

**A COMPARATIVE STUDY ON CONTRACTILE RESPONSES OF  
RABBIT AND GUINEA PIG VASA DEFERENTIA TO  
ELECTRICAL FIELD STIMULATION**

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**Abstract** : Contractile responses of rabbit and guinea pig vasa deferentia to electrical field stimulation (EFS) are compared. A muscarinic receptor blocking agent, 1  $\mu$ M atropine markedly reduced phasic and tonic contraction induced by EFS (20 Hz, 0.5 msec, 30 V, for 30 sec) in rabbit vas deferens, while it only slightly depressed those in guinea pig vas deferens. Further addition of an adrenergic  $\alpha_1$  receptor blocking agent, 1  $\mu$ M prazosin markedly depressed the second tonic contraction in both rabbit and guinea pig vasa deferentia. In the presence of atropine and prazosin, further addition of a P2X purinoceptor desensitizing agent, 10  $\mu$ M  $\alpha,\beta$ -methylene ATP ( $\alpha,\beta$ -MeATP) abolished the residual phasic contractile response in guinea pig vas deferens, while it partially depressed that in rabbit vas deferens. The administration of 10  $\mu$ M  $\alpha,\beta$ -MeATP in the absence of atropine and prazosin markedly potentiated the phasic contractile response of rabbit vas deferens to EFS, while it depressed that of guinea pig vas deferens. Contractile response of rabbit vas deferens to  $\alpha,\beta$ -MeATP was more potent than those of ATP and 2-methylthioATP (2-Me-thioATP), while these nucleotides had almost same potency in guinea pig vas deferens. These findings may indicate that contribution of cholinergic, adrenergic and purinergic neurotransmission to the contractile response of rabbit vas deferens to EFS is different from that of guinea pig vas deferens.

**Key words** : Rabbit vas deferens, Guinea pig vas deferens, Electrical field stimulation, Adenosine triphosphate, Norepinephrine

The contractile response of the guinea pig vas deferens or seminal vesicle to

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electrical field stimulation (EFS) is biphasic; viz. the first rapid phasic and the second slow tonic contractile response. The former is evoked by the release of adenosine 5'-triphosphate (ATP) and the latter is mainly evoked by the release of norepinephrine (NE) from the hypogastric nerve terminals<sup>1-3</sup>). In the presence of NE, the phasic contractile response of guinea pig vas deferens or seminal vesicle to ATP is markedly potentiated<sup>1,4,5</sup>).

On the other hand, the contractile response of rabbit vas deferens to EFS is also biphasic<sup>6,7</sup>), but the contractile profile was different from that in guinea pig. Therefore, the present investigation dealt with a comparison of pharmacological characteristics of the contractile responses of rabbit and guinea pig vasa deferentia to ATP, NE or EFS. In particular, effect of  $\alpha,\beta$ -methylene ATP ( $\alpha,\beta$ -MeATP), a selective P2X purinoceptor desensitizer<sup>8</sup>), on the contractile responses of rabbit and guinea pig vasa deferentia to ATP or EFS is focused.

#### MATERIALS AND METHODS

Male guinea pigs weighing 200-350 g and male rabbits weighing 1.5-3.0 kg were used. The animals were anesthetized with intramuscular injection of pentobarbital sodium (50 mg/kg) and exsanguinated. The vasa deferentia were removed. The middle portion of the vas deferens (approx. 1.5 cm, approx. 70 mg wet tissue weight) was suspended in an organ bath containing 10 ml of Krebs-Henseleit solution. The composition of the solution (mM) was NaCl, 119; CaCl<sub>2</sub> 2H<sub>2</sub>O, 2.5; KH<sub>2</sub>PO<sub>4</sub>, 1.2; KCl, 4.7; MgSO<sub>4</sub> 7H<sub>2</sub>O, 1.2; NaHCO<sub>3</sub>, 25; and dextrose, 11. The solution was gassed with a mixture of 95%O<sub>2</sub> and 5%CO<sub>2</sub>, 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 were applied through two platinum ring electrodes, using an electrical stimulator (Nihon Kohden MSE-3R) as previous report<sup>9</sup>). The stimulation parameters were as follows; 10 or 20 Hz stimulation frequency, 0.5 msec pulse duration, 30 V stimulation intensity, for 30 sec. The muscular strips were equilibrated for 90 min under resting tension of approx. 1 g. The study was performed 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),  $\alpha,\beta$ -methylene adenosine triphosphate ( $\alpha,\beta$ -MeATP; Sigma Co., St Louis, MO, USA), atropine sulfate (Wako Pure Chemicals, Osaka, Japan), 2-methyl-thio adenosine triphosphate (2Me-ThioATP; Sigma Co., St Louis, MO, USA), 1-norepinephrine bitartrate (Research Biochemicals International, Natick, MA, USA), Prazosin hydrochloride (Wako Pure Chemicals, Osaka, Japan).

