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Comparison of Bay K 8644, nitrendipine and atropine on spontaneous and pelvic-nerve-induced bladder contractions on rat bladder in vivo

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Summary. The effects of the dihydropyridine-type calcium antagonist (nitrendipine) and agonist (Bay K 8644) in comparison to atropine have been studied after intravenous administration on spontaneous and pelvic-nerve-induced contraction of rat urinary bladder. Bay K 8644 increased the basal internal bladder pressure as well as the amplitude of the spontaneous bladder contractions in a dose-dependent manner. In addition, an increase in systemic arterial blood pressure was noted for a period of about 20 min. In the presence of atropine the effects of Bay K 8644 on the urinary bladder were almost completely antagonized. Both nitrendipine and atropine reduced in a dose-dependent manner the amplitude of spontaneous and nerve-induced bladder contraction. The spontaneous and nerve-induced bladder contractions were significantly reduced by atropine or nitrendipine. Only nitrendipine caused a reduction of the spontaneous bladder contraction frequency. The systemic blood pressure was decreased significantly by nitrendipine but not after atropine administration. We suggest that both calcium antagonist and agonist can change the tension of the urinary bladder in vivo. As a side-effect the systemic blood pressure is altered. Atropine can antagonize the effect of BayK 8644 on the urinary bladder and reduces spontaneous and nerve-induced bladder contractions more specifically than nitrendipine.

Key words: Atropine – Bay K 8644 – Nitrendipine – Rat urinary bladder

In recent years a new group of drugs used in the treatment of bladder instability – 1,4-dihydropyridine derivatives – has come into clinical use. Their specific action is generally thought to be inhibition of calcium entry through the calcium channels [2]. A small modification of the dihydropyridine molecule produces a substance with effects diametrically opposed ("calcium agonist") to those of the calcium antagonist [15]. The effect of calcium channel modulation has been studied extensively in cardiac smooth muscle [5]. Various in vitro studies have shown that calcium channel agonists and antagonists have potent effects on the motility of smooth muscles of mammalian bladder [6, 11, 12] and upper urinary tract [7]. Thus far, however, little attention has been paid to the effect of these substances on bladder function in vivo.

To investigate the calcium channel modulation in vivo, we studied the effects of nitrendipine and Bay K 8644 in an acute rat model. In order to compare these pure calcium channel effects, we selected atropine and pelvic nerve stimulation as additional tools to modulate bladder motility.

Materials and methods

Surgical procedure

Female Han-Wistar rats, weighing 180–200 g, were anaesthetized with pentobarbital (50 mg/kg i.p.). The animals were placed in the supine position. The trachea was cannulated via a tracheotomy tube for undisturbed respiration throughout the experiment. One carotic artery and the external jugular vein were cannulated with polyethylene tube for arterial blood pressure monitoring, 0.9% saline injection (0.1–2 ml/h) and drug administration. The abdominal cavity was opened by a lower midline incision. Both ureters were cut and the urethra ligated to maintain a constant bladder volume. The bladder was then cannulated through a small incision of the dome and a polyethylene tube inserted and fixed in place. Fibres of the pelvic nerve were freed gently from adherent tissue about 2 mm lateral to the bladder wall. A bipolar hook electrode of suitable size, (made in our laboratory) was applied to the nerve fibres and connected to a simulator (Elpha 500, Biometer International, Odense, Denmark). The peritoneal cavity was filled with prewarmed neutral oil to prevent drying and current spread during neurostimulation. Finally, the skin of the abdominal wall was supported by clamps. Throughout the experiment, the body temperature of the animals was maintained at 37°C by an infra-red lamp (IR 150) except during the period of Bay K 8644 and nitrendipine administration. This process was monitored via a rectal probe (Ellab a-s, Kopenhagen, Denmark).

