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European Journal of Pharmacology 367 (1999) 59–66

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Different pathways for Ca^{2+} mobilization by angiotensin II and carbachol in the circular muscle of the guinea-pig ileum

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Received 20 August 1998; revised 1 December 1998; accepted 4 December 1998

Abstract

Ca^{2+} pathways activated by angiotensin II and carbachol were evaluated in the circular muscle of the guinea-pig ileum by recording mechanical and electrical activities. Transient contractions induced by angiotensin II were greatly reduced by Ca^{2+} removal from the medium whereas carbachol-induced responses were not significantly altered. Nifedipine had no effect on the responses to both agonists. A high concentration of tetrodotoxin (0.1 μM) inhibited angiotensin II-induced contractile responses without affecting the depolarization, whereas 1 mM Ni^{2+} inhibited the mechanical and electrical effects. Neither tetrodotoxin nor Ni^{2+} affected carbachol-induced effects. These results indicate that angiotensin II-induced phasic contractions depend on extracellular Ca^{2+} but not on voltage-dependent L-type Ca^{2+} channels. It is suggested that angiotensin II activates Ni^{2+}-sensitive Na^{+} and non-specific cationic channels, whereas the responses to carbachol are dependent on receptor-activated Ca^{2+} release. Furthermore the different response of the longitudinal and circular muscles to the inhibitory effects of tetrodotoxin and Ni^{2+} on the angiotensin II- and carbachol-induced contractions indicates that these agonists exert their own myogenic effects on each layer and are able to trigger different Ca^{2+} mobilization pathways.

Keywords: Angiotensin II; Carbachol; Ileal circular muscle; Mechanical activity; Electrical activity; Tetrodotoxin; Ni^{2+}

1. Introduction

Angiotensin II is the effector molecule of the renin-angiotensin system and exerts its actions via two distinct types of receptors which belong to the seven transmembrane receptor family. Most of the physiological functions of angiotensin II are mediated by the angiotensin AT_1 receptor coupled to heterotrimeric G proteins that activate signal transduction pathways which include an increase in phosphoinositide hydrolysis and inositol trisphosphate (IP_3) formation (Timmermans et al., 1993), and activation of Ca^{2+} channels (Hausdorff and Catt, 1988; Ohnishi et al., 1992). An important property of angiotensin II is its contractile effect in vascular and visceral smooth muscles.

In the guinea-pig ileum, important differences between the responses of the longitudinal and the circular muscle layers have been reported which were ascribed to the intrinsic properties of the respective smooth muscle cells (Kosterlitz and Robinson, 1957; Brownlee and Harry, 1963).

Angiotensin II-induced responses of isolated longitudinal muscle preparations are greatly reduced by the addition of Ca^{2+} channel blockers and abolished in Ca^{2+}-free medium, indicating that, besides Ca^{2+} release from intracellular stores, the major source of Ca^{2+} responsible for contraction in these cells is extracellular (Paiva et al., 1988; Shimuta et al., 1990). As for the circular muscle layer, all reported observations are for dispersed smooth muscle cells and not for intact tissues. These studies demonstrated that acetylcholine, cholecystokinin octapeptide and iloprost, a prostacyclin receptor agonist, induce contraction of the circular muscle cells by releasing intracellular Ca^{2+} rather than by increasing the influx of Ca^{2+} from the extracellular medium (Grider and Makhlouf, 1988; Botella et al., 1995). Murthy et al. (1991) measured the specific binding of $[^3H]IP_3$ to intracellular receptors in permeabilized muscle cells and suggested that circular, but not longitudinal, muscle cells are able to release intracellular Ca^{2+} since high-affinity IP_3 receptors are present predominantly in circular muscle. However galanin, a peptide receptor agonist, was shown to require extracellular Ca^{2+} to induce contractile responses of the circular muscle cells (Botella et al., 1992).

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0014-2999/99/$ - see front matter
PII: S0014-2999(98)00919-4

The contrasting pharmacomechanical responses of the two muscle layers to the agonists, and the fact that the effect of angiotensin II on circular smooth muscle from guinea-pig ileum has not yet been studied, led us to attempt to evaluate the pathways for Ca^{2+} mobilization activated by different receptors in this preparation. With this aim, the angiotensin II- and carbachol-induced responses were investigated by recording the mechanical and electrical activities of the circular smooth muscle cells of the guinea-pig ileum.

2. Materials and methods

2.1. Materials

Angiotensin II was a highly purified peptide synthesized in our laboratory. The angiotensin II receptor antagonist DuP753 (2-*n*-butyl-4-chloro-5-(hydroxymethyl)-1-[2′((1*H*-tetrazol-5-yl)biphenyl-4-yl)-methyl]imidazole) was kindly provided by DuPont/Merck Pharmaceutical, Wilmington, DE, USA, and PD123319 (1(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1*H*-imiazo[4,5-*c*]pyridine-6-carboxylic acid-2HCl) was from Parke-Davis, Ann Arbor, MI, USA. Carbachol, hemicholinium, nifedipine, tetrodotoxin and salts were from Sigma, St. Louis, MO, USA. Nicotine was from Aldrich Chemical, Milwaukee, WI, USA.

