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Mechanical and electrical responses to α-adrenoceptor activation in the circular muscle of guinea-pig stomach

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1 In the circular muscle of the corpus region of the guinea-pig stomach, the effects of catecholamines on mechanical activity were studied with simultaneous recording of membrane potential by use of intracellular microelectrodes. In order to investigate responses mediated through α-adrenoceptors, the β-adrenoceptors were blocked by propranolol (10^{-6} M) in most experiments.

2 Adrenaline ($<10^{-6}$ M) produced a monophasic contraction with little change in membrane potential. At higher concentrations ($>10^{-5}$ M), adrenaline usually produced an early transient contraction followed by a slow tonic contraction. During the early phase, the membrane was hyperpolarized and slow waves were reduced in amplitude.

3 The phasic contractions superimposed on the late slow phase of the contractile response were higher in frequency and larger in amplitude than in controls before adrenaline application. These changes were associated with an increase in the amplitude and frequency of slow waves, and with a spike-like component appearing on the top of each slow wave.

4 Adrenaline-induced changes in mechanical and electrical activity were inhibited by prazosin, but largely unaffected by yohimbine. In some preparations, there was a transient weak inhibition of phasic contraction before muscle tone was increased by adrenaline, and this became more pronounced in the presence of yohimbine.

5 Phenylephrine, selective for α_1-receptors, produced responses very similar to those of adrenaline, whereas agonists selective for α_2-receptors, clonidine and B-HT 920, produced only a small slow contraction without any clear change in membrane potential.

6 It is concluded that in the circular muscle of guinea-pig stomach, the contractile response and changes in the electrical slow wave activity produced by adrenaline are both mediated mainly through the activation of α_1-receptors.

Introduction

In stomach smooth muscle, the mechanical response to catecholamines is complicated because α-adrenoceptors mediate both inhibition and excitation, whilst β-adrenoceptor activation produces an inhibitory response. Furthermore, the relative degree of these effects varies in different regions and muscle layers of the stomach (Guimaraes, 1969; Bailey, 1971; Haffner, 1971; 1972; Yamaguchi & Tomita, 1974). In the circular muscle of the guinea-pig corpus, noradrenaline is considered to produce contraction at low concentrations by activating α_2-adrenoceptors, while at high concentrations α_1-adrenoceptors mediate relaxation (Sahyoun *et al.*, 1982a,b).

Although the effects of catecholamines on mechanical activity have been widely studied, the associated electrical changes have not. By use of a combination of sucrose-gap and intracellular microelectrode techniques in the circular muscle of guinea-pig stomach, it has been found that noradrenaline and isoprenaline prevent slow wave generation at a concentration of about 5×10^{-6} M, mainly by activating α-adrenoceptors (Magaribuchi *et al.*, 1972; Ito & Kuriyama, 1975). Ohkawa (1976) using pressure electrode recording (probably from longitudinal muscle), concluded that the inhibition was mediated through β-adrenoceptors whilst phenylephrine increased electrical spike activity by activating α-adrenoceptors.

In an attempt to clarify the situation, effects of catecholamines on membrane activity have been investigated by use of intracellular microelectrodes.

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We have also attempted to analyse the subtype of α-adrenoceptor responsible for mediating the action of adrenaline in the circular muscle in the corpus of guinea-pig stomach, using selective agonists and antagonists.

Methods

Hartley guinea-pigs (300–400 g) of either sex were stunned, then killed by exsanguination, and the stomach removed. Muscle strips from the corpus region were dissected in the direction of the circular muscle fibres, after careful removal of the mucosa. For simultaneous recording of electrical and mechanical activities, a small piece of tissue (0.5 × 5 mm) was mounted horizontally in a chamber (0.2 ml) by pinning down one end and attaching the other end to a strain gauge with thread. Conventional methods were used for measuring the membrane potential using microelectrodes filled with 3 M KCl (30–50 MΩ). To achieve stable penetrations, mechanical responses were minimized by applying very weak stretch to the preparation just sufficient to take up the slack of the thread. The preparations were superfused at a rate of 1.2 ml min^{-1} with a physiological salt solution which contained (mM): NaCl 132.3, $KHCO_3$ 5.9, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 11.8 and Tris-HCl 4.7, the pH being adjusted to 7.4 at 35°C.

