Effect of Calcium Entry Blockade on the Actions of Phenylephrine on the Taenia of the Guinea Pig Caecum

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Abstract. The interaction between phenylephrine and calcium entry blockers was studied on the taenia of the guinea-pig caecum using the double sucrose gap method.

Sustained hyperpolarization, relaxation and attenuation of evoked electrical and mechanical activity were induced by non-cumulative addition of phenylephrine (0.1 to 250 μmol.l^{-1}) for 2 to 4 min. When the α_1-adrenoceptor agonist was applied for a prolonged period (20 to 60 min) the initial inhibitory response gradually disappeared both at room temperature and at 32°C. The renewed action potentials were accompanied by a positive afterpotential.

The initial hyperpolarization and its delayed recovery in course of the phenylephrine effect were significantly reduced in calcium-free medium containing EDTA (2 mmol.l^{-1}), after pretreatment with nifedipine (0.1 to 1 μmol.l^{-1}), verapamil (10 to 100 μmol.l^{-1}) or procaine (0.5 to 2 mmol.l^{-1}). In contrast sodium nitroprusside (10 to 100 μmol.l^{-1}) which produced biphasic changes similar to those of phenylephrine, did not affect the initial and delayed phase of phenylephrine action.

Ba^{2+}(5 mmol.l^{-1}) could substitute for Ca^{2+} in the generation of action potentials but could not substitute for Ca^{2+} in the mechanisms responsible for the initial and delayed recovery phase of phenylephrine effects. In the presence of La^{3+} and Mn^{2+}(0.5 to 3 mmol. l^{-1}) the phenylephrine effects were reduced. In contrast, in the presence of extracellular Ca^{2+}, pretreatment with Mg^{2+}(12 mmol.l^{-1}) or Ba^{2+}(5 mmol.l^{-1}) did not affect the action of phenylephrine.

It is concluded that activation of α_1-adrenoceptors results in the release of Ca^{2+} from an intracellular store, which leads to the opening od TEA-sensitive potassium channels, causing the initial phase of α_1-adrenoceptor action. Ca^{2+} is loaded into this intracellular store by entering the cell through the potential sensitive calcium channels. Although the mechanisms responsible for the delay-

ed phase could not be clarified, its dependence on the presence of the initial phase is apparent.

Key words: α_1-adrenoceptors — Calcium entry blockade — Calcium activated potassium conductance — Taenia of the guinea-pig caecum

Introduction

It is well established that the activation of α-adrenergic receptors may either stimulate or inhibit smooth muscle (e.g. of blood vessel and intestine; Exton 1981; Den Hertog et al. 1984). It has been reported recently that the inhibitory α-adrenoceptors present in the taenia caeci of the guinea-pig are of the α_1-type (Török and Vizi 1980; Bauer 1982).

It has become increasingly evident that enhanced potassium conductance can play an important role in the inhibitory α-action of catecholamines in the taenia caeci of the guinea pig (Jenkinson and Morton 1967; Shuba and Klevetz 1967; Bülbring and Tomita 1969a). Moreover, TEA and apamine, blockers of calcium activated potassium conductance (Mironneau et al. 1977; Walsh and Singer 1980; Hermann and Gorman 1981; Shuba and Vladimirova 1981) were found to abolish the afterhyperpolarization of the spike (Bauer and Kuriyama 1982) and the hyperpolarization due to the inhibitory action of catecholamines (TEA — Bauer and Rusko 1982; apamin — Den Hertog 1981; Shuba and Vladimirova 1981). A similar role of the calcium activated potassium conductance in the α-action of catecholamines was proposed also in other tissues (Putney 1976; Haylett 1976; Banks et al. 1979; Egashira 1980; Grafe et al. 1980). The importance of calcium for the α-action of catecholamines in the guinea pig taenia caeci has been repeatedly reported (Bülbring and Tomita 1969b; Den Hertog 1981; Den Hertog and van den Akker 1986; Bauer and Rusko 1982) and it was suggested that extracellular (Bülbring and Tomita 1977; Fujihara et al. 1986) or both extracellular and intracellular (Den Hertog 1981, 1982; Rusko and Bauer 1985b, 1986) calcium are required for the inhibitory effect of α-adrenoceptor agonists.

The aim of the present experiments were therefore to analyse further the role of calcium also by using calcium entry blockers in the action of phenylephrine in the taenia of the guinea-pig caecum using the double sucrose gap method.

Materials and Methods

Male guinea pigs were sacrificed by a blow on the neck. Fine strips of the taenia were removed quickly from the caecum and the preparations were inserted into a double sucrose gap apparatus

(Bauer and Zakhari 1977). Any contact between the isotonic sucrose solution (270 mmol.l^{-1}) and the isotonic K_2SO_4 solution (77.5 mmol.l^{-1}) or the Krebs solution flowing on either side was prevented by using latex membranes through which the taenia was threaded. Constant current pulses of alternating polarity were applied and the membrane potential, spontaneous and electrically induced action potentials and isometric tension were recorded. The Krebs solution (Na^+ 136.6, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 133.3, HCO_3^- 15.4, $H_2PO_4^-$ 1.2 and glucose 11.5 mmol.l^{-1}) aerated by 95% and 5% CO_2 keeping pH $\doteq$ 7 was used. In experiment with La^{3+} and Mn^{2+} modified Krebs solution (Na^+ 120, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 133.3 and glucose 11.5 mmol.l^{-1}) aerated by O_2 was used. The pH of these solutions was maintained at 7.1 to 7.2 by Tris buffer. In experiments with Ca^{2+}-free solution $CaCl_2$ was omitted from the superfusion fluid and 2 mmol.l^{-1} of EDTA was added. In high Mg^{2+} solution the concentration of $MgCl_2$ was increased to 12 mmol.l^{-1}.