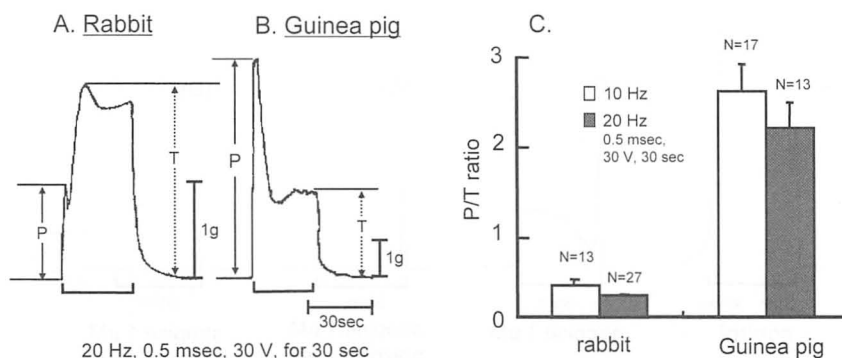


Fig. 1. Phasic contraction (P)/tonic contraction (T) ratio of the biphasic contractile response of rabbit and guinea pig vasa deferentia to electrical field stimulation (EFS). Left panel; Biphasic contractile responses of rabbit (A) and guinea pig (B) vasa deferentia to EFS (20 Hz, 0.5 msec, 30 V, for 30 sec). P: phasic contraction. T: tonic contraction. Right panel; P/T ratio of the biphasic contractile responses of rabbit and guinea pig vasa deferentia to EFS (10 Hz (open column) or 20 Hz (black column)). mean  $\pm$  s.e.m.. N means the number of experiments.

### Statistics

Experimental data are presented as mean  $\pm$  s.e.m.. Differences between obtained values were analyzed for statistically significant 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 produced biphasic contractions in rabbit (Fig. 1A) and guinea pig (Fig. 1B) vasa deferentia; viz. the first rapid phasic and the second tonic contraction. The amplitude of the tonic contraction (T) was higher than that of the phasic contraction in rabbit vas deferens. The ratios of P to T (P/T) in response to 10 Hz or 20 Hz stimulation frequency of EFS in rabbit vas deferens were  $0.35 \pm 0.06$  ( $n=13$ ) or  $0.26 \pm 0.03$  ( $n=27$ ), respectively. On the contrary, the amplitude of the phasic contraction was higher than that of the tonic contraction in guinea pig vas deferens. The ratios of P to T (P/T) in response to 10 Hz or 20 Hz stimulation frequency of EFS in guinea pig vas deferens were  $2.49 \pm 0.31$  ( $n=17$ ) or  $2.11 \pm 0.29$  ( $n=13$ ), respectively (Fig. 1C).

#### 2. Effects of atropine, prazosin and $\alpha, \beta$ -MeATP on the biphasic contractile responses of rabbit and guinea pig vasa deferentia to EFS

*Rabbit vas deferens*; The amplitude of the initial phasic contraction in response to EFS was reduced to  $63.8 \pm 10.0\%$ ,  $56.5 \pm 9.4\%$  and  $22.3 \pm 4.3\%$  ( $n=6$ ) by  $1 \mu\text{M}$  atropine,  $1 \mu\text{M}$  atropine plus  $1 \mu\text{M}$  prazosin, and  $1 \mu\text{M}$  atropine,  $1 \mu\text{M}$  prazosin plus

