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Purinergic innervation on the isolated renal artery of rabbit

Joo-heon Kim and Yong-keun Kim*

College of Veterinary Medicine, Gyeongsang National University and
*College of Medicine, Busan National University**

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가토 적출 신동맥에 있어서 purinergic 신경 분포

김 주 헌·김 용 근*

경상대학교 수의과대학 및 부산대학교 의과대학*

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초록 : 가토 적출 신동맥에 있어서 purinergic 신경 분포를 perivascular nerve stimulation을 통해서 확인하였다.

1. 가토 적출 신동맥은 perivascular nerve stimulation에 의해 수축성 반응을 나타내었다.
2. Perivascular nerve stimulation에 의한 수축현상은 자극의 크기와 자극의 빈도에 의존적이었으며, 내피세포의 유무에는 영향을 받지 않았다.
3. Perivascular nerve stimulation에 의한 수축현상은 prazosin(1 μM)에 의해 강하게 억제되어졌으며, tetrodotoxin(1 μM) 또는 α,β-methylene ATP(10 μM)에 의한 P_{2x}-purinoceptor desensitization에 의해 완전히 수축현상이 사라졌다.

이와같은 결과는 가토 신동맥에는 purinergic 신경이 분포되어 있으며, perivascular nerve stimulation에 의해 purinergic 신경전달물질이 norepinephrine과 함께 유리되어지는 것을 시사하고 있다.

Key words: Adenosine triphosphate, purinergic, perivascular nerve stimulation, rabbit.

Introduction

The existence of the purinergic nerve system was firstly suggested by Langley and colleagues[1,2] who observed atropine-resistant excitation of pelvic nerve stimulation in the bladder and atropine-resistant inhibition of stomach during vagal stimulation.

Thereafter, a third nerve component of the autonomic nervous system, which is neither adrenergic nor cholinergic nerve, was reported by a number of workers[3~8]. They proposed that active transmitter released from some of these nerves is the purine nucleotide and termed "purinergic" nerves. These nerves have been also observed in gastrointestinal tract[11~15] and a variety of other organs including bladder[16], seminal vesicle[17], uterus[18~20], vas deferens[21,22], vessel[23~28] and eye[29].

However, a little has been known about purinergic innervation on the renal artery. The present study was designed to investigate whether or not there exists a purinergic nerve in renal of rabbit.

Material and Method

Preparation of material

Adult New Zealand White rabbits(2.0~3.0kg) of either sex were anesthetized with pentobabital sodium (100mg/kg). Renal arteries were isolated and cleared of surrounding fatty tissue under a dissecting microscope. Care was taken to the vessels so as to preserve the endothelium[30]. The isolated arteries

were cut into segments, approximately 5mm in length. In some of the segments the endothelium was deliberately removed by pulling a silk thread through the lumen of the vessels.

Each segment was mounted horizontally under isometric conditions in 10 ml organ bath by inserting two tungsten wires through the lumen of the vessel according to the method of Beven & Osber[31]. The tissue was bathed in Krebs solution of the following composition(mM): NaCl 133, KCl 4.9, NaH_2PO_4 1.34, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8, $CaCl_2$ 2.52. The Krebs solution was kept at 37±1°C and bubbled with a gas mixture consisting of 95% O_2 and 5% CO_2. Preparation was allowed to equilibrated under a resting tension of approximately 1.0g for at least 1 hour before starting the experiments.

Contractions of the circular smooth muscle were recorded by use of a Grass FT03D transducer and displayed on a Grass polygraph(Model 79D).

Nerve stimulation

The perivascular nerves were electrically stimulated via two platinum wire electrodes placed parallel from approximately 5 mm apart either side of the vessel segment using a Grass stimulator(Model SD9BCD). Supramaximal voltage and pulse width (0.3 ms) were established and kept constant throughout the experiments.

The vessel segments were stimulated by train of pulse at 50 Hz for 1 sec. Under this electrical stimulation conditions, the contractile response was completely blocked by 1 μM of tetrodotoxin, indicating that stimulation was transmitted via nerve. Each stimulation was repeated at 5 min. interval.

Drug

Acetylcholine chloride(Sigma)
Noradrenaline bitartrate(Sigma)
Tetrodotoxin(Sigma)
Prazosin hydrochloride(Pfizer)
α, β-methylene ATP(Sigma)

Results

1) Response on exogenous acetylcholine(Ach)

Fig. 1 shows the effect of acetylcholine on the norepinephrine induced contraction in muscle strips with or without endothelium. Acetylcholine relaxed

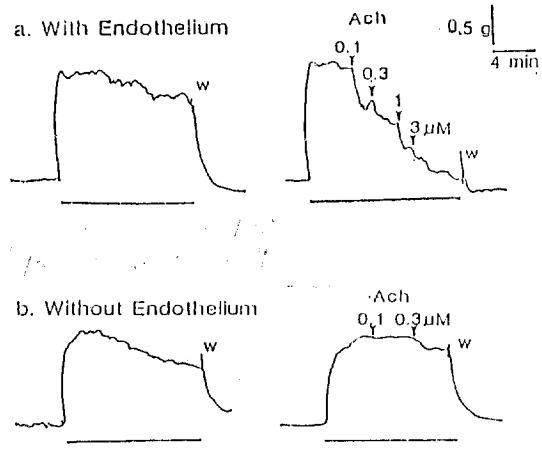

Fig 1. Effect of acetylcholine on 10^{-5}M noradrenaline-induced contraction in isolated rabbit renal artery with(a) or without(b) endothelium.