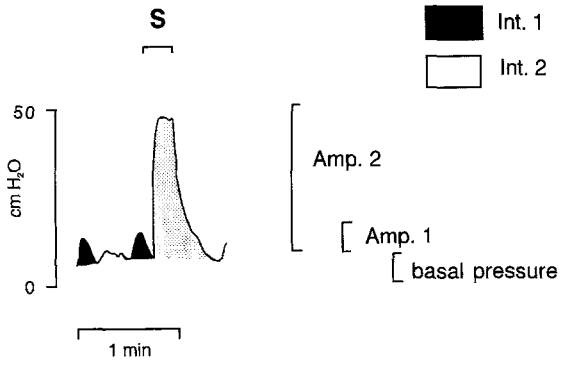

Fig. 1. Original tracing of rat bladder contractions in vivo; superimposed on the basal pressure are spontaneous contractions *(Amp. 1; Int. 1)* and one pelvic-nerve-induced *(S)* bladder contraction *(Amp. 2; Int. 2)*

Recordings

The tubes in the carotic artery and the bladder were connected to pressure transducers (Statham P23 ID, Gould, Oxnard, Calif., USA). Pressures were recorded by a Polygraph (Cardiopan 8R, Liecht, Ostermundigen, Switzerland). The bladder was filled with prewarmed 0.9% saline (0.03–0.05 ml) until the internal basal pressure increased to 10 cm H_2O. The preparations were allowed to equilibrate for 30 min. All further investigations were performed under pure sodium light (Osram L40W/62, Italy) to prevent a daylight-induced degradation of Bay K 8644 and nitrendipine [13].

Protocols

Cumulative dose-response curves were obtained using different protocols. In the first protocol the vesical and arterial blood pressure were recorded for at least 2 h. Every 15 min a stimulus (20 mA, 20 Hz, 0.125 ms; same parameters for further stimulations) was set for 10 s. In the second protocol, the cumulative concentration-response relationships of Bay K 8644 (1.5–1500 µg/kg), nitrendipine (1.5–1500 µg/kg) or atropine (0.5–50 µg/kg) were obtained as follows. After an initial neurostimulus, one of the drugs was injected i.v. at its lowest concentration. After 15 min another stimulus was set and after additional 5 min a cumulative drug dosage was administered in logarithmic increments. In the third protocol, after the first stimulus, 15 µg/kg and later 150 µg/kg Bay K 8644 was injected i.v. and additional dosages of atropine (50–500 µg/kg) were added every 20 min.

Drugs

Bay K 8644 and nitrendipine (Bayer, Leverkusen, FRG) were dissolved in Bay a 1040 (Bayer) and atropine sulphate (Braun, Melsungen, FRG) in 0.9% saline. Neutral oil (Miglyol 812, DAB 9) was purchased from Caesar and Loretz (Hilden, FRG) and pentobarbital sodium from Wirtschaftsgenossenschaft deutscher Tierärzte (Hannover, FRG).

Statistical analysis

Results are expressed as mean ± standard error of the mean (not listed in Table 1). An overall statistical comparison of independent mean values was based on the analysis of variance as described in Documenta Geigy [4]. This procedure was followed by standard *t* statistics. A *P* value of less than 0.05 (0.01) was considered significant (highly significant). All results are expressed as mean values if not mentioned otherwise.

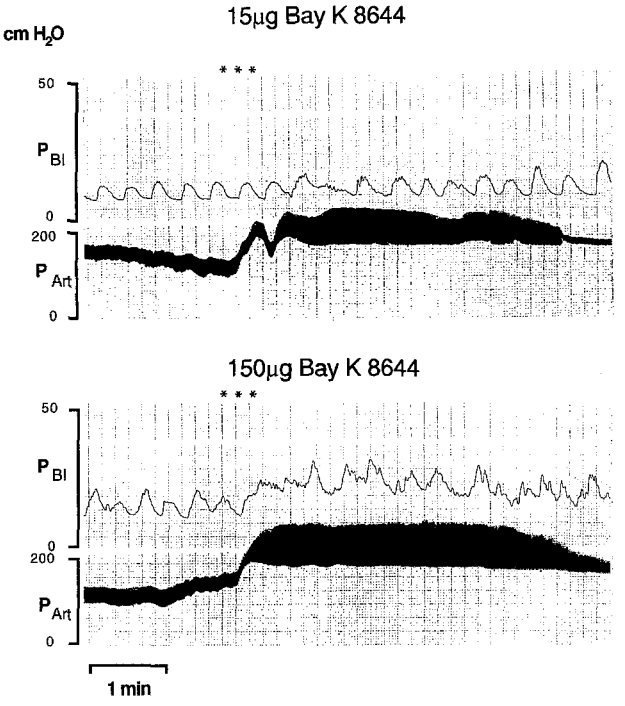

Fig. 2. Original tracing of rat bladder contractions and arterial blood pressure in vivo; effects of 15 and 150 µg Bay K 8644 on bladder pressure (P_{BI}) and arterial blood pressure (P_{Art}); time of injection (***)