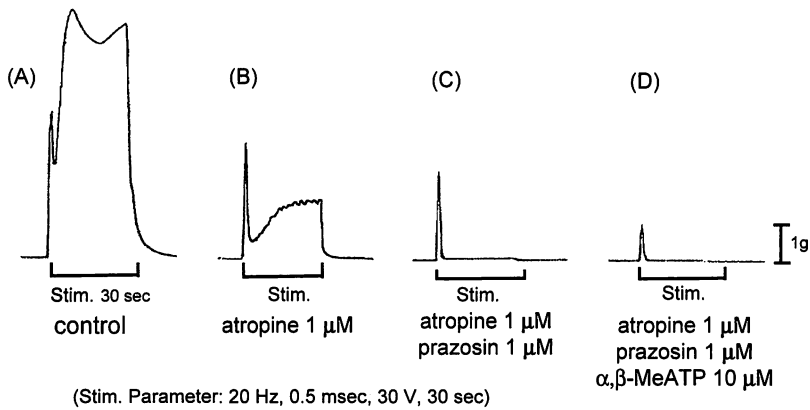


Fig. 2. Effects of atropine, prazosin and  $\alpha,\beta$ -MeATP on the biphasic contractile response of rabbit vas deferens to EFS (20 Hz, 0.5 msec, 30 V, for 30 sec) (A) control, (B) in the presence of 1  $\mu$ M atropine, (C) in the presence of 1  $\mu$ M atropine and 1  $\mu$ M prazosin, (D) in the presence of 1  $\mu$ M atropine, 1  $\mu$ M prazosin and 10  $\mu$ M  $\alpha,\beta$ -MeATP.

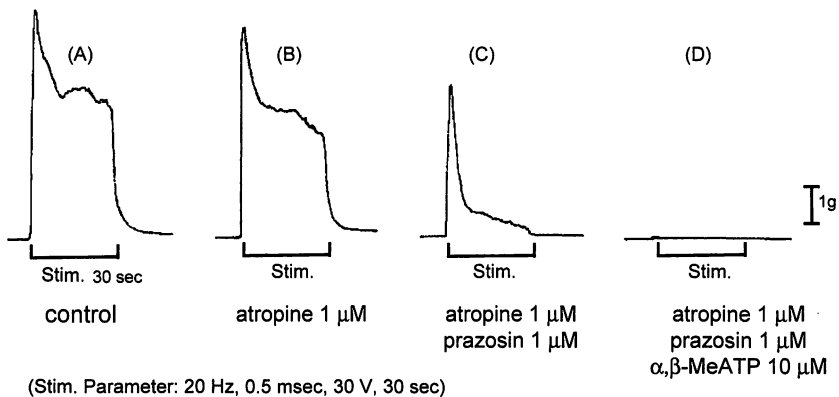


Fig. 3. Effects of atropine, prazosin and  $\alpha,\beta$ -MeATP on the biphasic contractile response of guinea pig vas deferens to EFS (20 Hz, 0.5 msec, 30 V, for 30 sec). (A) control, (B) in the presence of 1  $\mu$ M atropine, (C) in the presence of 1  $\mu$ M atropine and 1  $\mu$ M prazosin, (D) in the presence of 1  $\mu$ M atropine, 1  $\mu$ M prazosin and 10  $\mu$ M  $\alpha,\beta$ -MeATP.

10  $\mu$ M  $\alpha,\beta$ -MeATP, respectively. On the other hand, the amplitude of the second tonic contraction in response to EFS was reduced by  $49.0 \pm 11.1\%$  and  $1.2 \pm 0.9\%$  ( $n=6$ ) by 1  $\mu$ M atropine, and 1  $\mu$ M atropine plus 1  $\mu$ M prazosin, respectively, and abolished in the presence of 1  $\mu$ M atropine, 10  $\mu$ M prazosin plus 10  $\mu$ M  $\alpha,\beta$ -MeATP (Figs. 2 and 4).

