Cooperation of ATP and Norepinephrine in Inducing Contractile Responses in Guinea Pig Vas Deferens

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Abstract—Stimulation of the hypogastric nerve to the guinea pig vas deferens induced biphasic contraction consisting of a rapid transient phase (mediated by ATP) and a delayed tonic phase (mediated by norepinephrine, NE), whereas stimulations in the presence of selective antagonists caused each contractile phase separately. Stimulation in the absence of antagonist induced a larger rapid transient contraction than that induced by stimulation in the presence of $\alpha_1$-antagonist. Results obtained in separate or simultaneous additions of exogenous ATP and NE showed synergism in the rapid transient contraction. These findings indicate that NE assisted ATP in inducing the hypogastric nerve-mediated contractile response in guinea pig vas deferens, but not vice versa.

Electrical stimuli to the hypogastric nerve cause rapid transient contraction and subsequent tonic contraction of guinea pig vas deferens. Exogenous additions of ATP and norepinephrine (NE) cause rapid transient and tonic contraction, respectively (1-3). Iontophoretic or bath application of ATP causes an excitatory junction potential (e.j.p.) and an action potential when the e.j.p. reaches the threshold for the cell. The e.j.p. fairly closely resembles that induced by stimulation of the hypogastric nerve. The e.j.p.s elicited by nerve stimulation and the rapid transient contraction induced by exogenous ATP are inhibited after desensitization of P2-purinoceptors for ATP by $\alpha,\beta$-methylene ATP. Iontophoretic application of NE does not cause depolarization, although addition of NE to the bath causes slow depolarization, which is not followed by an action potential unless a very high concentration of NE is used (3, 4). Prazosin, a selective $\alpha_1$-antagonist, inhibits tonic contraction. These findings show that ATP and NE are co-released from the hypogastric nerve in guinea pig vas deferens and cause rapid transient and subsequent tonic contraction, resulting in a characteristic biphasic contractile pattern. Thus, the two neurotransmitters cause different mechanical responses and have different effects on the membrane potential of the cells. In this work, we studied the possible interaction between the two transmitters by examining the contractile responses induced by hypogastric nerve stimulation in the absence and presence of selective antagonists and those upon separate or simultaneous additions of ATP and NE to the bath.

Male Wistar guinea pigs (450-700 g) were used. The vas deferens from the prostatic end to the epididymal end with the hypogastric nerve which runs in the mesentery (5, 6) was removed and placed in Krebs-Ringer solution of the following composition: 119 mM NaCl, 4.5 mM KCl, 2.5 mM CaCl$_2$, 1.2 mM NaH$_2$PO$_4$, 0.5 mM MgCl$_2$, 25 mM NaHCO$_3$ and 11 mM glucose (7). A length of 3-4 cm of the hypogastric nerve was freed from adherent tissue and sucked into a suction electrode to a position about 8 mm from the vas deferens. The vas deferens connected to the nerve was suspended in an organ bath (15 ml) of a Magnus apparatus,
aerated with 95% O₂ - 5% CO₂ gas and equilibrated with Krebs-Ringer for 30 min at 37°C. The hypogastric nerve was stimulated by trains of 140 pulses of 0.1 msec duration at a frequency of 20 Hz and supramaximal voltage (50 V). The electrical pulses were confirmed to stimulate preganglionic neurons by showing that the contractile responses were blocked by pretreatment of the preparation with 500 µM hexamethonium. For studies on the contractile responses induced by addition of exogenous agonists to the bath, the vas deferens was freed from the serous coat of the mesentery. Contractile responses were recorded isometrically with a force displacement transducer (TB-651T, Nihon Kohden, Tokyo, Japan) on a rectiorder and an analyzing recorder (Model 3655-12, Yokogawa-hokushin, Tokyo, Japan). A load of 0.75 g was applied as a resting tension. Contractile responses were recorded successively with 10-min intervals between tests. Contraction is expressed as g per g wet wt. of the vas. The maximal contraction of the delayed tonic contraction is represented as the height of the contraction at the end of nerve stimulation. Results were analyzed statistically by Student's t-test, and a P value of <0.05 was regarded to indicate a significant difference. Prazosin hydrochloride and α,β-methylene ATP were from Sigma Chemical Co. (St. Louis, U.S.A.). All other chemicals were of analytical grade.

