

## CONTRACTILE RESPONSES OF PROSTATIC AND EPIDIDYMAL PORTIONS OF ISOLATED RABBIT VAS DEFERENS TO ELECTRICAL FIELD STIMULATION

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**Abstract :** The prostatic and epididymal portions of rabbit vas deferens were different in the reactivity to electrical field stimulation (EFS), adenosine triphosphate (ATP) and noradrenaline (NA). The EFS produced biphasic contractile response ; VIZ. the first rapid twitch like (phasic) and the second slow sustained (tonic) contraction. The ratio of the amplitude of the phasic contraction to that of the tonic contraction in response to the EFS was higher in the prostatic portion than that in the epididymal portion. The increase of stimulation frequency from 5 to 10 and 20 Hz more markedly augmented the tonic contractile response than the phasic contractile response. Then, the ratio of the amplitude of the phasic contraction to that of the tonic contraction decreased according to the increase of the stimulation frequency. The tension development of the phasic and the tonic contractile responses to the EFS in the prostatic portion was significantly larger than those in the epididymal portion. The administration of 1 mM ATP produced phasic contraction. The contractile response to 1 mM ATP was more remarkably appeared in the prostatic portion than in the epididymal portion. The administration of 10  $\mu$ M NA produced slow sustained tonic contraction, which also more markedly appeared in the prostatic portion than in the epididymal portion. The findings may indicate that the prostatic portion of rabbit vas deferens was more strongly innervated by purinergic nerves, and had higher reactivity to EFS, ATP and NA than the epididymal portion.

**Key words :** Rabbit vas deferens, electrical field stimulation, adenosine triphosphate, noradrenaline, purinergic nerve

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## INTRODUCTION

Electrical field stimulation (EFS) or hypogastric nerve stimulation of various species of vas deferens<sup>1-3)</sup> or seminal vesicle<sup>4-5)</sup> produced biphasic contractions; VIZ. the first rapid twitch like (phasic) and the second slow sustained (tonic) contractions. The phasic contraction was induced by adenosine 5'-triphosphate (ATP) and the tonic contraction was mainly induced by noradrenaline (NA) released from the nerve terminals. The cotransmission of ATP and NA has been generally accepted<sup>6)</sup>. On the other hand, the reactivity of vas deferens to EFS, ATP or NE was not uniform along the length of the tissue<sup>2,7,8)</sup>. For example, Sneddon and Machaly<sup>8)</sup> observed that NA was significantly more potent in epididymal segments than in prostatic segments, whereas ATP and  $\alpha, \beta$ -methylene ATP (a stable analog of ATP) was significantly more potent in prostatic segments than in epididymal segments of rat vas deferens.

The length of rabbit vas deferens is much longer than that of rat vas deferens. Then, it seems that rabbit vas deferens is suitable for the study on the regional variation of the reactivity to these stimulants. The present study compared the contractile responses of the prostatic portion of isolated rabbit vas deferens to EFS, ATP and NA with those of the epididymal portion. A preliminary account of these findings has been published as an abstract form<sup>9)</sup>.

## MATERIALS AND METHODS

Male rabbits weighing 1.5-3.0 kg were used. The animals were deeply anesthetized with intramuscular injection of pentobarbital sodium (50-60 mg/kg) and exsanguinated. The vasa deferentia were removed. The prostatic portion (the segment weight;  $69.2 \pm 4.4$  mg, the segment length;  $1.8 \pm 0.1$  cm; mean  $\pm$  S.E.;  $N=14$ ) or the epididymal portion (the segment weight;  $70.8 \pm 4.8$  mg, the segment length;  $1.8 \pm 0.1$  cm; mean  $\pm$  S.E.;  $N=14$ ) of the vas deferens was suspended in an organ bath containing 10 ml of Krebs-Henseleit solution. The differences of the weight and the length between prostatic and epididymal segments were statistically insignificant. The composition of the solution (mM) was NaCl, 110;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 2.5;  $\text{KH}_2\text{PO}_4$ , 1.2; KCl, 4.7;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.2;  $\text{NaHCO}_3$ , 25 and dextrose, 11. The solution was gassed with a mixture of 95%  $\text{O}_2$  and 5%  $\text{CO}_2$ , and kept at 37°C. Isometric tension development was recorded with a pen recorder (TOA Electronics Ltd. FBR-251A) via a force displacement transducer (Nihon Kohden TB612T) and a carrier amplifier (Nihon Kohden AP-600G). EFS was applied through two platinum ring electrodes, using an electrical square wave pulse generator (Nihon Kohden MSE-3R) as described in our previous report<sup>10)</sup>. 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. To avoid the influence of the preceding stimulation as much as

possible, the stimulation intervals were taken more than 15 min. The study was performed under the control of Animal Research Committee, in accordance with the guideline on Animal Experiment in Fukushima Medical University.

