Korean J Vet Res(1999) 39(4) : 702~709

Effect of Ca^{2+} on contractile responses induced by perivascular nerve stimulation in isolated coronary artery of pig

Yong-geun Hong, Cheol-soo Shim*, Joo-heon Kim**

Department of Biochemistry, College of Natural Sciences, Gyeongsang National University
*Eastern Branch, Gyeongnam Livestock Promotion Institute**
*College of Veterinary Medicine and Institute of Animal Medicine, Gyeongsang National University, Chinju 660-701, Korea***

(Received Mar 9, 1999)

Abstract : The present study was performed to elucidate the effects of extracellular Ca^{2+} on contractile responses in isolated porcine coronary artery ring using by perivascular nerve stimulation (PNS). Especially, the study was focused on the source of Ca^{2+} on P_{2X}-purinoceptor mediated muscle contraction which one of P_2-purinoceptor subtypes.

The following results can be drawn from these studies :

1. The phasic contractions induced by PNS were inhibited with muscarinic receptor antagonist, atropine (10^{-6}M).

2. The phasic contractions induced by PNS were significantly inhibited by sequential treatment with atropine and adrenergic neural blocker, guanethidine (10^{-6}M).

3. The phasic contractions induced by PNS were inhibited with P_{2X}-purinoceptor desensitization by repetitive application of α,β-Me ATP (10^{-4}M).

4. The phasic contractions induced by PNS were so weakened in calcium-free medium.

5. The phasic contractions induced by PNS were inhibited with calcium channel blocker, verapamil (10^{-6}~5×10^{-6}M).

6. The phasic contractions induced by PNS on pretreated with verapamil (10^{-6}~5×10^{-6}M) were not changed by α,β-Me ATP (10^{-4}M).

These results demonstrate that the neurogenic phasic contractions induced by PNS are due to adrenergic-, cholinergic- and P_{2X}-purinergic receptors and the origin of Ca^{2+} on P_{2X}-purinoceptor mediated muscle contraction is extracellular Ca^{2+} through plasmalemmal Ca^{2+} channels.

Key words : perivascular nerve stimulation, Ca^{2+}, P_{2X}-purinoceptor, coronary artery, pig.

Address reprint requests to Dr. Joo-heon Kim, College of Veterinary Medicine, Gyeongsang National University, Chinju 660-701, Republic of Korea. Tel : 0591-751-5819, Fax : 0591-751-5803, E-mail : jhkim@nongae.gsnu.ac.kr

Introduction

Burnstock *et al*[1] reported that blockade of adrenergic and cholinergic nerve did not completely abolish the stimulation-evoked responses in the guinea-pig taenia coli, and they suggested that these responses are mediated with nonadrenergic, noncholinergic(NANC) nerve fibres which may act on NANC nerves transmitters substances involving histamine[2], serotonine[3], prostaglandin[4], purine nucleoside and nucleotide[5]. Among the substances of putative NANC neurotransmitters, purine nucleotides are considered as the most likely candidate for NANC neurotransmitter. Thus, Burnstock[5] nomenclatured so called as "purinergic nerve fibre".

The receptors mediating responses to purines have been categorized in two types, P_1 and P_2, with selectivity for adenosine and adenosine triphosphate, respectively. Among the responses mediated by P_1-purinergic receptors are relaxations of vascular smooth muscle. P_2-purinergic receptors can have either contractile or relaxant effects on different blood vessels[7], as was the cause as described above, Burnstock & Kennedy[7] proposed a subdivision of the P_2-purinoceptor(so called as ATP receptor) into P_{2X} and P_{2Y}-subtypes.

Previously we reported purinergic innervation and contractile action of P_{2X}-purinoceptor from urinary bladder detrussor muscle. We also investigated the neuromodulatory function of P_{2X}-purinoceptor agonist α,β-methylene ATP on the neurogenic contraction in the isolated renal artery of rabbit.

Recently, many investigators demonstrated that purinergic receptors are widely distributed and mediate several responses, including vasodilation, proliferation, muscle contraction or relaxation, secretion and modulation of ion transport[8~12]. In the nervous tissue, ATP can either act as a neurotransmitter or modulate the release and action of other neurotransmitters[13].

