Effect of α,β-methylene ATP on the potentiation of contractions to field stimulation of rat vas deferens by eledoisin

LEE Chi-Ming (Department of Biochemistry, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong)

ABSTRACT Eledoisin potentiated contractions to field stimulation of rat vas deferens. This effect was antagonized by D-Prop, D-Trp\(^{\text{39}}\)-substance P (0.01 mmo/L), a tachykinin receptor antagonist but not by zotaxin (1 μm/L), an α₁-adrenoceptor antagonist. Desensitization of P_2\text{,P}_{2\text{,P}}\text{-purinergic receptors in rat vas deferens by α,β-methylene ATP (0.03 mmo/L, 2 min) attenuated the contractile response of the tissue to field stimulation and markedly reduced the potentiating effect of eledoisin. α,β-methylene ATP (0.002-0.02 mmo/L) had no significant effect on the Bolton Hunter reagent conjugate of eledoisin \((1^\text{111}I\text{-BHE})\) binding to NK\(_2\) tachykinin receptors in the rat vas deferens. It is concluded that the potentiating effect of eledoisin on the contractions to field stimulation in the rat vas deferens may be the result of an enhancement of purinergic rather than adrenergic neurotransmission.

KEY WORDS α,β-methylene adenosine triphosphate; eledoisin; vas deferens; prazosin; substance P; tachykinin receptors

Substance P (SP) and eledoisin are members of the tachykinin family which share remarkable homology in their carboxyl-terminal sequence: Phe-X-Gly-Leu-Met-NH\(_2\)\(^{10}\). Eledoisin is about a hundred fold more active than SP in potentiating contractions to field stimulation of rat vas deferens\(^{11,12}\). The molecular mechanism underlying this action is unknown. Neurogenic contractions in the rat vas deferens has been reported to comprise 2 components with an α-adrenergic component being predominant in the epididymal segment and a 'non-adrenergic' component being predominant in the prostatic segment\(^{13}\). Although the transmitter responsible for this 'nonadrenergic' component has not been unequivocally identified, there is evidence to suggest that a P\(_2\)-purinergic system involving adenosine triphosphate (ATP) may be involved\(^{14}\). The present study was undertaken to evaluate whether eledoisin potentiated the contractions of vas deferens to field stimulation via an enhancement of adrenergic or purinergic transmission.

MATERIALS AND METHODS

Materials Eledoisin and [D-Pro\(^{\text{2}}\), D-Trp\(^{\text{39}}\)-SP were from Bachem Biochemicals and Peninsula Laboratories respectively. Prazosin HCl was a gift from Pfizer, \((-\text{-})\)-Noradrenaline bitartrate and α,β-methylene ATP were purchased from Sigma. 3-(4-Hydroxy-3-(1^\text{11}^\text{111}I\text{-iodophenyl})propionyl) eledoisin \((1^\text{111}I\text{-BHE})\) (7.1 TBq/mmol) was synthesized and purified according to the method of Casieri and Liang\(^{15}\). The purity of the radioisotopes and peptides used was >95% by HPLC Analyses.

Field Stimulation and spasmodogenic test of isolated rat vas deferens Vas deferens from adult Sprague-Dawley rats were injected into prostatic and epididymal halves. Each tissue was suspended in oxygenated Krebs-bicarbonate solution in a 5 ml water jacketed organ bath at 37°C under a resting load of 1 g. The tissue was stimulated transmurally at supramaximal voltage (60-80 V, 0.1 Hz, 1 ms or 2.5 Hz, 0.5 ms), and the contractions were recorded isometrically as previously described\(^{16}\). Desensitization of P\(_2\)-purinceptors by α,β-methylene ATP To obtain a long-lasting desensitization of the P\(_2\)-purinoceptors, the vas deferens was exposed to α,β-methylene ATP (0.03 mmo/L) for 2 min.
No contractile response to subsequent application of the same drug was obtained as long as this metabolically stable analogue of ATP remained in the organ bath. The tissue, however, was still responsive to noradrenaline indicating a selective denitrogenation of the PGH₂-parasympathetic response by this treatment.

Radioligand binding experiments. The binding of [³H]-Bolton Hunter reagent conjugated edoisin (¹³¹I-BHE) to NK₁ receptors (mSP-E) receptors in fresh membranes of rat vas deferens was performed as previously described [13]. The effects of α, β-methylene ATP (0.002–0.2 mM/L) on the equilibrium specific binding of [³H]-BHE (0.2 mM/L) at 20°C were examined. Nonspecific binding was defined in the presence of SP (0.01 mM/L).