In a separate series of experiments the action of phenylephrine on the spontaneous mechanical activity of taenia caeci was also studied. Segments of the taenia, about 2.5 cm in length, were placed in an organ bath containing Krebs solution. The isometric muscle activity was recorded using a strain gauge transducer at 37°C. One end of the tissue was tied to the bottom of a 20 ml tissue chamber and the other one to the transducer. The tissue was equilibrated under 20 mN tension for 30 min and the actual experiments were carried out under a basal tension of about 10 mN.

Following drugs were used: nifedipine (Sigma), phenylephrine hydrochloride (Boehringer Ingelheim), procaine hydrochloride (Spofa), sodium nitroprusside (Lachema), verapamil chloride (Lääketehdas Orion).

Fresh stock solutions were prepared just before the experiments. A period of 40 to 60 min was allowed to elapse after mounting the preparations in the sucrose gap chamber to stabilize the preparations, under a tension of 15 mN. If not stated otherwise the experiments were carried out at 32°C under an initial tension of 5 mN.

The results are expressed as the arithmetic mean ($\pm$ SEM) of at least six measurements. Differences were tested by Student's *t*-test.

Results

Effects of phenylephrine

Superfusion of taenia caeci with phenylephrine (0.1 to 250 µmol.l^{-1}, non-cumulative addition) for 2 to 4 min induced a concentration-dependent increase of both membrane potential and membrane conductance, a decrease of the frequency of spontaneous action potentials, of basal tension of the muscle and of the amplitude of both evoked action potentials and contractions. In the presence of high concentrations of the agonist, in parallel with reduction of the number of action potentials, their amplitude was transiently enlarged and then gradually abolished. At this stage even stronger pulses (up to 4 µA) did not evoke action potentials. The amplitude of the evoked contractions reflected the number of evoked spikes and not their amplitude (Fig. 1).

Continuous exposure of taenia to phenylephrine (1 to 100 µmol.l^{-1}) for 20 to 60 min at room temperature (20 to 22°C) or at 32°C caused biphasic changes in electrical and mechanical activity (Fig. 2). Despite the presence of phenylephrine in the superfusion solution the initial inhibitory phase neither developed further nor did remain sustained. Within 7 to 15 min the membrane potential

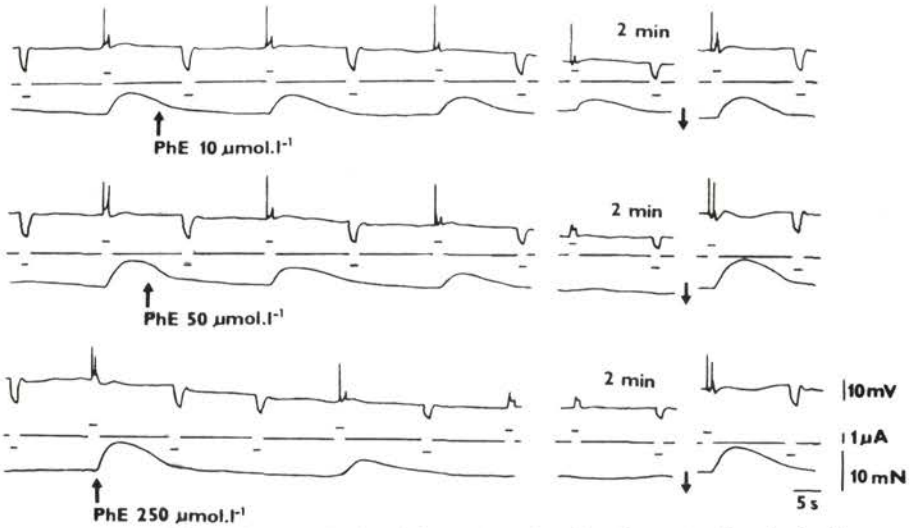

Fig. 1. Taenia caeci. Records of elecrical activity (*top*) and tension (*bottom*) using the double sucrose gap method. 32°C. The middle trace represents the applied current. Phenylephrine (PhE: 10, 50 and 250 µmol . l^{-1}) was applied non-cumulatively for 2 min to the same tissue and at the same level of the resting membrane potential. Note concentration dependent and more rapid onset of hyperpolarization, increase in membrane conductance attenuation of evoked spikes and contractions.

and the evoked membrane and mechanical activity were gradually restored. The renewed evoked action potentials were frequently accompanied by a pronounced positive afterpotential (Fig. 5). The recovery was more pronounced at room temperature than at 32°C but these differences were statistically insignificant (Fig. 3).

