

## Effects of $\alpha,\beta$ -methylene ATP on potentiation of contractions to field stimulation of rat vas deferens by carbachol<sup>1</sup>

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**ABSTRACT** Carbachol (0.1–300  $\mu\text{mol/L}$ ) potentiated contractions to field stimulation (0.1 Hz, 1 ms, supramaximal V) in the rat epididymal and prostatic vas deferens. Desensitization of  $P_2$ -purinoceptors by exposure to  $\alpha,\beta$ -methylene ATP (30  $\mu\text{mol/L}$ ) markedly reduced (>80%) the potentiating effect of carbachol in the prostatic vas deferens but only moderately reduced (about 20%) the maximal stimulated response to carbachol in the epididymal segment. The presence of prazosin (10  $\mu\text{mol/L}$ ) and yohimbine (10  $\mu\text{mol/L}$ ), being selective  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor antagonists, did not modify the attenuation of carbachol potentiation caused by  $\alpha,\beta$ -methylene ATP treatment. At 0.1 mmol/L,  $\alpha,\beta$ -methylene ATP had no significant effect on the binding of [<sup>3</sup>H]QNB to muscarinic cholinergic receptors.

It is concluded that carbachol may potentiate the contractions to field stimulation in the prostatic vas deferens via an enhancement of purinergic neurotransmission. The molecular mechanism of carbachol potentiation in the epididymal vas deferens remains to be established.

**KEY WORDS** vas deferens; prostaglandins; epididymis;  $\alpha,\beta$ -methylene adenosine triphosphate; carbachol; purinergic neural transmission;

Cholinoceptor agonists can enhance the contractile response to nerve stimulation in rat vas deferens<sup>(1,2)</sup>. The molecular mechanism of this action is unknown.

Neurogenic contractions in the rat vas deferens have been reported to comprise 2 components with an  $\alpha$ -adrenergic component being predominant in the epididymal

segment and a 'non-adrenergic' component being predominant in the prostatic segment<sup>(3,4)</sup>. The potentiating effect of carbachol can be antagonized by atropine and is more pronounced in the epididymal segment but it is not attenuated by prazosin, an  $\alpha_1$ -adrenoceptor antagonist<sup>(2)</sup>. Furthermore, carbachol neither significantly modified the direct contractile effect of noradrenaline nor altered the field-stimulation evoked release of noradrenaline from the rat vas deferens. We, therefore, concluded that the potentiating effect of carbachol in the rat vas deferens is not a result of an enhancement of adrenergic neurotransmission<sup>(2)</sup>. Although the transmitter responsible for the 'non-adrenergic' component has not been unequivocally identified, there is evidence suggesting that ATP may be involved<sup>(5-7)</sup>.

Since exposure to a large dose of  $\alpha,\beta$ -methylene ATP can produce a continuous, complete desensitization of the  $P_2$ -purinoceptors<sup>(8)</sup>, it was employed in the present study to examine the role of purinergic mechanism in this potentiating action of carbachol.

### Materials and methods

**Field stimulation and spasmogenic test of isolated rat vas deferens** Vasa deferentia from adult male Sprague-Dawley rats (276  $\pm$  SD 18 g) were bisected into prostatic and epididymal halves. Each tissue was suspended in a 5 ml water-jacketed organ bath at 37°C containing oxygenated modified Krebs-bicarbonate solution, under a resting load of 1 g. After an equilibration period of 30 min with frequent changes of the bathing medium, the vas deferens was

Received 1988 Apr 2 Accepted 1988 Jul 22

<sup>1</sup> A preliminary account of these results were presented at the IUPHAR 10th International Congress of Pharmacology, Sydney, Australia,

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stimulated by means of a Grass stimulator with 2 platinum ring electrodes at supra-maximal voltage (60–80 V, 0.1 Hz, 1 ms) and the contractions were recorded isometrically. The dose–response relationship for carbachol was determined in a cumulative fashion as previously described<sup>(2)</sup>. Net stimulated response induced by the presence of carbachol was calculated by subtracting the basal contractile response to field stimulation in the absence of carbachol.

