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Intrathecal α-trinositol facilitates the flexor reflex but does not block the depressive effect of neuropeptide Y

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Abstract

We have studied the effects of α-trinositol (D-*myo*-inositol-1,2,6-trisphosphate, PP56), a putative antagonist of neuropeptide Y receptors, on the nociceptive flexor reflex in decerebrate, spinalized rats after intrathecal and intravenous administration. Intrathecal α-trinositol caused strong and prolonged facilitation of the flexor reflex, which was usually associated with an increase in spontaneous motoneuron activity. The reflex depressive effect of intrathecal neuropeptide Y was neither blocked nor reversed by α-trinositol. Intravenous α-trinositol at low doses had no effect on the flexor reflex and at high dose, reflex facilitation was sometimes observed. It is concluded that α-trinositol acts as a spinal excitant and is not an antagonist of the neuropeptide Y receptor in the rat spinal cord.

Keywords: Flexor reflex; Inositol trisphosphate; Motoneuron; Neuropeptide Y; Nociception; Spinal cord

1. Introduction

α-Trinositol (D-*myo*-inositol-1,2,6-trisphosphate, also known as PP56) is an inositol trisphosphate isomer related to the intracellular second messenger, Ins(1,4,5)P_3. It is produced through the enzymatic degradation of phytic acid and it is purified via a chromatographic process. α-Trinositol produces some interesting pharmacological effects after peripheral administrations, including inhibition of inflammatory reactions (Claxson et al., 1990) and prevention of diabetic complications (Ruf et al., 1991). α-Trinositol, however, generated most interest by its property as a rather specific, but non-competitive, antagonist of neuropeptide Y in a variety of peripheral and central vascular beds (Edvinson et al., 1990; Edvinson and Adamsson, 1992; Adamsson et al., 1992; Wahlestedt et al., 1992; Donoso et al., 1993), although this compound appears not to bind to either neuropeptide Y Y_1 or Y_2 receptors (Heilig et al., 1991; Wahlestedt et al., 1992; Feth et al., 1993).

In the spinal cord a rich system of neuropeptide Y-like immunoreactivity (LI), particularly in the superficial layers of the dorsal horn and in the intermediolateral cell columns has been described (Hökfelt et al., 1981; Gibson et al., 1984). Neuropeptide Y-LI was not detected normally in sensory neurons in rat lumbar dorsal root ganglia, but peripheral nerve section caused a dramatic increase in the synthesis of neuropeptide Y and neuropeptide Y-LI in rat sensory neurons (Wakisaka et al., 1990; Noguchi et al., 1993). High density neuropeptide Y receptor binding sites have been described in the superficial layers of the rat dorsal spinal cord, which were partially depleted following neonatal capsaicin treatment, dorsal rhizotomy and sciatic nerve section (Kar and Quirion, 1992), indicating that at least some of these neuropeptide Y-binding sites are located on the terminals of primary sensory afferents. It is therefore possible that neuropeptide Y, like a host of other neuropeptides, may have a role in sensory transmission and modulation. Functional studies conducted with neuropeptide Y receptor agonists support this hypothesis. Thus, Hua et al. (1991) had reported that intrathecal (i.t.) administration of neuropeptide Y and *C*-terminal neuropep-

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tide Y fragments elicited strong antinociception in rats in the hot plate and paw pressure tests.

We have also recently reported that i.t. neuropeptide Y had a biphasic effect on the spinal nociceptive flexor reflex with excitation at low doses and depression at high doses (Xu et al., 1994). Finally, neuropeptide Y inhibits the release of substance P, a major candidate of nociceptive transmitter, from primary sensory afferent in vivo (Duggan et al., 1991) and in vitro (Walker et al., 1988). The present experiment was designed to examine the effect of systemically and spinally administered α-trinositol on the spinal nociceptive flexor reflex and the depression of the reflex induced by i.t. neuropeptide Y in order to validate the possibility of using this compound as a tool to study the physiological role of neuropeptide Y in spinal nociceptive mechanisms.

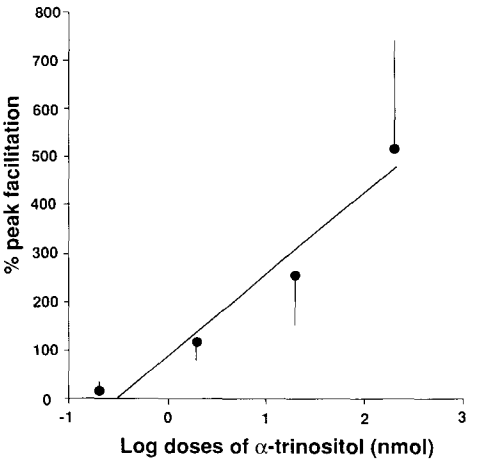

Fig. 1. Dose-dependent facilitatory effect of i.t. α-trinositol on the flexor reflex in decerebrate, spinalized, unanesthetized rats. Four experiments were performed for each dose of α-trinositol and the data are expressed as means ± S.E.M. The dose-response curve was drawn according to the regression line $Y = 167X + 89$ ($r = 0.58$). ANOVA indicate that the regression was significant [$F(1,14) = 6.6$, $P < 0.05$].