2.2. Measurement of mechanical response

Guinea-pigs of 250–350 g body weight, of either sex, were killed by decapitation and bled. A portion of the ileum near the caecum was excised, dissected free of fat and the mucosal layer was thoroughly flushed with Tyrode solution (in mM: NaCl, 137; KCl, 2.7; $CaCl_2$, 1.4; $MgCl_2$, 0.5; $NaHCO_3$, 12; NaH_2PO_4, 0.4; glucose, 5.5, pH 7.6). Rings were mounted either transversally or longitudinally, under 1-g load for isometric recording of the tension due to the circular or longitudinal muscle layer, respectively. The preparations were incubated at 37°C in chambers containing Tyrode solution bubbled with a mixture of 95% O_2 and 5% CO_2. In order to check the possible cholinergic mediation of the responses to angiotensin II in circular muscle, the contribution of acetylcholine released from the myenteric plexus was prevented by using 0.1 mg/ml hemicholinium and 120-μM nicotine (Paiva et al., 1976). Under this condition, angiotensin II maximally contracted the preparation. Moreover, a similar result was obtained when the intramural ganglia were destroyed by cold treatment (4°C, 48 h). The isometric tension was recorded by means of a force–displacement transducer (Hewlett–Packard, model FTA-10) through an amplifier recorder (ECB, model RB102). Concentration–response curves were made by administering different concentrations of angiotensin II at 15-min (for low concentrations) or 30-min intervals (higher concentrations, to avoid tachyphylaxis). Agonist potency is expressed as pD_2, the $-\log$ of the effective concentration inducing 50% of the maximum response (EC_{50}), which was determined by using nonlinear regression Inplot software (Graph-Pad software, San Diego, CA). In the experiments in which the angiotensin II receptor antagonist DuP753 was used, the antagonist potency was determined by the parallel displacement of the angiotensin II concentration–response curves in a competitive manner. Schild analysis (Arunlakshana and Schild, 1959) was applied by using three antagonist concentrations and the potency (pA_2, the log of the apparent dissociation constant) was determined by linear regression analysis (Inplot software) of the Schild plot. Concentration–response curves were also made to evaluate the effect of a 5-min preincubation with tetrodotoxin on the responses of the circular and longitudinal muscles to the agonists and to KCl. Similarly, a 5-min incubation with antagonists of Ca^{2+} channels was followed by the addition of the stimulants. When Ca^{2+} was omitted from the incubation medium, it was not replaced by any other ion and the

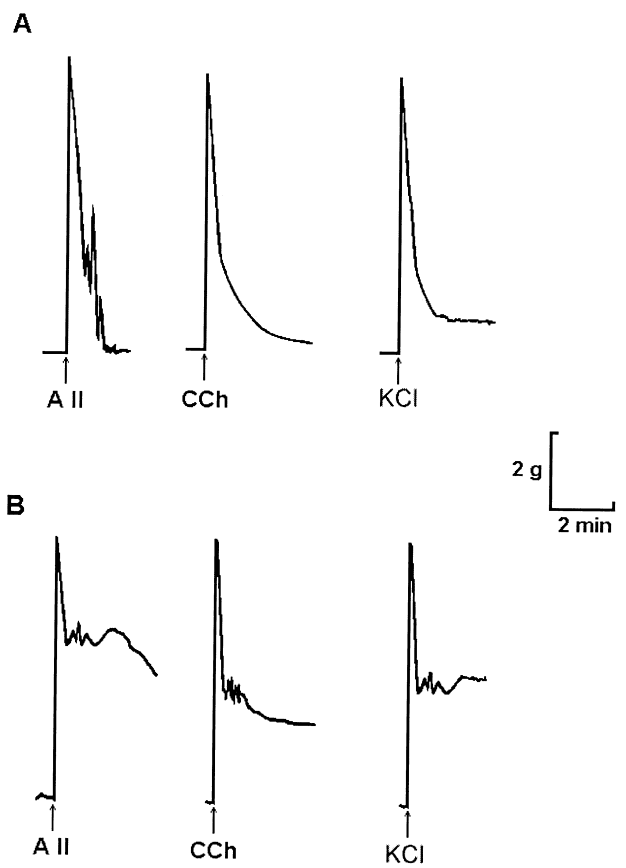

Fig. 1. Isometric recordings of the contractile responses induced by angiotensin II, carbachol and KCl in circular and longitudinal muscles. Responses to 0.1 μM angiotensin II (AII), to 1 μM carbachol (CCh) and to 40 mM KCl added at 30-min intervals of circular (A) or longitudinal (B) muscle preparations. Similar results were obtained in 4–6 experiments. Upward arrows indicate addition of the stimulants.

