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The effects of Bay K 8644 and nifedipine on the responses of rat urinary bladder to electrical field stimulation, β,γ-methylene ATP and acetylcholine

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1 Bay K 8644 (0.33 nM to 1 μM) greatly increased the contractions of rat urinary bladder detrusor muscle induced by β,γ-methylene ATP (β,γ-MeATP, 10 μM) and by electrical field stimulation of the purinergic component (the cholinergic response was blocked by atropine).

2 The contractions induced by acetylcholine (ACh, 10 μM) and by electrical field stimulation of the cholinergic component (the purinergic response was blocked following desensitization by α,β-MeATP) were also potentiated by Bay K 8644, although to a lesser extent than the purinergic responses.

3 Nifedipine (1 nM to 3.3 μM) inhibited all the contractions induced by β,γ-MeATP, ACh and electrical field stimulation. However, while the responses to β,γ-MeATP and electrical field stimulation of the purinergic component were almost abolished, a substantial proportion of the responses to ACh and electrical field stimulation of the cholinergic component were nifedipine resistant.

4 The concentration-effect curves for the potentiation by Bay K 8644 of the responses to β,γ-MeATP, ACh and electrical field stimulation were shifted to the right by nifedipine (10 nM). At concentrations greater than 1 μM, Bay K 8644 inhibited contraction.

5 It is concluded that voltage-sensitive calcium channels play an important role in the excitatory mechanical action of P_{2x}-purinoceptor-mediated purinergic responses in the rat urinary bladder, while cholinergic-mediated responses are less dependent on such channels.

Introduction

The actions of adenosine 5'-triphosphate (ATP) as a neurotransmitter or a neuromodulator have been studied in various tissues (Burnstock, 1972; 1986; Gordon, 1986; White, 1988). However, the mechanisms coupling the activation of purinoceptors to the electrophysiological and biochemical changes in the target cells are less clear. It has been observed that ATP can affect the ionic permeability of the plasma membrane of many cell types (Kolb & Wakelam, 1983; Marchenko *et al.*, 1987; Inoue, 1990). Among the ions, calcium may be of importance in mediating the action of ATP (Yatani *et al.*, 1982; Dorn *et al.*, 1989; Nonotte *et al.*, 1989), and in guinea-pig and rat urinary bladder the contractions induced by ATP have been demonstrated to be dependent on extracellular calcium (Iacovou *et al.*, 1988; Bhat *et al.*, 1989). Nifedipine, a 1,4-dihydropyridine, blocks calcium channels and can preferentially inhibit the non-cholinergic component of motor transmission in rat urinary bladder (Iravani *et al.*, 1988). Recently, nifedipine was shown to antagonize the contraction and calcium influx evoked by ATP in guinea-pig urinary bladder (Katsuragi *et al.*, 1990).

In the present study, we have investigated the effects of Bay K 8644, a 1,4-dihydropyridine, which is a calcium channel activator, and nifedipine on the excitatory mechanical response of rat urinary bladder to electrical field stimulation of the purinergic and cholinergic components, and on the contractions elicited by β,γ-methylene ATP (β,γ-MeATP) and acetylcholine (ACh). The aim was to evaluate the relative calcium-dependence of purinergic and cholinergic actions in urinary bladder. In this experiment β,γ-MeATP, a stable analogue of ATP, was chosen to induce contractions instead of ATP since previous experiments have shown that β,γ-MeATP acts more selectively on P_{2X}-purinoceptors, the dominant subclass of purinoceptors in urinary bladders (Brown *et al.*, 1979; Hourani *et al.*, 1985).

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Methods

Male Wistar rats (200–250 g) were killed by asphyxiation with CO_2. Bladders were quickly removed. Four mucosa-free detrusor strips (length 8–10 mm, width 1.5–2 mm) were cut from each bladder. Each strip was threaded through a pair of platinum-ring electrodes, with one end attached to a holder and the other to a Dynamometer UF1 isometric force transducer. The strips were equilibrated for 1 h in organ baths containing modified Krebs solution (mM: NaCl 133, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.6, NaH_2PO_4 1.4, $NaHCO_3$ 16.3, glucose 7.7, pH 7.4) bubbled with 95% O_2 and 5% CO_2 at 37°C. The muscle strips were initially loaded to a tension of 1 g. Mechanical activity was displayed on a Grass polygraph. Electrical field stimulation was achieved by delivering pulses of 0.3 ms duration at supramaximal voltage from Grass SD 9 stimulators. The strips were stimulated at frequencies of 2 Hz and 16 Hz for 10 s. Two of the four strips from the same bladder were incubated with atropine (1 μM) for 10 min to block the cholinergic response. The other two strips were exposed to α,β-MeATP (10 μM) to desensitize the purinergic response (Kasakov & Burnstock, 1983). Guanethidine (5 μM) was present in all the experiments. The responses to β,γ-MeATP (10 μM) and ACh (10 μM) were tested before and after the exposure to atropine and α,β-MeATP. β,γ-MeATP and ACh were washed away immediately after the maximum responses were obtained in order to avoid desensitization. (When the control responses were being measured 0.1% acetone was present in the incubation solution.) The effects of Bay K 8644 (0.33 nM to 3.3 μM) and nifedipine (1 nM to 3.3 μM) were measured after the strips had been incubated with the agents for 10 min. The interaction between Bay K 8644 and nifedipine was demon-