**Desensitization of P<sub>2</sub>-purinoceptors by  $\alpha,\beta$ -methylene ATP** A long lasting desensitization of the P<sub>2</sub>-purinoceptors can be obtained by exposing the non-stimulated vas deferens to  $\alpha,\beta$ -methylene ATP 30  $\mu\text{mol/L}$  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 norepinephrine indicating a selective desensitization of the P<sub>2</sub>-purinergic response by this treatment.

**Radioligand binding experiments** The binding of 1-quinuclidinyl [*phenyl*-4-<sup>3</sup>H]-benzilate ([<sup>3</sup>H]QNB) to muscarinic cholinergic receptors in fresh membranes of rat cerebral cortex and vas deferens was performed according to reference (9) with modifications. Male Sprague–Dawley rats (320  $\pm$  25 g) were killed by cervical dislocation. Cerebral cortex and vas deferens were homogenized in 10 vol (wt/vol) of ice cold Tris–HCl buffer 50 mmol/L (pH 7.4) using a Brinkmann Polytron PF 10 (setting 6, 10–15 s). The homogenate was centrifuged at 45 000  $\times$  g for 10 min, and the pellet was washed 3 times in 20 volumes of the same buffer. For muscarinic cholinergic receptor binding assays, the membrane pellets were resuspended in the same Tris–HCl buffer to give a protein concentration of about 1 mg/ml (vas deferens) or 0.3 mg/ml (cerebral cortex), and 0.35 ml of the membrane preparation was incu-

bated with [<sup>3</sup>H]QNB at 0.2–2 nmol/L in duplicate. The incubation was carried out in a total volume of 0.5 ml for 1 h at 37°C with or without  $\alpha,\beta$ -methylene ATP. The reaction was terminated by filtration on a glass fibre filter (Whatman GF/B). The filters were washed 4 times with 3 ml aliquots of ice cold Tris–HCl buffer. Radioactivity retained on the filters was determined by liquid scintillation spectrometry. Nonspecific binding was defined in the presence of atropine 1  $\mu\text{mol/L}$ . Specific binding was calculated by subtracting nonspecific binding from total binding.

**Data analysis** The results, unless otherwise stated, were expressed as  $\bar{x} \pm \text{SD}$ , othwhere (n) is the number of separate experiments. Data, where appropriate, were compared using *t* test.

**Drug sources** Atropine sulphate, (–) noradrenaline bitartrate, carbachol HCl, yohimbine HCl and  $\alpha,\beta$ -methylene ATP were purchased from Sigma Chemical Company. Prazosin HCl was a gift from Pfizer. [<sup>3</sup>H]QNB (1406 GBq/mmol) was from Amersham.

## Results and discussion

**Attenuation of carbachol potentiation by  $\alpha,\beta$ -methylene ATP in the prostatic vas deferens** In agreement with our previous observation<sup>(2)</sup>, carbachol, a muscarinic receptor agonist, caused a dose-dependent (0.1–300  $\mu\text{mol/L}$ ) potentiation of the muscle contractions to field stimulation (0.1 Hz, 1 ms) in the rat prostatic vas deferens (Fig 1). This potentiating effect was reversible, in that the field stimulated response returned to control levels after the carbachol was washed out. Twenty minutes after constructing the first carbachol dose-response curve, the non-stimulated prostatic vas deferens was treated with  $\alpha,\beta$ -methylene ATP 30  $\mu\text{mol/L}$ . A pronounced transient contraction of the tissue was observed which gradually declined to the