However, the mechanism by which P_{2X}-purinoceptor drives calcium movement in neurogenic constriction remains to be resolved.

Chang *et al*[14] demonstrated that very high concentrations of verapamil completely abolished a phasic contraction induced by carbachol on Ca^{2+}-free media, so they suggested that verapamil can enter into the cytoplasm, where they may exert secondary actions on internal sites of the muscle, such as the sarcoplasmic reticulum. Thus, the present study carried out using by verapamil in order to investigate either intracellular or extracellular Ca^{2+} mobilization.

As described above, Ca^{2+} is so important in modulation of vascular resistance because it acts as a final substance. Thus, the objectives of this study were (a) to define the sources of Ca^{2+} on contractile responses in isolated porcine coronary artery ring induced by perivascular nerve stimulation (PNS) and (b) to understand the origin of Ca^{2+} on P_{2X}-purinoceptor mediated muscle contraction using by PNS method.

Materials and Methods

Animals : Coronary arteries were obtained from Landrace porcine of either sex (90~100kg) which were collected at a nearby slaughter house and were immediately put in 4℃ ice-cold Krebs ringer solution of the following composition (mM) : NaCl, 120 ; KCl, 4.75 ; Glucose, 6.4 ; $NaHCO_3$, 25 ; KH_2PO_4, 1.2 ; $MgSO_4$, 1.2 ; $CaCl_2$, 1.7

Artery ring preparation : The branches of the left circumflex artery were excised[10,12]. The arteries were cut into rings (approximately 4~5mm length) and the surrounding fat and connective tissue were removed in 4℃ ice-cold Krebs ringer solution and then those were used for organ bath studies[9~12,14].

Recording system : The rings were then suspended horizontally between two parallel platinum wire electrodes, lower end fixed at basement of a water-jacketed organ bath (volume 10ml) maintained at 37±0.5℃, and upper end attached to an isometric force transducer (FT03, Grass). The tension of the coronary artery rings was recorded on an ink-writing curvilinear polygraph (79, Grass). The initial tension applied was 2g, and the vessels were allowed to equilibrate at least 60 min. All experiments were performed with gassing with 95% O_2 and 5% CO_2.

Perivascular nerve stimulation : Platinum wire electrodes were placed parallel approximately 5mm apart from either side of artery ring were connected with stimulator (SM-1, Narco Biosystems). Artery rings were stimulated for

20 sec with 0.5 msec duration on 2~64Hz, 20V or 40V.

Drugs and procedure : The following drugs were used : Atropine sulfate (ATR), Guanethidine sulfate (GUA), Tetrodotoxin (TTX), Verapamil, α,β-methylene 5'-adenosine triphosphate (α,β-Me ATP), Ethylene glycol-bis(β-aminoethyl ether)N,N,N',N'-tetraacetic acid (EGTA). These drugs were purchased from Sigma Chemical Co. Other chemicals used were of analytical grade. The final concentration of drugs were diluted more than 100 times using under 100μl in 10ml volume organ bath. 3× rinsing procedure and 60 min equilibrating period were applied between each step.

Results

Effect of atropine (ATR) on frequency-response in isolated coronary artery of pig : Perivascular nerve stimulation (PNS) (2~64Hz, 0.5msec, 20sec, 20 or 40V) caused phasic contraction as frequency-dependent manner. Figure 1 shown that the frequency-dependent (2~64Hz) phasic contractions were inhibited with muscarinic receptor blocker, atropine (10^{-6}M). In orther to study neurogenic effects, we treated a drug tetrodotoxin (TTX : 10^{-6}M)) which was known as neural blocker. The phasic contraction induced by PNS were completely blocked in the presence of neural blocker, TTX (data not shown). These result indicated that PNS induced phasic contractions were nerve-mediated response.