For recording of mechanical responses only, to obtain the concentration-tension curve for adrenaline, preparations (1 × 6 mm) were suspended vertically in a small tube (5 mm in diameter) and superfused with physiological salt solution at a rate of 1.2 ml min^{-1} and at 35°C.

Preparations were equilibrated for at least 1 h before starting the experiments. Drugs were applied to the superfusing solution, and the intervals between agonist application were 15–20 min. Propranolol (10^{-6} M) was usually added to the solution to eliminate responses mediated through β-adrenoceptors, although their contribution was generally very small. When the effects of antagonists were tested, preparations were pretreated for 10 min before agonist-induced responses were re-examined in the continued presence of the antagonist.

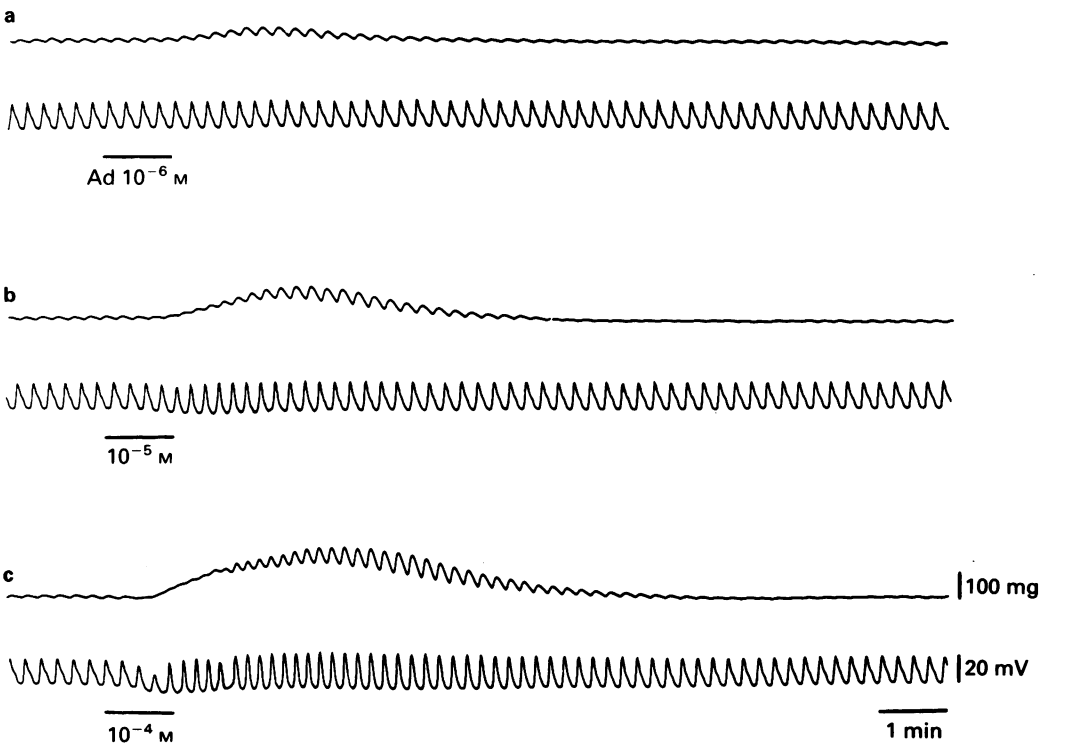

Figure 1 Effects of adrenaline on mechanical (upper) and electrical (lower trace) activities of the circular muscle of guinea-pig stomach (lower trace: continuous recordings from the same cell). Adrenaline (Ad) was applied for 1 min as indicated by horizontal bars, at intervals of 15 min: (a) 10^{-6} M; (b) 10^{-5} M, and (c) 10^{-4} M.

Drugs used were adrenaline, phenylephrine, isoprenaline, propranolol, yohimbine (all HCl salts from Sigma), clonidine, and B-HT 920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d) azepine, Boehringer).

Results

The circular muscle of guinea-pig stomach exhibited spontaneous waves of mechanical activity which were superimposed on a basal muscle tone. The rhythmic contractions were associated with electrical slow waves, as previously described (Ohba *et al.*, 1977). In the present experiments, their frequency was 4.5 ± 0.2 waves min^{-1} (mean $\pm$ s.e.mean, $n = 13$) and the membrane potential measured at the highest level between slow waves was -61.9 ± 1.3 mV ($n = 7$). There was a tendency for larger phasic contractions to be associated with a larger spike component superimposed on the slow wave. However, the rhythmic contractions could be observed even when the spike component was apparently lacking.