2. Materials and methods

The experiments were conducted in female Sprague-Dawley (B&K Universal, Stockholm) rats weighing 200–250 g. The magnitude of the polysynaptic hamstring flexor reflex in response to activation of high threshold afferents was examined. The animals were briefly anesthetized with methohexital, ventilated and decerebrated by aspiration of the forebrain and midbrain. In some experiment, an intravenous cannula was implanted in the jugular vein. The spinal cord was exposed by a laminectomy at mid-thoracic level and sectioned at Th8-9. An i.t. catheter (PE 10) was implanted in some experiments caudally to the transection with its tip on the lumbar spinal cord (L4-5). The flexor reflex was elicited by supramaximal electric shocks to the sural nerve or its innervation area in the left foot (0.5 ms, 10 mA, 1/min) that activated A- and C-afferents. The flexor reflex was recorded as E.M.G. activity via stainless steel needle electrodes inserted into the ipsilateral posterior biceps femoris/semitendinosus muscles. The number of action potentials elicited during the reflex was integrated over 2s and recorded on a chart recorder. During the experiments the heart rate and rectal temperature of the rat were monitored. α-Trinositol in monosodium salt was the generous gift of Dr. T. Gustafsson (Perstorp Pharma, Lund, Sweden) and neuropeptide Y was obtained from Cambridge Research Biochemicals. They were dissolved in 0.9% saline and injected i.t. in a volume of 10 μl followed by 10 μl saline to flush the catheter.

3. Results

The effect of i.t. α-trinositol on the flexor reflex was evaluated under a wide dose range from 0.2 nmol to 200 nmol. The minimal dose required to have a clear

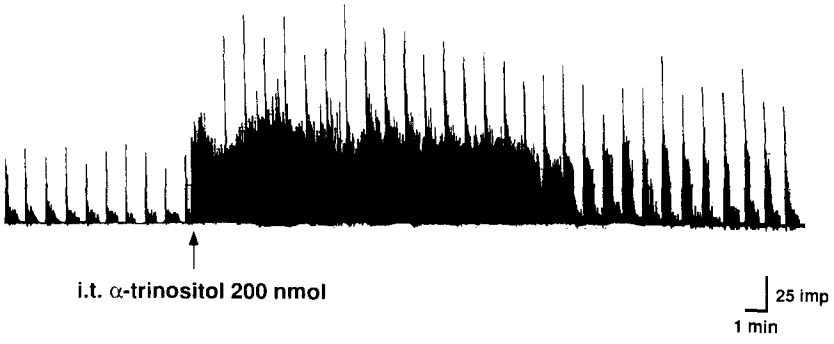

Fig. 2. Chart recording of the effect of 200 nmol i.t. α-trinositol on the flexor reflex in one experiment. The reflex was integrated for 1 s intervals. The stimulus was applied 1/min. Note the increase in background activity as well as reflex magnitude, in association with the i.t. injection of α-trinositol.

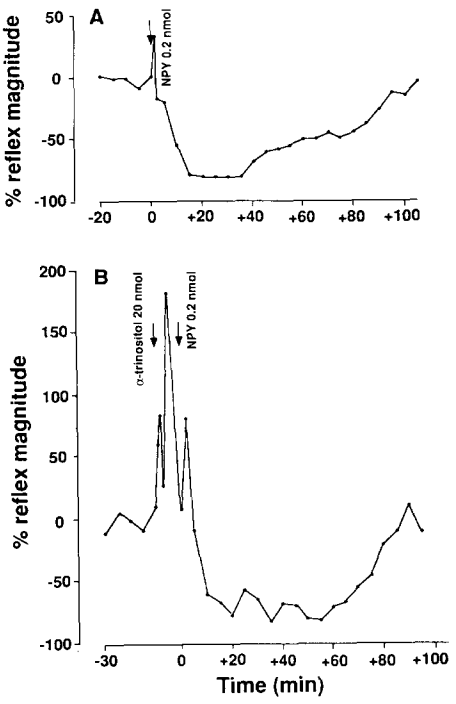

Fig. 3. Illustration of the effect of 0.2 nmol i.t. NPY on the flexor reflex alone in one experiment (A) or after pretreatment with 20 nmol α-trinositol i.t. in another experiment (B). The lack of effect of α-trinositol on i.t. NPY induced reflex depression was observed in two further experiments where 200 nmol of α-trinositol was injected i.t.