*Guinea pig vas deferens*; The amplitude of the initial phasic contraction in response to EFS was reduced to  $86.5 \pm 2.0\%$ ,  $72.0 \pm 4.5\%$  and  $2.5 \pm 1.8\%$  ( $n=6$ ) by 1  $\mu$ M atropine, 1  $\mu$ M atropine plus 1  $\mu$ M prazosin, and 1  $\mu$ M atropine, 1  $\mu$ M prazosin

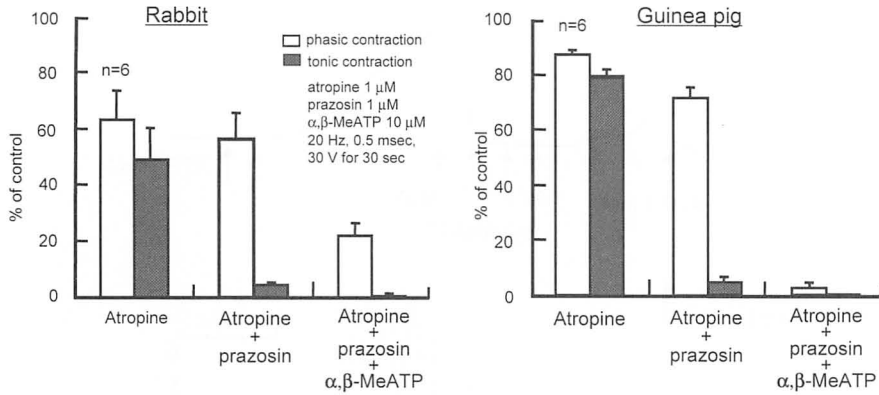


Fig. 4. Effects of  $1 \mu\text{M}$  atropine,  $1 \mu\text{M}$  prazosin and  $10 \mu\text{M}$   $\alpha, \beta$ -MeATP on the biphasic contractile responses of rabbit (left panel) and guinea pig (right panel) vasa deferentia to EFS (20 Hz, 0.5 msec, 30 V, for 30 sec). Open column; phasic contraction. Black column; tonic contraction. Ordinates: % of control. The height of the contractile response to EFS before drug administration was taken as 100%. mean  $\pm$  s.e.m. ( $n=6$ ).

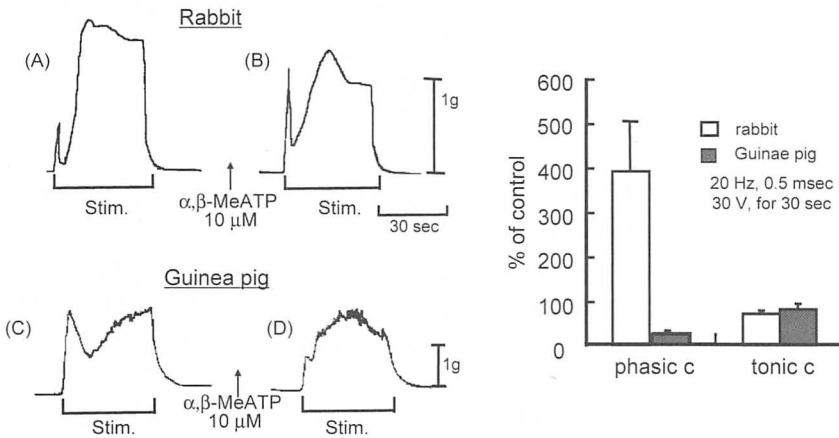


Fig. 5. Effect of  $\alpha, \beta$ -MeATP alone on the contractile responses of rabbit and guinea pig vasa deferentia to EFS (20 Hz, 0.5 msec, 30 V, for 30 sec). Left panel; upper trace: rabbit vas deferens. (A) control, (B) in the presence of  $10 \mu\text{M}$   $\alpha, \beta$ -MeATP. Lower trace: guinea pig vas deferens. (C) control, (D) in the presence of  $10 \mu\text{M}$   $\alpha, \beta$ -MeATP. Right panel; Effect of  $\alpha, \beta$ -MeATP alone on the biphasic contractile responses of rabbit (open column) and guinea pig (black column) vasa deferentia to EFS. Ordinate; % of control (the height of contraction in response to EFS before  $\alpha, \beta$ -MeATP administration was taken as 100%). mean  $\pm$  s.e.m. ( $n=7$ ).

plus  $10 \mu\text{M}$   $\alpha, \beta$ -MeATP, respectively. On the other hand, the amplitude of the second tonic contraction in response to EFS was reduced by  $77.2 \pm 2.7\%$  and  $4.7 \pm 1.8\%$  ( $n=6$ ) by  $1 \mu\text{M}$  atropine, and  $1 \mu\text{M}$  atropine plus  $1 \mu\text{M}$  prazosin, respectively, and abolished in the presence of  $1 \mu\text{M}$  atropine,  $1 \mu\text{M}$  prazosin plus  $10 \mu\text{M}$   $\alpha, \beta$ -MeATP (Figs. 3 and 4).