Stimulation of the hypogastric nerve induced a biphasic contraction, consisting of a rapid transient contraction (first phase) and a delayed tonic contraction (second phase). As the biphasic contraction is mediated by ATP and NE (3, 8), the two phases could be obtained separately by inducing the contractile response in the presence of selective antagonists. Treatment with 1 µM prazosin resulted in significant decrease in the second phase, but also decreased the first phase compared with that induced in the absence of the antagonist (Fig. 1). The contractions in the first phase in the absence and presence of prazosin were 166.0±10.6 (n=14) and 118.3±9.1 (n=14) g/g vas, respectively (P<0.01). After desensitization of P₂-purinoceptors by treatment of 10 µM α,β-methylene ATP, the first phase was scarcely detectable, but the second phase was less affected (Fig. 1). The contractions in the second phase before and after the α,β-methylene ATP treatment were 112.7±13.0 (n=12) and 87.4±9.0 (n=12) g/g vas, respectively (P>0.05). Both the treatment with 1 µM prazosin and that with 10 µM α,β-methylene ATP completely inhibited the contractile response (not shown).

Addition of 50 µM ATP to the organ bath induced a rapid transient contraction. Addition of 50 µM NE induced a delayed contraction that was preceded by a small, shoulder-like contraction. Simultaneous additions of ATP and NE resulted in a larger response in the rapid phase than the simple sum of the contraction induced by ATP and

![Fig. 1. Effects of selective antagonists on the contractile responses induced by hypogastric nerve stimulation. Contractile responses before (Control) and after pretreatment for 8 min with 1 µM prazosin (A) or 10 µM α,β-methylene ATP (mATP) (B). The first upward twitch marks the time of onset of nerve stimulation.](image-url)
the shoulder-like contraction induced by NE (Fig. 2A). The rapid contractions induced by 50 μM ATP without and with 50 μM NE were 49.7±1.7 (n=4) and 79.2±10.5 (n=4) g/g vas, respectively (P<0.05). This synergism was completely inhibited in the presence of 10 μM α,β-methylene ATP (Fig. 2A). There was no difference between the delayed contractions induced by NE without and with ATP. The delayed contractions induced by 50 μM NE without and with 50 μM ATP were 92.9±18.1 (n=4) and 89.8±16.2 (n=4) g/g vas, respectively. Molar ratios of NE and ATP stored in the sympathetic nervous system vary in different organs (9). The ratio in the hypogastric nerve terminals in the guinea pig has not been shown, but synergism of effects on the rapid phase was observed upon the simultaneous additions of ATP and NE over wide concentration ranges (Fig. 2B).

As already shown (3), the exogenous addition of ATP and NE mimicked the first and the second phases, respectively, of the contractile responses induced by hypogastric nerve stimulation. However, there was a significant difference between the first phases of the contraction induced by hypogastric nerve stimulation in the absence and presence of prazosin, which inhibits only the adrenergic component. This result indicates that NE has a helping role in the effect of ATP, but ATP is not involved in the effect of NE. The synergism of ATP and NE observed upon their simultaneous additions supports this helping role of NE. Nakanishi and Takeda (10) first showed that NE markedly potentiates the contractile responses of the guinea pig seminal vesicle induced by exogenously added ATP and by electrical transmural stimulation, and they suggested that a complex of ATP and NE might act as a chemical transmitter. Holck and Marks (11) observed the potentiating effect of NE on the contractile response of guinea pig vas deferens to exogenously added ATP and suggested an interaction between purinergic and alpha adrenoceptor mechanisms.

The concept of co-transmission mediated by ATP and NE in guinea pig vas deferens is now widely accepted. Interaction between the two neurotransmitters in the presynaptic site has been reported; that is, ATP inhibits the release of NE induced by sympathetic nerve stimulation in the rat (12) and mouse (13) vas deferens. However, the physiological significance of co-transmission is still uncertain. The cooperation of ATP and NE suggested in the present study indicates the physiological significance of this co-transmission.

Fig. 2. Cooperative effects of exogenous ATP and NE in inducing the rapid phase. (A): Typical contractile responses induced by exogenous addition of 50 μM ATP, 50 μM NE, and both together. An arrow indicates potentiated rapid phase. The tissue was treated with 10 μM α,β-methylene ATP (mATP) for 8 min. (B): Dose-response curves on simultaneous addition of ATP and NE. The contractions (rapid phase) induced by the indicated concentrations of ATP with and without various concentrations of NE (○) and heights of the shoulder induced by various concentrations of NE alone (●) are shown. Vertical bars indicate S.E.M. (n=4).

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