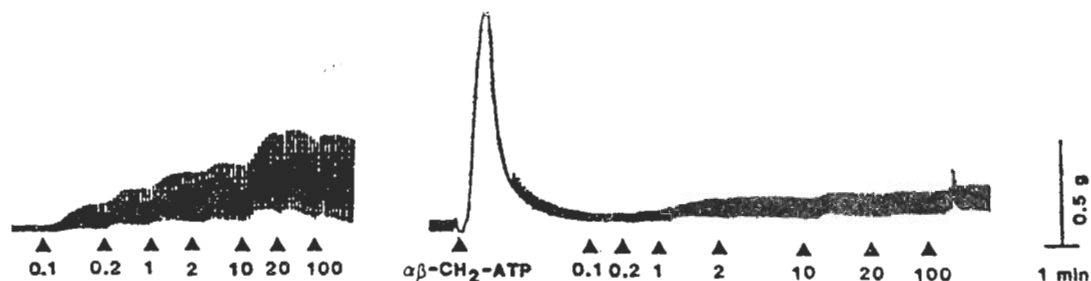


Fig 1. Typical tracing to illustrate the effect of  $\alpha,\beta$ -methylene ATP ( $30 \mu\text{mol/L}$ ) on the potentiating effect of carbachol on contractions to field stimulation in the prostatic rat vas deferens ( $0.1 \text{ Hz}$ ,  $1 \text{ ms}$ , supramaximal voltage). Drugs were added at ( $\blacktriangle$ ). Numerals indicate the concentrations of carbachol in  $\mu\text{mol/L}$ .

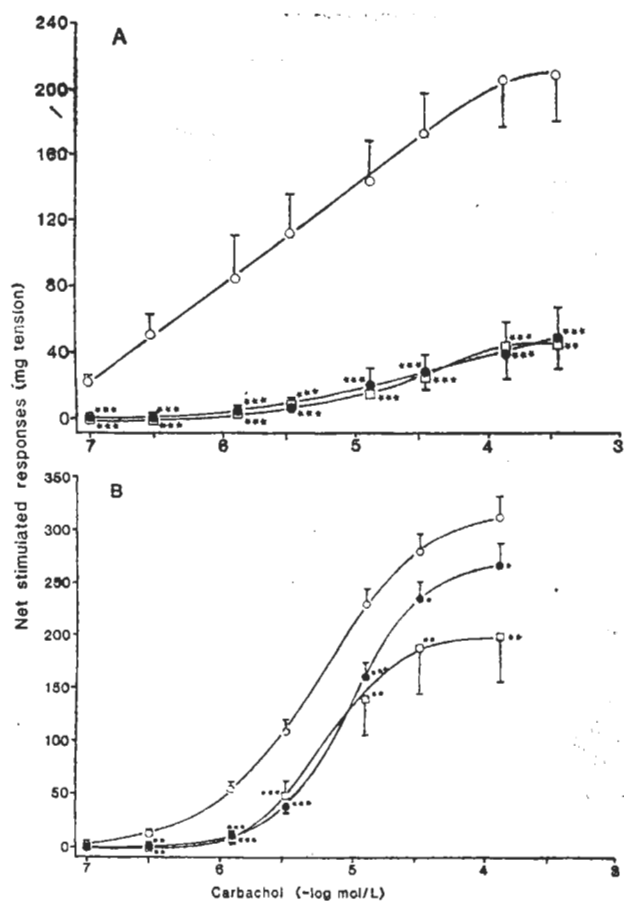


Fig 2. Attenuation of the potentiating effects of carbachol on contractions to field stimulation ( $0.1 \text{ Hz}$ ,  $1 \text{ ms}$ , supramaximal voltage) in the prostatic (A) and epididymal (B) rat vas deferens. ( $\circ$ ) control, ( $\bullet$ )  $\alpha,\beta$ -methylene ATP  $30 \mu\text{mol/L}$ , ( $\square$ )  $\alpha,\beta$ -methylene ATP  $30 \mu\text{mol/L}$  plus prazosin  $10 \mu\text{mol/L}$ ,  $n=5$  (A),  $n=4$  (B) separate experiments,  $\bar{x} \pm \text{SD}$ . \* $P > 0.05$ , \*\* $P < 0.05$ , \*\*\* $P < 0.01$  vs control.

basal level in 2–3 min, presumably as a result of tachyphylaxis. As long as the  $\alpha,\beta$ -methylene ATP remained in the bath, the tissue stayed unresponsive to subsequent application of the same drug (data not shown). While this treatment itself did not alter the contractile response of the tissue to field stimulation significantly, it markedly reduced ( $>80\%$ ) the potentiating effect of carbachol (Fig 1,2). The  $\text{EC}_{50}$  (drug concentration which produce 50% of maximal response) of carbachol was increased from  $4 \pm 3$  in the controls to  $30 \pm 29 \mu\text{mol/L}$  ( $n=5$ ) after desensitization with  $\alpha,\beta$ -methylene ATP. The attenuation of carbachol potentiation induced by  $\alpha,\beta$ -methylene ATP desensitization was reversible. Two hours after washout of the nucleotide analogue, the potentiating effect of carbachol and the contractile response to  $\alpha,\beta$ -methylene ATP were restored (data not shown).