### Drugs

Drugs used were adenosine 5'-triphosphate disodium (ATP; Wako Pure Chemicals, Osaka Japan) and 1-noradrenaline bitartrate (NA; Research Biochemicals International, Natick, MA, USA).

### Statistics

Experimental data were presented as  $\text{mean} \pm \text{s.e.m.}$ . Differences between obtained values were analyzed for statistically changes using unpaired Student's *t*-test and  $P < 0.05$  were considered significant.

## RESULTS

### 1. Ratio of amplitude of phasic contraction (P) to that of tonic contraction (T) in response to EFS

EFS with stimulation frequency in 5 Hz (Fig. 1A) or 20 Hz produced biphasic contractile response of the rabbit vas deferens; VIZ. the first rapid phasic (twitch like)(P) and the second tonic (T) contractile response. The amplitude of the tonic contraction was higher than that of the phasic contraction. The ratios of P to T (P/T) in responses of the prostatic segment to 5 Hz and 20 Hz stimulation frequency of

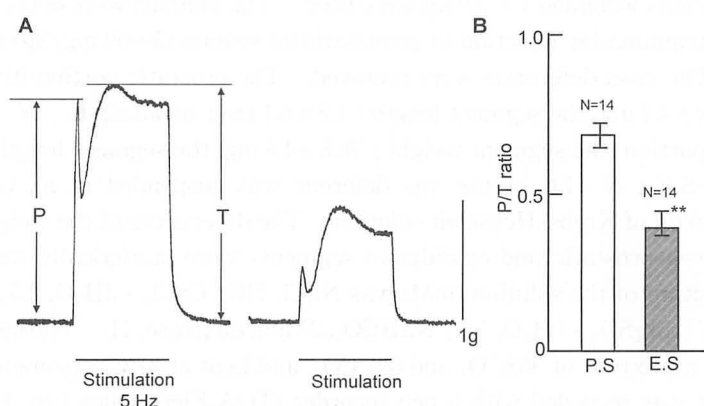


Fig. 1. A; Phasic contraction (P)/tonic contraction (T) ratio of the biphasic contractile response of prostatic (left panel) and epididymal (right panel) segments of rabbit vas deferens to electrical field stimulation (EFS 5 Hz, 0.5 msec, 30 V, for 30 sec). B; P/T ratio of the biphasic contractile response of ptostatic segments (P. S; open column) and epididymal segments (E.S; obliquely striped column) of rabbit vas deferens to the EFS. Ordinate; P/T ratio. The difference between the values obtained in the prostatic and the epididymal segments was statistically significant (\*\* $P < 0.01$ ).  $\text{mean} \pm \text{s.e.m.}$ . N means the number of experiments.

EFS were  $0.69 \pm 0.04$  ( $N=14$ ) and  $0.49 \pm 0.05$  ( $N=14$ ), respectively. The difference between the P/T ratios in response of the prostatic segments to EFS with the stimulation frequency of 5 Hz and 20 Hz was statistically significant ( $P < 0.05$ ). The P/T ratios in response of the epididymal segments to EFS with stimulation frequency in 5 Hz and 20 Hz were  $0.39 \pm 0.07$  ( $N=14$ ) and  $0.33 \pm 0.03$  ( $N=14$ ), respectively. The difference between the P/T ratios in response of the epididymal segments to EFS with the stimulation frequency of 5 Hz and 20 Hz was statistically significant ( $P < 0.05$ ). The difference of P/T ratios between the prostatic segments and the epididymal segments was statistically significant ( $P < 0.01$ ) (Fig. 1B). The tension development in response to EFS was also dependent on the stimulation frequency. The increase of the stimulation frequency from 5 Hz to 20 Hz increased the tension development in both prostatic and epididymal segments (Figs. 2 and 3). The second tonic contractions were more markedly increased than the first phasic contractions in both prostatic and epididymal segments according to the increase of stimulation frequency from 5 Hz to 20 Hz. The finding may indicate that the effective stimulation frequency for the contractile response to adrenergic nerve stimulation is more higher than that for the contractile response to purinergic nerve stimulation. The