Repeated applications of phenylephrine (10 µmol . l^{-1}) into the organ bath or sucrose gap chamber for 1 to 2.5 min with washouts lasting for 20 and 60 s, respectively, which allowed partial reappearance of the spontaneous and evoked activity, resulted in relaxation and hyperpolarisation reaching the same level as on the first application.

To examine the possible desensitisation of α_1-adrenergic receptors phenylephrine was applied in a test concentration (10 µmol . l^{-1}) before and after the desensitising concentration (250 µmol . l^{-1} for 5 min). The testing phenylephrine concentration remained effective even at shortlasting washouts (for 20 to 30 s) (Fig. 4).

Effects of [Ca_0^{2+}] on phenylephrine action

At excess Ca_0^{2+} (10.8 mmol . l^{-1}) the basic features of the initial and the delayed recovery phase of phenylephrine action were preserved. Since phenylephrine was applied when the membrane had already been hyperpolarized and the stimulation threshold increased by excess Ca_0^{2+} the final hyperpolarization (the

Table 1. The action of phenylephrine (50 μmol . l^{-1}) on the guinea-pig taenia caeci in different $[Ca^{2+}]_0$ for 40 to 50 min in the superfusion solution. The results are given as mean percentage of the control responses and the resting membrane potential ±S.E.M.

Drug	Action potential amplitude	Electrotonic potential amplitude	Evoked contraction amplitude	Membrane potential	*n*
2.7 mmol . l^{-1} $CaCl_2$	100.0	100.0	100.0	100.0	13
+ Phenylephrine	15.0 ± 4.0	25.0 ± 3.2	0.0	115.0 ± 2.0	
	(74.3 ± 16.2)	(57.2 ± 20.0)	(47.3 ± 36.2)	(102.2 ± 3.0)	
10.8 mmol . l^{-1} $CaCl_2$	70.1 ± 4.4	72.9 ± 7.4	87.7 ± 9.4	108.4 ± 2.1	9
+ Phenylephrine	21.7 ± 4.8	35.9 ± 7.3	0.0	122.1 ± 2.1	
	(52.0 ± 8.5)	(51.8 ± 5.9)	(42.7 ± 13.9)	(114.6 ± 1.8)	
1.35 mmol . l^{-1} $CaCl_2$	120.0 ± 7.1	101.8 ± 6.1	115.5 ± 29.7	104.2 ± 1.4	6
+ Phenylephrine	13.6 ± 2.5	31.3 ± 7.5	0.0	127.3 ± 12.2	
	(73.4 ± 13.5)	(51.3 ± 9.4)	(92.4 ± 47.5)	(113.3 ± 9.1)	
$CaCl_2$-free + 2 mmol . l^{-1} EDTA	0.0	0.0	0.0	85.4 ± 4.8	5
+ Phenylephrine	could not be measured	could not be measured	could not be measured	89.9 ± 4.1	
				(89.4 ± 4.9)	
$CaCl_2$-free + 5 mmol . l^{-1} $BaCl_2$	162.2 ± 25.3	69.6 ± 11.05	0.0	99.5 ± 5.2	6
+ Phenylephrine	172.7 ± 13.9	55.6 ± 13.6	could not be	102.2 ± 5.5	
	(173.4 ± 24.1)	(61.1 ± 11.9)	measured	(104.9 ± 6.2)	

In the phenylephrine action the open figures indicate the initial phase (at the top of its inhibitory action) and those in parentheses the delayed recovery phase (20 to 30 min after begining of superfusion with phenylephrine).

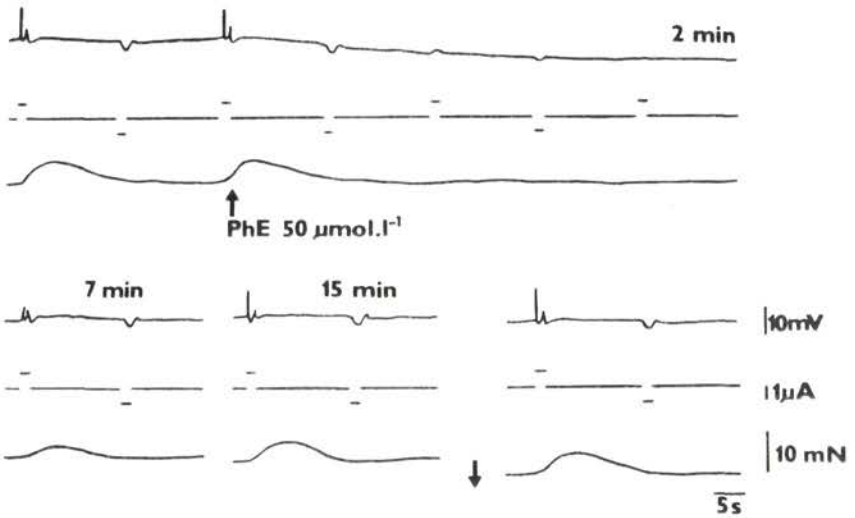

Fig. 2. Taenia caeci. Records of electrical activity (*top*) and tension (*bottom*) using the double sucrose gap method. 32°C. The middle trace represents the applied current. Phenylephrine (PhE 50 µmol . l^{-1}) was applied for 15 min. The interval after PhE injection is indicated. Note the gradual reappearance of evoked spikes, contractions, recovery of membrane resistance and repolarisation of the membrane in spite of the presence of PhE in the bathing fluid.

