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Research Report

Effect of electrical stimulation of sciatic nerve on synaptic plasticity of spinal dorsal horn and spinal c-fos expression in neonatal, juvenile and adult rats

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ABSTRACT

To explore the response to nociceptive stimuli in spinal cord of immature rat and observe the electrical stimulation of the sciatic nerve on synaptic plasticity of the spinal dorsal horn and spinal c-fos expression in rats of different ages, MK-801 was added to the spinal cord of rats, and the resulting changes in field potential as well as c-fos expression were recorded. LTP in neonatal rats was mainly evoked by A-type nerve fibers, whereas LTP in juvenile and adult rats was mainly evoked by C-type nerve fibers. C-fos expression was significantly increased in the superficial and deep layers of the spinal dorsal horn and in the ventral horn in neonatal rats indicating that the pain signal changed with age.

1. Introduction

Long-term potentiation (LTP) is a type of synaptic plasticity that reflects persistent potentiation of synaptic intensity mainly involved in hippocampus(Bliss and Gardner-Medwin, 1973). Recent studies have shown that LTP is also involved in other central nervous system (CNS) regions, such as spinal dorsal horn, thalamus, amygdala, and cerebellum. Current evidence indicates that electrical stimulation of C-fibers by natural nociceptive stimuli or acute nerve injury could evoke C-fiber-induced LTP of spinal dorsal horn. (Liu and Sandkuhler, 1995; Sandkuhler and Liu, 1998). Because LTP of C-fiber-evoked field potentials plays a critical role in the transduction and modulation of hyperalgesia and pain sensation, the "memory" might be formed in spinal dorsal horn (Klein et al., 2004; Liu et al., 2009; Sandku hler, 2000). Although the primary afferent C-fibers of neonatal rats have started to penetrate into the gray matter of the spinal dorsal horn, C-fibers and crude myelinated A-fibers are gradually involved in the superficial layer of spinal dorsal horn. Until postnatal day 21, C-fibers start to occupy the superficial layer of the spinal dorsal horn. In adult rats, A-fiber synapses are mainly located and distributed in layers III–IV of the spinal dorsal horn, whereas they are found in layers I-II during the neonatal stage. On postnatal day 21, C-fibers compete with A-fibers to occupy the superficial layer of the spinal dorsal horn, and thereby determine which fiber mainly performs the transmission of nociceptive information and processing. Repeated painful stimulation during the neonatal stage could cause long-term adverse effects on the formation of individual pain perception, response function, transduction pathway and neural growth, thereby affecting pain sensitization. However, peripheral pain receptors in neonates are immature, and there is no integrated myelin sheath in Aδ fibers. How pain

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sensitization changes during development, and which fibers mediate these changes have been the sources of debate, especially regarding which fibers mediate LTP with different ages(Fitzgerald and Gibson, 1984; Klein et al., 2004).

In hippocampus, LTP-induction is dependent on activation of NMDA (*N*-methyl-D-aspartate) receptors. A previous report has demonstrated that LTP of C-fiber-evoked field potentials in spinal dorsal horn can also be blocked by NMDA receptor blocker MK-801, which inhibited or blocked central sensitization (Fitzgerald and Gibson, 1984). A recent report showed that c-fos, a member of the proto-oncogene family, is highly expressed and correlated with the activation of NMDA receptors during the maintenance of hippocampal LTP (Zhang et al., 2006). To explore whether a similar phenomenon is also involved in spinal dorsal horn and to verify whether c-fos expression in the spinal dorsal horn is correlated with activation of NMDA receptors during development, we performed electrical stimulation of nerve fibers in rats from different age groups to find the differences in LTP of evoked field potentials and analyzed the effects of stimulation on c-fos expression in the spinal dorsal horn.

2. Results

2.1. *Field potential in spinal cord of rats were evoked by different fibers depending on age*

The first evoked potential had a short latency and large amplitude. Subsequent evoked potentials had a gradually increasing mean amplitude, mean latency and area. The amplitude, latency and area of evoked potentials in juvenile rats ($n=10$, potentials were 8–10 V) were significantly different ($P<0.05$) from those of neonatal rats ($n=8$, potentials were 3–5 V; Table 1). There were significant differences between the latency and the area in adult and neonatal groups ($P<0.05$). (Table 2). Because A- and C-fibers coexist, and A-type fibers in neonatal rats are mainly located in layers I and II of the spinal dorsal horn (Coggeshall et al., 1996; Fitzgerald et al., 1994; Klein et al., 2004), the juvenile and adult groups were compared with the neonatal group, which showed evoked potentials with short latencies and large amplitudes and areas ($P<0.05$) that were preliminarily confirmed to be C-fiber-evoked potentials.