Table 1
pD_2-values for the contractile responses of the circular and longitudinal muscles to agonists and to KCl in normal medium and in the presence of 100 nM tetrodotoxin

Stimulant	Circular		Longitudinal	
	Control	TTX	Control	TTX
AII	8.1 ± 0.1 (5)[a]	ND	8.9 ± 0.2 (5)	8.6 ± 0.2 (5)
CCh	6.4 ± 0.2 (4)	6.4 ± 0.1 (4)	6.5 ± 0.1 (5)	6.6 ± 0.1 (5)
KCl	1.8 ± 0.2 (4)	1.6 ± 0.3 (4)	1.5 ± 0.3 (3)	1.7 ± 0.3 (4)

Angiotensin II (AII, 0.1 μM), carbachol (CCh, 1 μM) and KCl (40 nM) were used to stimulate the preparations in the absence (Control) or in the presence of tetrodotoxin (TTX, 0.1 μM) preincubated for 5 min. Values are means ± S.E.M.; the number of experiments is given in parentheses. ND indicates that pD_2 was not determined because the contractile effect was completely inhibited.
[a] Significantly different ($P < 0.05$) from pD_2-values for angiotensin II in longitudinal smooth muscle.

preparation was stimulated after a 5-min preincubation in Ca^{2+}-free medium.

2.3. Measurement of membrane potential

Microelectrodes were constructed as previously described (Harvey and Kernan, 1984) by pulling capillaries in a horizontal puller (Narishige, model PN3). The electrodes were filled with 2 M KCl (tip resistance: 30–40 MΩ), mounted in Ag/AgCl half-cells on a micromanipulator (Leitz) and connected to an electrometer (Biodyne, model AM-2). The signals were recorded with a potentiometric chart recorder (ECB, model RB102).

The preparations were placed in a 2-ml perfusion chamber and superfused at a rate of 3 ml/min with Tyrode solution at 37°C, bubbled with a mixture of 95% O_2 and 5% CO_2. The impalements were made directly from the side of the circular muscle layer with the longitudinal muscle layer still attached.

Membrane potentials were measured as previously described (Silva et al., 1994). The successful impalement of the electrode was evidenced by a sharp drop in voltage when the microelectrode penetrated a cell, by a stable potential (± 3 mV) for at least 1 min after impalement, by a sharp return to zero upon exit, and by the minimal change (< 10%) in microelectrode resistance after impalement. Measurements of the membrane potential of circular muscles were obtained in Tyrode solution in the absence and in the presence of angiotensin II (0.1 μM) or carbachol (1 μM). Some measurements of membrane potential were also carried out in preparations that had been preincubated with Ni^{2+} (1 mM) or tetrodotoxin (0.1 μM) for 5 min.

2.4. Statistical analysis

Results were analysed for statistical significance by using Student's *t*-test and a *P*-value less than 0.05 was considered to be statistically significant.