Responses to adrenaline

Figure 1 shows the effect of three different concentrations of adrenaline each applied for 1 min at intervals of 15 min. At 10^{-6} M, both the basal tone and rhythmic contractions were increased, but there was no clear change in the associated electrical activity. When the concentration was increased, the contractile response was increased and a transient hyperpolarization of the membrane appeared (3.2 ± 0.4 mV at 10^{-5} M, $n = 5$, and 5.7 ± 0.6 mV at 10^{-4} M, $n = 6$). At a concentration of 10^{-4} M, phasic contractions associated with slow waves were transiently reduced by adrenaline during the rising phase of muscle tone. When high concentrations of adrenaline were applied, the slow wave was reduced before the hyperpolarization had reached its peak (Figure 1c). Subsequently the amplitude of slow waves became larger and their duration shorter than during the control period. In consequence, slow wave frequency and that of the accompanying phasic contractions were increased and a small spike-like com-

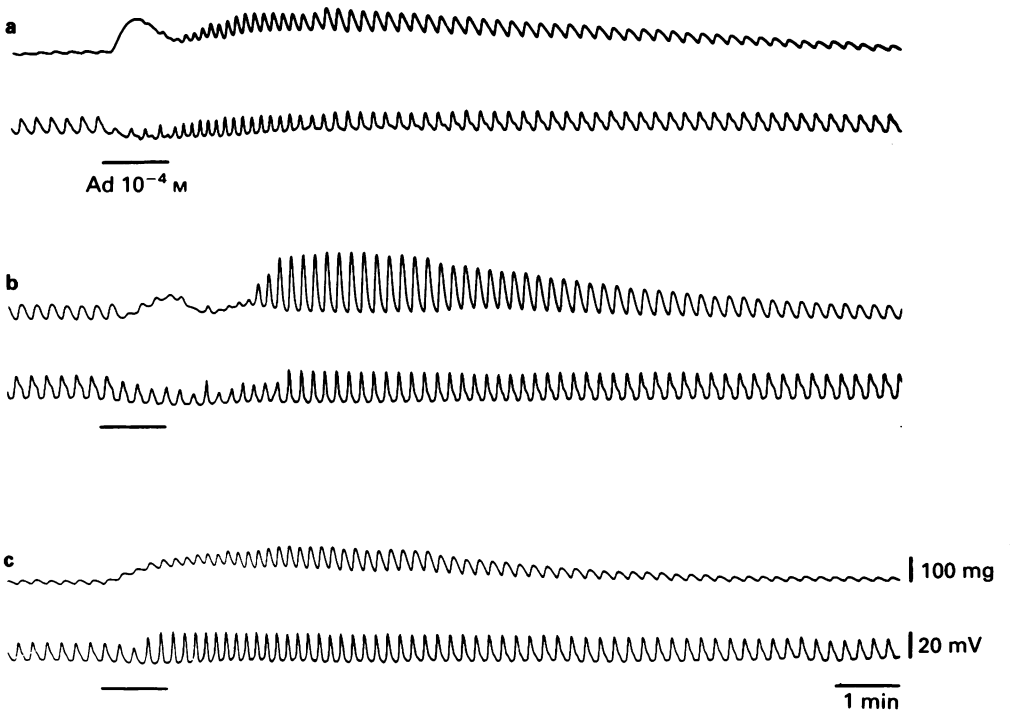

Figure 2 Examples of the response to adrenaline (Ad) 10^{-4} M in three different preparations. See text for further explanation. Upper trace mechanical response and lower trace electrical response.

ponent usually appeared on the top of each slow wave. These changes in slow wave configuration were independent of, and lasted longer than, the membrane hyperpolarization. The increase in frequency was concentration-dependent, being 4.4 ± 3.8, 8.1 ± 3.5, and $50.6 \pm 12.7\%$ ($n = 5$) at 10^{-6}, 10^{-5} and 10^{-4} M adrenaline, respectively. Thus, for a demonstration of the full effect of adrenaline on electrical activity, a high concentration (10^{-4} M) was necessary. The response to adrenaline was not modified by atropine (5×10^{-6} M), confirming the previous results with hyoscine (Yamaguchi & Tomita, 1974). This suggests that a contribution of cholinergic nerves to the response is negligible.

