NITRIC OXIDE MODULATES BIPHASIC CONTRACTILE RESPONSE OF GUINEA PIG VAS DEFERENS TO ELECTRICAL FIELD STIMULATION

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Abstract: Electrical field stimulation (EFS) produced a biphasic contractile response; viz. initial rapid phasic contraction and second slow tonic contraction, in isolated guinea pig vas deferens. Pretreatment with the substrate of nitric oxide (NO) synthase (NOS), 1 mM L-arginine (L-ARG), augmented both the initial rapid and the second slow contractile responses to EFS (5 Hz, 0.5 msec, 30 V, for 30 sec). The increase of stimulation frequency from 5 Hz to 10 Hz or 20 Hz tended to attenuate the augmented responses. On the contrary, pretreatment with an inhibitor of NOS, 0.1 mM N⁰-nitro-L-arginine (L-NNA) suppressed both the initial rapid and the second slow contractile responses to EFS. The suppressive effect on the initial rapid contraction was also attenuated by the increase of stimulation frequency from 5 Hz or 10 Hz to 20 Hz. Contractile response to exogenously administered 1 mM adenosine triphosphate (ATP) tended to be slightly increased and decreased by the treatment with 1 mM L-ARG and 0.1 mM L-NNA, respectively. Contractile response to exogenously administered 10 μM noradrenaline (NA) was almost unaffected by the treatment with 1 mM L-ARG, while the treatment with 0.1 mM L-NNA slightly depressed the response. Potentiated contractile response to 1 mM ATP in the presence of 10 μM NA was further potentiated by the treatment with 1 mM L-ARG, while the response was almost unaffected by the treatment with 0.1 mM L-NNA. These findings may indicate that NO acts mainly on presynaptic site and increases the release of chemical transmitter, ATP or prevents the inactivation of ATP. Also, NO may act, at least in part, on postsynaptic site and potentiates the contractile response to ATP in the presence of NA.
Key words: Nitric oxide, Adenosine triphosphate, Noradrenaline, Guinea pig vas deferens, Electrical field stimulation

There is much evidence that noradrenaline (NA) and adenosine triphosphate (ATP) are cotransmitters in neurotransmission between hypogastric nerve terminals and vas deferens\(^1\)\(^-\)\(^4\) or seminal vesicle\(^5\)\(^-\)\(^6\). On the other hand, nitric oxide (NO) synthase (NOS)-immunoreactive nerve fibres exists in rat vas deferens\(^7\)\(^-\)\(^9\). Many investigators demonstrated that NO acted as second messenger, neurotransmitter or neuromodulator in various tissues\(^10\)\(^-\)\(^15\). However, there are conflicting findings on the effect of NO on the contractile response of vas deferens to electrical field stimulation (EFS). For example, Vladimirova \textit{et al.}\(^16\) demonstrated that the contractile response of rat vas deferens to EFS was suppressed by the treatment with a NOS blocker, N\(^\text{G}\)-nitro-L-arginine methylester (L-NAME), while Allawi \textit{et al.}\(^17\) showed that neither a precursor of NO, L-arginine (L-ARG) nor L-NAME affected the contractile response. The conflict of the results may be due to the difference of the stimulation parameters used. Vladimirova \textit{et al.}\(^16\) used following parameters of EFS; 12 V in stimulation intensity, 0.5 msec in stimulation duration, 1-10 Hz in stimulation frequency, for 10 sec with 10-15 min intervals, while Allawi \textit{et al.}\(^17\) used; 100V, 0.5 msec, 0.1-60 Hz, for 20 sec with 5 min intervals, respectively. To avoid the influence of previous electrical stimulation as possible, the stimulation intervals were taken more than 15 min in the present study. The present study dealt with effects of L-ARG and L-NNA on the contractile responses to ATP, NA or ATP in the presence of NA, and EFS, under the above-mentioned experimental condition. A part of the content of the article has been published as an abstract form\(^18\).