Effect of atropine (ATR) and guanethidine (GUA) on frequency-response in isolated coronary artery of pig : To investigate the involvement of autonomic nerve in the neurogenic contraction, atropine and guanethidine were applied. Figure 2 shown that the frequency-dependent (20 or 40V, 2~64Hz) phasic contractions were inhibited at the treat-

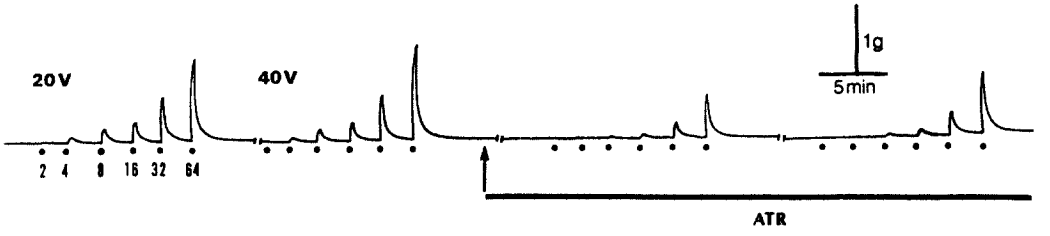

Fig 1. Figure showing effects of atropine (ATR : 10^{-6}M) on phasic contraction induced by perivascular nerve stimulation (20 or 40V, 2~64Hz).

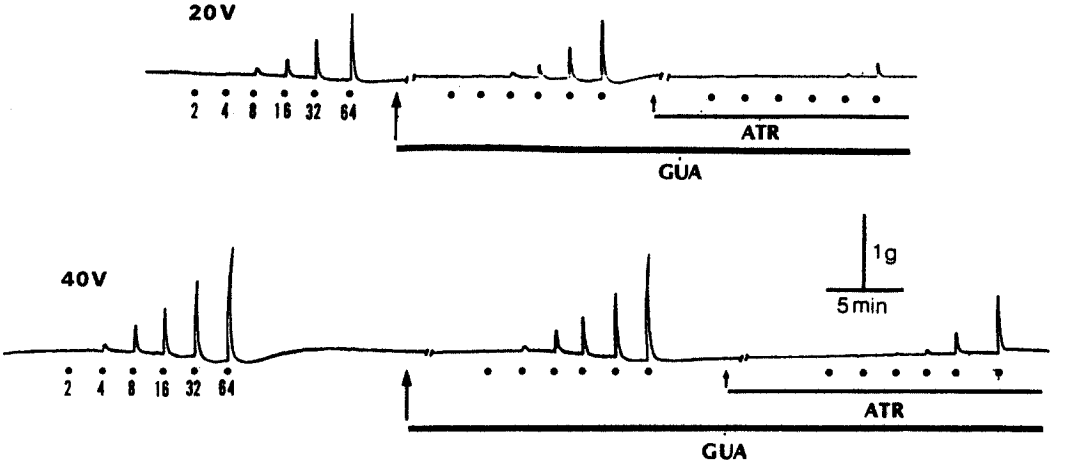

Fig 2. Figure showing effects of atropine (ATR : 10^{-6}M) and guanethidine (GUA : 10^{-6}M) on phasic contraction induced by perivascular nerve stimulation (20 or 40V, 2~64Hz).

ed with atropine and these neurogenic phasic contractions were significantly inhibited by sequential application of atropine (ATR : 10^{-6}M) and adrenergic neural blocker, guanethidine (GUA : 10^{-6}M).

Effect of atropine (ATR), sequential application of atropine and guanethidine (GUA) to frequency-response in isolated coronary artery of pig : Electrical perivascular nerve stimulation (PNS) (2~64Hz, 0.5msec, 20sec, 40V) caused phasic contraction as frequency-dependent manner, and these contractile responses were inhibited in functional cholinergic blocked porcine coronary artery ring, and the frequency-dependent (2~64Hz) neurogenic contractions were significantly inhibited by combination of atropine (10^{-6}M) and adrenergic neural blocker, guanethidine (10^{-6}M). As shown in Figure 3, PNS evoked neurogenic contractions were increased in frequency-dependent manner (upper line). These responses were decreased, shifting the neurogenic contraction curve to the right and downward, it was due to treated with atropine (middle line) or with combination of atropine and guanethidine (bottom line).