2.2. *Induction of LTP in spinal dorsal horn in rats of different ages*

After conditioned electrical stimulation, the evoked potential in each group changed. The mean amplitude, latency and area of the evoked potentials after recording stably for 30 min were set as control.

Table 1 – Comparisons of the parameters of A-fiber-evoked potentials in neonatal, juvenile and adult rats.

	Mean amplitude (μV)	Mean latency (ms)	Mean area (μV s)
Neonatal rats	48.53±7.39	10.73±2.48	57.68±2.68
Juvenile rats	97.53±9.48#	7.84±2.57#	124.62±6.82#
Adult rats	163.79±8.64*	4.58±1.92*	198.62±5.73*

# $P<0.05$ comparing juvenile and neonatal rats, * $P<0.05$ comparing adult and neonatal rats.

Table 2 – Comparison of the parameters of C-fiber-evoked potentials in neonatal, juvenile and adult rats.

	Mean amplitude (μV)	Mean latency (ms)	Mean area (μV s)
Neonatal rats	6.84±1.87	104.84±4.38	6.72±1.83
Juvenile rats	27.29±4.8 #	94.49±7. 46#	30.65±5.38#
Adult rats	32.68±5.63*	91. 73±4. 65*	46.74±5.68*

#$P<0.05$ comparing juvenile and neonatal rats, * $P<0.05$ comparing adult and neonatal rats.

2.2.1. *Amplitude of field potential*

After high-frequency stimulation (HFS) in the neonatal group, the average amplitude of field potential between 65 and 120 min was increased to 123.78±16.79% ($P<0.01$). When MK-801 was added 10 min before the conditioned stimulus was administered, LTP was completely blocked (Fig. 1A, B), indicating that LTP in neonatal rats depends on the NMDA receptor. C-fiber-evoked potentials showed no significant changes. Although the amplitude of A-fiber-evoked potentials changed slightly in juvenile rats, there was no significant difference (Fig. 2A, B). After HFS, the number of nerve fibers that showed features of C-fibers increased (Fig. 3A), and the mean amplitude of field potentials between 65 and 120 min increased to 238.36±14.76% ($P<0.01$), whereas there were no changes in A-fiber-evoked field potentials. When MK-801 was added before the conditioned stimulus, LTP was almost completely blocked after 10 min (Fig. 3B). This result indicates that LTP in spinal dorsal horn is mediated by different fiber typesin rats of different ages, but is consistently dependent on the NMDA receptor.

2.2.2. *Latency of field potentials*

The latency of A-fiber-evoked field potentials in neonatal rats 0–60 min, 60–120 min, and 125–175 min after the conditioned stimulus was significantly different in juvenile rats compared to adult rats ($P<0.05$; $P<0.01$; $P<0.01$, Supplemental Fig. 1A, B, C). Additionally, the latency of C-fiber-evoked field potentials in juvenile and adult rats was significantly different compared to neonatal rats 0–60 min, 65–120 min and 125–175 min after the conditioned stimulus ($P<0.05$, Supplemental Fig. 1A, B, C).

2.2.3. *Area of evoked field potentials*

The area of A-fiber-evoked field potentials 0–60 min, 65–120 min and 125–175 min after the conditioned stimulus was significantly different in juvenile rats compared to adult rats ($P<0.05$, Supplemental Fig. 2A, B, C). Additionally, 0–60 min, 65–120 min and 125–175 min after the conditioned stimulus, the area of C-fiber-evoked field potentials in juvenile and adult rats was significantly different compared to neonatal rats ($P<0.05$, $P<0.01$ for both, Supplemental Fig. 2A, B, C).

2.3. *Immunohistochemistry of the superficial layer of spinal dorsal horn before and after conditioned electrical stimulation*

The C-fos expression (buffy particles) in L4–L5 section of spinal cord of neonatal rats, juvenile rats and adult rats in