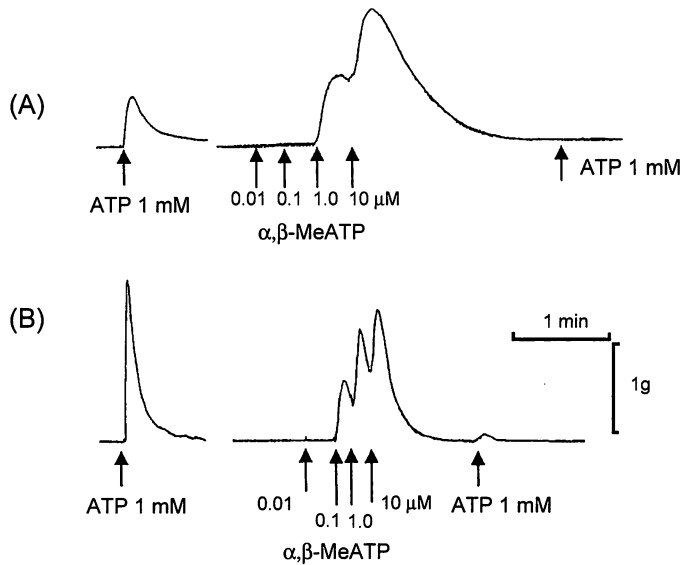


Fig. 6. Effect of  $\alpha,\beta$ -MeATP on the contractile responses of rabbit (A) and guinea pig (B) vasa deferentia to exogenously administered ATP. Alpha, $\beta$ -MeATP was cumulatively administered from  $0.01\ \mu\text{M}$  to  $10\ \mu\text{M}$ . Note that the contractile responses of both vasa deferentia to  $1\ \text{mM}$  ATP was almost completely suppressed in the presence of  $\alpha,\beta$ -MeATP.

3. *Effect of  $\alpha,\beta$ -MeATP on the biphasic contractile responses of rabbit and guinea pig vasa deferentia to EFS*

*Rabbit vas deferens*; The initial phasic contractile response to EFS was markedly augmented by  $387.5 \pm 109.8\%$  ( $n=7$ ) by the treatment with  $10\ \mu\text{M}$   $\alpha,\beta$ -MeATP, while the second tonic contractile response was reduced by  $70.2 \pm 6.9\%$  ( $n=7$ ) (Fig. 5).

*Guinea pig vas deferens*; The initial phasic and the second tonic contractile responses to EFS were reduced to  $22.6 \pm 7.5\%$  and  $78.9 \pm 14.7\%$  ( $n=7$ ) by the treatment with  $10\ \mu\text{M}$   $\alpha,\beta$ -MeATP, respectively (Fig. 5).

4. *Effect of  $\alpha,\beta$ -MeATP on the contractile responses of rabbit and guinea pig vasa deferentia to ATP*

In rabbit vas deferens, ATP at the concentration of  $1\ \text{mM}$  produced phasic contraction, of which duration was somewhat longer than that produced in guinea pig vas deferens. In rabbit vas deferens, more than  $1\ \mu\text{M}$  of  $\alpha,\beta$ -MeATP produced phasic contraction, of which duration was also longer than that produced in guinea pig vas deferens, in concentration-dependent manner (Fig. 6A).

In guinea pig vas deferens, more than  $0.1\ \mu\text{M}$  of  $\alpha,\beta$ -MeATP produced phasic contraction in concentration-dependent manner. In the presence of more than  $10\ \mu\text{M}$  of  $\alpha,\beta$ -MeATP, the contractile response to ATP  $1\ \text{mM}$  was abolished in both