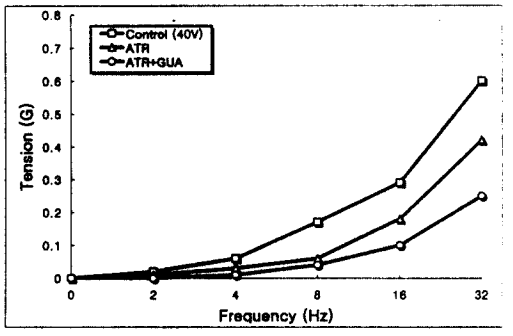

Fig 3. Graph showing effects of atropine (ATR : 10^{-6}M) and atropine plus guanethidine (GUA : 10^{-6}M) on muscle contractile tension induced by perivascular nerve stimulation (40V, 2~32Hz). Each graph represents the mean of performed in 3 separated expriments. Data are expressed as a tension (Gram : G).

Effect of Ca^{2+}-free medium to neurogenic contraction in isolated coronary artery of pig : To understand the origin of calcium involved in the neurogenic contraction induced by PNS, specifically, to examining the role of exogenous Ca^{2+}, Ca^{2+}-free medium were applied. Figure 4 shown that the neurogenic contractile responses were weaken compared with contractile responses (20 or 40V) in calcium containing normal Krebs. These data suggest that neurogenic contraction induced by PNS is mediated by extracellular Ca^{2+} movement as well as intracellular Ca^{2+}.

Effect of exogenous Ca^{2+} with concentration gradients and effect of atropine (ATR) on these responses to the optimal PNS (40V, 32Hz) in isolated coronary artery of pig : To identify the correlation of exogenous Ca^{2+} and cholinergic nerve in the neurogenic contraction, the effect of the exogenous Ca^{2+} were investigated by treatment with calcium concentration gradients on PNS induced neurogenic contraction (40V, 32Hz). Figure 5 shown that contractile tension was significantly increased between 1.75×10^{-3} and 2.0×10^{-3}M calcium concentration (upper line). However, when the atropine was treated to these neurogenic contraction, contractile responses were slightly increased as shown in lower line. This result implicated that the extracellular Ca^{2+} as well as intracellular Ca^{2+} may involved in the cholinergic mediated neurogenic contraction.

Effect of verapamil (VERA) on frequency-response in isolated coronary artery of pig : As it has been suggested that very high concentrations of Ca^{2+} channel blocker, vera-

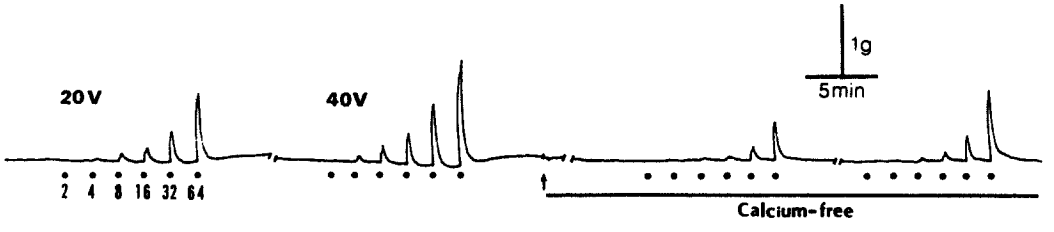

Fig 4. Figure showing effects of Ca^{2+} on phasic contraction induced by perivascular nerve stimulation (20 or 40V, 2~64Hz). After neurogenic stimulation as 3 minutes interval.

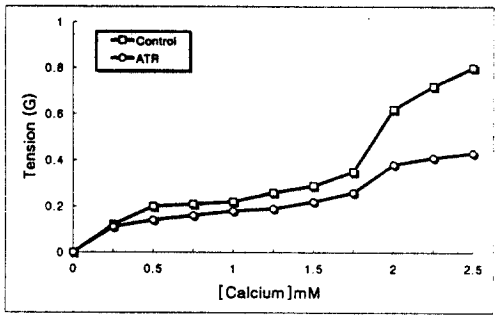

Fig 5. Graph showing effects of atropine (ATR : 10^{-6}M) on muscle contractile tension induced by perivascular nerve stimulation (40V, 32Hz) as the indicated Ca^{2+} concentration gradients.

pamil (5×10^{-6}M) completely abolished a phasic contraction induced by carbachol on Ca^{2+}-free media, suggesting that verapamil can enter into the cytoplasm, where they may exert secondary actions on internal sites of the muscle, such as the sarcoplasmic reticulum[14]. Thus, applied verapamil concentration was 10^{-6} to 5×10^{-6}M, and PNS were carried out into about 5 min after treated with verapamil to blockade only calcium channels placed on plasmalemmal membrane. Figure 6 shown that the effect of Ca^{2+}-channel blocker, verapamil on frequency-response induced by PNS. Verapamil significantly inhibited neurogenic phasic contraction induced by PNS.