Results

In all animals we found a permanent and homogeneous alteration of the inner bladder pressure. Superimposed on a basic or tonic bladder pressure which was set by the amount of bladder volume at 10 cm H_2O, rhythmic or phasic contractions were recorded with an amplitude of 7 cm H_2O (Fig. 1). The planimetry of these contraction curves showed an area of 32.5 mm^2. These pressure peaks were followed by two to three major peaks of 2–3 cm H_2O. The frequency of the main peaks was 2.8/ min. By neurostimulation of the pelvic nerve fibres the inner bladder pressure increased rapidly (Fig. 1) to a peak of 36.4 cm H_2O and decreased within 23 s (area 250.5 mm^2) to the basal pressure. No significant differences in basal pressure, peak pressure or neurostimulation responses could be recorded throughout the experiments (eight animals) for at least 2 h after the recovery time.

Figure 2 shows the concentration-response curve of Bay K 8644 on the spontaneous and Figs. 3 and 4 on neurostimulation-induced contractions. Increasing the drug concentration resulted in an increase of basal and superimposed peak pressure (Figs. 3, 4) as well as the corresponding areas (Table 1). The amplitude of the rhythmic contractions decreased to the previous resting pressure after 15–20 min. The increase of tonic contraction was not altered during this period. In comparison to

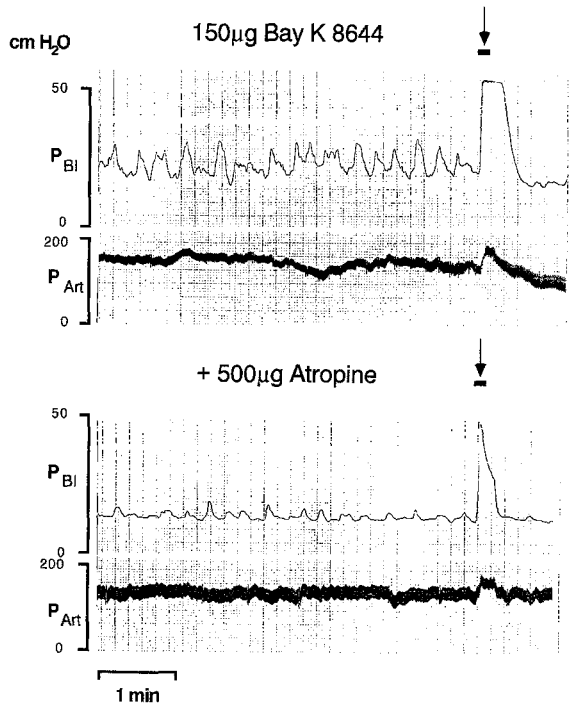

Fig. 3. Original tracing of rat bladder contractions and arterial blood pressure (P_{Art}) in vivo; effects of 150 µg Bay K 8644 (*upper trace;* 15 min after i.v. injection of Bay K 8644) and 500 µg atropine (*bottom trace;* 35 min after i.v. injection of Bay K 8644 and 20 min after i.v. injection of atropine) on spontaneous and pelvic-nerve-induced (↓) bladder contractions (bladder pressure: P_{Bl}) as well as P_{Art}

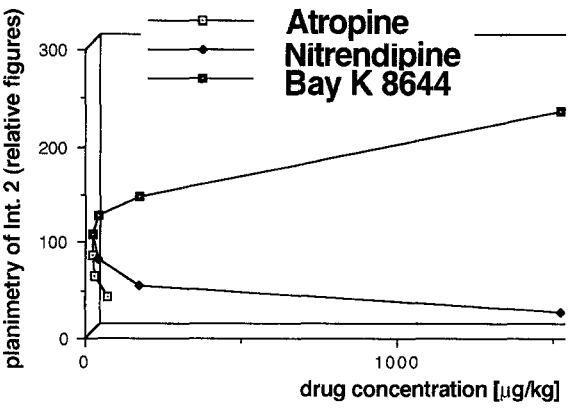

Fig. 4. Cumulative concentration-response relationships of atropine, nitrendipine and Bay K 8644 on pelvic-nerve-induced bladder contractions expressed as relative planimetric changes of the contraction curves (*Int. 2;* control = 100)

peak pressure amplitudes the arterial blood pressure showed a similar temporary increase (Fig. 2).