strated by incubating the strips with different concentrations of Bay K 8644 and nifedipine (10 nM).

All the drugs were dissolved in distilled water except Bay K 8644 and nifedipine, which were dissolved in acetone. As Bay K 8644 and nifedipine solutions are sensitive to light they were kept in the dark and the organ baths were tightly wrapped with black plastic sheets. The total volume of added solutions was less than 0.5% of the capacity of the organ bath.

Drugs

β,γ-MeATP (Sigma), α,β-MeATP (Sigma), Bay K 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate, a gift from Bayer AG), nifedipine (Bayer AG), atropine sulphate (Antigen Ltd), guanethidine (Ciba).

Statistical analysis

The responses were calculated as a percentage increase or reduction compared with the relevant control groups. The results are expressed as mean ± s.e.mean. EC_{50} and IC_{50} values were calculated with Litchfield and Wilcoxon methods. Student's *t* test was used to test for significant differences between groups. Probability values of less than 0.05 were considered significant.

Results

Bay K 8644 potentiated the responses of detrusor strips to electrical field stimulation, β,γ-MeATP and ACh in a concentration-dependent manner in the range 0.33 nM to 1 μM (Figure 1a–f). When the concentration of Bay K 8644 was above 1 μM, the maximal responses were reduced, so the concentration-effect curve became bell-shaped. Bay K 8644 had a much greater effect on the contractions induced by β,γ-MeATP than on those induced by ACh (Table 1). The responses of atropine-treated detrusor strips to electrical field stimulation were more strongly increased by Bay K 8644 than were those of α,β-MeATP-desensitized strips (Table 1). The EC_{50} values showed no significant differences (Table 2). Bay K 8644 also increased the resting tension and spontaneous activity in a concentration-dependent manner (in the range 0.33 nM to 1 μM).

Nifedipine inhibited all the responses tested (Figure 2a and b). The responses to β,γ-MeATP and electrical field stimulation in atropine-treated strips were nearly or completely abolished, while a proportion of the responses to ACh and electrical field stimulation in α,β-MeATP-desensitized strips was nifedipine-resistant (Table 1). The nifedipine-resistant contractions were completely abolished by atropine. There was no significant difference in IC_{50} values, except that the effect on the responses to electrical field stimulation at 16 Hz showed a higher IC_{50} because the response included a higher proportion of the nifedipine-resistant component. In contrast to the effect of Bay K 8644, nifedipine reduced the resting tension and spontaneous activity. When the concentration was above 100 nM, all the spontaneous contractions were abolished.