MATERIALS AND METHODS

Male guinea pigs weighing 300-400 g were used. The animals were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg) and exsanguinated. The vas deferens was extirpated. The muscular strip (approx. 1.5 cm length, approx. 70 mg wet tissue weight) was suspended in an organ bath containing 10 ml of Krebs-Ringer solution. The composition of the solution (mM) was NaCl, 119; CaCl\(_2\)•2H\(_2\)O, 2.5; KH\(_2\)PO\(_4\), 12; KCl, 4.7; MgSO\(_4\)•7H\(_2\)O, 12; NaHCO\(_3\), 25 and dextrose, 11. The solution was gassed with a mixture of 95% O\(_2\) and 5% CO\(_2\) and kept at 37°C. Isometric tension development was recorded with a pen recorder (TOA electrics Ltd. FBR-251A) via a force-displacement transducer (Nihon-Kohden TB-612T) and a carrier amplifier (Nihon-Kohden AP-600G). Electrical field stimulation (EFS) was applied through two platinum ring electrodes, using an electrical stimulator (Nihon Kohden MSE-3R) as in the previous report\(^19\). The stimulation parameters were as follows: 5, 10 or 20 Hz stimulation frequency, 0.5 msec pulse duration, 30 V stimulation intensity, for 30 sec. The stimulation inter-
vals were taken more than 15 min. The muscular strips were equilibrated for 90 min under the initial resting tension of approx. 1 g. The maximal height of the phasic contraction and the height of tonic contraction induced by EFS 30 sec after beginning of the stimulation were measured, respectively\(^{20}\). The study was conducted under the control of the Animal Research Committee, in accordance with the Guideline on Animal Experiments in Fukushima Medical University.

**Drugs**

The following drugs were used: adenosine triphosphate disodium (ATP; Wako Pure Chemicals, Osaka, Japan), L-arginine (L-ARG; Sigma Chemicals Co., MO, USA), l-norepinephrine bitartrate (NA; RBI Research Biochemicals International, MA, USA), N\(^\circ\)-nitro-L-arginine (L-NNA; sigma Chemical Co., MO, USA).

**Statistics**

Experimental data are presented as mean±s.e.m. Differences between obtained values were analyzed statistically using unpaired Student's \(t\)-test and \(P < 0.05\) was considered to be significant.

**RESULTS**

1. **Effect of L-ARG on the biphasic contractile response of guinea pig vas deferens to EFS**

EFS elicited biphasic contraction in guinea pig vas deferens; viz. the first rapid phasic and the second tonic contraction (Fig. 1A). The former is elicited by the release of ATP and the latter is mainly elicited by the release of NA from the nerve terminals of the short adrenergic neuron\(^{21-23}\) innervating guinea pig vas deferens\(^{1,2}\).
Fig. 2. Effect of 1 mM L-ARG on the initial phasic contractile response of guinea pig vas deferens to EFS. ○; 20 Hz stimulation frequency, ●; 10 Hz stimulation frequency, □; 5Hz stimulation frequency. N means the number of experiment. Ordinate; % of the control (The height of the contraction in response to EFS before the drug administration was taken as 100%). Abscissa; time (min) after 1 mM L-ARG administration. Mean±s.e.m. Asterisks mean statistically difference from the control (*P<0.05, **P<0.01).