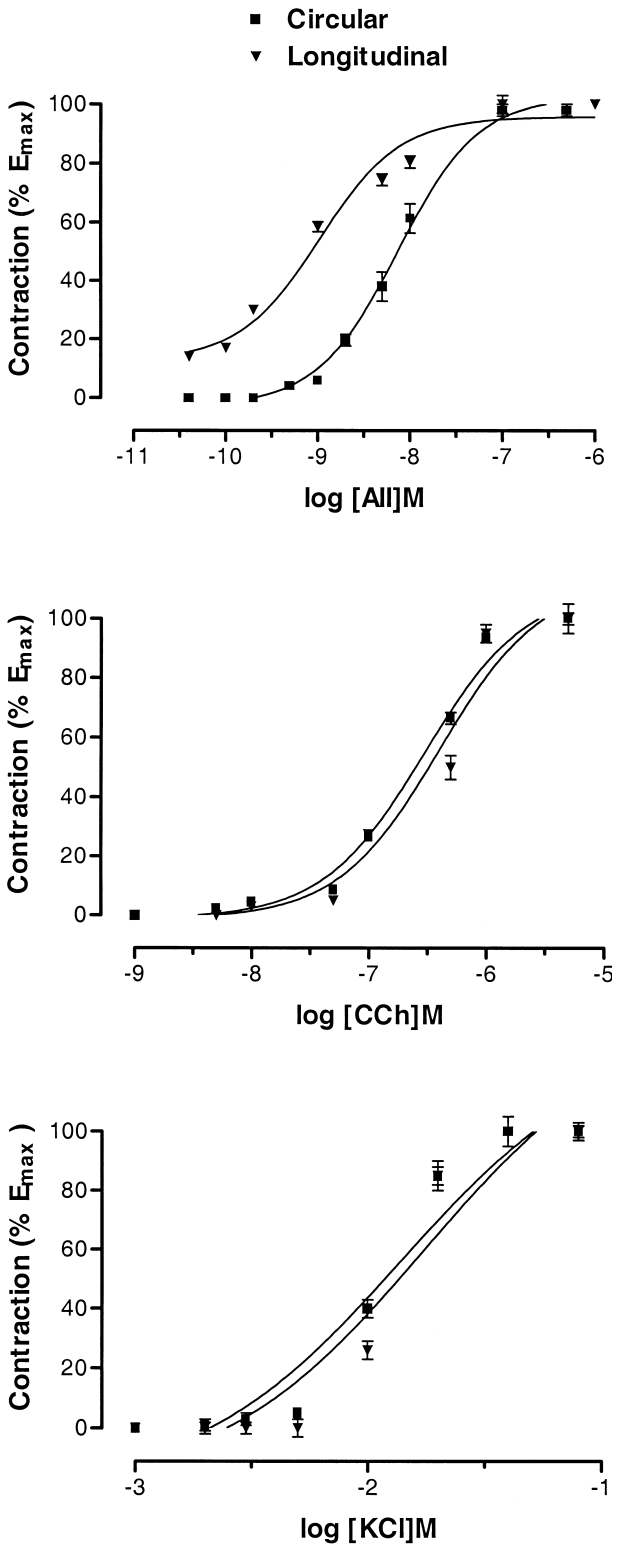

Fig. 2. Isometric contraction induced by the agonists angiotensin II (AII) and carbachol (CCh) and by KCl in isolated guinea-pig ileum. The circular (A) or longitudinal (B) muscle preparations were incubated for 90 s at 37°C with increasing concentrations of the stimulants. Results are expressed as the percentage of maximal effect (E_{max}). Values are means and ± S.E.M. of 4–6 separate experiments.

3. Results

3.1. Contractile effect of angiotensin II and carbachol on circular and longitudinal smooth muscles

The circular muscle responded to maximum concentrations of angiotensin II (0.1 μM), carbachol (1 μM) and KCl (40 mM) with equipotent phasic contractions which, in the case of angiotensin II, were followed by a train of small transient contractions that faded to the basal tonus in the presence of the agonist, whereas the phasic contraction induced by KCl was followed by a small tonic component (Fig. 1A). In contrast to the circular muscle, the responses of the longitudinal muscle to these stimulants had both phasic and tonic components (Fig. 1B).

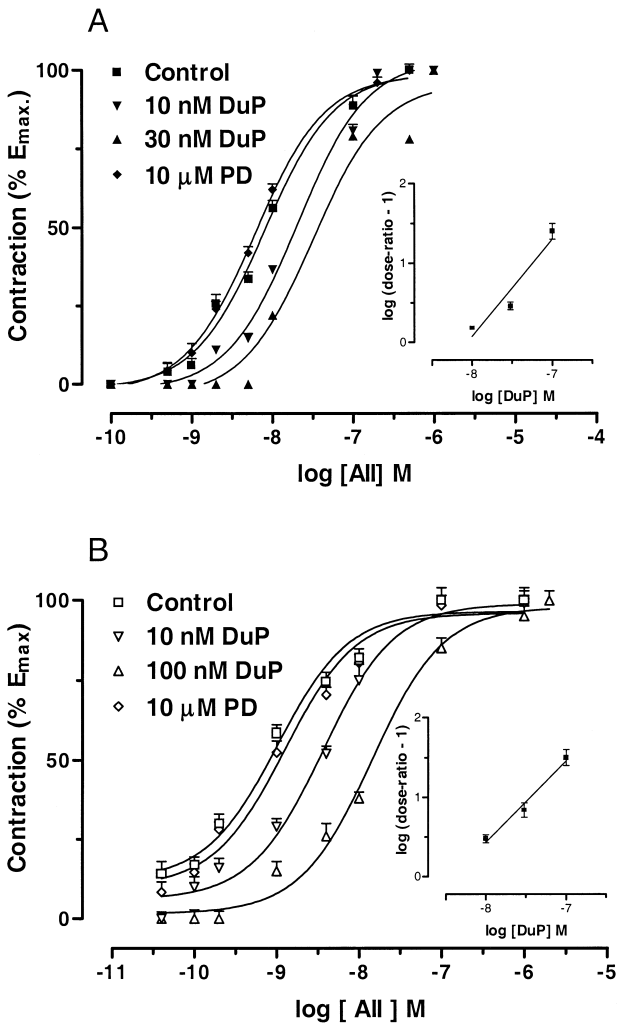

Fig. 3. Inhibition by DuP753 (DuP) of angiotensin II (AII)-induced contractions in ileal circular and longitudinal muscle preparations. Control (without the antagonist) and with 10 nM or 30 nM DuP753, in circular (A) and 30 nM or 100 nM DuP753 in longitudinal (B) muscle preparations. 100% corresponds to the maximal contraction (E_{max}) induced by angiotensin II under normal conditions. Each curve is the mean (±S.E.M.) of at least three sets of experiments. Inset: Schild plot. Effect of 10 μM PD123319 (PD) on the angiotensin II-induced contraction of circular and longitudinal muscles is also shown.