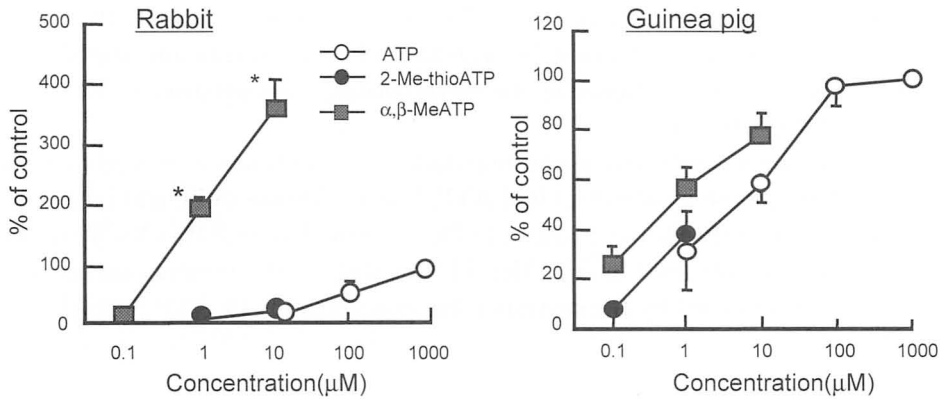


Fig. 7. Contractile responses of rabbit (Left panel) and guinea pig (Right panel) vasa deferentia to ATP (○), 2-Me-thioATP (●) or  $\alpha,\beta$ -MeATP (■). Ordinate; % of control. The height of contraction in response to exogenously administered ATP 1,000  $\mu$ M was taken as 100%. Abscissae; final concentration of administered drugs. mean  $\pm$  s.e.m. ( $n=6-7$ ). \*Statistically significant difference from the values obtained by the corresponding concentration of ATP ( $p < 0.001$ ).

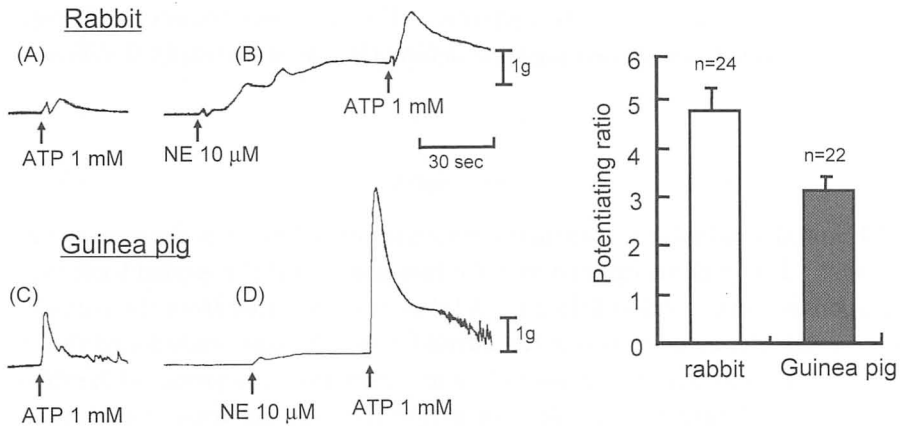


Fig. 8. Potentiating effect of NE on contractile responses of rabbit and guinea pig vasa deferentia to ATP. Left panel; upper trace: rabbit vasa deferentia. (A) ntractile response to 1 mM ATP. (B) ntractile response to 1 mM ATP in the presence of 10  $\mu$ M NE. lower trace; guinea pig vasa deferentia. (C) ntractile response to 1 mM ATP. (D) ntractile response to 1 mM ATP in the presence of 10  $\mu$ M NE. Right panel; Ordinate: Potentiating ratio (the height of contraction elicited by 1 mM ATP in the presence of 10  $\mu$ M NE / the height of contraction elicited by 1 mM ATP in the absence of NE. mean  $\pm$  s.e.m.. N means number of experiments.

rabbit and guinea pig vasa deferentia (Fig. 6).

##### 5. Comparison of contractile responses of ATP, $\alpha,\beta$ -MeATP and 2-Me-thio ATP

In rabbit vasa deferentia, as the amplitude of the contraction induced by 1 mM ATP was 100%, those induced by 10  $\mu$ M ATP, 10  $\mu$ M 2-Me-thioATP and 10  $\mu$ M

$\alpha,\beta$ -MeATP were  $9.7\pm 5.8\%$  ( $n=7$ ),  $11.7\pm 5.6\%$  ( $n=7$ ) and  $357.3\pm 46.2\%$  ( $n=7$ ), respectively. The value obtained by  $\alpha,\beta$ -MeATP was statistically significantly different from the value obtained by the corresponding concentration of ATP or 2-Me-thio ATP ( $p<0.001$ ).