or seminal vesicle). The muscular tension development of the first phasic contractile response to EFS with 5, 10 and 20 Hz was 1.78±0.24, 3.82±0.43 and 5.67±0.57 g (N=15), respectively. The muscular tension development of the second tonic contractile response to EFS with 5, 10 and 20 Hz was 0.61±0.12, 1.65±0.18 and 2.44±0.24 g (N=15), respectively. The administration of 1 mM L-ARG did not affect on the muscular tone and motility of the vas deferens. The first rapid phasic contractile response to EFS (5 Hz and 10 Hz stimulation frequency) was significantly enhanced 15, 30 and 60 min after the treatment with 1 mM L-ARG (Fig. 1B, C and D). The repeated washout of the preparation disappeared the enhancing effect of L-ARG (Fig. 1E). The first phasic contractile response to EFS with 5 Hz stimulation frequency was more markedly enhanced than those to EFS with 10 Hz and 20 Hz stimulation frequencies (Fig. 2). The second tonic contractile response was also enhanced by the treatment with 1 mM L-ARG. The second tonic contractile response to EFS with 5 Hz stimulation frequency was also more markedly enhanced than those to EFS with 10 Hz and 20 Hz stimulation frequencies. The findings may indicate that the effective stimulation frequency for the enhancing effect of NOS immunoreactine nerve fibers is lower than those for the contractile responses to the
Fig. 3. Effect of 1 mM L-ARG on the second tonic contractile response of guinea pig vas deferens to EFS. ○; 20 Hz stimulation frequency, •; 10 Hz stimulation frequency, □; 5 Hz stimulation frequency. N means the number of the experiment. Ordinate; % of the control (The height of the contraction in response to EFS before the drug administration was taken as 100%). Abscissa; time (min) after 1 mM L-ARG administration. Mean±s.e.m. Asterisks mean statistically significant difference from the control (*P < 0.05, **P < 0.01).

purinergic and adrenergic nerve fibers. On the other hand, the enhancing effect of 1 mM L-ARG on the second tonic contractile response lasted shorter than that on the first phasic contractile response (Fig. 3).

2. Effect of L-NNA on the biphasic contractile response of guinea pig vas deferens to EFS

The administration of 0.1 mM L-NNA did not affect on the muscular tone and motility of the vas deferens. The first phasic contractile responses to EFS with 5 Hz and 10 Hz stimulation frequencies were significantly reduced by the treatment with 0.1 mM L-NNA. The inhibitory effects of 0.1 mM L-NNA were prevented 30 min after the addition of 1 mM L-ARG (Figs. 4 and 5). However, the first contractile response to EFS with 20 Hz stimulation frequency was almost unaffected by the treatment with 0.1 mM L-NNA. The first phasic contractile response to EFS with 20 Hz stimulation frequency in the presence of 0.1 mM L-NNA was augmented 30 min after the addition of 1 mM L-ARG (Fig. 5). The second tonic contractile response to EFS with 20 Hz stimulation frequency was significantly reduced by the
Fig. 4. Effect of 0.1 mM L-NNA on the biphasic contractile response of guinea pig vas deferens to EFS (10 Hz in stimulation frequency). A. control, B, C and D. 15, 30 and 60 min after 0.1 mM L-NNA administration, respectively. E. 30 min after 1 mM L-ARG addition. Stimulation time; 30 sec.

Fig. 5. Effect of 0.1 mM L-NNA on the initial phasic contractile response of guinea pig vas deferens to EFS. ○; 20 Hz stimulation frequency, ●; 10 Hz stimulation frequency, □; 5 Hz stimulation frequency. N means the number of the experiment. Ordinate; % of control (the height of the contraction in response to EFS before the drug administration was taken as 100%). Abscissa; time (min) after 0.1 mM L-NNA administration. Mean ± s.e.m. Asterisks mean statistically significant difference from the control (*P < 0.05, **P < 0.01). *§P < 0.05 significant difference from the values obtained 60 min after 0.1 mM L-NNA administration.
treatment with 0.1 mM L-NNA. The inhibitory effect of 0.1 mM L-NNA was not prevented 30 min after the addition of 1 mM L-ARG. The second tonic contractile responses to EFS with 5 Hz and 10 Hz stimulation frequencies were almost unaffected by the treatment with 0.1 mM L-NNA. The second tonic contractile responses to EFS with 5 Hz and 10 Hz stimulation frequencies in the presence of 0.1 mM L-NNA were almost unaffected 30 min after the addition of 1 mM L-ARG (Fig. 6).