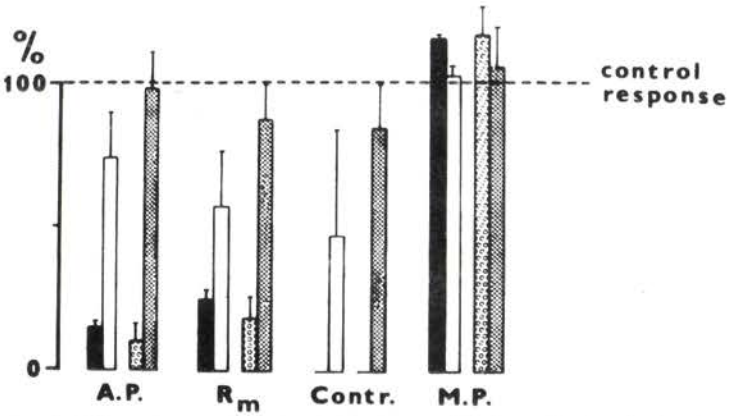

Fig. 3. Effects of phenylephrine (PhE: 50 µmol . l^{-1}) on the electrical and mechanical activity of the taenia caeci at 32°C (full and open columns, $n = 13$) and at room temperature (columns with open circles and stippled columns, $n = 9$). The membrane potential (M. P.) level, amplitude of evoked contractions (Contr.), anelectrotonic potentials (R_m) and action potentials (A.P.) recorded under untreated conditions were taken as 100%. Note the similarity of changes induced by PhE at both temperatures studied. The initial phase (full columns and colums with open circles) at min 4 and the delayed recovery phase (open columns and stippled columns) at min 20 of PhE action are shown.

sum resulting from both excess Ca_0^{2+} and phenylephrine) reached a higher membrane potential than that recorded under control conditions. The reduction of the amplitude of evoked action potentials and electrotonic potentials reached the same final inhibition as in controls. The recovery of the evoked action potentials and membrane potential during the delayed phase of phenylephrine action was slightly reduced in high compared with normal $[Ca_0^{2+}]$ (Table 1).

Reduction of $[Ca_0^{2+}]$ to 1.35 mmol.l^{-1} did not influence the electrical and mechanical responses of the taenia caeci, except for a 20% augmentation of the amplitude of evoked action potentials, nor were the phenylephrine effects significantly affected (Table 1).

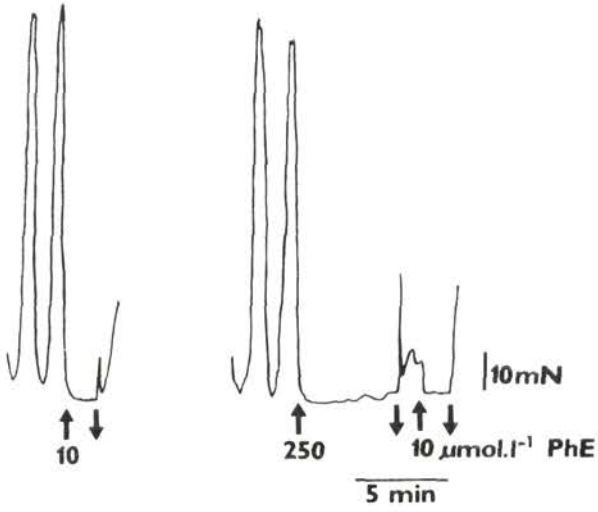

Fig. 4. Effects of phenylephrine (PhE) in testing concentration (10 µmol.l^{-1}) before and after 5-min exposure of the taenia to high PhE concentration (250 µmol.l^{-1}).

In Ca^{2+}-free superfusion solution containing 2 mmol.l^{-1} EDTA, after a transient (10 to 15 min) acceleration of spontaneous action potential discharges, increased amplitude of evoked action potentials and increased basal muscle tension, the membrane potential decreased and the spontaneous and evoked activity disappeared. Thus the effect of phenylephrine only on the membrane potential could be analysed. Phenylephrine under these conditions did not produce any significant change in the membrane potential.

Effects of verapamil, nifedipine, sodium nitroprusside and procaine on phenylephrine action

Verapamil (10 to 100 µmol.l^{-1}) and nifedipine (0.1 to 1 µmol.l^{-1}) markedly reduced the amplitude of both evoked action potentials and contractions,

without significantly changing the membrane potential, yet verapamil slightly decreased and nifedipine increased the membrane conductance. In their presence, both the initial and the delayed recovery phase of phenylephrine action were suppressed (Table 2). During the delayed phase of phenylephrine action pronounced afterhyperpolarization following the spike was recorded even in verapamil (up to 25 $\mu mol.l^{-1}$) pretreated preparations (Fig. 5).