All the concentration-effect curves for Bay K 8644-induced

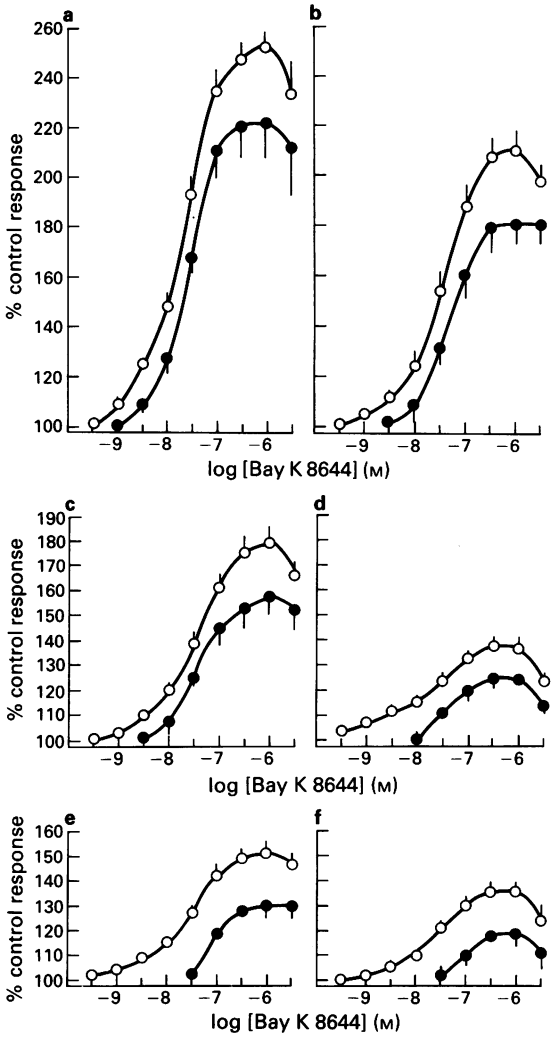

Figure 1 Concentration-response relationship for the effect of Bay K 8644 on the contraction of rat urinary bladder detrusor muscle induced by (a) β,γ-methylene ATP (β,γ-MeATP, 10 μM), (b) field stimulation of purinergic component at 2 Hz, (c) field stimulation of purinergic component at 16 Hz, (d) acetylcholine (ACh, 10 μM), (e) field stimulation of cholinergic component at 2 Hz, and (f) field stimulation of cholinergic component at 16 Hz, in the absence (○) and presence (●) of nifedipine (10 nM). Electrical field stimulation parameters: supramaximal voltage, 0.3 ms duration and 10 s stimulation. Cholinergic responses were blocked by atropine (1 μM), purinergic responses were desensitized by α,β-MeATP (10 μM). Each point represents the mean of 7 experiments; vertical lines show s.e.mean.

Table 1 The maximal effects of Bay K 8644 (0.33 nM to 3.3 μM) and nifedipine (1 nM to 3.3 μM) on the responses of rat urinary bladder detrusor to β,γ-methylene ATP (β,γ-MeATP, 10 μM), acetylcholine (ACh, 10 μM) and electrical field stimulation and the maximal effects of Bay K 8644 in the presence of nifedipine (10 nM)

			Field stimulation			
			Purinergic		*Cholinergic*	
	β,γ-MeATP	*ACh*	*2 Hz*	*16 Hz*	*2 Hz*	*16 Hz*
Bay K 8644	252 ± 5***	138 ± 4	210 ± 8***	179 ± 7***	151 ± 5	136 ± 4
Bay K 8644 + nifedipine	221 ± 4***	125 ± 2	181 ± 8***	158 ± 8***	130 ± 5	119 ± 5
Nifedipine	2 ± 1***	16 ± 2	0 ± 0***	2 ± 1***	6 ± 1	23 ± 3

Data shown are mean ± s.e.mean of the percentage of the control response, $n = 7$.
Significant difference between the relevant groups of β,γ-MeATP and ACh, field stimulation of purinergic and cholinergic components; *** $P < 0.001$.

Table 2 The EC_{50} values for the effect of Bay K 8644 (0.33 nM to 3.3 μM) on the responses of rat urinary bladder detrusor to β,γ-methylene ATP (β,γ-MeATP, 10 μM), acetylcholine (ACh, 10 μM) and electrical field stimulation in the absence and presence of nifedipine (10 nM) and the IC_{50} values for nifedipine (1 nM to 3 μM) on the response

			Field stimulation			
			Purinergic		*Cholinergic*	
	β,γ-MeATP	*ACh*	*2 Hz*	*16 Hz*	*2 Hz*	*16 Hz*
Bay K 8644	25 ± 3	17 ± 5††	26 ± 6	31 ± 5	25 ± 4†††	27 ± 3†††
Bay K 8644 + nifedipine	34 ± 5	43 ± 5	40 ± 9*	36 ± 6*	87 ± 9	71 ± 13
Nifedipine	33 ± 7	34 ± 4	44 ± 6	45 ± 6*	40 ± 5	69 ± 9

Data shown are mean values (in nM) ± s.e.mean, $n = 7$.
Significant differences between the relevant groups of β,γ-MeATP and ACh, field stimulation of purinergic and cholinergic components are indicated by * $P < 0.001$.
Significant differences between the groups in the absence and presence of nifedipine are indicated by †† $P < 0.01$, ††† $P < 0.001$.

potentiation of the responses to β,γ-MeATP, ACh and electrical field stimulation were shifted to the right in the presence of nifedipine (10 nM) (Figure 1a–f). The maximal responses were also suppressed. The EC_{50} values for the effect of Bay K 8644 on β,γ-MeATP and stimulation of purinergic nerves in the presence of nifedipine (10 nM) showed no significant difference compared with those in the absence of nifedipine, owing to the suppression of the maximal responses. Nevertheless, the EC_{50} values for the effect of Bay K 8644 on the responses to ACh and electrical field stimulation of the cholinergic component were still significantly increased in the presence of nifedipine (10 nM).