The electrical responses to adrenaline were essentially the same in all preparations examined. In contrast, mechanical responses were quite variable in different preparations (Figure 2). In 33 out of 42 preparations, a biphasic (early transient and late slow prolonged) contraction appeared (Figure 2a,b), while in remaining preparations, only a monophasic contraction occurred (Figure 2c). Due to the faster increase in contraction, stable electrical recordings were more difficult from preparations exhibiting biphasic contractions.

During the slow increase in basal tone produced by adrenaline (Figure 2), there was an increase in the amplitude and frequency of phasic contractions. These were usually accompanied by corresponding increases in slow wave amplitude and the appearance of a spike-like component on top of the slow wave. However, there was no clear relationship between the size of the phasic contraction and the increase in basal tone. The early component of biphasic contractions (Figure 2a,b) lasted for about 1 min and its peak roughly coincided with the maximum hyperpolarization of the membrane. There was often an inhibition of phasic contractions just before and during the rising phase of the early component.

The duration of application of adrenaline had little effect on the characteristics of the evoked responses. The records shown in Figure 3 were obtained from the same preparation as used for Figure 1 in which the separation of early and late components of the contractile response was poor. The early component was not apparently affected, but the slow component was prolonged with longer application of adrenaline. However, muscle tone and phasic contractions tended to decline slowly even in the continued presence of adrenaline (Figure 3b).

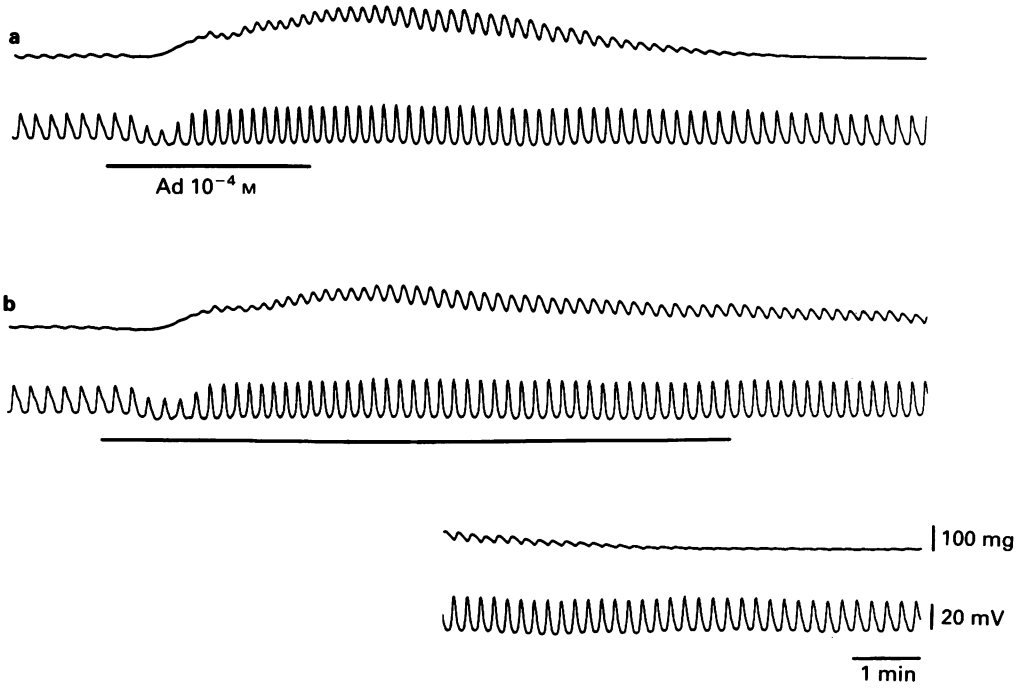

Figure 3 Effects of prolonged application of adrenaline (Ad 10^{-4} M), obtained from the same preparation as in Figure 1. The response pattern was not essentially modifed by prolonging adrenaline application.

Receptor subtypes involved in adrenaline response

The mechanical and electrical responses to adrenaline (10^{-4} M) were not apparently affected by propranolol (10^{-6} M). The hyperpolarization of the membrane and increased frequency of rhythmic activity caused by 10^{-4} M adrenaline were 4.5 ± 0.3 mV and $40.5 \pm 7.2\%$, respectively, in controls and 5.1 ± 0.5 mV and $33.9 \pm 6.0\%$ ($n = 7$) after 10 min exposure to propranolol (10^{-6} M). These changes were not statistically significant. However, it was possible to demonstrate a propranolol-sensitive relaxant effect of adrenaline after α-receptor blockade with phentolamine (10^{-6} M). Furthermore, isoprenaline (10^{-6}–10^{-4} M) caused relaxation without clear hyperpolarization of the membrane. Thus, although β-adrenoceptors are present in the preparation, the responses to adrenaline were mainly mediated through α-adrenoceptors. The following experiments were carried out in the presence of propranolol (10^{-6} M).