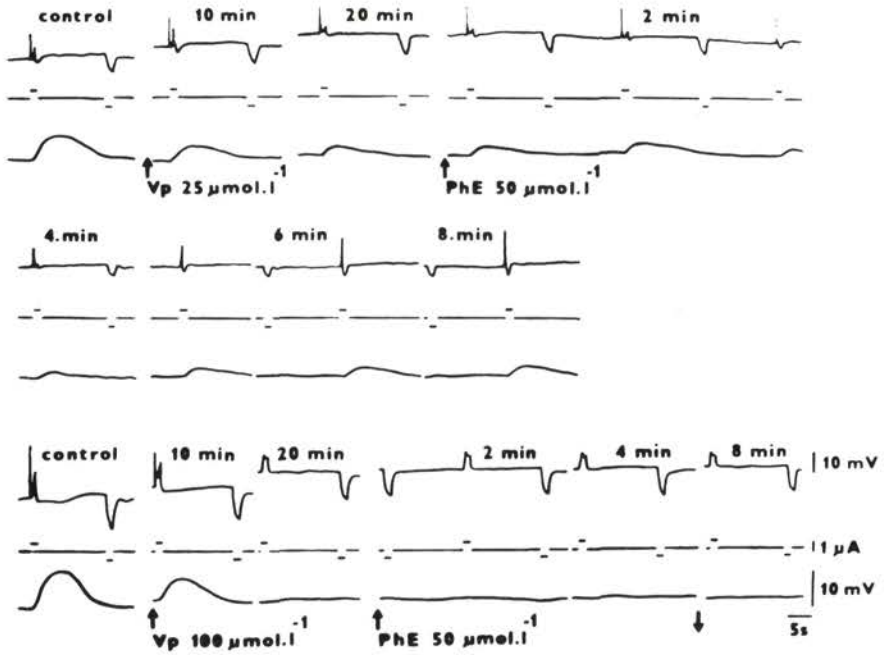

Fig. 5. Records of electrical activity (*top*), applied current (*middle*) and tension (*bottom*) of guinea-pig taenia caeci at 32°C. Upper and middle records: responses to phenylephrine (PhE: 50 $\mu mol.l^{-1}$) after 20 min Verapamil (Vp: 25 $\mu mol.l^{-1}$) pretreatment. Lower record: response to PhE after 20 min Vp (100 $\mu mol.l^{-1}$) pretreatment. The intervals after Vp and PhE injection are indicated. Note the complete impairment of the PhE effect in the presence of the higher Vp concentration and a pronounced positive afterpotential in the delayed phase of the PhE action in the presence of the lower Vp concentration.

The effects of sodium nitroprusside (10 to 100 $\mu mol.l^{-1}$) on electrical and mechanical activities of the taenia caeci were virtually the same as those of phenylephrine (Table 2). The action of the former, however, developed more slowly than that of phenylephrine. As it is evident from Fig. 6 and Table 2 longlasting exposure of the taenia to sodium nitroprusside did not prevent phenylephrine from eliciting initial and delayed recovery changes in electrical and mechanical activities.

Superfusion with procaine (0.5 and 2 $mmol.l^{-1}$) caused a concentration-dependent enhancement of the amplitude and duration of evoked action poten-

Table 2. The action of phenylephrine (50 μmol . l^{-1}) on the guinea-pig taenia caeci in the presence of nifedipine, verapamil, procaine and sodium nitroprusside in the superfusion solution for 30 to 40 min. The results are given as mean percentage of the control responses and the resting membrane potential ± S.E.M.

Drug	Action potential amplitude	Electrotonic potential amplitude	Evoked contraction amplitude	Membrane potential	*n*
1 μmol . l^{-1} Nifedipine	43.2 ± 7.8	85.2 ± 8.1	0.0	95.2 ± 6.1	6
+ Phenylephrine	20.7 ± 5.4	34.9 ± 7.3	could not be	101.5 ± 6.9	
	(34.2 ± 10.3)	(52.1 ± 5.3)	measured	(98.2 ± 6.5)	
100 μmol . l^{-1} Verapamil	55.2 ± 6.1	109.1 ± 7.9	14.3 ± 8.5	93.2 ± 3.5	9
+ Phenylephrine	51.3 ± 6.8	106.5 ± 8.7	5.4 ± 4.9	93.5 ± 3.9	
	(45.7 ± 5.0)	(103.6 ± 10.1)	(0.0)	(93.0 ± 3.7)	
0.5 mmol . l^{-1} Procaine	103.4 ± 11.6	106.6 ± 5.7	115.8 ± 8.4	98.9 ± 4.1	5
+ Phenylephrine	23.4 ± 3.5	42.1 ± 2.9	0.0	105.1 ± 3.9	
	(85.8 ± 9.8)	(78.8 ± 3.7)	(86.8 ± 9.5)	(98.4 ± 4.4)	
2 mmol . l^{-1} Procaine	139.7 ± 18.6	102.5 ± 8.6	153.5 ± 20.8	104.4 ± 1.1	8
+ Phenylephrine	142.6 ± 13.3	101.2 ± 8.3	124.1 ± 22.4	104.7 ± 1.0	
	(151.4 ± 37.3)	(90.5 ± 6.4)	(132.2 ± 18.1)	(108.4 ± 4.0)	
100 μmol . l^{-1} Nitroprusside	41.5 ± 9.0	53.5 ± 8.1	0.0	108.3 ± 2.7	5
	(54.9 ± 8.6)	(71.7 ± 6.7)	46.2 ± 2.8	(99.7 ± 2.8)	
+ Phenylęphrine	11.0 ± 3.1	32.3 ± 2.1	0.0	115.7 ± 5.1	
	(38.4 ± 4.7)	(54.9 ± 5.7)	39.3 ± 3.4	(103.9 ± 6.2)	

In phenylephrine and sodium nitroprusside the open figures indicate the initial phase and those in parentheses the delayed recovery phase of their action. For control effects of phenylephrine and further detailes see Table 1.