3. Effect of L-ARG on the contractile response of guinea pig vas deferens to ATP, NA or ATP in the presence of NA

The administration of 1 mM ATP produced a phasic contractile response. The contractile response to 1 mM ATP had a tendency to be augmented 30 min after the treatment with 1 mM L-ARG, but the augmentation was statistically insignificant. The administration of 10 μM NA produced a slow tonic contractile response. The tonic contractile response to 10 μM NA was unaffected by the treatment with 1 mM L-ARG. The phasic contractile response to 1 mM ATP was markedly potentiated
Fig. 7. Effect of 1 mM L-ARG on the contractile response of guinea pig vas deferens to ATP or ATP in the presence of NA. A; contractile response to 1 mM ATP. B; contractile response to 1 mM ATP 15 min after 1 mM L-ARG administration. C; contractile response to 1 mM ATP in the presence of 10 μM NA. D; contractile response to 1 mM ATP in the presence of 10 μM NA 15 min after 1 mM L-ARG administration.

Fig. 8. Effect of 1 mM L-ARG on the contractile response of guinea pig vas deferens to 1 mM ATP (left column), 10 μM NA (middle column) and 1 mM ATP in the presence of 10 μM NA (right column). N means the number of the experiment. Ordinate; % of control (The height of the contraction in response to 1 mM ATP, 10 μM NA or 1 mM ATP in the presence of 10 μM NA before 1 mM L-ARG administration was taken as 100%). Mean±s.e.m. Asterisks mean statistically significant difference from the control (**P<0.01).
Fig. 9. Effect of 0.1 mM L-NNA on the contractile response of guinea pig vas deferens to 1 mM ATP or 1 mM ATP in the presence of 10 μM NA. A; contractile response to 1 mM ATP. B; contractile response to 1 mM ATP 15 min after 0.1 mM L-NNA administration. C; contractile response to 1 mM ATP in the presence of 10 μM NA. D; contractile response to 1 mM ATP in the presence of 10 μM NA 15 min after 1 mM L-NNA administration.

Fig. 10. Effect of 0.1 mM L-NNA on the contractile response of guinea pig vas deferens to 1 mM ATP (left column), 10 μM NA (middle column) and 1 mM ATP in the presence of 10 μM NA (right column). N means the number of the experiment. Ordinate; % of the control (The height of the contraction in response to 1 mM ATP, 10 μM NA or 1 mM ATP in the presence of 10 μM NA before 0.1 mM L-NNA administration was taken as 100%). Mean±s.e.m. Asterisks mean statistically significant difference from the control (*P<0.05).
in the presence of 10 \mu M NA. The potentiated contractile response to 1 mM ATP in the presence of 10 \mu M NA was further potentiated 15 min after the addition of 1 mM L-ARG (Figs. 7 and 8).

4. Effect of L-NNA on the contractile response of guinea pig vas deferens to ATP, NA, or ATP in the presence of NA

The phasic contractile response to 1 mM ATP had a tendency to be suppressed 15 min after the treatment with 0.1 mM L-NNA, but the suppression was statistically insignificant. The tonic contractile response to 10 \mu M NA was significantly depressed 15 min after the treatment with 0.1 mM L-NNA. The potentiated phasic contractile response to 1 mM ATP in the presence of 10 \mu M NA was almost unaffected 15 min after the treatment with 0.1 mM L-NNA (Figs. 9 and 10).