RESULTS AND DISCUSSIONS

In agreement with our previous observation [21], edoisin (0.1 mM/L) caused a marked potentiation of the muscle contractions to field stimulations (2.5 Hz, 5.5 ms and 0.1 Hz, 1 ms) in the rat vas deferens (Fig 1). This was accompanied by a transient increase in basal tone at higher concentrations of edoisin (0.3–1 mM/L). This effect on the resting tension was particularly pronounced in the epididymal segment (Fig 2). Both of these actions of edoisin appeared to be mediated by specific tachykinin receptors as they were antagonized by the presence of a tachykinin receptor antagonist. [D-Pro², D-Trp⁶⁷⁸⁷]-SP

Fig 1. Effects of edoisin (0.1–1 mM/L), prazosin (1 μM/L) and [D-Pro², D-Trp⁶⁷⁸⁷]-SP (0.2) mM/L on the contractions to field stimulation, 2.5 Hz, 5.5 ms, 5.5 Hz, 1 ms, and the direct contractile effects of noradrenaline (NE, 0.01 mM/L) in the rat epididymal vas deferens. Horizontals has indicated the period of field stimulation. Edoisin was applied at (△) while NE was added at (▲) and washed out at (▼).

Fig 2. Effects of α,β-methylene ATP (A, 0.02 mM/L) pretreatment on the potentiation by edoisin (E, 0.3 μM/L) of contractions to field stimulation (0.1 Hz, 1 ms) of prostastic and epididymal segments of the rat vas deferens,

Fig 3. Influence of α,β-methylene ATP (0.55 mM/L, dotted lines) pretreatment on the potentiation by edoisin (1 mM/L) of contractions to field stimulation (0.1 Hz, 1 ms) of prostastic (●, ○) and epididymal (■, ∘) segments of the rat vas deferens.
To examine the role of adrenergic transmission in the potentiation of muscle contractions to field stimulation in rat vas deferens by eleidinol, prazosin, an α₁-adrenoceptor antagonist, was used. In the presence of prazosin (1 μmol/L), the direct contractile response to noradrenaline 0.01 μmol/L was completely blocked (Fig 1). It also markedly attenuated the contractile response to field stimulations (2.5 Hz, 0.1 ms and 0.3 Hz, 1 ms). The same concentration of prazosin, however, did not attenuate the potentiating effect of eleidinol (Fig 1).

Eleidinol (0.1-1 μmol/L) produced a dose-dependent potentiation of the muscle contractions to field stimulation (0.1 Hz, 1 ms) in both epididymal and prostatic vas deferens. The net stimulated response caused by eleidinol was more pronounced in the prostatic segment (Fig 3). There is pharmacological evidence suggesting that both adrenergic and purinergic systems may be involved in the motor innervation in the rat vas deferens, with the purinergic system being predominant in the prostatic segment (4,5). The following experiment was therefore undertaken to evaluate the role of purinergic transmission in the potentiating action of eleidinol in both epididymal and prostatic segments of the rat vas deferens. α,β-methylene ATP caused a dose-dependent transients contraction of the non-stimulated tissue. After exposing the tissues to α,β-methylene ATP 0.03 μmol/L for 2 min, they became refractory to subsequent application of the same drug at the same concentration (Fig 2). This desensitization of the P2-purinoceptors was maintained as long as the drug was not washed out. This treatment attenuated the contractile response of the tissue to field stimulation (0.1 Hz, 1 ms). It also greatly attenuated the potentiating effect of eleidinol (Fig 2). The attenuation being more pronounced in the prostatic than in the epididymal segment.

To examine whether α,β-methylene ATP acts as a NK, (SP-E) tachykinin receptor antagonist, its effect on the binding of [125I]-BHE in fresh rat vas deferens membrane was studied. The specific binding of [125I]-BHE 0.2 nmo/L in the presence of 2, 20 and 200 μmol/L of α,β-methylene ATP were 104 ± 4, 99 ± 3 and 95 ± 6 % (±SD of 3 separate experiments) of the control respectively, indicating little or no direct interaction of this stable nucleotide analogue with NK, tachykinin receptor.

These results suggest that eleidinol may potentiate the contractile responses to field stimulation in the rat vas deferens by enhancing purinergic rather than adrenergic transmission.

REFERENCES
α,β-次甲基腺苷三磷酸对章鱼肽质增强电场刺激大鼠输精管收缩的影响

李志明 （香港中文大学医学院生物化学系，香港）

摘要  通过电场刺激大鼠输精管收缩并评估其效果。实验中使用了α,β-次甲基腺苷三磷酸（0.002-0.2 mmol/L）和L-精氨酸（L-Arg）混合液。结果显示，α,β-次甲基腺苷三磷酸能显著增加输精管的收缩反应，而L-精氨酸单独使用则无明显作用。这些结果表明，α,β-次甲基腺苷三磷酸在增强输精管收缩方面具有潜在的临床应用价值。

关键词 α,β-次甲基腺苷三磷酸；章鱼肽质；输精管；收缩；L-精氨酸；电场刺激