Table 3. The action of phenylephrine (50 μmol . l^{-1}) on the guinea-pig taenia caeci in the presence of Ba^{2+}, Mn^{2+}, La^{3+} and Mg^{2+} in the superfusion solution for 35 to 45 min. The results are given as mean percentage of the control responses and the resting membrane potential ±S.E.M.

Drug	Action potential amplitude	Electrotonic potential amplitude	Evoked contraction amplitude	Membrane potential	*n*
5 mmol. l^{-1} $BaCl_2$	183.9 ± 26.3	118.0 ± 8.7	72.9 ± 8.9	93.6 ± 5.7	5
+ Phenylephrine	42.8 ± 8.7	46.2 ± 6.3	8.6 ± 1.2	118.4 ± 6.0	
	(235.5 ± 48.0)	(101.7 ± 12.0)	(91.6 ± 8.6)	(99.9 ± 4.8)	
0.5 mmol. l^{-1} $MnCl_2$	106.1 ± 11.1	105.7 ± 13.6	43.3 ± 5.4	92.4 ± 1.7	7
+ Phenylephrine	18.4 ± 3.1	39.6 ± 5.8	0.0	102.9 ± 3.7	
	(105.8 ± 12.2)	(72.6 ± 2.6)	(37.8 ± 7.6)	(98.1 ± 3.1)	
3 mmol. l^{-1} $MnCl_2$	27.2 ± 4.6	56.9 ± 9.1	0.0	94.5 ± 1.7	7
+ Phenylephrine	19.1 ± 3.1	42.5 ± 5.4	could not be	97.1 ± 2.1	
	(18.7 ± 4.5)	(39.1 ± 8.6)	measured	(104.7 ± 1.1)	
0.5 mmol. l^{-1} $LaCl_3$	20.3 ± 4.3	32.8 ± 7.8	0.0	98.2 ± 1.9	5
+ Phenylephrine	7.4 ± 5.2	11.0 ± 7.1	could not be	105.5 ± 2.3	
	(0.0)	(0.0)	measured	(101.0 ± 3.1)	
3 mmol. l^{-1} $LaCl_3$	8.2 ± 2.5	24.8 ± 6.1	0.0	111.8 ± 4.8	6
+ Phenylephrine	1.1 ± 0.7	5.0 ± 3.4	could not be	108.6 ± 0.9	
	(0.0)	(0.0)	measured	(127.0 ± 8.1)	
12 mmol. l^{-1} $MgCl_2$	61.1 ± 7.2	105.1 ± 19.2	22.0 ± 10.1	119.3 ± 3.6	5
+ Phenylephrine	21.4 ± 2.9	41.8 ± 8.1	0.0	127.1 ± 4.1	
	(33.1 ± 2.4)	(65.4 ± 10.8)	8.8 ± 4.0	(120.3 ± 2.7)	

In the phenylephrine action the open figures indicate the initial phase and those in parentheses the delayed recovery phase of its effects. For control effects of phenylephrine and further detailes see Table 1.

tials. At the lower concentrations used it only reduced the initial hyperpolarization, whereas at the higher concentrations it completely abolished all the effects of phenylephrine studied (Table 2).

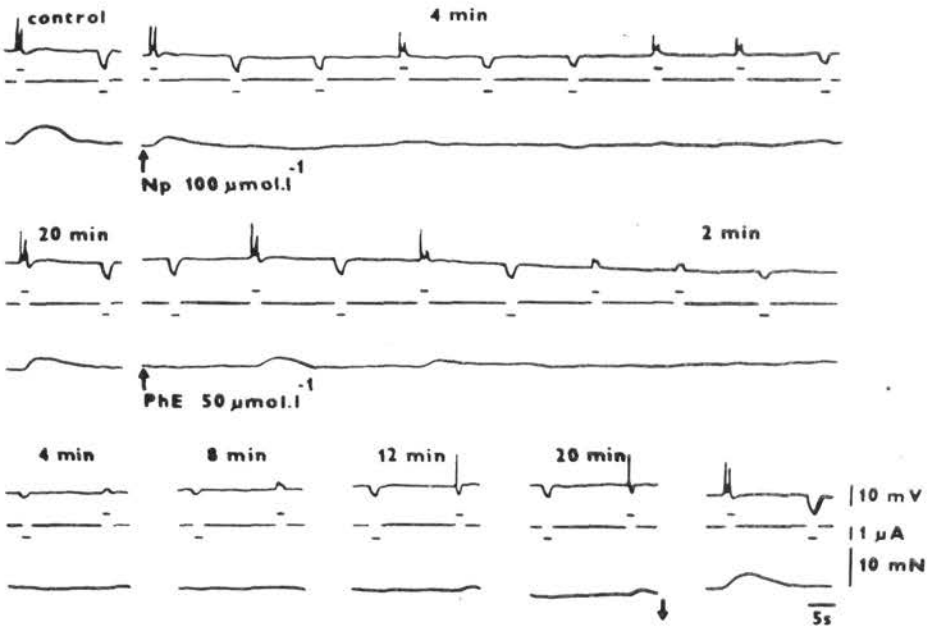

Fig. 6. Continuous records of the electrical activity (*top*), applied currents (*middle*) and tension (*bottom*) of the guinea-pig taenia caeci at 32°C. The intervals after sodium nitroprusside (Np: 100 µmol . l^{-1}) and phenylephrine (PhE: 50 µmol . l^{-1}) injection in the presence of Np are indicated. Note the minimal influence of Np pretreatment on the PhE effects in spite of the similar action of Np and PhE.

