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

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ABSTRACT Eledoisin potentiated contractions to field stimulation of rat vas deferens. This effect was antagonized by [D-Pro^2, D-Trp^{7,9}]-substance P (0.01 mmol/L), a tachykinin receptor antagonist but not by prazosin (1 μmol/L), an α_1-adrenoceptor antagonist. Desensitization of P_2-purinoceptors in rat vas deferens by α, β-methylene ATP (0.03 mmol/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 (0.002-0.2 mmol/L) had no significant effect on ^{125}I-Bolton Hunter reagent conjugate of eledoisin (^{125}I-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)[1]. Eledoisin is about a hundred fold more active than SP in potentiating contractions to field stimulation of rat vas deferens[2]. 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[3,4]. 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[5]. 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.

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MATERIALS AND METHODS

Materials Eledoisin and [D-Pro^2, D-Trp^{7,9}]-SP were from Bachem Biochemicals and Peninsula Laboratory respectively. Prazosin HCl was a gift from Pfizer. (−)-Noradrenaline bitartrate and α, β-methylene ATP were purchased from Sigma. 3-(4-hydroxy, 3-[^{125}I]iodophenyl) propionyl eledoisin (^{125}I-BHE, 74 TBq/mmol) was synthesized and purified according to the method of Cascieri and Liang[6]. The purity of the radioligand and peptides used was >95% by HPLC analyses.

Field Stimulation and spasmogenic test of isolated rat vas deferens Vas deferens from adult ♂ Sprague-Dawley rats were bisected into prostatic and epididymal halves. Each tissue was suspended in oxygenated Krebs-bicarbonate solution in a 5 ml water-jacketed organ bath at 37℃ 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[2,7].

Desensitization of P_2-purinoceptors 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 mmol/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 desensitization of the P_2-purinergic response by this treatment.

Radioligand binding experiments The binding of ^{125}I-Bolton Hunter reagent conjugated eledoisin (^{125}I-BHE) to NK_2(≡SP-E) receptors in fresh membranes of rat vas deferens was performed as previously described[8]. The effects of α, β-methylene ATP (0.002-0.2 mmol/L) on the equilibrium specific binding of ^{125}I-BHE (0.2 nmol/L) at 20℃ were examined. Nonspecific binding was defined in the presence of SP (0.01 mmol/L).

RESULTS AND DISCUSSIONS

In agreement with our previous observation[2], eledoisin (0.1 μmol/L) caused a marked potentiation of the muscle contractions to field stimulations (2.5 Hz, 0.5 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 eledoisin (0.3-1 μmol/L). This effect on the resting tension was

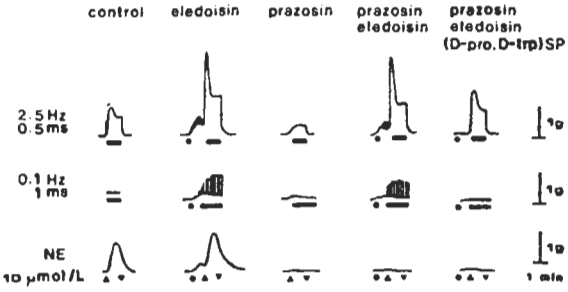

Fig 1. Effects of eledoisin(0.1 μmol/L), prazosin (1 μmol/L) and [D-Pro², D-Trp⁷,⁹]-SP (0.01 mmol/L) on the contractions to field stimulation, 2.5 Hz, 0.5 ms; 0.1 Hz, 1 ms and the direct contractile effects of noradrenaline (NE, 0.01 mmol/L) in the rat epididymal vas deferens. Horizontal bar indicated the period of field stimulation. Eledoisin was applied at (●) while NE was added at (▲) and washed out at (▼).

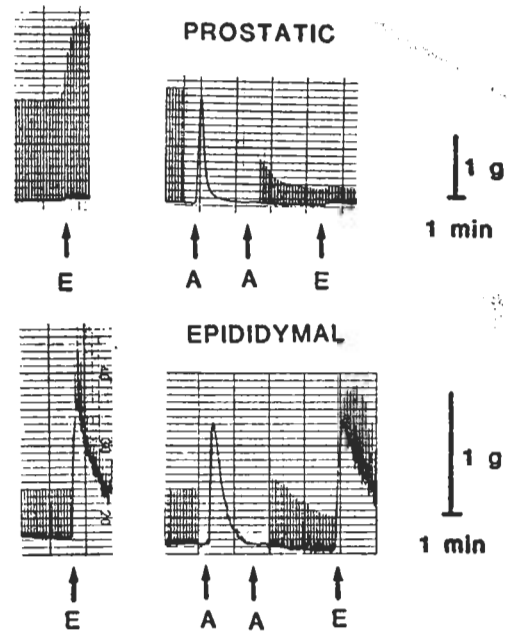

Fig 2. Effects of α,β-methylene ATP (A, 0.03 mmol/L) pretreatment on the potentiation by eledoisin (E, 0.3 μmol/L) of contractions to field stimulation (0.1 Hz, 1 ms) of prostatic and epididymal segments of the rat vas deferens.