Table 2
Role of extracellular Ca^{2+} on the contractile responses to angiotensin II, carbachol or KCl in circular (CM) and longitudinal muscles (LM)

Tissue	Ca^{2+}-free	Nifedipine		Ni^{2+}	
		Phasic	Tonic	Phasic	Tonic
CM					
AII	16.0±2.0[a]	93.0±6.0	ND	2.0±0.5[a]	ND
CCh	84.0±4.0[a]	96.0±5.0	ND	90.0±2.0[a]	ND
KCl	1.0±0.3[a]	87.0±3.0[a]	1.0±0.5[a]	67.0±2.0[a]	76.0±2.0[a]
LM					
AII	10.0±0.8[a]	90.0±2.0[a]	2.0±0.5[a]	40.0±1.0[a]	45.0±0.5[a]
CCh	12.0±1.0[a]	92.0±3.0[a]	1.0±0.5[a]	42.0±2.0[a]	65.0±1.0[a]
KCl	1.0±0.5[a]	78.0±4[a]	3.0±0.5[a]	80.0±3.0[a]	51.0±0.5[a]

The preparations were stimulated with angiotensin II (AII, 0.1 μM) or carbachol (CCh, 1 μM) or KCl (40 mM) in normal medium or, after a 5-min incubation in Ca^{2+}-free medium, in normal medium + 100 nM nifedipine or in normal medium + 1 mM Ni^{2+}. Results are expressed as the percentage of the response in relation to the maximum response to each contracting agent in the normal medium. ND means not detectable. Values are means ± S.E.M. of 4–6 separate experiments.
[a] Significantly different ($P < 0.05$) from the control values.

The pD_2-values shown in Table 1 were obtained from the concentration–response curves for angiotensin II, carbachol and KCl shown in Fig. 2. The pD_2 estimates for angiotensin II revealed significant differences in the longitudinal and circular muscles, which were not observed in the case of carbachol- and KCl-induced responses.

3.2. Effect of antagonists on the responses to angiotensin II in circular and longitudinal muscle preparations

The effect of the angiotensin AT_1-selective nonpeptide receptor antagonist DuP753 on the responses to angiotensin II is shown in Fig. 3. DuP753 produced a parallel, concentration-dependent rightward displacement of the angiotensin II response curve. Schild analysis yielded a slope that was not significantly different from unity (1.2 ± 0.3, $n = 5$) and the pA_2-value was 8.0 ± 0.3 ($n = 3$), as shown in the inset of Fig. 3A. Similarly, DuP753 depressed the contractions induced by angiotensin II in longitudinal muscle preparations (Fig. 3B), yielding a pA_2-value of 8.4 ± 0.3 ($n = 4$) and a Schild plot's slope of 1.03 ± 0.14 ($n = 4$). The angiotensin AT_2 selective receptor antagonist PD123319 (up to 10 μM) failed to inhibit the responses to angiotensin II in the circular and in the longitudinal muscles (Fig. 3).

3.3. Role of extracellular Ca^{2+} in the contractile responses induced by the agonists angiotensin II and carbachol and by KCl

After the removal of Ca^{2+} from the medium for 5 min, the response induced by 0.1 μM angiotensin II in the circular muscle was drastically inhibited whereas the response to 1 μM carbachol was only slightly affected.

Under the same condition, almost no response was observed when a depolarizing concentration (40 mM) of KCl was used (Table 2). However the contractile responses of the longitudinal muscle to both agonists and to the depolarizing concentration of KCl were markedly reduced (Table 2), in agreement with previous observations (Paiva et al., 1988; Grider and Makhlouf, 1988).

In order to verify the Ca^{2+} pathways involved in the responses induced by angiotensin II, carbachol or KCl, Ca^{2+} channel antagonists were used. A 5-min preincubation of circular muscle preparations with nifedipine (0.1 μM), an inhibitor of the voltage-dependent L-type Ca^{2+} channels, had no significant effect on the phasic responses induced by the agonists whereas the tonic component of the KCl-induced contractile effect was blocked (Table 2). However the tonic component present in the responses of the longitudinal muscle to all stimulants, which is due to Ca^{2+} influx through L-type Ca^{2+} channels, was totally inhibited by nifedipine, leaving the phasic component of the responses partially affected (Table 2). A similar effect was observed when 1 μM verapamil or 0.01 μM isradipine was used (not shown).