Application of 50 µg atropine/kg in addition to 30 µg Bay K 8644/kg reduced the tonic pressure, spontaneous pressure peaks and the corresponding areas to previous levels (500 µg atropine/kg; Table 1, Fig. 3). Using 500 µg/kg atropine without a previous Bay K 8644 injection resulted in severe respiratory depression. The amplitude and intensity of spontaneous (induced) bladder contraction were reduced by atropine to 45% (37%) and 57% (65%) respectively (Table 1). The frequency of bladder

Table 1. Effects of nitrendipine, Bay K 8644 and atropine on arterial blood pressure (BP), frequency of spontaneous bladder contractions (F), amplitude and intensity of spontaneous bladder contractions (Amp. 1, Int. 1) and amplitude and intensity of pelvic nerve induced bladder contractions (Amp. 2, Int. 2) in rats

	BP	F	Amp. 1	Int. 1	Amp. 2	Int. 2
Nitrendipine						
Control	164.6	2.6	8.6	42.8	37.0	277.1
15 µg/kg	141.9*	2.9	6.0	21.5*	28.6	206.3
150 µg/kg	113.6**	2.8	4.2**	14.1**	23.3*	132.8*
1500 µg/kg	94.4**	1.3*	1.4**	3.9**	15.3**	57.4**
Bay K 8644						
Control	171.3	3.5	5.2	18.8	37.0	238.0
15 µg/kg	187.2	3.4	8.0*	37.0	40.0	286.1
150 µg/kg	192.5	3.8	11.3**	48.7*	44.9*	366.0*
1500 µg/kg	197.5	3.3	14.5**	59.5**	46.3*	543.0**
Atropine						
Control	190.0	4.8	6.7	14.3	29.3	156.7
0.5 µg/kg	203.3	6.5	4.6	7.7**	26.3	125.0
5 µg/kg	197.5	5.8	5.2	6.3**	21.0**	90.0**
50 µg/kg	193.3	5.9	3.7**	6.2**	18.7**	55.0**
Bay K 8644 (30 µg = control) + atropine (10 µg; 100 µg)						
Control	192.5	3.8	11.3	48.7	44.9	366.0
10 µg/kg	150.0	4.7	6.3	11.0**	36.3	165.0*
100 µg/kg	146.7*	4.8	4.0*	6.3**	32.7*	115.0**

* P value less than 0.05; ** P value less than 0.01

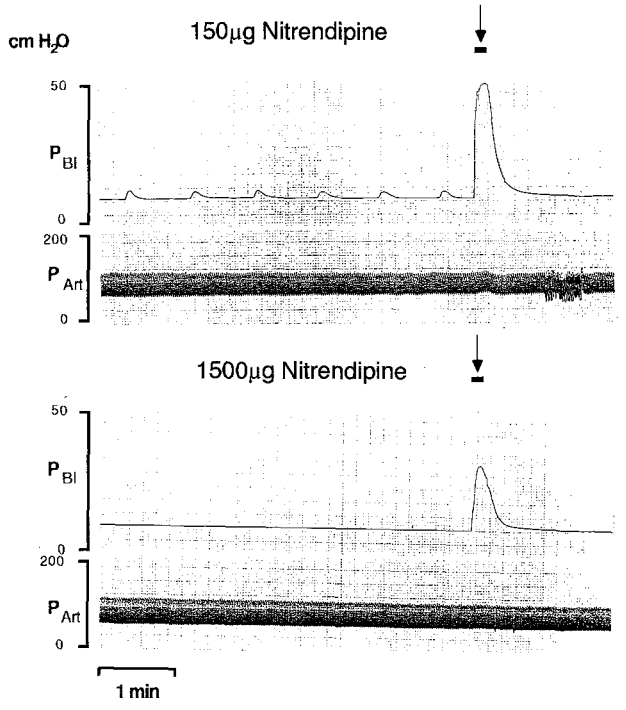

Fig. 5. Original tracing of rat bladder contractions and arterial blood pressure (P_{Art}) in vivo; effects of 150 µg *(upper trace)* and 1500 µg nitrendipine (*bottom trace;* each time 15 min after i.v. injection of nitrendipine) on spontaneous and pelvic-nerve-induced (↓) bladder contractions (bladder pressure: P_{Bl}) as well as P_{Art}

contraction and systemic blood pressure were not altered by atropine.