The presence of prazosin  $10 \mu\text{mol/L}$  did not modify the attenuation of carbachol potentiation caused by the  $\alpha,\beta$ -methylene ATP treatment (Fig 2 A). The increase in the  $\text{EC}_{50}$  for carbachol by  $\alpha,\beta$ -methylene ATP was not affected by prazosin ( $20 \pm 5 \mu\text{mol/L}$ ,  $n=5$ ). In control experiments, the replacement of  $\alpha,\beta$ -methylene ATP by vehicle did not significantly alter the second carbachol dose–response curve, indicating little or no desensitization of cholinergic receptors under these experi-

mental conditions (data not shown).

#### Attenuation of carbachol potentiation by $\alpha,\beta$ -methylene ATP in the epididymal vas deferens

As in the prostatic segment,  $\alpha,\beta$ -methylene ATP 30  $\mu\text{mol/L}$  caused a transient contraction of the epididymal vas deferens and rendered the tissue unresponsive to subsequent application of the same drug (data not shown). This treatment reduced the maximal stimulated response to carbachol by about 20% (Fig 2 B). The attenuation of carbachol potentiation was accompanied by a significant change in the  $\text{EC}_{50}$  of carbachol, being  $6 \pm 3 \mu\text{mol/L}$  ( $n=14$ ) in the control versus  $11 \pm 2 \mu\text{mol/L}$  ( $n=7$ ;  $P<0.002$ ) in the presence of  $\alpha,\beta$ -methylene ATP 30  $\mu\text{mol/L}$ .

The presence of prazosin 10  $\mu\text{mol/L}$ , an  $\alpha_1$ -adrenoceptor antagonist, did not further modify the attenuation of carbachol potentiation or the  $\text{EC}_{50}$  of carbachol induced by  $\alpha,\beta$ -methylene ATP treatment (Fig 3). The presence of yohimbine 10  $\mu\text{mol/L}$ , an  $\alpha_2$ -adrenoceptor antagonist, also did not modify the effect of  $\alpha,\beta$ -methylene ATP on the potentiation by carbachol (data not shown).

**Effect of  $\alpha,\beta$ -methylene ATP on [ $^3\text{H}$ ]QNB binding** To examine whether  $\alpha,\beta$ -methylene ATP may act as a muscarinic cholinergic receptor antagonist, its effect on the binding of [ $^3\text{H}$ ]QNB in rat cerebral cortex and vas deferens was studied. When fresh membranes from rat cerebral cortex; epididymal and prostatic vas deferens were

incubated with [ $^3\text{H}$ ]QNB 2 nmol/L, the specific equilibrium binding were  $66 \pm 12$  ( $n=13$ ),  $3.1 \pm 0.2$  ( $n=4$ ) and  $6.7 \pm 0.9$  ( $n=4$ ) pmol/g tissue respectively. There was a gradual reduction of the specific binding as a function of the concentration of atropine ( $\text{IC}_{50} = 25 \pm 15$  nmol/L,  $n=3$ ). However, the presence of  $\alpha,\beta$ -methylene ATP 0.1 mmol/L had no significant effect on [ $^3\text{H}$ ]QNB binding in all 3 tissues (Tab 1), indicating little or no direct interaction of this nucleotide analogue with muscarinic cholinergic receptors.

In the present study, we have demonstrated the selective desensitization of ATP receptors in the rat vas deferens upon exposure to  $\alpha,\beta$ -methylene ATP 30  $\mu\text{mol/L}$ . Moreover, this treatment greatly attenuated the potentiating effect of carbachol in the prostatic segment (Fig 1,2). The possibility that  $\alpha,\beta$ -methylene ATP may act as a muscarinic cholinergic antagonist was ruled out as it did not affect the equilibrium binding of [ $^3\text{H}$ ]QNB in rat cerebral cortex and vas deferens membranes even when tested at a concentration of 0.1 mmol/L (Tab 1). We, therefore, conclude that carbachol may potentiate the contractions to field stimulation in the rat prostatic vas deferens by enhancing the purinergic neurotransmission. Whether carbachol acts presynaptically to enhance the stimulated release of ATP from nerve terminals or postsynaptically to potentiate the contractile response to ATP remains to be established. Preliminary results in our laboratory, however, suggest the site of action of carbachol may be presynaptic rather than postsynaptic as the presence of carbachol 1 and 100  $\mu\text{mol/L}$  did not significantly potentiate the direct contractile response of the prostatic vas deferens to  $\alpha,\beta$ -methylene ATP 3  $\mu\text{mol/L}$  (Lee and Cheung, unpublished observations).

