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Sympathectomy induces novel purinergic sensitivity in sciatic afferents[1]

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KEY WORDS adenosine triphosphate; sympathectomy; nerve fibers; pain

ABSTRACT

AIM: To test the hypothesis that sympathectomy could induce novel purinergic sensitivity in sciatic afferents. **METHODS**: Teased-fiber recordings were made from 32 spontaneously active A afferents from the sciatic nerves in surgically sympathectomized rats and 30 spontaneously active A afferents from the sciatic nerves in intact rats. Adenosine 5′-triphosphate (ATP) was injected via a cannula in jugular vein. **RESULTS**: Twenty eight percent of the spontaneously active afferent fibers from sciatic nerves in the sympathectomized rats responded to ATP, either with an increase or with a decrease in spontaneous firing. However, none of the fibers from the sciatic nerves in the intact rats was activated by ATP. **CONCLUSION**: Sympathectomy induces novel purinergic sensitivity in A afferents from sciatic nerve.

INTRODUCTION

Normally type A sciatic afferents are not activated by adenosine 5′-triphosphate (ATP), however, a novel purinergic sensitivity develops in the injury site after partial injury of sciatic nerve[1]. As a result, ATP excites or inhibits most of the injured type A afferents in sciatic nerve. It is thought that this novel purinergic sensitivity may contribute to neuropathic paraesthesia and pain[1].

Most peripheral nerves contain sympathetic post-ganglionic fibers. It is well known that an adrenergic denervation supersensitivity develops after interruption of sympathetic innervation to effector organs[2]. Bossut and coworkers have reported that sympathectomy alone induced adrenergic excitability of cutaneous C fiber nociceptors[3]. They believed that this sympathectomy-induced excitability might be a part of the mechanism underlying sympathetically related pain states. Since ATP is one of the co-transmitters in the sympathetic fibers[4], we hypothesize that sympathectomy may alter the responses of type A afferents to ATP and hence partially contribute to the novel purinergic sensitivity observed following peripheral nerve injury. In this study we test this hypothesis in surgical sympathectomized rats.

MATERIALS AND METHODS

Sympathectomy Male Sprague-Dawley rats (Grade II, Certificate № 003, weighing 250 – 300 g) were obtained from Shanghai Experimental Animal Center, Chinese Academy of Sciences). All experimental protocols followed the guidelines of the International Association for the Study of Pain (IASP) concerning the use of laboratory animals. Surgical sympathectomy was performed in 6 rats. Under sodium pentobarbital anesthesia (50 mg/kg, ip), sympathetic chain from paravertebral ganglia $L_2 - L_5$ was exposed using a lateral retroperitoneal approach[5]. The ganglia and connectives were excised bilaterally, and the surgical wound was closed in layers. This protocol destroyed the sympathetic efferents[6].

Electrophysiology Between 7 – 35 d, the 6 sympathectomized rats and 6 intact rats weighing 250 – 350 g were used for the acute electrophysiological experiments. Under sodium pentobarbital anesthesia (50 mg/kg, ip initially, followed by 20 mg/kg as and when required), trachea and jugular vein were cannulated. Heart rate and electrocardiogram were monitored. Core temperature was maintained at 37 ℃ by an automated heating blanket. The animal was mounted pronely in a metal frame with legs extended. The left sciatic nerve was exposed from the point of sciatic trifurcation until the

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nerve passed under the pelvic ischium. Exposed nerves were covered with warmed (35 ℃) mineral oil.

To eliminate impulses from the spinal cord, including sympathetic efferent activity, the sciatic nerve was crushed as it passed into the pelvis. Just distal to this point, about 20 mm proximal to the sciatic trifurcation, the perineurium was opened. Fine fiber bundles (microfilaments), containing one or more axons, were teased from the nerve using specially honed № 5 Dumont forceps. Microfilaments, cut centrally were placed on a single Ag/AgCl recording electrode referenced to a nearby indifferent electrode. In experiments with 6 intact rats the sciatic nerves were exposed and a similar recording procedure was followed.

The nerve impulses were recorded with conventional electrophysiological apparatus[7]. Discharges were assigned to a single unit if they were of the same shape and amplitude. Discharges were identified as A-fibers based solely on waveform criteria[8].

After recording the basal firings for at least 2 min, ATP was intravenously injected in a volume of 0.2 mL and washed in with 0.2 mL saline. The drugs were administered within 5 s at an interval of 10 min or more.

Data analysis The criteria for determining a drug-induced effect was either a change in the firing rate of at least three times the SD of the baseline rate, or a 30 % increase in the number of impulses recorded in 60 s before the drug administration as compared to the 60 s after the drug administration. All means are given $\bar{x} \pm s$. Statistical evaluations were based on t test or chi-square test, with significance criterion of $P < 0.05$.

RESULTS

Thirty-two and 30 spontaneously active afferent fibers in the sciatic nerves were recorded from the sympathectomized and the intact rats, respectively. All of the fibers examined were A fibers as judged by their spike sizes and durations[8]. Most of the spontaneous discharges were rhythmic, with a constant interspike interval (isi) ranging from 25ms – 80 ms (30ms ± 16 ms). In most of the units the rhythmic discharge was tonic and, thus, presumably primarily represented proprioceptive afferents. The remainder consisted of bursts (on-off). There was no difference between the mean isi of the rhythmic discharge of fibers from sympathetomized and intact sciatic nerves ($P > 0.05$). A few fibers firing at a low rate with irregular isi were discarded.