To determine whether T-type Ca^{2+} channels were involved in the contractile responses induced by angiotensin II, carbachol and KCl, an antagonist of these channels, Ni^{2+} (Ertel and Ertel, 1997), which is also known to be a

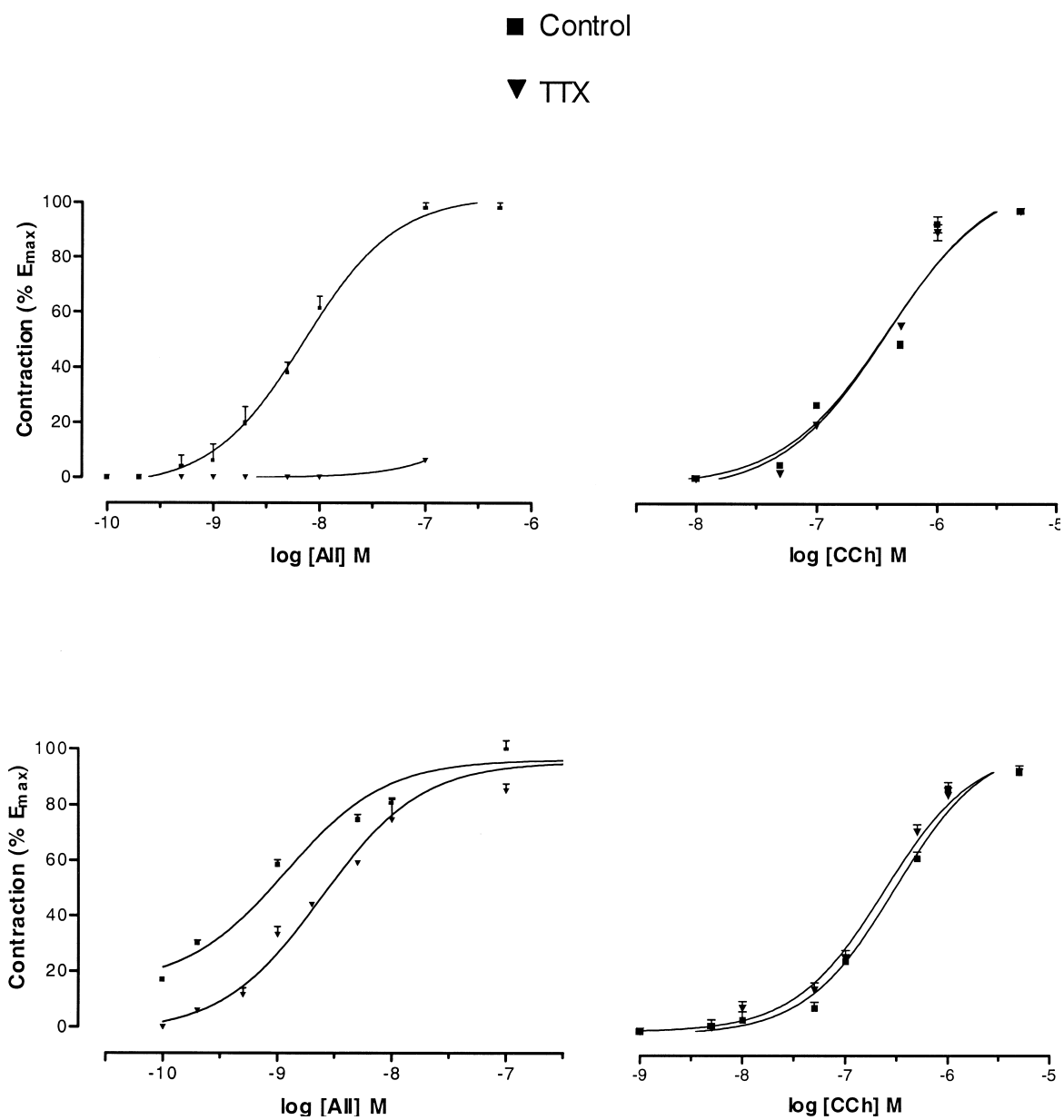

Fig. 4. Effect of tetrodotoxin (TTX) on the contractile responses to angiotensin II (AII) and carbachol (CCh) in the guinea-pig ileum. Concentration-dependent contractions induced by angiotensin II or carbachol in circular (A) or in longitudinal (B) muscles in the absence or in the presence of 100 nM tetrodotoxin added 5 min before each concentration of the agonist tested. The values are means ± S.E.M. of 4–6 experiments.

nonselective cation channel inhibitor (Inoue, 1991) was used. The maximum effect of angiotensin II in the circular muscle was drastically inhibited at 1 mM Ni^{2+}. Under the same experimental condition, Ni^{2+} slightly inhibited the contraction induced by 1 μM carbachol and the responses to 40 mM KCl were markedly inhibited (Table 2).

Preincubation of the longitudinal muscle preparation for 5 min with 1 mM Ni^{2+} inhibited both the phasic and the tonic components of the contractions induced by 0.1 μM angiotensin II, 1 μM carbachol and 40 mM KCl (Table 2).