In guinea pig vas deferens, as the amplitude of the contraction induced by 1 mM ATP was 100%, those induced by 1 mM ATP, 1 mM 2-Me-thioATP and 1 mM  $\alpha,\beta$ -MeATP were  $30.4\pm 10.0\%$  ( $n=6$ ),  $24.8\pm 10.7\%$  ( $n=6$ ) and  $55.8\pm 8.4\%$  ( $N=6$ ), respectively. The value obtained by  $\alpha,\beta$ -MeATP was statistically insignificant different from the value obtained by the corresponding concentration of ATP or 2-Me-thio ATP (Fig. 7).

#### 6. Potentiating effect of NE on contractile responses of rabbit and guinea pig vasa deferentia to ATP

Contractile responses of both rabbit and guinea pig vasa deferentia to 1 mM ATP were potentiated in the presence of  $10\ \mu\text{M}$  NE. As the amplitude of the contraction induced by 1 mM ATP in the absence of NE was 1.0, those obtained in the presence of  $10\ \mu\text{M}$  NE in rabbit and guinea pig vasa deferentia were  $4.82\pm 0.48$  ( $n=24$ ) and  $3.16\pm 0.29$  ( $n=22$ ), respectively. The difference between the potentiating ratios of rabbit and guinea pig vasa deferentia was statistically significant ( $p<0.01$ ) (Fig. 8).

### DISCUSSION

EFS produced biphasic contractile responses of isolated vas deferens in various species<sup>9,10</sup>. It is well recognized that the first phasic and the second tonic contractile responses were mainly elicited by ATP and NE released from the nerve terminals, respectively, as described in the introduction. Present study found that smaller first phasic contraction than second tonic contraction was produced in rabbit vas deferens, while larger first phasic contraction than second tonic contraction was produced in guinea pig vas deferens. Therefore, it seems that rabbit vas deferens was predominantly controlled by adrenergic neurotransmission, while guinea pig is predominantly controlled by purinergic neurotransmission. Furthermore, the present study showed that atropine considerably depressed both the first phasic and the second tonic contractile responses of rabbit vas deferens. Then, considerable contribution of cholinergic innervation could not exclude in rabbit vas deferens, besides adrenergic and purinergic innervation. The first phasic (twitch-like) contractile response of rabbit vas deferens to EFS could not be completely depressed by the combined administration of even high concentrations of atropine, prazosin and  $\alpha,\beta$ -MeATP. Therefore, there is possibility that different type of purinoceptor or another neurotransmitter may be involved in the phasic (twitch-like) contraction. The duration of the contraction induced by exogenously administered ATP or  $\alpha,\beta$ -MeATP was longer in the rabbit vas deferens than that in guinea pig vas deferens.



The difference may be due to the different characteristics of ecto-ATPases or the different distribution of heteromultimeric P2X purinoceptors between the rabbit and guinea pig vasa deferentia<sup>11</sup>). The administration of  $\alpha,\beta$ -MeATP potentiated the first phasic (twitch-like) contractile response of rabbit vas deferens, while the first phasic contractile response of guinea pig vas deferens was depressed. The contractile responses of both rabbit and guinea pig vasa deferentia to ATP were abolished (desensitized) in the presence of  $\alpha,\beta$ -MeATP.

Therefore, there is possibility that another neurotransmitter may be involved in the first phasic (twitch-like) contractile response of rabbit vas deferens to EFS, as described before. Presumably,  $\alpha,\beta$ -MeATP also acts on the presynaptic purinergic receptor, which is different subtype of purinergic receptor existing in postsynaptic site, and accelerates the release of another neurotransmitter. It has been reported that various kinds of neurotransmitters are released by the stimulation of purinergic receptors existing in presynaptic site in various tissues<sup>12,15</sup>). There is possibility that purinergic receptor, which regulates the release of an excitatory transmitter, exists in presynaptic site of the nerve innervating rabbit vas deferens, and is different with that of guinea pig vas deferens. Further study needs to clarify the subtype of the purinergic receptor.

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