery for the monitoring of the arterial blood pressure was the only surgery performed on intact animals. In sham- and fully-spinalized animals, cannulations were followed by a small laminectomy from thoracic 10 to 8 vertebrae and the opening of the dura mater. Laminectomy was made carefully to avoid intense bleeding, and vagal and nociceptive inputs were reduced with the infiltration of lidocaine (1%) with adrenaline (10 μg/ml). No further surgery was made in the group of sham-spinalized animals and the incision was closed. In the group of spinalized animals, a transection of the spinal cord was made at thoracic segment 8 or 9, where the level of vascularization was lower, using cauterization to minimize the bleeding.

After the surgery the animal was transferred to a standard frame for electrophysiological recordings, where the right hind limb was fixed with plaster into a Perspex block in inframaximal extension. Halothane was discontinued and anaesthesia maintained with α-chloralose (Sigma; 50 mg/kg for induction and 20 mg/kg/h by a perfusion pump for maintenance). Core body temperature was maintained at $37 \pm 0.5^{\circ}$ C by means of a homeothermic blanket throughout the surgery and the experiment. The preparation was left to rest for at least 1 h in intact and sham-spinalized animals and 2 h in spinalized animals before the experiment started. Blood pressure was monitored constantly and in all cases the systolic blood pressure was above 100 mmHg before the administration of the drugs.

Inflammation was induced in all animals by the intraplantar injection of 100 μl of carrageenan λ (Sigma, 10 mg/ml in distilled water) into the right hind paw 16 h before the experiment under brief halothane anaesthesia (5% in oxygen for induction and 2% for maintenance).

The recording of withdrawal reflexes as single motor units has been described in detail several times and has been used to study the phenomenon of wind-up and to test the analgesic activity of different drugs, including opiates, neurokinins, nonsteroidal antiinflammatory drugs and CPA [11,18,25,28]. Very briefly, units were recorded by means of a bipolar tungsten electrode from muscles of the right hind limb involved in the withdrawal reflex. The units were activated in 3-min cycles, each cycle consisting of 10 s of noxious mechanical stimulation (0.2 N above the threshold over an area of 14 mm^2) and 16 electrical stimuli (2 ms width, 1 Hz, twice the threshold intensity for the recruitment of long latency responses; [16,17]). The mean intensities of electrical stimulation used were 4 ± 0.6 mA in intact animals, 5 ± 0.7 mA in spinalized animals and 3.6 ± 1 mA in sham-spinalized animals. A computer-controlled pincher device (Cibertec) was used to apply noxious mechanical stimuli and to determine the threshold force required to trigger the withdrawal response using a constantly increasing pressure ramp stimulation. The intensity of the stimulation used in the experiments was 200 mN above the threshold found for each single motor unit. The mean forces used in the three experimental groups were 0.86 ± 0.1 N in intact animals, 1.26 ± 0.1 N in spinalized animals and 1.07 ± 0.1 N in sham-operated animals. The animals were killed with an overdose of sodium pentobarbital at the end of the experiment (Euta-Lender, Normon). All the experimental procedures conformed to the institutional, national and European guidelines for the use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

The drugs studied were prepared fresh everyday, immediately before administration, and were injected intravenously in a total volume of 0.3 ml. CPA (Sigma), when injected previous to CPT, was studied at doses of 10 to 320 μg/kg, dissolved in dimethyl sulfoxide (DMSO, Sigma) to 0.5 μg/μl and diluted in saline and injected in cumulative $\log_2$ regime every two cycles of stimulation (6 min). The administration of each dose of CPA was made very slowly, over a minimum of 3 min, so as to minimize its effect on blood pressure [25]. CPA was injected as a single bolus of 160 μg/kg when studied after CPT. Preliminary experiments showed that a bolus dose of 160 μg/kg of CPA was sufficient to induce a complete inhibition of SMU responses while minimizing the decrease in blood pressure caused by higher doses. The effect of CPT (Sigma) was studied at doses of 10 and 20 mg/kg [25] and naloxone (Sigma) at a dose of 1 mg/kg. CPT was dissolved in NaOH 0.1 M (1 mg/50 μl) and diluted in saline, whereas naloxone was dissolved in saline. CPT was always applied 30 min before CPA since that was the peak time of effect ob-