3.4. Effect of tetrodotoxin on the contractile responses induced by angiotensin II, carbachol and KCl in circular and longitudinal muscle preparations

Since studies with cardiac cells demonstrated that Ca^{2+} ions may permeate tetrodotoxin-sensitive Na^{+} channels after their stimulation by agonist (Sorbera and Morad, 1990), we investigated whether a similar channel is involved in the contractile responses induced by angiotensin II, carbachol and KCl in the circular muscle. Fig. 4A shows that 100 nM tetrodotoxin completely inhibited the contractile responses to angiotensin II but not to carbachol in the circular muscle. Similar results were obtained (not shown) under conditions where the cholinergic mediation was pharmacologically blocked (see Section 2.2). Tetrodotoxin did not affect KCl-induced contractions (not shown). The pD_2-values determined for the effects of carbachol and KCl in the absence and in the presence of 100 nM tetrodotoxin were not significantly different (Table 1). In contrast to the drastic inhibitory effect of tetrodotoxin on angiotensin II-induced responses observed in circular muscle, in the longitudinal muscle tetrodotoxin had a partial effect on the responses to low concentrations of angiotensin II (Fig. 4B), and the pD_2-value obtained in the presence of tetrodotoxin was not significantly different from the control (Table 1). The responses of the longitudinal muscle to carbachol were not significantly affected by the presence of tetrodotoxin whereas the responses elicited by low concentrations of KCl were inhibited without significant changes in the pD_2-value (Table 1).

3.5. Electrical responses of the circular smooth muscle to angiotensin II and carbachol

To investigate the mechanism underlying the inhibition by Ni^{2+} or tetrodotoxin of the contractile responses induced by angiotensin II, the membrane potential of the circular muscle layer was measured when the muscle was still attached to the longitudinal muscle. The resting potential was found to be -50.5 ± 2 mV ($n = 57$), and marked depolarizations were observed upon addition of 0.1 μM angiotensin II ($\Delta V_m = 30.9 \pm 3.7$ mV, $n = 28$) or 1 μM carbachol ($\Delta V_m = 32.6 \pm 4.2$ mV, $n = 16$), as shown in Fig. 5.

Ni^{2+} (1 mM) completely inhibited the depolarizing effect induced by angiotensin II (0.1 μM), as shown in Fig. 5B, but no inhibition was observed in the case of carbachol (Fig. 5B), in agreement with the lack of inhibition of the response of the latter by Ni^{2+}.

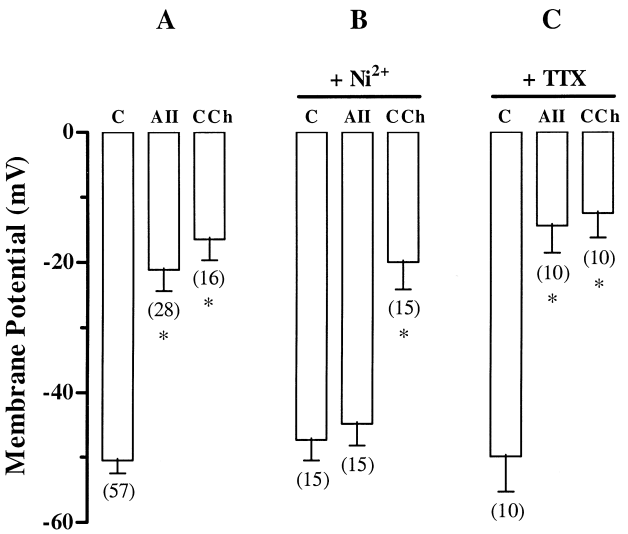

Fig. 5. Effect of angiotensin II and carbachol on the electrical activity of circular muscles. (A) Membrane potentials in the absence (control) and in the presence of angiotensin II (0.1 μM AII) or carbachol (1 μM CCh). (B) Effect of 1 mM Ni^{2+}. (C) Effect of tetrodotoxin (0.1 μM TTX). Values shown represent the means ± S.E.M. of the number of impalements, given below the columns. * Significantly different from the controls ($P < 0.05$).

In contrast, the inhibition induced by tetrodotoxin of the contractile response to angiotensin II (Fig. 4A) was not paralleled by an inhibition of the depolarizing effect. In preparations pretreated with tetrodotoxin, angiotensin II caused depolarization of the circular muscle cells similar to that found in untreated controls (Fig. 5C). Similarly, the depolarization induced by carbachol was unaltered by pretreatment with tetrodotoxin (Fig. 5C), which also did not affect the contractile response to carbachol (Fig. 4A).

4. Discussion

In the circular muscles of the guinea-pig ileum, angiotensin II and carbachol induced phasic responses, whereas biphasic responses were observed in longitudinal muscles. Angiotensin II was significantly less pontent in circular smooth muscle than in longitudinal muscle. The angiotensin II-induced effect was antagonized by DuP753 but not by PD123319, indicating that the angiotensin AT_1 receptor is involved in both preparations. The finding that the agonist potency but not the antagonist potency was different in the two preparations indicates that probably the signaling mechanisms involved in the contractile responses to the peptide were distinct. In the case of carbachol, however, no significant differences were found in the potency of the agonist in circular and longitudinal muscles, indicating that probably the same mechanism was respon-

sible for the carbachol-induced effect in both preparations. Like the carbachol-induced response, the contractile effect of KCl was not significantly different in circular and longitudinal muscle preparations. The finding that angiotensin II produced equivalent maximum responses in the two muscle layers contrasts with the report by Schinke et al. (1991), who showed that in the rat ileum the contractile response of the circular muscle to angiotensin II amounted to 16% of that to methacholine, whereas both agonists were equipotent in the longitudinal muscle. The difference between the results is probably due to species differences.