Effect of α,β-Me ATP on frequency-response of neurogenic contraction in isolated coronary artery of pig : At the P_{2X}-purinoceptor, α,β-Me ATP is the highest potency agonist among the purine nucleotide derivatives, therefore, it has been suggested that α,β-Me ATP is a selective desensitizing agent for P_{2X}-purinoceptors[15]. As shown in figure 7, the phasic contractions induced by PNS were inhibited in consequence of P_{2X}-purinoceptor desensitization by 3-times repetitive introduction with α,β-Me ATP. Subsequently, when additionally sequential treated with atropine (ATR) and guanethidine (GUA), the neurogenic contraction was severely inhibited. This result gives a suggestion that P_{2X}-purinoceptors exist independent on adrenergic- and cholinergic-receptors in coronary artery of pig.

Effect of α,β-Me ATP after pretreated with verapamil

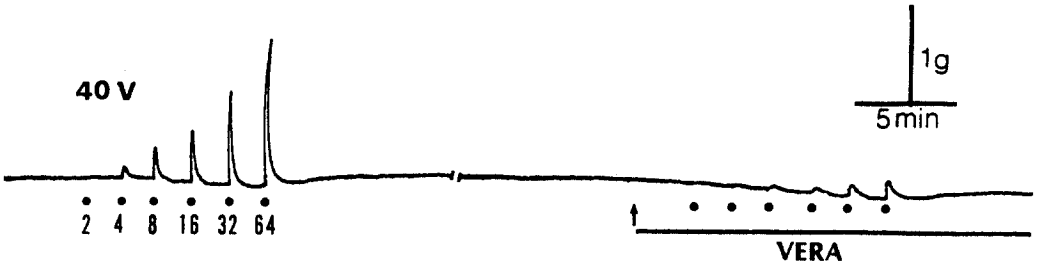

Fig 6. Figure showing effects of verapamil (VERA : 10^{-6}~5×10^{-6}M) on phasic contraction induced by perivascular nerve stimulation (40V, 2~64Hz).

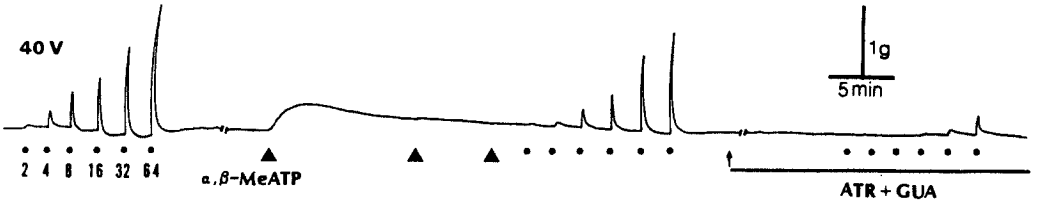

Fig 7. Figure showing effects of 3-times repetitive administration of high dose α,β-Me ATP (10^{-4}M) on phasic contraction induced by perivascular nerve stimulation (40V, 2~64Hz). Atropine (ATR : 10^{-6}M) and guanethidine (GUA : 10^{-6}M) were applied to sample containing α,β-Me ATP. Verapamil (VERA : 10^{-6}~5×10^{-6}M) on phasic contraction induced by perivascular nerve stimulation (40V, 2~64Hz).