Discussion

The present study shows that contractions of rat urinary bladder elicited by β,γ-MeATP and electrical field stimulation of the purinergic nerves can be greatly potentiated by Bay K 8644, and nearly abolished by nifedipine. This provides further evidence that the excitatory mechanical action of purinergic nerves is dependent on extracellular calcium, and that the activation of P_{2X}-purinoceptors may lead to the opening of calcium channels. In guinea-pig and rat urinary bladder contractions induced by ATP are greatly reduced in low calcium calcium channels. In guinea-pig and rat urinary bladder the contractions induced by ATP are greatly reduced in low calcium or calcium-free solutions (Iacovou *et al.*, 1988; Bhat *et al.*, 1989). A recent study found that the contraction and calcium-influx evoked by ATP in guinea-pig urinary bladder smooth muscle can be antagonized by nifedipine (Katsuragi *et al.*, 1990), which is in good agreement with the results presented in this paper. Intracellular recording techniques have revealed that extracellular ATP can elicit fast inward currents in smooth muscle cells isolated from guinea-pig bladder (Marchenko *et al.*, 1987; Inoue, 1990). In other tissues, the role of calcium in the action of ATP and purinergic nerve stimulation has also been studied. In guinea-pig vas deferens it has been shown that, in contrast to the action of noradrenaline, nifedipine can block the action potentials and contractions elicited by electrical field stimulation and ATP, while Bay K 8644 augments the action potentials and contractions (Burnstock *et al.*, 1986; MacKenzie *et al.*, 1988). In the dog mesenteric artery, nifedipine can selectively inhibit the purinergic component of the contractions evoked by sympathetic nerve stimulation (Omote *et al.*, 1989), and in snail neurones nanomolar concentrations of extracellular ATP can activate membrane calcium channels (Yatani *et al.*, 1982). Also, P_2-purinoceptor-mediated calcium mobilization across the cell membrane has been observed in rat isolated alveolar type II cells (Dorn *et al.*, 1989), murine thymocytes (El-Moatassim *et al.*, 1989), human promyelocyteic cell line HL60 (Nonotte *et al.*, 1989), Ehrlich ascites tumour cells (Dubyak & De Young, 1985), pancreatic β-cells (Gylfe & Hellman, 1987) and rat parotid acinar cells (McMillian *et al.*, 1987).

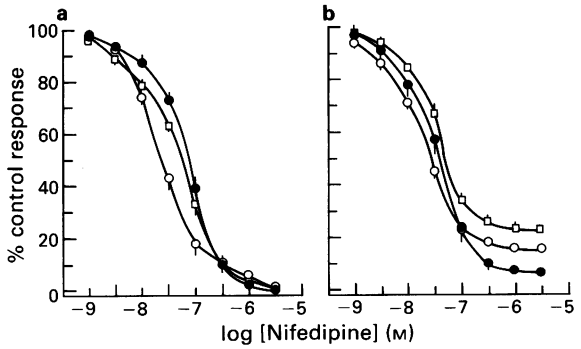

Figure 2 Concentration-response relationship for the effect of nifedipine on the contraction of rat urinary bladder detrusor muscle induced by (a) β,γ-methylene ATP (β,γ-MeATP, 10 μM, ○) and field stimulation of the purinergic component at 2 Hz, (●) and 16 Hz (□), (b) acetylcholine (ACh, 10 μM, ○) and field stimulation of the cholinergic component at 2 Hz (●) and 16 Hz (□). Electrical field stimulation parameters: supramaximal voltage, 0.3 ms duration, and 10 s stimulation. In (a) cholinergic responses had been blocked by atropine (1 μM); in (b) purinergic responses had been desensitized by α,β-MeATP (10 μM). Each point represents the mean of 7 experiments and vertical lines show s.e.mean.