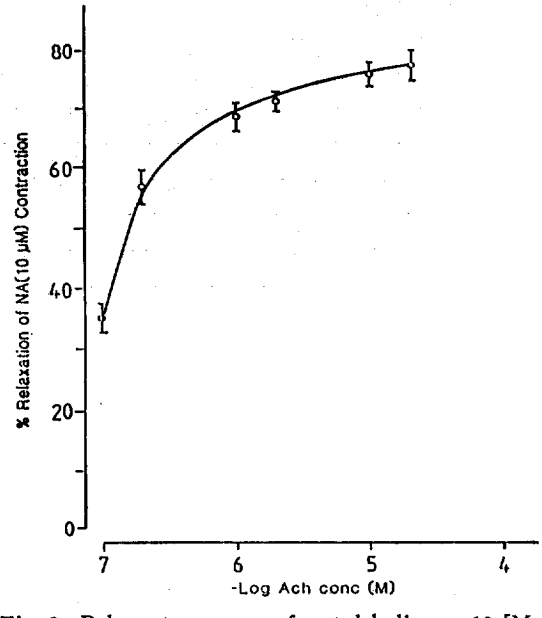

Fig 2. Relaxant responses of acetylcholine on 10^{-5}M noradrenaline-induced contraction in isolated rabbit ranl artery with endothelium.

the norepinephrine-induced contraction in muscle strips with endothelium, but did not alter the tension of muscle strips without endothelium. When acetylcholine was added cumulatively to the norepinephrine induced contraction in muscle strips with endothelium, the relaxation showed a dose-dependent manner in the range of 0.1 to 300 μM (Fig. 2).

2) Effect of electrical stimulation of nerve

While the perivascular nerve of the isolated renal artery was stimulated electrically, the voltage-response(10~100 voltage, 0.3 ms. pulse width, supramaximal frequency) and frequency-response(supramaximal voltage, 2~65Hz, 0.3 ms. pulse width) examined.

A 1 sec train of stimulation produced rapid and monophasic contraction which is voltage-dependent (Fig. 3) and frequency-dependent (Fig. 4) with immediate onset and maximal responses at 90 voltage and 60 Hz. So the electrical parameter was set up usually to 80 voltage, 50 Hz, 0.3 ms pulse width and 1 sec duration.

Neurogenic contraction by perivascular nerve stimulation showed no difference between the vessel segment with endothelium and the vessel segment without endothelium.

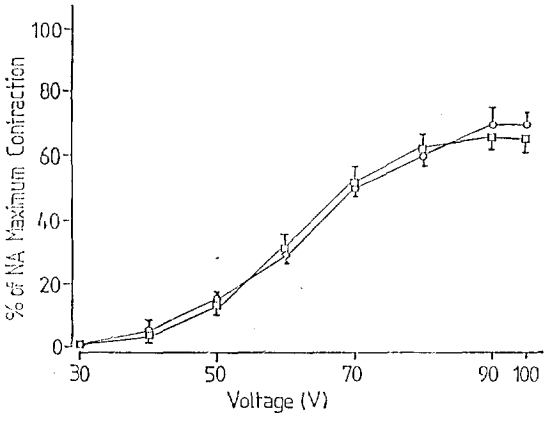

Fig 3. Voltage responses for perivascular nerve stimulation on isolated rabbit renal arteries when endothelium is either intact(○) or removed(□).

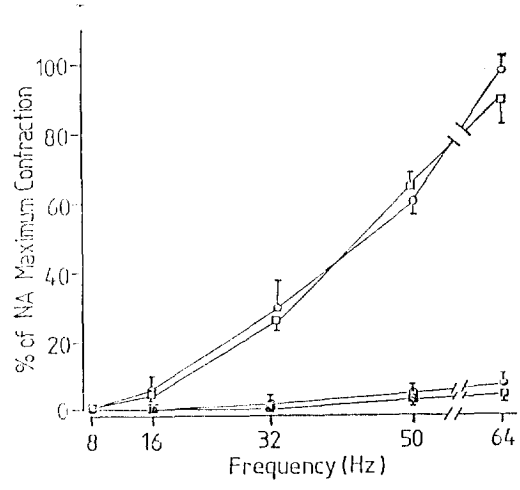

Fig 4. Frequency responses for perivascular nerve stimulation on isolated rabbit renal arteries when endothelium is either intact(○) or removed(□). Filled symbols represent contractions in the presence of prazosin(10^{-6}M).