particularly pronounced in the epididymal segment (Fig 2). Both of these actions of eledoisin appeared to be mediated by specific tachykinin receptors as they were antagonized by the presence of a tachykinin receptor antagonist, [D-Pro², D-Trp⁷,⁹]-SP

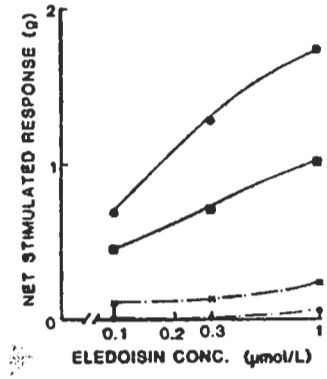

Fig 3. Influence of α,β-methylene ATP (0.03 mmol/L; dotted lines) pretreatment on the potentiation by eledoisin(0.1 to 1 μmol/L) of contractions to field stimulation (0.1 Hz, 1 ms) of prostatic (●, ○) and epididymal (■, ×) segments of the rat vas deferens.

0.01 mmol/L (Fig 1).

To examine the role of adrenergic transmission in the potentiation of muscle contractions to field stimulation in rat vas deferens by eledoisin, prazosin, an α_1-adrenoceptor antagonist was used. In the presence of prazosin (1 μmol/L), the direct contractile response to noradrenaline 0.01 mmol/L was completely blocked (Fig 1). It also markedly attenuated the contractile response to field stimulations (2.5 Hz, 0.5 ms and 0.1 Hz, 1 ms). The same concentration of prazosin, however, did not attenuate the potentiating effect of eledoisin (Fig 1).

Eledoisin (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 eledoisin 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[3,4]. The following experiment was therefore undertaken to evaluate the role of purinergic transmission in the potentiating action of eledoisin. In both epididymal and prostatic segments of the rat vas deferens, α,β-methylene ATP caused a dose-dependent transient contraction of the non-stimulated tissue. After exposing the tissues to α, β-methylene ATP 0.03 mmol/L for 2 min, they became refractory to subsequent application of the same drug at the same concentration (Fig 2). This desensitization of the P_2-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 eledoisin (Fig 2). The attenuation being more pronounced in the prostatic than in the epididymal segment (Fig 3). To examine whether α,β-methylene ATP acts as a NK_2 (SP-E) tachykinin receptor antagonist, its effect on the binding of [^{125}I]-BHE in fresh rat vas deferens membrane was studied. The specific binding of ^{125}I-BHE 0.2 nmol/L in the presence of 2, 20 and 200 μmol/L of α,β-methylene ATP were 104 ± 4, 99 ± 3 and 95±6 % ($\bar{x}$±SD of 3 separate experiments) of the control respectively, indicating little or no direct interaction of this stable nucleotide analogue with NK_2 tachykinin receptors.

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

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α,β-次甲基腺苷三磷酸对章鱼涎肽增强电场刺激大鼠输精管收缩的影响

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提要　章鱼涎肽对电场刺激大鼠输精管收缩有增强作用．此作用可被 0.01 mmol/L 的[D-脯氨酸^{2}, D-色氨酸^{7,9}]-p 物质(一种速激肽受体阻断剂）所拮抗，但 α_1 肾上腺能受体阻断剂哌唑嗪(1μmol/L)则无拮抗效果．用 0.03 mmol/L 的 α，β-次甲基腺苷三磷酸把大鼠输精管的 P_2 嘌呤能受体脱敏 2 min 后，该组织对电场刺激的收缩反应明显减弱，而章鱼涎肽对电场刺激的增强作用亦大幅下降．α，β-次甲基腺苷三磷酸（0.002-0.2 mmol/L）对于 ^{125}I-章鱼涎肽与大鼠输精管 NK_2 速激肽受体的结合作用无显著影响．以上结果显示章鱼涎肽增强电场刺激大鼠输精管收缩的作用可能是通过增强嘌呤能神经传导引起的．

关键词　α,β-次甲基腺苷三磷酸；章鱼涎肽；输精管；哌唑嗪；P 物质；速激肽受体

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