DISCUSSION

The vas deferens and seminal vesicle are innervated by short adrenergic neurons. It has been widely recognized that the short adrenergic neurons release cotransmitters, ATP and NA\(^1\)\(^-\)\(^6\). The vas deferens is also innervated by nitric oxide synthase (NOS)-immunoreactive nerve fibers\(^5\)\(^-\)\(^8\), which is identical with nicotinamide dinucleotide phosphate (NADPH) diaphorase positive fibers\(^9\). There are conflicting findings on the effect of NO on the contractile response of vas deferens to EFS, ATP or NA as mentioned before. For example, Vladimirova et al.\(^16\) reported that a NOS blocker, L-NAME, depressed the fast, non-adrenergic and slow, adrenergic contraction in response to EFS, in rat vas deferens. The depressive effect of L-NAME was antagonized by the treatment with L-ARG. Contrary to the contractions elicited by EFS, the contractions elicited by ATP and NA were not depressed, rather potentiated by L-NAME. On the other hand, Pinto et al.\(^24\) showed that the contractile response of rat vas deferens to NA was reduced by L-NNA. Allawi et al.\(^17\) demonstrated that neither L-ARG (1 mM) nor L-NAME (100 \mu M) affected the contractile response of rat vas deferens to EFS (0.1 Hz or 2 Hz stimulation frequency). Allawi et al.\(^17\) also showed that another NOS inhibitor, 7-nitro-imidazole (7-N1) caused a dose and stimulation frequency dependent inhibition of the phasic contractile response of rat vas deferens to EFS. However, the inhibitory effect of 7-N1 was due to the mechanisms unrelated to inhibition of NOS.

In guinea pig vas deferens, Crederqvist and Gustafsson\(^25\) showed that NO which was applied as acid nitrite inhibited the "twitch" responses to prolonged transmural nerve stimulation, and tonic "hump" responses were either enhanced or unaffected. On the other hand, a putative donor of NO, sodium nitroprusside enhanced the contractions evoked by EFS (100V, 0.04-1 msec, 1-5 Hz, 10 sec train every 60 sec) in guinea pig vas deferens\(^28\). The sodium nitroprusside-induced enhancement was unaffected by L-NAME\(^28\). Sodium nitroprusside did not alter the magnitude of the
associated excitatory junction potentials). The contractions induced by exogenously administered ATP or NA were not affected by sodium nitroprusside.

Sunano showed that high concentration of sodium nitroprusside potentiated the contraction induced by NA, probably due to the depolarizing action of the drug. L-ARG or another NO donor, S-nitroso-N-acetyl-DL-penicillamine did not affect the contractions evoked by EFS.

In order to be metabolized, L-ARG must enter the intracellular space of NOS-immunoreactive nerve fibers through an active transport system. There, L-ARG may be converted to NO and L-citrulline through the action of neuronal NOS. The generated NO may act on the presynaptic and postsynaptic sites. Then, NO activates the soluble guanylyl cyclase and increases cyclic guanylyl monophosphate (cGMP) in both neuronal and smooth muscle cells. In the present study, L-ARG incubated for more than 15 min, and the interval of EFS was taken more than 15 min. Under the experimental condition, L-ARG alone enhanced the initial phasic and the second tonic contractile responses of guinea pig vas deferens to EFS. Also, L-ARG augmented the contractile response to ATP in the presence of NA. On the contrary, L-NNA suppressed the initial phasic and the second tonic contractile responses to EFS. The suppressive effect of L-NNA on the initial phasic contractile response was antagonized by the treatment with L-ARG. Fujita et al. demonstrated that NA in the presence of guanylyl triphosphate (GTP) caused contraction at a constant Ca²⁺ level in β-escin-permeabilized smooth muscle strips of guinea pig vas deferens (Ca²⁺ sensitization). Fujita et al. suggested that cooperation of ATP and NA in inducing a rapid contraction of guinea pig vas deferens is mainly due to the "Ca²⁺ sensitization" effect of NA. Therefore, it is interesting to study whether NO further increase the "Ca²⁺ sensitization" or not. Concerning the mechanism of the enhancing effect of L-ARG on the contractile response to EFS, the generated NO-induced release of the neurotransmitters (ATP and NA) from nerve terminals through Ca²⁺ and cGMP system may be involved. NO is a cell membrane permeable substance. Therefore, it seems that NO, at least in part, acts on the smooth muscle cells and potentiates the contractions induced by EFS. Also, the possibility that NO inactivates ecto-nucleotidase, neuronally released nucleotidase or increase the sensitivity of P2X receptors can not be excluded. Further study would be required to clarify the precise mechanism of the augmenting effect of NO on the contractile response of guinea pig vas deferens to EFS.

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