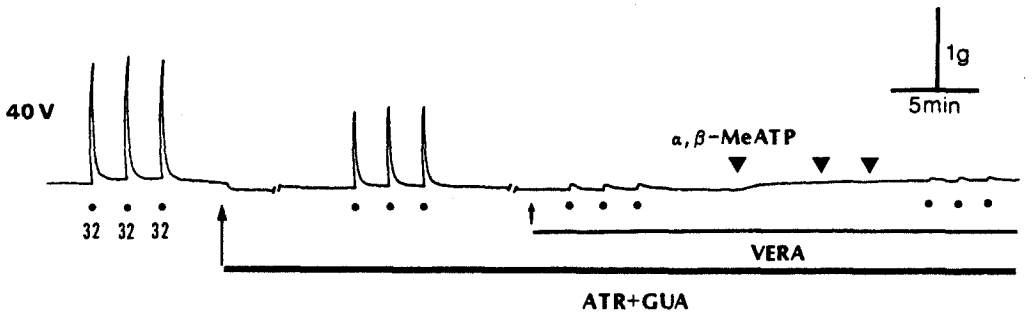

Fig 8. Figure showing effects of α,β-Me ATP (10^{-4}M) on phasic neurogenic contraction induced by perivascular nerve stimulation (40V, 32Hz) under preincubation with verapamil (VERA : 10^{-6}M~5×10^{-6}M).

(VERA), on neurogenic contraction in isolated coronary artery of pig : The neurogenic contractions induced by PNS were significantly inhibited with Ca^{2+}-channel blocker, verapamil (10^{-6}~5×10^{-6}M). Subsequently, α,β-Me ATP did not evoke contractile responses after 3× repetitive administration, and then neurogenic contraction was not changed compared to control under the presence of verapamil (Fig 8). This result implicated that P_{2X}-purinoceptor mediated contractile responses are not dependent on intracellular Ca^{2+} in isolated coronary artery of pig.

Discussion

Stimulation of the autonomic nerves to the heart can affect coronary blood flow in two ways-directly and indirectly. The direct effects result from direct action of the neurotransmitters, acetylcholine from the vagus nerves and norepinephrine from the sympathetic nerves, on the coronary vessels themselves. Cholinergic nerves are generally considered to be dilator to blood vessels in those few vascular beds where they are presumed to operate[13]. In contrast, the coronary arteries of the heart in most mammalian species so far tested respond to exogenous acetylcholine with contraction[15], via muscarinic M_2 receptors[15], and may have a cholinergic constrictor component as well[16,17].

This study showed that PNS induced phasic contractions were completely blocked with neural blocker, tetrodotoxin (TTX), indicating PNS induced phasic contractions were neurogenic responses. Therefore, from this observation the authors considered that the the phasic contraction induced by PNS method appears as a result of releasing endogenous neurotransmitters. Also, the present study revealed that the contractile reponse evoked by PNS was partially inhibited by treatment with atropine, and guanethidine act as synergist on inhibited contractile response with atropine. This result implicated that adrenergic and cholinergic nerve cause excitatory responses induced by PNS. However, these data cannot exclude that adrenergic and cholinergic act as inhibitory nerves because atropine and guanethidine are nonselective antagonist of adrenergic, cholinergic nerve, respectively.

In addition, this study gives a suggestion that the remaining contractile responses after with atropine and guanethidine are due to the other neural activation beyond adrenergic, cholinergic nerve. The striking result were that neurogenic contractile responses in Ca^{2+}-free medium were weaken compared with contractile response in normal Krebs solution. This responses give a suggestion that PNS induced contractile response use the extracellular Ca^{2+} as well as the stored intracellular Ca^{2+} in isolated coronary artery of pig. These observations are consistent with the suggestion of the recent publications, who indicated that both α_1- and α_2-adrenoceptor mediated contractions vascular smooth muscles can activate both Ca^{2+} influx from the extracellular space and Ca^{2+} release from internal stores[18,19]. However, contractions induced by α_1-adrenergic stimulation seem to rely mainly on Ca^{2+} release from agonist-sensitive internal stores, whereas contractions induced by α_2-adrenergic agonists rely

preferentially on Ca^{2+} influx from the extracellular space[19].

There is the suggestion that only a small fraction of cholinergic receptor sites need to be occupied by an agonist like acetylcholine in the guinea-pig ileum to promote maximal Ca^{2+} transport across the plasma membrane and that the activation of the Ca^{2+} channels is not directly coupled to the cholinergic receptor occupation[20,21].