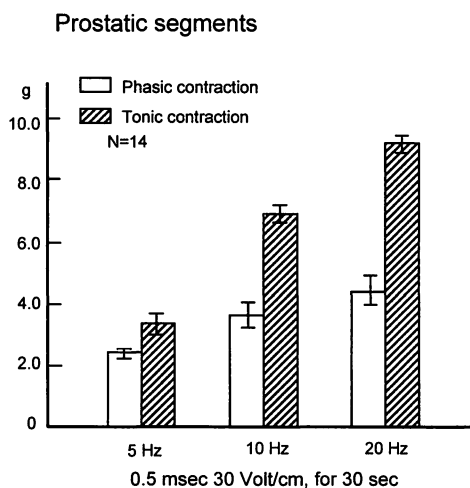


Fig. 2. Phasic (open column) and tonic (obliquely striped column) contractile responses of prostatic segments of rabbit vas deferens to EFS. Stimulation frequency was 5 Hz (left columns), 10 Hz (middle columns) and 20 Hz (right columns). Ordinate; tension development(g). Abscissa; stimulation frequency. Mean  $\pm$  s.e.m.. N means the number of experiments.

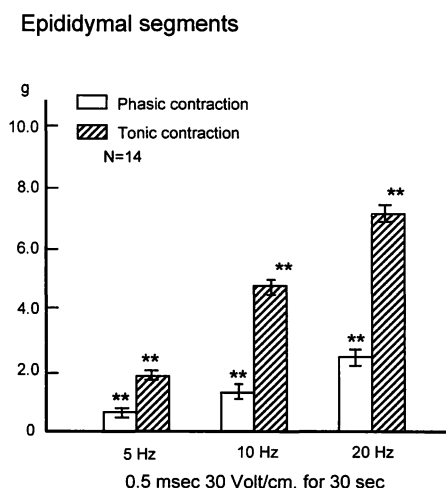


Fig. 3. Phasic (open column) and tonic (obliquely striped column) contractile responses of epididymal segments of rabbit vas deferens to EFS. Stimulation frequency was 5 Hz (left columns), 10 Hz (middle columns) and 20 Hz (right columns). Ordinate; tension development (g). Abscissa; stimulation frequency. Mean  $\pm$  s.e.m.. N means the number of experiments. The difference between the corresponding values obtained in the prostatic and the epididymal segments was statistically significant (\*\* $P < 0.01$ ).

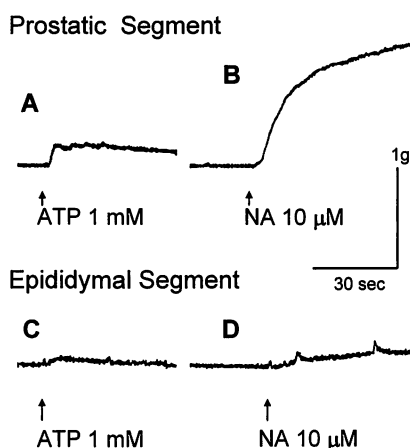


Fig. 4. Contractile responses of prostatic segment (upper trace) and epididymal segment (lower trace) of rabbit vas deferens to 1 mM ATP (A and C) and 10 μM NA (B and D).

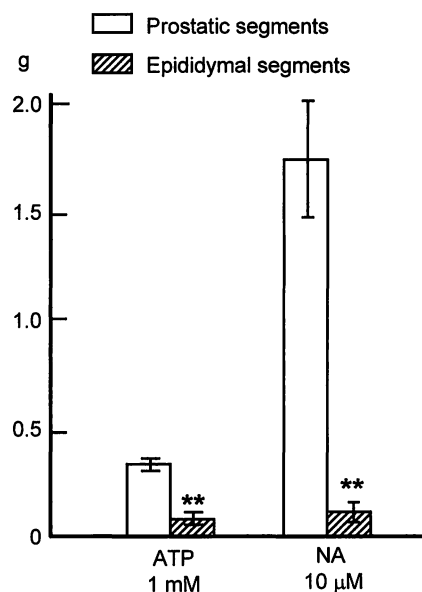


Fig. 5. Contractile responses of prostatic segments (open column) and epididymal segments (obliquely striped column) of rabbit vas deferens. Ordinate; tension development (g). Contractile responses to 1 mM ATP (left columns) and 10 μM NA (middle columns). Mean  $\pm$  s.e.m.. N means the number of experiments. The difference between the corresponding values obtained in the prostatic and the epididymal segments was statistically significant (\*\* $P < 0.01$ ).