In Figure 4, the effects of adrenaline, phenylephrine, and clonidine, each at 10^{-4} M, were compared in the presence of propranolol (10^{-6} M). Phenylephrine, a selective α_1-adrenoceptor agonist, produced a very similar response to adrenaline. On the other hand, clonidine, a relatively selective α_2-adrenoceptor agonist, had a weak effect with a slow onset. Another α_2-adrenoceptor agonist, B-HT 920 (10^{-4} M) produced similar effects (results not shown). Clonidine and B-HT 920 had almost no effect on membrane potential and slow wave configuration. The frequency of electrical and mechanical activities was also not apparently affected by these agents.

Figure 5 shows the effects of prazosin (an α_1-adrenoceptor antagonist) which strongly reduced the adrenaline response. In the presence of prazosin (10^{-6} M), adrenaline (10^{-4} M) produced only a slow increase in contractile response with a reduced effect on electrical activity. Thus, the effects of adrenaline became very similar to those of clonidine (Figure 4c, Figure 5b). Paradoxically, however, the effects of clonidine were abolished by prazosin (Figure 5d).

Yohimbine (an α_2-adrenoceptor antagonist, 10^{-6} M) reduced the amplitude of spontaneous phasic contractions without altering electrical activity (the slow wave) (Figure 6). In the presence of yohimbine, adrenaline (10^{-4} M) still produced membrane hyperpolarization, but the degree of potentiation of elec-

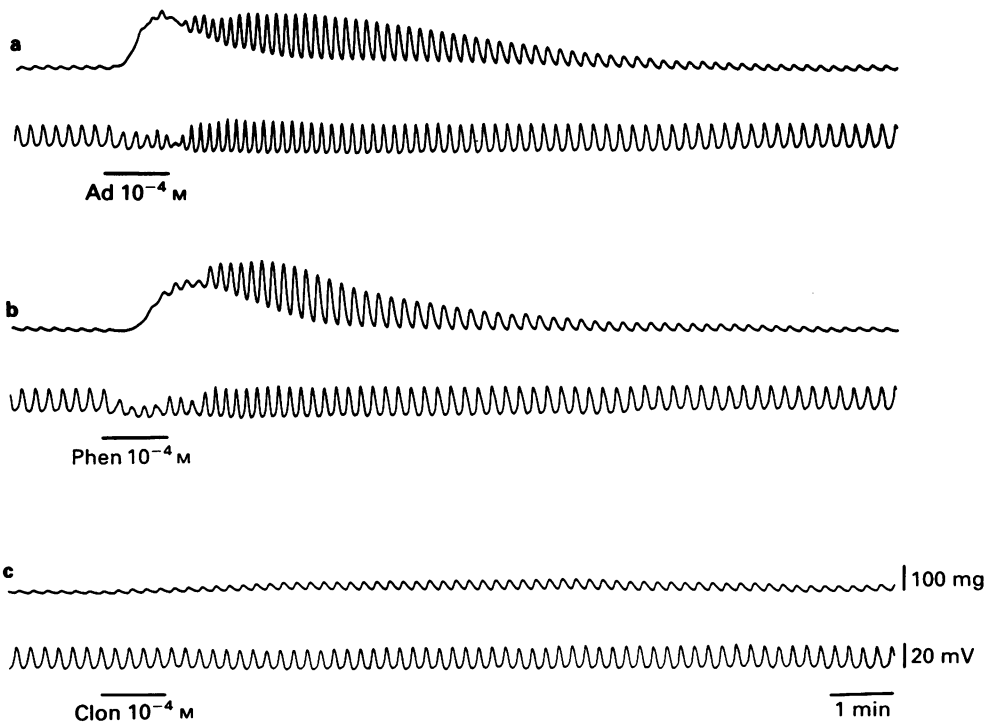

Figure 4 Comparison of the effects of (a) adrenaline (Ad), (b) phenylephrine (Phen) and (c) clonidine (Clon). Continuous intracellular recordings from the same cell in the presence of propranolol (10^{-6} M) in lower trace. Mechanical responses in upper traces.