It has been suggested that, in the rabbit bladder muscle, not only there a marked excess of receptor-regulated Ca^{2+} permeable sites in the muscle membrane but also an excess of cholinergic binding sites[22]. Thus, it postulate that the pretreatment for vascular rings with the adrenergic neural blocker guanethidine, the presence during stimulation of the cholinergic muscarinic receptor antagonist atropine or both compounds together, has inhibitory action on the release of intracellular stored Ca^{2+} and extracellular Ca^{2+} influx causing contractile response elicited by PNS.

However, Burnstock *et al*[1] recently suggested that presence of nonadrenergic, noncholinergic (NANC) nerves which contain the neurotransmitters such as histamine[2], serotonine[3], prostaglandin[4], purine nucleoside and nucleotide[5]. Among the substances of putative NANC neurotransmitters, purine nucleotides are considered as the most likely candidate for NANC neurotransmitter. Thus, Burnstock[5] nomenclatured so called as "purinergic nerve fibre" which acts on purine nucleotides. Furthermore, result of effort of many investigators, it demonstrated that ATP can either act as a neurotransmitter or modulate the release and action of other neurotransmitters[13]. The receptors mediating responses to purines have been catagorized in two types, P_1 and P_2, with selectivity for adenosine and adenosine triphosphate, respectively. Among the responses mediated by P_1-purinergic receptors are relaxations of vascular smooth muscle. Whereas, P_2-purinergic receptors can have either contractile or relaxant effects on different blood vessels[6]. Thus Burnstock & Kennedy[7] proposed a subdivision of the P_2-purinoceptor into P_{2X} and P_{2Y}-subtypes.

Recently we reported that the contractile response induced by electrical transmural nerve stimulation was partially blocked by treatment with atropine, and was completely blocked by the desensitization of the P_{2X}-purinoceptor using α,β-Me ATP in isolated urinary detrussor muscle of pig[9]. In the porcine coronary artery, Jeon *et al*[10]. suggested that the neurogenic contractile responses induced by perivascular nerve stimulation was inhibited by the desensitization of the P_{2X}-purinoceptor using 3 times repetitive administration of α,β-Me ATP.

As like the above two reports, the phasic contraction induced by PNS was appeared that it activated smooth muscle contraction by the cholinergic excitatory responses and P_{2X}-purinoceptor mediated excitatory responses.

The present study also revealed that the phasic contractions induced by PNS were inhibited with P_{2X}-purinoceptor desensitization using α,β-Me ATP, subsequently, when sequential treated with atropine and guanethidine, the neurogenic contraction was severely inhibited. These observations are consistent with the suggestions by Park[9] and Jeon[10].

Calcium channel blockers containing diltiazem, verapamil and nifedipine have key role in voltage-dependent Ca^{2+} channels on vascular smooth muscle cells. Chang *et al*[14]. suggested that verapamil can enter into the cytoplasm, where they may exert secondary actions on internal sites of the muscle, such as the sarcoplasmic reticulum.

Most striking were that PNS evoked phasic contractions were significantly inhibited with verapamil, it implicated that neurogenic contractions are mainly due to influx of extracellular Ca^{2+}. At the pretreatment with verapamil, α,β- Me ATP induced nonneurogenic contractions were absence and neurogenic phasic contractions were not changed. It postulated that the origin of Ca^{2+} on P_{2X}-purinoceptor mediated muscle contraction is extracellular Ca^{2+} through Ca^{2+} channels.

In summary, these results demonstrate that the neurogenic phasic contractions induced by PNS are due to adrenergic-, cholinergic- and P_{2X}-purinergic receptors and the origin of Ca^{2+} on P_{2X}-purinoceptor mediated muscle contraction is extracellular Ca^{2+} through plasmalemmal Ca^{2+} channels.

Acknowledgments : We thank to Dr. I-K Park's help at the Gyeongnam Livestock Promotion Institute for supplement of experimental animals.