first phasic and the second tonic tension development in response of the prostatic segments (Fig. 2) to EFS was more remarkable than that in response of the epididymal segments (Fig. 3). The difference between the tension development induced by the EFS in the prostatic and the epididymal segments was statistically significant ( $P < 0.01$ ).

## 2. Effects of ATP and NA on the prostatic and epididymal segments

ATP at the concentration of 1 mM produced phasic contraction and NA at the concentration of 10 μM produced tonic, long lasting contraction in both prostatic and epididymal segments. The contractile responses to 1 mM ATP and 10 μM NA were more remarkable in the prostatic segments than in the epididymal segments (Figs. 4 and 5). The differences of the tension development induced by 1 mM ATP and 10 μM NA between the prostatic and the epididymal segments were statistically significant, respectively ( $P < 0.01$ ) (Fig. 5).

## DISCUSSION

There is a considerable species variation in the biphasic contractile responses of the vasa deferentia to EFS, and in the contractile responses to ATP and NA<sup>11,12</sup>. Indeed, the amplitude of the phasic contraction elicited by EFS was higher than that of the tonic contraction in the middle portion of guinea pig vas deferens, while the amplitude of the tonic contraction was higher than that of the phasic contraction in the middle portion of rabbit vas deferens<sup>10</sup>.

Furthermore, a considerable amount of cholinergic component existed in both phasic and tonic contractile responses of rabbit vas deferens to EFS<sup>10</sup>. Also, there is a considerable regional variation in purinergic and adrenergic responses of the vasa deferentia<sup>2,7,8,13</sup>. For example, in rat vas deferens, the rapid phasic contractile response to EFS was more prominent at the prostatic portion, while the slow tonic contractile response was more remarkable at the epididymal portion<sup>14</sup>.

Therefore, the present study dealt with a comparison between the contractile responses of the prostatic and epididymal portions of rabbit vas deferens to EFS, ATP and NA using almost equal weight and length of both segments. The present study showed that the amplitude of the first phasic (twitch like) contractile response (P) to EFS was smaller than that of the second slow tonic contractile response (T) in both prostatic and epididymal portions of the rabbit vas deferens. The ratio of P to T (P/T) was significantly higher in the prostatic portion than the epididymal portion. Anton and McGrath<sup>13</sup> demonstrated that the response of rabbit vas deferens in the two portions to EFS was quantitatively different, with the 'secondary' component dominating in the epididymal portion as in the case of rats<sup>14</sup>. The present study also showed that the phasic contractile response of rabbit vas deferens to exogenously administered ATP was more pronounced in the prostatic portion than the epididymal portion. The finding was consistent with those of other species<sup>9</sup>. The difference in potency of ATP between the prostatic and epididymal portion can not be explained by the difference in degradation by ectonucleotidases<sup>15</sup>. The present study showed that the slow tonic contractile response of rabbit vas deferens to exogenously administered NA was weaker in the epididymal portion than the prostatic portion. The finding was contrast with those of other species. For example, Pennefather *et al.*<sup>7</sup> and Sneddon and Machaly<sup>8</sup> observed that NA was significantly more potent in producing contraction in the epididymal segments of rat vas deferens than in the prostatic segments. The difference may be due to the histological variation of the two ends as described by Pennefather *et al.*<sup>7</sup> and the higher availability of adrenergic  $\alpha_1$ -receptor linked  $G_{q/11}$  protein in the epididymal portion<sup>16</sup>. Therefore, it seems that species difference between rat and rabbit vas deferens exists in the potency of the effect of NA on the prostatic and epididymal portion. The regional variation of the reactivity along the length of the vas deferens may contribute to the control of the transport of the sperm. Further study

is needed to clarify the physiological significance.

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