Nitrendipine was used to study the calcium channel blocking properties. All parameters were significantly reduced by nitrendipine (Table 1, Fig. 5). Spontaneous bladder contractions were depressed by nitrendipine to a greater extent than by induced contractions (Table 1). A significant decrease in blood pressure was observed even after small doses of nitrendipine. Two animals died after the injection of 1500 µg/kg nitrendipine as a result of a drastic fall in blood pressure.

Discussion

Basal bladder pressure, spontaneous and induced bladder contractions were enhanced by Bay K 8644 but reduced by nitrendipine. The effect of BayK 8644 on bladder and arterial pressure was antagonized by atropine. The present study showed that both spontaneous and electrically induced bladder contractions were sensitive to both calcium channel agonist and antagonist in vivo.

There is evidence that transmembrane flux of calcium is responsible for spontaneous contractions of rat urinary bladder in vivo [9]. Using nifedipine and verapamil Maggi et al. [9] observed different relaxing properties of these two calcium channel blockers. Both substances reduced the frequency of spontaneous contractions of rat bladder while having minor effects on their amplitude. This corresponded to only a small reduction in amplitude of micturition contraction by nifedipine [10]. In contrast, we found that amplitude intensity was reduced by 50% after the administration of 15 µg and by 90% after 1500 µg nitrendipine. A reduction of frequency, however, could only be observed in our experiments using high dosages of nitrendipine. These differences may be explained by our different experimental design as compared with other investigators or by a different potency of nifedipine, verapamil and nitrendipine on the calcium channel blocking property. Maggi et al. [9] reported a spontaneous rhythmic contraction rate in only 40%. We observed a rhythmic contraction in each animal. The effects of nitrendipine suggested a physiological mobilization of extracellular calcium for spontaneous rhythmic bladder contractions. Bay K 8644 induced a temporary increase of spontaneous and a persisting increase of tonic bladder contraction which is known from in vitro experiments [11]. These observations seem to be evidence of a strong dependence of tonic and rhythmic contraction of normal rat bladder on calcium channels. However, atropine showed a complete abolition of the effects of Bay K 8644. In addition, the reduction of spontaneous bladder contractions was more specific to atropine as compared with nitrendipine. Therefore, tonic and rhythmic bladder contractions in rats can be modulated by calcium channels and muscarinic receptors in vivo. The present data do not suggest a clear correlation for a frequency dependence on muscarinic or on calcium channels.

In the present study, pelvic nerve stimulation was used for contractile activation of rat urinary bladder in accordance with Carpenter and Rubin [3]. The stimulation parameters were used to obtain a reproducible bladder contraction at approximately 80% of the maximum. The stimulation interval was set at 10 s. Extending the length of this stimulation interval was not followed by an increase of contraction amplitude. Only the decrease of the response could be prolonged. This contractile response cannot be totally abolished by muscarinic receptor blockers [1]. With atropine, we observed a similar decrease of induced bladder contraction as has been reported previously in vivo [14]. In our animal model, 50 µg/kg atropine was the most effective dosage without causing severe cardiopulmonary side-effects. This maximal concentration was less effective on induced contractions than the maximal tolerable concentration of nitrendipine. However, higher concentrations of nitrendipine were necessary to decrease the induced contraction amplitude. From these findings it is suggested that the calcium-channel-blocking property of nitrendipine is rather non-specific compared to the muscarin receptor blockage caused by atropine. Recently Lacovou et al. [8] suggested that muscarin receptor stimulation mobilizes intracellular calcium via hydrolysis of inositol phospholipid and production of the second messenger inositol triphosphate. Mobilization of intracellular calcium seems to play a major role in spontaneous and neurostimulation-induced bladder contractions.