References

1. Burnstock G, Campbell G, Bennet M, *et al*. Inhibition of the smooth muscle of the taenia coli. *Nature (London)*, 200:581-583, 1963.
2. Satchell DG, Lynch A, Bourke PM, *et al*. Potentiation of the effects of exogenously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridamol and hexobendine. *Eur J Pharmacol*, 19:343-350, 1972.
3. Gershon MD. Nonadrenergic, noncholinergic, autonomic neurotransmission mechanisms. In NRD Bulletin. G. Burnstock., T. Hokefelt., M. D. Gershon., L. L. Iverson., H. W. Kosferlitz., T. H. Szurseweski. *Cambridge Mass Mitt*, 17:414-424, 1979.
4. Bennett T. Innervation of nerve mediated excitation and inhibition of single smooth muscle cells of the avian gizzard. *Comp Biochem Physiol*, 32:669-680, 1970.
5. Burnstock G. Neural nomenclature. *Nature (London)*, 229:282-283, 1971.
6. Kennedy C, Burnstock G. Evidence for two types of P_2-purinoceptors in longitudinal muscle of the rabbit portal vein. *Eur J Pharmacol*, 111:49-56, 1985.
7. Burnstock G, Kennedy C. Is there a basis for distinguishing two types of P_2-purinoceptor? *Gen Pharmacol*, 16:433-440, 1985.
8. Berti-Mattera LN, Wilkins PL, Madhun Z, *et al*. P_2-purinergic receptors regulate phospholipase C and adenylate cyclase activities in immortalized Schwann cells. *Biochem J*, 314:555-561, 1996.
9. Park SE, Hong YG, Shim CS, Jeon SC, Kim JH. Action of P_{2X}-purinoceptor on urinary bladder smooth muscle of pig. *Kor J Vet Res*, 37(1):103-110, 1997.
10. Jeon SC, Shim CS, Kim JH. Effect of purinoceptor to perivascular nerve stimulation on isolated coronary artery of pig. *Kor J Vet Res*, 38(4):730-736, 1998.
11. Kim JH, Hong YG. Neuromudulation on neurogenic contraction of electrical nerve stimulation on isolated renal artery of rabbit. *Kor J Vet Res*, 36(4):821-828, 1996.
12. Kim JH, Shim CS, Jeon SC. Effect of purinergic nerve on relaxation of pig coronary artery. *Kor J Vet Res*, 37(3):533-540. 1997.
13. Dubyak GR, SL-Moatassim C. Signal transduction via P_2-purinergic receptors for extracellular ATP and other nucleotides. *Am J Physiol*, 265:C577-C606, 1993.
14. Chang KC, Ko HJ, Cho SD, Yoon YJ, Kim JH. Pharmacological characterization of effects of verapamil and GS 283 on isolated guinea pig and rat trachealis. *Eur J Pharmacol*, 236:51-60, 1993.
15. Duckles SP. Vascular muscarinic receptors: Pharmacological characterization in the bovine coronary artery. *J Pharmacol Exp Ther*, 246:929-934, 1988.
16. Kalsner S. Prostaglandin mediated relaxation of coronary artery strips under hypoxia. *Prostaglandins Med* 1:231-239, 1978.
17. Kalsner S. Cholinergic constriction in the general circulation and its role in coronary artery spasm. *Brief Review Circ Res*, 65:237-257, 1989.
18. Daly CJ, Dunn WR, McGrath JC, *et al*. An eximination of the sources of calcium for contractions mediated by postjunctional α_1- and α_2-adrenoceptors in several blood vessels isolated from the rabbit. *Br J Pharmacol*, 99:253-260, 1990.
19. Guan YY, Kwan CY, Daniel EE. Does *in vitro* activation of postsynaptic α_2-adrenoceptor utilize intracellular Ca^{2+} for contraction in dog saphenous vein? *Can J Physiol Pharmacol*, 67:1086-1091, 1989.
20. Dunn WR, McGrath JC, Wilson VG. Expression of functional postjunctional α_2-adrenoceptors in rabbit isolated distal saphenous artery-A permissive role for angiotensin II. *Br J Pharmacol*, 96:259-261, 1989.
21. Hurwitz L, Hubbard W, Little S. The relationship between the drug-receptor interaction and calcium transport in smooth muscle. *J Pharmacol Exp Ther*, 183: 117-125, 1972.
22. Hurwitz L, Weissinger J. Effects of variations in extracellular acetylcholine and calcium ion concentration on the operational level of calcium channels in intestinal smooth muscle. *J Pharmacol Exp Ther*, 214: 581-588, 1980.