Effects of different cations on phenylephrine action

Ba^{2+} (5 mmol . l^{-1}) increased the frequency of spontaneous and the amplitude of evoked action potentials, contracted the taenia caeci and reduced the amplitude of evoked contractions which were superimposed on the increased basal tension. The membrane was slightly depolarized and the membrane resistance increased. The effects of phenylephrine were unchanged when Ba^{2+} was present in the superfusion solution, with only the amplitude of evoked action potentials in the delayed recovery phase being more pronounced (Table 3).

Distinct differences were observed, however, when Ca^{2+} was omitted from the Ba^{2+} (5 mmol . l^{-1}) containing bathing medium: the membrane conductance

increased, the smooth muscle was further contracted and spontaneous mechanical activity appeared preventing us to measure exactly the evoked contractions. Exposure of taenia caeci to Ca^{2+}-free Ba^{2+} containing solution for 30 min blocked all the effects of phenylephrine (Table 1).

Mn^{2+} (0.5 and 3 mmol.l^{-1}) reduced both the amplitude of evoked action potentials and anelectrotonic potentials, and only sligtly the membrane potential. Both phases of the phenylephrine action were elicited in the presence of low Mn^{2+} concentration, while at its high concentration phenylephrine failed to induce any significant effect (Table 3).

La^{3+} (0.5 and 3 mmol.l^{-1}) reduced the amplitude of evoked action and electrotonic potentials, it completely suppressed the evoked contractions, and at the highest concentration used, it also increased the membrane potential. Despite the presence of La^{3+} in the superfusion solution for at least 20 min, phenylephrine further depressed the remaining action potentials and membrane resistance, whereas in the initial phase the phenylephrine induced membrane hyperpolarisation was abolished in a concentration dependent way. The delayed phase of phenylephrine action did not develop in the presence of La^{3+} (Table 3).

When Mg^{2+} (12 mmol.l^{-1}) was applied to the superfusion solution the membrane of the taenia was hyperpolarised, the amplitudes of its evoked action potentials and contractions were significantly reduced. Under Mg^{2+} pretreatment phenylephrine remained effective (Table 3).

Discussion

Den Hertog (1981, 1982) and Rusko and Bauer (1985b, 1986) showed that α-action of adrenaline on the membrane potential and muscle tension is sustained in the presence of Ca^{2+}. The present results, in agreement with our recent observations (Rusko and Bauer 1985b, 1986), showed that even in the presence of Ca^{2+} the electrical and mechanical activities of the taenia caeci are only transiently affected by phenylephrine upon prolonged contact of the α_1-agonist with the receptor. The initial phase of phenylephrine action corresponded well with the sustained changes found in the case of adrenaline (Den Hertog 1981, Rusko and Bauer 1985b, 1986). Following the initial inhibitory effects the electrical and mechanical activity of taenia partially or completely returned to the respective control values, even though phenylephrine and Ca^{2+} were still present in the superfusion solution. These delayed recovery changes, once fully developed (within about 20 min), persisted throughout the action of the agonist (20 to 60 min). Several mechanisms may account for the gradual reappearence of the mechanical and electrical activity: (1) desensitisation of α_1 adrenoceptors to their agonists, (2) inactivation of processes which are res-

ponsible for the development of the initial phase (i.e. inactivation of potassium conductance), (3) activation of processes independent of those responsible for the development of the initial phase (i.e. active calcium extrusion or sequestration, changes in the sodium pump activity, etc.).

As described earlier (Rusko and Bauer 1985a) and shown in the present experiments the tachyphylaxis or desensitisation of α_1-adrenoceptors does not participate in the appearence of the delayed recovery phase of phenylephrine action. The large positive afterhyperpolarization which appeared during the delayed recovery phase might suggest that this part of the phenylephrine action could result from an increased sodium pump activity. The more pronounced recovery at room temperature than at 32°C however does not suggest that metabolically active processes play a significant role in the development of the delayed phase of the α_1-adrenergic effect.

It is obvious that the initial phase of the action of α_1-adrenoceptor agonists is the result of an increased potassium conductance of smooth muscle cell membrane of the taenia caeci (Jenkinson and Morton 1967; Shuba and Klevetz 1967; Bülbring and Tomita 1969a) due to the activation of TEA-sensitive potassium conductance (Bauer and Rusko 1982). Some local anaesthetics, such as procaine seem to block not only potassium channels open at the resting membrane potential, but also those responsible for the repolarising phase of action potentials. In this respect the action of procaine observed in the present study resembles that of TEA (Bauer and Rusko 1982; Bauer 1985). Moreover, procaine can block also the calcium influx and its microsomal accumulation (Tomiyama et al. 1975; Bolton 1979).