Compared with the contractions evoked by β,γ-MeATP and electrical field stimulation of the purinergic component, the responses to ACh and electrical field stimulation of the cholinergic component were less affected by Bay K 8644. The inhibitory effects of nifedipine on the responses to ACh and cholinergic nerve stimulation were significant, but a proportion of the contraction was nifedipine-resistant. Previous results have indicated that nifedipine reduces the amplitude of nerve-mediated rat bladder contraction by about 70% (Maggi *et al.*, 1988) and the amplitude of nerve-mediated guinea-pig urinary bladder contractions by 62% (Adamson *et al.*, 1986). The nifedipine-resistant contractions were still cholinergic because they were abolished by atropine. In gastrointestinal systems, such as the mouse isolated distal colon, contractile activity induced by ACh is barely enhanced by Bay K 8644 and partially inhibited by nifedipine (Fontaine & Lebrun, 1988), and in rat duodenal smooth muscle the response induced by ACh is not modified by Bay K 8644 but can be significantly decreased by nifedipine (Coruzzi *et al.*, 1986). This indicates that the contractile response to ACh is only partially dependent on the dihydropyridine-sensitive calcium channels. However, the reason for the discrepancy between the sensitivities to Bay K 8644 and nifedipine is not clear at present.

All the concentration-effect curves for the potentiation by Bay K 8644 of the responses to β,γ-MeATP, ACh and electrical field stimulation were shifted to the right by nifedipine. The interaction between Bay K 8644 and nifedipine was not typically competitive because the maximal response to Bay K 8644 was suppressed. Increasing the concentration of Bay K 8644 did not surmount the inhibitory effect of nifedipine, on the contrary, when the concentration of Bay K 8644 exceeded

1 μM its effect became negative. This characteristic of Bay K 8644 has been observed in many experiments (Bechem & Schramm, 1987). The inhibitory effect of Bay K 8644 may not result from the use of a racemic compound because there is no obvious difference between the effects of the racemic compound and (−)-Bay K 8644 (Kass, 1987). One explanation is that there are two dihydropyridine receptors, i.e., excitatory and inhibitory, and when the concentration of Bay K 8644 is above a certain level it also activates the inhibitory receptors (Thomas *et al.*, 1984; Lee *et al.*, 1987). In guinea-pig heart, nifedipine even shows a small positive inotropic effect at low concentrations (about 4 nM) (Thomas *et al.*, 1984). These results suggest that Bay K 8644 and nifedipine are not pure agonists or antagonists of calcium channels. At high concentrations Bay K 8644 has been shown to interfere with intracellular activity, which may also account for the bell-shaped concentration-response curve of Bay K 8644 (Movsesian & Adelstein, 1984).

The voltage-sensitive calcium channels on excitable cell membranes have been classified into three subtypes, i.e., T, N and L. T- and L-type channels are important in smooth muscle activities (Miller, 1987). 1,4-Dihydropyridines primarily act on L-type channels (Nowycky *et al.*, 1985). From the present experiments the involvement of the L-type channels in the action of purinergic responses seems obvious, but a process linking the activation of P_{2X}-purinoceptors and the opening of calcium channels remains to be established. Receptor-operated calcium permeable channels activated by ATP have been found on the smooth muscle membrane of rabbit ear artery (Benham & Tsien, 1987). These channels, with a selectivity of 3:1 for calcium over sodium, can be opened at very negative potentials and are nifedipine-resistant. Purinoceptor-operated channels, which are permeable to many kinds of cations, have also been found in the single isolated cells of guinea-pig urinary bladder (Inoue, 1990). Thus, one possible mechanism is that ATP depolarizes the membrane by acting on the receptor-operated channels leading to the influx of calcium and other cations. Depolarization of the membrane will promote the opening of the voltage-sensitive calcium channels and elicit smooth muscle contraction. The existence of receptor-operated calcium permeable channels may also account for the phenomenon in guinea-pig vas deferens, that excitatory junction potentials and ATP- and α,β-MeATP-elicited depolarizations at low concentrations are insensitive to both Bay K 8644 and nifedipine, while action potential discharges and contractile responses are greatly affected by the two dihydropyridines (MacKenzie *et al.*, 1988). However, a direct link between P_{2X}-purinoceptors and the voltage-sensitive calcium channels cannot be excluded at present. The effect of P_{2Y}-purinoceptor activation on membrane ion permeability, which leads to hyperpolarization of the smooth muscle cell by selectively opening potassium channels (White, 1988; Burnstock, 1990), seems to be different from that of P_{2X}-purinoceptor activation.

In conclusion, the results indicate that voltage-sensitive calcium channels play an important role in the excitatory mechanical effect of P_{2X}-purinoceptor-mediated responses in rat urinary bladder, while the action of cholinergic nerves is less dependent on such channels.

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