Whereas the contractile responses of the circular muscle to carbachol were not affected in Ca^2-free medium, in agreement with previous observations (Grider and Makhlouf, 1988; Botella et al., 1995), the angiotensin II responses were dependent on extracellular Ca^{2+} since they were greatly reduced upon removal of this ion from the medium. Thus, in spite of the similar transient contractions induced by angiotensin II and carbachol in circular muscle, the mechanisms underlying these responses are different, involving Ca^{2+} influx in the case of angiotensin II and Ca^{2+} release from intracellular stores in the case of carbachol.

The finding that nifedipine did not inhibit the circular muscle responses to either angiotensin II or carbachol indicates that these agonists are not able to activate L-type Ca^{2+} channels, although these channels are present in this preparation, as was indicated by the complete inhibition of the tonic component of the KCl response by nifedipine. Therefore, the angiotensin II-induced contraction is dependent on Ca^{2+} influx via nifedipine-insensitive Ca^{2+} channels.

Our finding that relatively high concentrations of tetrodotoxin inhibited the contractile responses of circular muscle induced by angiotensin II suggests that this peptide stimulates a Na^+ channel in which the cationic selectivity site was altered from Na^+ to Ca^{2+} ions by an agonist, as has been described in cardiac myocytes (Sorbera and Morad, 1990). However, the inhibitory effect of tetrodotoxin on the contractile response to angiotensin II occurred without the depolarization, which is mainly due to Na^+ influx induced by this peptide (Paiva et al., 1988), being affected. This suggests that angiotensin II can activate a non-selective cationic channel in circular muscle cells, such as that shown to be present in longitudinal muscle cells (Nouailhetas et al., 1994). This could be responsible for the angiotensin II-induced depolarization, which occurred even in the presence of tetrodotoxin. Ni^2, which is known to block non-specific cationic channels (Inoue, 1991), in addition to the T-type Ca^{2+} channel (Ertel and Ertel, 1997), markedly inhibited the electrical and the contractile responses of the circular muscle to angiotensin II, suggesting that it can block both channels. In agreement with this hypothesis, Yamamoto et al. (1993), using the whole cell voltage clamp method, described a Na^+ channel in smooth muscle cells which is relatively resistant to tetrodotoxin and much more sensitive to divalent cations. The possibility that a T-type Ca^{2+} channel could also be involved in the responses induced by angiotensin II and by KCl in circular muscle cannot be ruled out since the phasic contractions elicited by both agonists were inhibited by Ni^{2+}.

In spite of the evidence that angiotensin II exerts a direct contractile effect on smooth muscles (Ohashi et al., 1967; Paiva et al., 1976), the finding that this effect can be inhibited by tetrodotoxin has been interpreted as an indication that nervous mediation is involved in the response of the guinea-pig ileum to angiotensin II (Godfraind et al., 1966; Hawcock and Barnes, 1993). However, our present results indicate that the effect of tetrodotoxin is due to the inhibition of angiotensin II-induced Ca^{2+} influx through the channels proposed here since nervous mediation was impaired.

In longitudinal muscle, where the response to angiotensin II, carbachol or KCl is due to the influx of extracellular Ca^{2+}, Ni^{2+} inhibited both the phasic and tonic components of the responses to the three agents. This is probably due to inhibition by Ni^{2+} of extracellular Ca^{2+} influx through both L-type Ca^{2+} channels and Ni^{2+}-sensitive non-specific cationic channels whose depolarizing effect was described in longitudinal muscle (Inoue, 1991).

In contrast to what was observed in longitudinal muscle, in the circular muscle only the responses to angiotensin II and KCl, which depend on external Ca^{2+}, were inhibited by Ni^{2+}. The responses to carbachol, which depend on intracellular Ca^{2+} release, were not affected by this ion.

In conclusion, the present study provides evidence that, in the guinea-pig ileum circular smooth muscle, angiotensin II induces phasic contractions that depend on Ca^{2+} influx by activating non-specific cationic channels, whereas the responses to carbachol are dependent on receptor-activated intracellular Ca^{2+} release. Voltage-dependent L-type Ca^{2+} channels do not appear to be activated by either agonist in circular muscle.

Furthermore, since both muscle layers were present in the preparations, the different response of the longitudinal and circular muscles to the inhibitory effects of tetrodotoxin and Ni^{2+} on the angiotensin II- and carbachol-induced contractions indicates that these agonists exert their own myogenic effects on each layer and are able to trigger different Ca^{2+} mobilization pathways.

Acknowledgements

We are grateful to Nelson Mora and to Silvana Aparecida Alves Correa for competent technical assistance. Supported by FAPESP, CNPQ and CAPES grants.

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