From in vitro studies Bay K 8644 is known to increase the sensitivity of bladder smooth muscle contraction to electrical field stimulation [6, 12]. In addition, Bay K 8644 can restore the suppressed muscle contraction of a

calcium channel blocker [6] as well as nifedipine reduces the Bay K 8644 response on smooth muscle [12]. In our experiments, the effects of Bay K 8644 on induced contractions (tonic and amplitude of rhythmic contractions) were antagonized by atropine. These observations contribute to the hypothesis that calcium channel modulation and modulation of intracellular calcium stores are potent pathways for alterations of bladder smooth muscle tone [6]. Finally, we observed that nitrendipine and atropine did not totally abolish pelvic-nerve-induced bladder contractions as has been shown with tetrodotoxin [1, 6, 14]. The so-called atropine or nitrendipine resistance was of a similar degree throughout our experiments.

References

1. Brading A (1987) Physiology of bladder smooth muscle: In: Torrens M, Morrison JFB (eds) The physiology of lower urinary tract. Springer, Berlin Heidelberg New York, p 161
2. Caine M (1984) Non-autonomic drugs acting on the urinary tract. In: Caine M (ed) The pharmacology of the urinary tract. Springer, Berlin Heidelberg New York, p 31
3. Carpenter FG, Rubin RM (1967) The motor innervation of the rat urinary bladder. J Physiol (Land) 192:609
4. Documenta Geigy (1968) In: Diem D, Lentner C (eds) Wissenschaftliche Tabellen, 7th edn. Geigy, Basel
5. Fleckenstein A (1985) Calcium antagonists and calcium agonists: fundamental criteria and classification. In: Fleckenstein A, Breemen C van, Groß R, Hoffmeister F (eds) Cardiovascular effects of dihydropyridine-type calcium antagonists and agonists. Springer, Berlin Heidelberg New York, p 3
6. Fovaeus M, Andersson KE, Batra S, Morgan E, Sjögren C (1987) Effects of calcium, calcium channel blockers and Bay K 8644 on contractions induced by muscarinic receptor stimulation of isolated bladder muscle from rabbit and man. J Urol 137:798
7. Hertle L, Nawrath H (1989) Stimulation of voltage-dependent contractions by calcium channel activator Bay K 8644 in the human upper urinary tract. J Urol 141:1014
8. Lacovou JW, Hill SJ, Birmingham AT (1990) Agonist-induced contraction and accumulation of inositol phosphates in the guinea-pig detrusor: evidence that muscarinic and purinergic receptors raise intracellular calcium by different mechanisms. J Urol 144:775
9. Maggi CA, Grimaldi G, Meli A (1982) The effects of nifedipine and verapamil on spontaneous and carbachol-stimulated contractions of rat urinary bladder "in vivo". Arch Int Pharmacodyn 257:288
10. Maggi CA, Manzini S, Parlani M, Conte B, Giuliani S, Meli A (1988) The effect of nifedipine on spontaneous, drug-induced and reflexly-activated contractions of the rat urinary bladder: evidence for the participation of an intracellular calcium store to micturition contraction. Gen Pharmacol 19:73
11. Maggi CA, Giuliani S, Patacchini R, Turini D, Barbanti G, Giachetti A, Meli A (1989) Multiple sources of calcium for contraction of the human urinary bladder muscle. Br J Pharmacol 98:1021
12. Malmgren A, Andersson KE, Andersson PO, Fovaeus M, Sjögren C (1988) Effects of nifedipine and Bay K 8644 on normal and hypertrophied rat detrusor. Neurourol Urodyn 7:27
13. Mikkelsen E, Kazda S, Nyborg NCB (1985) Effects of light and Bay K 8644, a new 1,4-dihydropyridine, on mechanical response of rat thoracic aorta. Acta Pharmacol Toxicol 56:126
14. Sato S, Hayashi H, Garfield RE (1989) Mechanical response of the rat uterus, cervix, and bladder to stimulation of hypogastric and pelvic nerves in vivo. Biol Reprod 40:209
15. Schramm M, Thomas G, Towart R, Frankowiak G (1983) Novel dihydropyridines with positive inotropic action through activation of Ca^{2+} channels. Nature 303:535

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