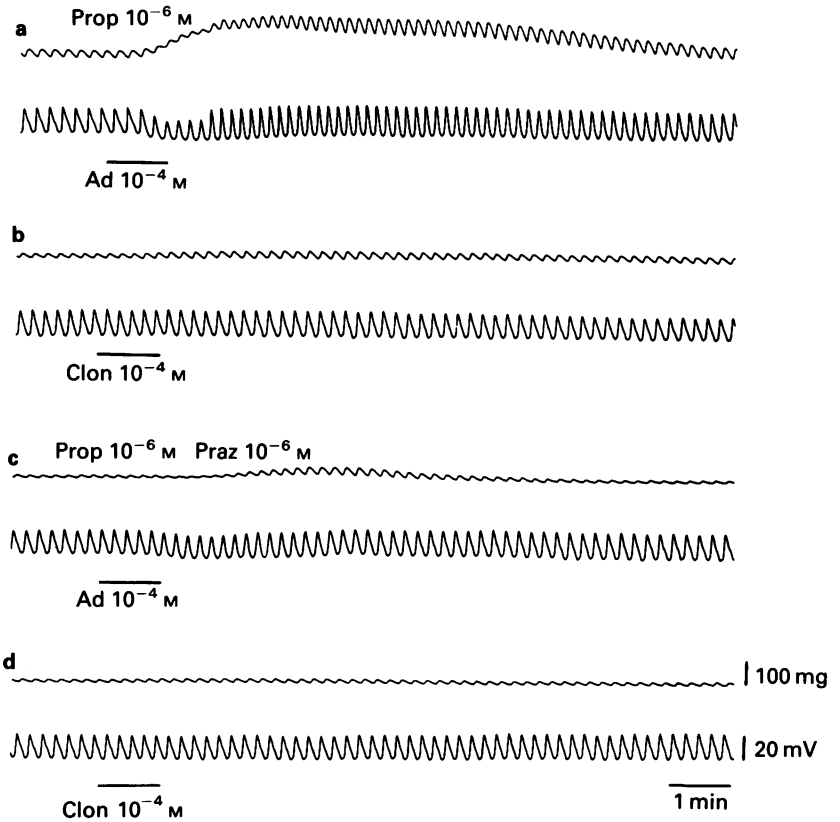

Figure 5 Effects of prazosin on responses to adrenaline and clonidine in the presence of propranolol (Prop, 10^{-6} M). After observing the control response to adrenaline (Ad) (a) and clonidine (Clon) (b), prazosin (Praz, 10^{-6} M) was applied 10 min before the second application of adrenaline (c) and exposure to clonidine (d). Successive recordings from the same cell in lower traces.

trical slow wave amplitude was slightly less than the control. The early inhibition of phasic contractions by adrenaline was very marked and the enhancement of phasic contractions was transient in the presence of yohimbine. Due to the early mechanical inhibition, the excitatory action of adrenaline appeared with some delay. Clear electrical and mechanical responses were produced by phenylephrine in the presence of yohimbine. However, the early inhibition of mechanical activity also observed with adrenaline was strong whilst any slow increase in muscle tone was very weak. It is not clear whether these apparent differences in the actions of adrenaline and phenylephrine are the result of α_2-adrenoceptor blockade or the consequence of a slowly developing non-specific action of high concentrations of yohimbine.

The effects of prazosin and yohimbine on membrane hyperpolarization and acceleration of rhythmic activity caused by adrenaline are summarized in Table 1. It is clear that both responses were strongly inhibited by prazosin, but not by yohimbine.

