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Research report

Intrathecal administration of yohimbine impairs locomotion in intact rats

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Abstract

The effects of upper lumbar level intrathecal injection of yohimbine, an α_2-noradrenergic antagonist, on overground locomotion in intact rats was studied. This treatment caused dose-dependent impairment of hindlimb locomotor movement, which varied from transient hindlimb paralysis at a dose of 200 μg/20 μl to transient trunk instability at 50 μg/20 μl. Repetitive (every 48 h) injections of yohimbine at high (200 μg/20 μl) and medium (100 μg/20 μl) doses caused tachyphylaxis, which usually led to a lack of reaction to the third injection. This phenomenon was not observed after repetitive injections of the low (50 μg/20 μl) dose of the drug. These results show that the noradrenergic system is involved in the control of locomotion, since intrathecal administration of a specific antagonist affects this activity in intact rats.

Keywords: Noradrenergic system; Intrathecal administration; Intact rat; Spinal cord; Overground locomotion; Hindlimb movements; Tachyphylaxis

1. Introduction

The neural mechanisms involved in the initiation and modulation of locomotion have been extensively investigated over recent decades. Many studies have demonstrated that monoaminergic (mainly serotonergic and noradrenergic) systems play an important role in mammalian locomotor activity [6,43–45]. In cats, following complete thoracic spinal cord transection, both intraperitoneal [6] and intrathecal [21] application of clonidine, an α_2-noradrenergic agonist, was found to initiate treadmill locomotion shortly after spinalization (as early as the second postoperative day). Clonidine also improved locomotor training in adult spinal cats and substantially accelerated the recovery of hindlimb locomotion [4]. In spinal cats that had spontaneously recovered stable locomotion, clonidine modulated locomotor activity by lengthening the step cycle and increasing flexor and extensor muscle burst duration [22]. On the other hand, intrathecal application of an α_2-noradrenergic antagonist (yohimbine), caused serious locomotor difficulties in intact cats such as asymmetric stepping, stumbling and poor lateral stability [22]. Moreover, intraspinal injection of yohimbine at the midlumbar level blocked spontaneous hindlimb locomotion in decerebrated cats tested on a treadmill [31].

In contrast to these findings, activation of serotonergic receptors in cats led to less pronounced effects. Injections of serotonin (5-HT) precursor failed to initiate locomotion in acute spinal cats but the 5-HT_2 agonist, quipazine, could modulate well retrained locomotor movements by increasing the amplitude and duration of EMG bursts in hindlimb flexors and extensors [6].

In spinal rats, the serotonergic system seems to play a more important role than the noradrenergic system in the recovery of hindlimb locomotor activity. A sublesional transplant of serotonergic cells from fetus raphe nuclei was found to improve treadmill locomotion of spinal rats [17,35,42,46]. This was reflected by better timing of muscle activity during locomotor-like hindlimb movements elicited by tail pinching, improved interlimb coordination and increased responsiveness of the spinal cord circuitry to stimulation of the hindlimb skin or proprioceptive receptors. Essentially similar results were obtained after intraperitoneal or intrathecal injections of 5-HT or some of its agonists in spinal animals [1,16]. On the other hand, as demonstrated recently, a 5-HT_2 receptor antagonist, cyproheptadine, delivered intraspinally at the L_2–L_3 level had a severe effect on locomotion in intact rats [34].

The role of the noradrenergic system in rat locomotion has received less attention. In spinal rats, clonidine, an α_2-

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noradrenergic agonist, injected intraperitoneally, in contrast to various serotonergic agonists, failed to elicit activity in either the flexor or extensor muscles of the hindlimbs [5]. In spinal cord preparations from neonatal rats examined *in vitro*, noradrenaline (NA) also failed to evoke locomotor activity, but when added to the bath, it decreased the frequency of ongoing rhythmic activity [47]. Contrary to the above results, embryonic noradrenergic cells grafted to the spinal cord below the lesion improved hindlimb locomotor movements in spinal rats [50].

Although several studies have shown that the administration of monoaminergic agonists can improve locomotor movements in spinal animals, there are also data that question the essential role of monoaminergic systems in evoking the locomotor pattern in these animals. For example, spinal cats, despite the lack of monoaminergic innervation below the spinal cord transection, could recover the ability to walk on a treadmill as a result of locomotor training without the aid of any drugs [8,32]. Moreover, intrathecal administration of the noradrenergic α_2 antagonist, yohimbine, in spinal cats that had recovered the ability to walk, did not produce any impairment of their hindlimb movements [22].

In view of the available data it was of interest to examine whether blocking of α_2-noradrenergic receptors could influence the locomotion of intact rats, as was the case in our previous study using cyproheptadine, a 5-HT antagonist [34]. We hoped that such experiments would permit us to compare the roles played by noradrenergic and serotonergic systems in rat locomotion as well as to see whether interspecies differences exist in these systems [43]. Extending some preliminary results presented earlier in abstract form [33], we confirmed that blocking of the noradrenergic system in intact rats elicited similar effects to blocking the serotonergic system, which indicates that both systems play an essential role in rat locomotion.