On the other hand, unlike the pros-

**Tab 1. Effects of  $\alpha,\beta$ -methylene ATP 0.1 mmol/L on the binding of [ $^3\text{H}$ ]QNB (1-quinuclidinyl [*phenyl-4- $^3\text{H}$ ] benzilate) in rat vas deferens and cerebral cortex,  $n=3$  separate determinations in duplicate,  $\bar{x} \pm \text{SD}$***

[ $^3\text{H}$ ]QNB (nmol/L)	Binding (% of control)		
	Vas deferens		Cerebral cortex
	Prostatic	Epididymal	
0.2	98 $\pm$ 3	101 $\pm$ 5	96 $\pm$ 5
0.5	102 $\pm$ 5	95 $\pm$ 6	102 $\pm$ 6
2.0	105 $\pm$ 6	100 $\pm$ 3	101 $\pm$ 7

tatic segment, desensitization of ATP receptors in the epididymal vas deferens only moderately reduced the stimulated response to carbachol (Fig 2 B). The presence of prazosin 10  $\mu\text{mol/L}$ , a selective  $\alpha_1$ -adrenoceptor antagonist, did not modify the attenuation of carbachol potentiation induced by  $\alpha,\beta$ -methylene ATP (Fig 2 B). Similar result was also obtained in the presence of yohimbine 10  $\mu\text{mol/L}$ , a selective  $\alpha_2$ -adrenoceptor antagonist. Thus the bulk of the carbachol potentiation observed in the epididymal vas deferens can neither be accounted for by the potentiation of purinergic nor adrenergic neurotransmission. This is very different from eledoisin where its potentiating effect on the contractions to field stimulation in both the prostatic and epididymal rat vas deferens can be markedly attenuated by  $\alpha,\beta$ -methylene ATP pretreatment<sup>(10)</sup>.

In conclusion, the present result supports the notion that carbachol may potentiate the contractions to field stimulation in the prostatic vas deferens via an enhancement of purinergic mechanism. The molecular mechanism of carbachol potentiation in the epididymal vas deferens, on the other hand, remains to be established.

**ACKNOWLEDGMENTS** We are grateful to Miss May Look for her excellent technical assistance and to Mrs Flora Tang for her efficient preparation of the manuscript.

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# $\alpha, \beta$ -次甲基腺苷三磷酸对卡巴胆碱增强电场刺激大鼠输精管收缩的影响

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**提要** 卡巴胆碱(0.1—300  $\mu\text{mol/L}$ )对电场刺激大鼠前列腺和附睾输精管收缩有增强作用。采用30  $\mu\text{mol/L}$ 的 $\alpha, \beta$ -次甲基腺苷三磷酸把嘌呤能受体脱敏后, 前列腺输精管对卡巴胆碱增强电场刺激的收缩反应大幅减弱(>80%), 而附睾输精管的反应只是稍微减弱(约20%)。 $\alpha_1$ 和 $\alpha_2$ 肾上腺能受体阻断剂哌唑嗪(10  $\mu\text{mol/L}$ )和育亨宾(10  $\mu\text{mol/L}$ )均不能加强 $\alpha, \beta$ -次甲基腺苷三磷酸对卡巴胆碱的作用。 $\alpha, \beta$ -次甲基腺苷三磷酸(100  $\mu\text{mol/L}$ )对于 $[^3\text{H}]\text{QNB}$ 与大脑皮层和输精

管M胆碱受体的结合作用无显著影响。

以上结果显示卡巴胆碱增强电场刺激大鼠前列腺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。卡巴胆碱增强电场刺激大鼠附睾输精管收缩的机理则仍有待探讨。

**关键词** 输精管; 前列腺; 附睾;  $\alpha, \beta$ -次甲基腺苷三磷酸; 卡巴胆碱; 嘌呤能神经传递