In search of a possible calcium-dependent mechanism of adrenaline induced membrane potential changes Den Hertog (1981, 1982) and Den Hertog and van den Akker (1986) discovered a sustained and transient membrane hyperpolarisation, the former being dependent on, and the latter independent of, the presence of extracellular calcium. In our previous (Rusko and Bauer 1986) and present experiments using phenylephrine, a selective α_1-agonist, both intra- and extracellular calcium was found to be essential for membrane hyperpolarisation.

The fact that a reduction of extracellular calcium to 1.25 mmol.l^{-1} or an increase to 10.8 mmol.l^{-1} only slightly affected the intensity of phenylephrine action and did not change its characteristics suggests that intracellular calcium is probably the main calcium source for the initiation of an enhanced potassium conductance, or that calcium entry depends on calcium binding to a site with a high affinity for calcium. Reduction of the phenylephrine effects in calcium-free solution and the inhibitory action of calcium entry blockers underline that this intracellular calcium pool is supplied by a mechanism sensitive to the action of organic calcium entry blockers. Blockade of calcium entry resulted therefore in gradual reduction of the available intracellular calcium source and thus indirect-

ly in reduced phenylephrine effect. When the smooth muscle was regularly stimulated in absence of extracellular calcium, thus reducing the intracellular calcium source, there was a more pronounced attenuation of the phenylephrine induced hyperpolarisation than seen under the unstimulated conditions (Rusko and Bauer 1986) which is also in favour of a significant participation of intracellularly bound calcium in the phenylephrine effect.

Some calcium entry blockers, such as D600 and verapamil, can interact with the α_1-adrenoceptors (Glossmann and Hornung 1980) as well as enter the cell (Pang and Sperelakis 1983) and hence competitively inhibit the binding of α_1-adrenoceptor ligand, as observed with D600, or exert an action on intracellular calcium sites, as reported for verapamil. The findings that the action of phenylephrine is reduced also by nifedipine, which is devoid of the above mentioned effects, as well as in calcium-free solution, and in particular that it is suppressed by La^{3+} and Mn^{2+} in concentrations high enough to markedly suppress the action potentials (3 mmol . l^{-1}) suggest that the action of verapamil is the result of the blockade of voltage sensitive calcium channels rather than of some other mechanisms. The fact that the resting membrane conductance was not significantly affected by nifedipine and verapamil, also supports this assumption since calcium channels probably contribute but negligibly to the resting conductance as shown also in other smooth muscles (Godfraind and Miller 1982).

The more pronounced effects of La^{3+} and Mn^{2+} on the delayed recovery phase than on the initial phase of the phenylephrine action suggest that the delayed recovery phase is more dependent on the superficially bound calcium and calcium entry through the so called "leak" channels than the initial phase.

Calcium entry evoked by receptor activation is generally considered to occur through receptor operated channels (for review see Bolton 1979). Sodium nitroprusside was proposed to inhibit the receptor activated calcium entry into smooth muscle cells (Weiss 1981) and to act mainly on intracellular binding sites of calcium (Kreye et al. 1975; Hester et al. 1979). This smooth muscle relaxant had a similar transient effect as did phenylephrine on all the studied activities of the taenia caeci. In spite of the development of the delayed phase of sodium nitroprusside action, however, phenylephrine remained effective. This together with the fact that simultaneous superfusion with phenylephrine and sodium nitroprusside elicited the same biphasic change in electrical and mechanical activity of the taenia (unpublished observations of the authors) suggests that both substances activate the same final mechanisms but probably through different pathways. Thus in the taenia, in contrast to blood vessels, it appears that either sodium nitroprusside does not block the receptor operated calcium channels, or their importance for the action of phenylephrine is minimal.

It has been widely reported that an increase in extracellular concentration

of Mg^{2+} causes smooth muscle to relax but permits continuation of action potentials of intestinal smooth muscle (Sperelakis 1962) as observed also in the present experiments. The presence of Mg^{2+} in the bathing fluid in a relatively high concentration however did not influence the action of phenylephrine when Ca^{2+} was also present, as also observed with Ba^{2+}. Ba^{2+} can replace Ca^{2+} only in entering the cell during action potentials but cannot substitute for Ca^{2+} in the action of adrenaline (Bülbring and Tomita 1969b) and phenylephrine. The present results moreover suggest that Ba^{2+} can potentiate the delayed recovery phase of phenylephrine action, but cannot substitute for Ca^{2+} in the mechanisms responsible for the initiation of both phases of the phenylephrine action.

Our results imply that the activation of α_1-adrenoceptors in the taenia is probably primarily linked with the release of Ca^{2+} which leads to the opening of TEA-sensitive potassium channels and results in the initial phase of the α_1-adrenergic action. We presume that the development of the delayed recovery phase is not only dependent on the presence of the initial phase but also on the presence of superficially bound calcium. Further experiments should be done, however, to clarify the role of other mechanisms, such as receptor operated channel activation in the initial phase or delayed active processes in the delayed recovery phase of phenylephrine action.

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