3) Effect of prazosin and tetrodotoxin on neurogenic contraction

Neurogenic contraction by perivascular nerve stimulation was reduced markedly by prazosin(1 μM)

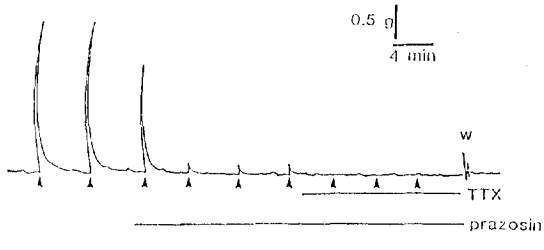

Fig 5. Effect of prazosin(10^{-6} M) and tetrodotoxin (10^{-6} M) on neurogenic contraction in isolated rabbit renal artery: perivascular nerve stimulation at 50 Hz, supramaximal voltage, 0.3 ms pulse width, for 1 sec and 5 min interval.

Fig 6. Effect of prazosin(10^{-6} M) and α,β-methylene ATP(10^{-5} M) on neurogenic contraction in isolated rabbit renal artery: perivascular nerve stimulation at 50 Hz, supramaximal voltage, 0.3 ms pulse width, for 1 sec and 5 min interval.

and completely abolished by tetrodotoxin(1 μM) (Fig. 5).

These results indicate that the neurogenic contraction was due to sympathetic nerve stimulation.

4) Effect of prazosin and the desensitization of P_{2x}-purinoceptor by α,β-methylene ATP on neurogenic contraction

Contraction by electrical stimulation was completely abolished by the desensitization of P_{2x}-purinoceptor through the addition of high concentration of α,β-methylene ATP(10 μM) after neurogenic contraction was suppressed by prazosin(Fig. 6).

These results suggest that the neurogenic contraction suppressed by prazosin conducted through purinergic nerve stimulation.

Discussion

The cell body of purinergic neurons is localized in the gut wall, probably in Auerbach's plexus. Terminal axons of these neurons are supplied to the smooth muscle of both longitudinal and circular smooth muscle coats[10].

ATP was released in the rabbit ear vessel during stimulation of the great auricular nerve[32]. However ATP was shown to be released together with catecholamines from adrenal medullary vesicle and whole glands[33]. Furthermore relatively large amounts of ATP was known to be present in adrenergic nerves[34] and to be involved in both uptake and release of noradrenaline in isolated adrenergic nerve granules[35].

The excitatory autonomic innervation of mammalian urinary bladder is thought to be comprised of cholinergic component and purinergic component[20]. Exposure to atropine or desensitization to α,β-methylene ATP caused a significant decrease in the response to field stimulation. This lack of response reduced after α,β-methylene ATP blokade may be due to the absence of a non-cholinergic component in excitatory response to electrical stimulation, and both atropine and α,β-methylene ATP desensitization were shown to block contractions to field stimulation in urinary bladder, this evidence suggest that acetylcholine and ATP may be coreleased by the parasympathetic neurons supplying the smooth muscle of the bladder[36].

The contractile response induced by electrical stimulation in this study was powerfully suppressed by α_1-antagonist, and was abolished by the addition of neural blocker and the desensitization of P_{2x}-purinoceptor by α,β-methylene ATP.

The small neurogenic contraction powerfully suppressed by α_1-antagonist may by appeared through stimulation of purinergic nerve, and the contractile response was abolished after addition of neural blocker.

These results indicate that the contractile response by electrical stimulation was through perivascular nerve stimulation.

The rapid monophasic responses to neurogenic stimulation observed in this study mimics closely the response of uterine smooth muscle to exogenous ATP[18,20].

There is now considerable evidence in favour of the coexistence of established transmitters with various substance and with purine nucleotide in nerve terminal in both central and autonomic nervous system. The evidence has been presented for coexistance and release of ATP and noradrenaline from sympathetic nerves supplying the vas deferens[37,38], blood vessels[28,39,40] and seminal vesicle[41,42].

Purinergic neurotransmitter and norepinephrine may be a function as cotransmitters from sympathetic perivascular nerve innervating the rabbit renal arteries. This results produce evidence that rabbit renal arteries are innervated by purinergic nerves.

Conclusion

The study was carried out to a purinergic innervation on the rabbit renal artery.

1. Electrical perivascular nerve stimulation produced the contractile responses in isolated rabbit renal artery.

2. The contractile response induced by electrical perivascular nerve stimulation was voltage(10~100 volt.)-dependent and frequency(2~65 Hz)-dependent manner, and showed no difference between the vessel segment with endothelium and the vessel segment without endothelium.

3. The contractile response induced by electrical nerve stimulation mas powerfully suppressed by

prazosin(1 μM) and abolished by the addition of tetrodotoxin(1 μM) or the desensitization of P_{2x}-purinoceptor by α,β-methylene ATP(10 μM).

These findings suggest that the rabbit renal arteries are innervated by purinergic nerve and a purinergic neurotransmitter is released with norepinephrine by electrical nerve stimulation.

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