None of the spontaneously active afferent fibers from the sciatic nerves in the intact rats were activated by ATP ($n = 30$, Tab 1). In contrast, 28 % (9/32) of the spontaneously active afferent fibers from the sciatic nerves in the sympathectomized rats responded to ATP (Tab 1, Fig 1), the firing was transiently increased in 6 out of the 9 responding fibers and decreased in the remaining three. The mean latency from the beginning of the ATP injection to the initiation of change in firing rate was (10 ± 6) s, and there was no relation between spontaneous firing pattern and latency of ATP-induced response ($P > 0.05$).

Tab 1. ATP-sensitive spontaneously active A fibers in sympathectomized and intact rats.

	Sympathetomized rat	Intact rat
Excitation	6/32	0/30
Inhibition	3/32	0/30

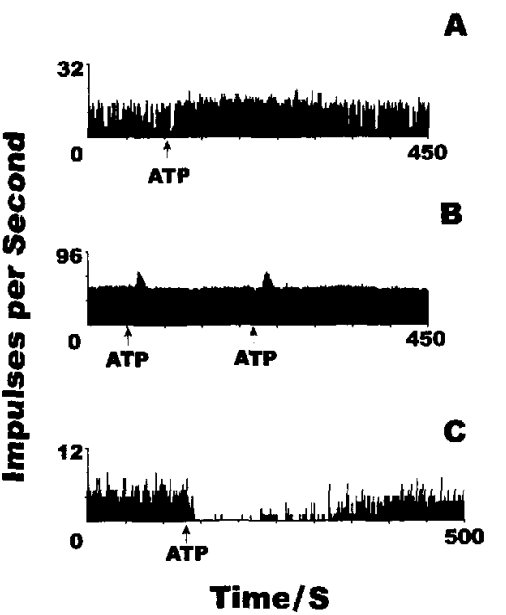

Fig 1. Novel purinergic sensitivity developed in sciatic A afferents in the surgically sympathectomized rats. Spontaneous activities from sciatic A afferents were recorded. A: systemic injection of ATP (2 μmol) evoked excitation in a fiber from a rat 9 d after sympathectomy. B: repeated injection of ATP (2 μmol) in a fiber from a rat 35 d after sympathectomy elicited similar excitatory effect. C: ATP (2 μmol) induced inhibitory effect in a fiber from a rat 9 d after sympathectomy.

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DISCUSSION

The main finding of this experiment is the induction of novel purinergic sensitivity in A type sciatic afferents following surgical lumbar sympathectomy. About one third of the fibers tested were responded to ATP after removal of the sympathetic chain from $L_2 - L_5$. However, no detectable responses to ATP were found in fibers from intact animals under equivalent experimental conditions.

The mechanism underlying this novel purinergic sensitivity remains obscure. It is unlikely that the effects of ATP on the afferents were secondary to its effects on the cardiorespiratory system[9] since ATP could not cause any detectable effects on fibers from intact rats under equivalent experimental conditions. mRNA of both P2X and P2Y receptors and P2X receptor protein were found on rat dorsal root ganglia[10-12]. P2 receptor antagonists could well block the excitatory effects of ATP on injured A afferents[1]. We therefore prefer to hypothesize that ATP may be directly acting on P2 receptor on afferents in our experiments. If this is the case, the P2 receptors which responded to ATP in our study may probably be P2Y receptors since the response latency was considerably long. Subcutaneous injection of ATP excites large sensory fibers with a latency period up to 3 min via P2Y receptors[11]. In light of the existence of the subtypes of P2X and P2Y receptors, it was also possible that different subtypes of these receptors accounted for different response patterns of type A afferents to ATP in the present study: excitation and inhibition.

The response patterns of the fibers from sympathectomized rats to ATP were similar to those we found in sciatic nerve injury units. Most of the responses were excitatory and the remainders were inhibitory. However, the response rate of ATP in mixed nerve (which contains somatic afferent and sympathetic efferent fibers) injury in the previous study was much higher than that in the present study. Thus, it is conceivable that the removal of sympathetic fibers in the mixed nerve might partly contribute to the development of the novel purinergic sensitivity in injured afferents following mixed nerve injury and hence contribute to neuropathic pain.

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交感神经切除术在坐骨神经传入纤维中诱导新生的嘌呤能敏感性[1]

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关键词 腺苷三磷酸; 交感神经切除术; 神经纤维; 神经损伤; 疼痛

目的: 观察交感神经切除术是否可在大鼠坐骨神经传入纤维中诱导新生的嘌呤能敏感性. **方法**: 应用单纤维记录技术在行交感神经切除术的大鼠坐骨神经上记录了 32 根 A 型传入纤维的自发放电活动; 在正常鼠坐骨神经上记录了 30 根 A 类传入纤维的自发放电活动. 通过颈静脉注射 ATP (2 μmol). **结果**: 行交感神经切除术的大鼠坐骨神经中 28 % 自发放电纤维对 ATP 起反应, 表现为放电频率增加或减少; 而正常鼠坐骨神经中具有自发放电的纤维对 ATP 却均无反应. **结论**: 交感神经切除术可在坐骨神经 A 类传入纤维中诱导新生的嘌呤能敏感性.

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