and consistent effect on the flexor reflex was 2 nmol where it elicited substantial reflex facilitation (peak facilitation 117 ± 40% over baseline level, duration 11 ± 3 min, $n = 4$) (Fig. 1). The flexor reflex was strongly facilitated by 20 and 200 nmol of α-trinositol (peak facilitation 251 ± 100% and 500 ± 220% for 13 ± 4 and 12 ± 7 min respectively, $n = 4$) (Fig. 1). The increased reflex response was sometimes associated with increase in background activity (Fig. 2). At the highest dose, α-trinositol caused obvious motor excitation expressed as spontaneous tail movement and muscle contraction. Injected after the initial phase of excitation by α-trinositol had passed, the reflex facilitatory and depressive effect of 0.2 nmol neuropeptide Y was not blocked by 20 or 200 nmol α-trinositol ($n = 3$. Fig. 3). Two hundred nmol α-trinositol also failed to reverse the established inhibition of the flexor reflex by 0.2 nmol i.t. neuropeptide Y in two experiments (not shown).

Systemic injection of 10 mg/kg α-trinositol i.v. did not produce a measurable effect on the flexor reflex in three of four experiments. In one experiment, this dose of α-trinositol briefly depressed the reflex (−30% for 15 min). Increasing the dose of α-trinositol to 30 or 50 mg/kg still produced no consistent effect, with increase in reflex magnitude observed in three of four experiments (65 ± 17% for 18 ± 4 min) and depression in one experiment (−30 min, 20 min).

4. Discussion

The present results showed that i.t. α-trinositol potently facilitated the flexor reflex, but did not block the either the facilitatory or depressive effect of neuropeptide Y. It is unlikely that the lack of antagonism by α-trinositol of neuropeptide Y-induced effects is due to inadequate doses used as previous studies have shown that the dose of α-trinositol used to antagonise neuropeptide Y's vascular effects was at most 10–100 times higher than that of neuropeptide Y (Edvisson et al., 1990; Edvinsson and Adamsson, 1991; Adamsson et al., 1992; Wahlestedt et al., 1992; Donoso et al., 1993). It appears that α-trinositol may not function as an antagonist for neuropeptide Y in the spinal cord despite its high degree of potency and selectivity in a variety of blood vessel bioassays. Our findings are very similar to another report where the central effect of α-trinositol has been studied. Thus, Heilig et al. (1991) found that intracerebroventricular administration of α-trinositol increased locomotor activity, but did not influence neuropeptide Y-induced hypoactivity. It is unclear whether these results reflect the presence of different neuropeptide Y receptor subtypes in the blood vessels and central nervous systems or the difference in drug penetration under in vitro and in vivo situations. The systems where α-trinositol has been found to function as an antagonist usually involve activation of the neuropeptide Y Y_1 receptor (Edvisson et al., 1990; Edivinsson and Adamsson, 1991; Adamson et al., 1992; Wahlestedt et al., 1992; but see Donoso et al., 1993). The spinal receptor subtypes mediating the reflex depressive effect of NPY have not been fully characterized. However, we have recently found that it may also be related to activation of Y_1 receptors as it can be mimicked by the selective Y_1 receptor agonist [Leu^{31},Pro^{34}] neuropeptide Y (Xu et al., unpublished observations). Furthermore, Y_1 receptor mRNA has been found in dorsal root ganglion cells and dorsal horn interneurons in the rat (Zhang et al., 1994).

Although failing to antagonise neuropeptide Y's effect, it is however interesting to note that α-trinositol exerted potent biological effects on its own in the spinal cord, inducing facilitation of the flexor reflex. The associated increase in spontaneous EMG activity and the direct observation of the existence of motoneuron activation indicated that the increase in reflex magnitude may have resulted from an excitation of motoneurons by α-trinositol. The exact mechanisms for such effect is unclear, but may be related to an interaction with the endogenous Ins(1,4,5)P_3 system due to its molecular structure and ligand binding characteristics (Yoo et al., 1994). α-Trinositol exerted antinociceptive effect in mice after systemic injection in the hot plate and writhing tests (T. Gustafsson, personal communications). As systemic α-trinositol failed to produce

consistent depression of the flexor reflex and i.t. α-trinositol was associated with increased reflex magnitude, it is unlikely that the antinociceptive effect of α-trinositol is mediated through spinal mechanisms.

Acknowledgments

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