2. Methods

2.1. Subjects

The experiments were carried out on nine intact adult female Wistar rats aged 3 months at the beginning of the study and weighing between 230 and 320 g. The animals were kept in a room with a 12:12 h dark/light cycle and received water and food *ad libitum*. The intrathecal injection procedure and the testing of changes in overground locomotion were the same as in our previous study [34]. All experiments were conducted with the approval of the Local Ethics Committee and followed EU guidelines on animal care.

2.2. Surgical procedure

In all animals, an intrathecal cannula was implanted under Equithesin (0.35 ml/100 g) anesthesia in aseptic conditions. The thoracic vertebra T_9 was exposed and a small window was opened in the dorsal part of the arcus vertebra using a microrongeur. Then a small incision was made in the dura mater and a thin polyethylene cannula (PE-10) was carefully inserted into the subarachnoid space. With reference to marks drawn on the cannula according to calculations made before insertion, it was gently pushed until the tip was positioned over the L_2–L_3 spinal cord segments. The cannula was then fixed by sewing it to the T_8 spinous process and back muscles. The other end of the cannula was guided under the skin to reach the skull where it was connected to a custom-made adaptor. The adaptor was then fixed to the bone using dental acrylic cement. The paravertebral muscles and the fascia were closed in layers using sterile sutures (MERSILK 6/0) and the skin was closed with stainless-steel surgical clips. After surgery, the animals received non-steroidal anti-inflammatory and analgesic treatment (Tolfedine 0.4 mg/100 g). During the following 7 days the animals were routinely given antibiotics (Gentamicin 0.2 mg/100 g and Oxacillin 0.3 mg/100 g).

2.3. Drug application

The stainless-steel inlet of the cannula adaptor was capped to prevent leakage of cerebrospinal fluid and to reduce infection. The cap was removed only at the time of drug or saline administration. The dead space of the cannula, measured before implantation, was about 15 μl.

Two to 5 days after surgery the patency and correct placement of cannulae were verified by injection of 15 μl of 2% lidocaine followed by 15 μl of saline [27].

Yohimbine, prepared with sterile saline (0.9%), was injected into the subarachnoid space as a bolus of 20 μl, followed by a bolus of about 15 μl of sterile saline to wash out the cannula. The injection was made using a 50 μl Hamilton syringe connected to the inlet of the adaptor via silastic tubing. In each animal, injections were always separated by an interval of 1 week to avoid interaction of drug application. Experiments were generally performed once a week for at least 3 weeks starting about 1 week after cannula implantation. During each experiment, only one drug injection was made. The lowest yohimbine dose (50 μg/20 μl) was established empirically, as that which evoked only minor locomotor impairment (see below). The higher doses (100 and 200 μg/20 μl) were chosen arbitrarily as the lowest dose multiples. Injections of sterile 0.9% saline were used as the control.

To study whether repetitive injections of the same dose of yohimbine elicited similar effects, the rats were randomly separated into three groups and the drug was administered three times in succession with a 48 h interval between each injection. In each group a different dose of yohimbine was administered. This experiment started at least 2 weeks after the end of the main experiment, i.e. about 5 weeks after cannula implantation.

2.4. Experimental conditions

2.4.1. Open field locomotion

Overground locomotion was tested in an open field and on a runway. In the former testing situation rats moved freely within an area of 120 cm × 80 cm. Their locomotion was evaluated by three investigators (for one of whom the experiments were performed blind) and additionally recorded on a digital video camera. The animals were tested for 2 min before and for several minutes after drug administration, up to the moment when they regained plantar walking ability with hindquarter support and only minor trunk instability. Locomotor impairment was evaluated by the presence and duration of arbitrarily chosen stages characterizing animal behavior. These stages corresponded to respective scores in the 21-point BBB locomotor rating scale [7]. A more detailed rating of hindlimb locomotor performance was not possible due to the speed of recovery (see below), but the order of recovery of hindlimb movements was generally similar to that described in the BBB scale. Three stages of impairment of locomotor movements were distinguished [34]:

- *Stage I*. Complete hindlimb paraplegia with limbs passively extended and pads facing upwards; rats were able to move around using their forelimbs, while the hindquarters and hindlimbs were dragged behind. This stage corresponded to a score of 0 in the BBB locomotor rating scale [7].
- *Stage II*. Some movement in one or more hindlimb joints in the absence of body weight support; rats continued to move around using only their forelimbs. During this stage rats improved their locomotion score from 1 to 7.
- *Stage III*. Plantar stepping followed shortly by the reappearance of hindlimb weight support and quadrupedal walking, albeit with major trunk instability. At the end of this stage rats walked with full plantar stepping and hindquarter support but with some residual trunk instability. During this stage the animals improved their locomotion score from 8 to 19 or 20 in the BBB scale, except for those treated with the low dose of yohimbine, which had higher locomotor scores (at least 12) at the beginning of this stage and improved their scores to 19 or 20.