In another series of experiments, the effects of prazosin and yohimbine were studied only on the mechanical response to adrenaline in the presence of propranolol (10^{-6} M). An example of such an experiment is shown in Figure 7. The response to adrenaline ($<10^{-6}$ M) was monophasic, and the maximum tension development was usually obtained at 10^{-6} M (Figure 7a). As the concentration was increased to 10^{-4} M, the response was prolonged and became biphasic (Figure 7b,c). Prazosin (10^{-6} M) strongly inhibited the effects of adrenaline and, in the presence of prazosin, the response was always monophasic (Figure 7d–f). When adrenaline (10^{-4} M) produced the biphasic response, the amplitude of contraction was not significantly reduced by prazosin, although the rate of contraction was slowed and the late phase of the response was markedly inhibited. In preparations in which the mechanical response to 10^{-4} M adrenaline was monophasic, prazosin had a strong inhibitory

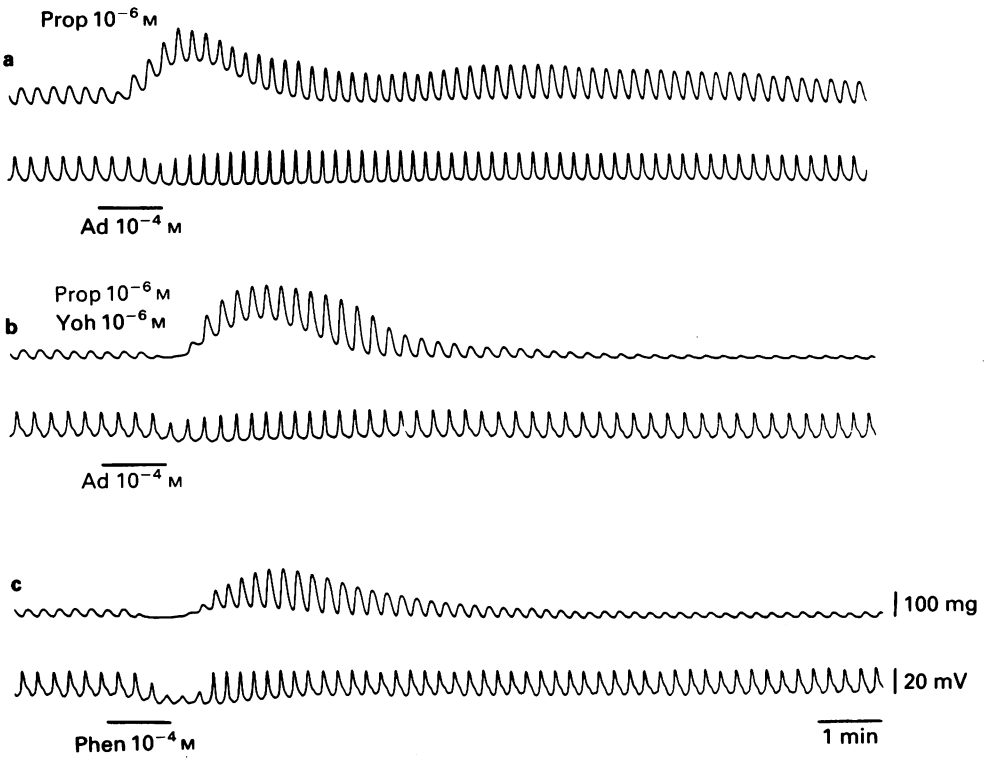

Figure 6 Effects of yohimbine on responses to adrenaline and phenylephrine in the presence of propranolol (10^{-6} M). After observing the control response to adrenaline (Ad) (a), yohimbine (Yoh 10^{-6} M) was applied 10 min before the second application of adrenaline (b) and exposure to phenylephrine (Phen) (c). Successive recordings from the same cell in lower traces.

effect, as shown in Figure 5. Yohimbine (10^{-6} M) had much weaker effects than prazosin and the biphasic response could still be produced by adrenaline (10^{-5}–10^{-4} M) in the presence of yohimbine (results not shown).

The effects of prazosin and yohimbine on concentration-contraction curves for adrenaline are shown in Figure 8. The peak tension development in the presence of adrenaline was plotted irrespective of the phase of contraction during which it occurred and the control response produced by 10^{-6} M adrenaline in the absence of the antagonists was expressed as 100%. Compared with yohimbine, prazosin produced a large shift of the curve to the right, the potency being

Table 1 Effects of prazosin and yohimbine on membrane electrical responses to adrenaline (10^{-4} M)

	Membrane hyperpolarization (mV)	*% increase in frequency of slow wave*
Control	5.7 ± 0.6	43.9 ± 14.7
Prazosin (10^{-6} M)	1.8 ± 0.7*	2.1 ± 1.3**
Yohimbine (10^{-6} M)	6.7 ± 0.4	34.4 ± 8.4

Values are mean ± s.e.mean, $n = 6$. Inhibition by prazosin was significant, *$P < 0.05$ and **$P < 0.01$ (Student's t test).

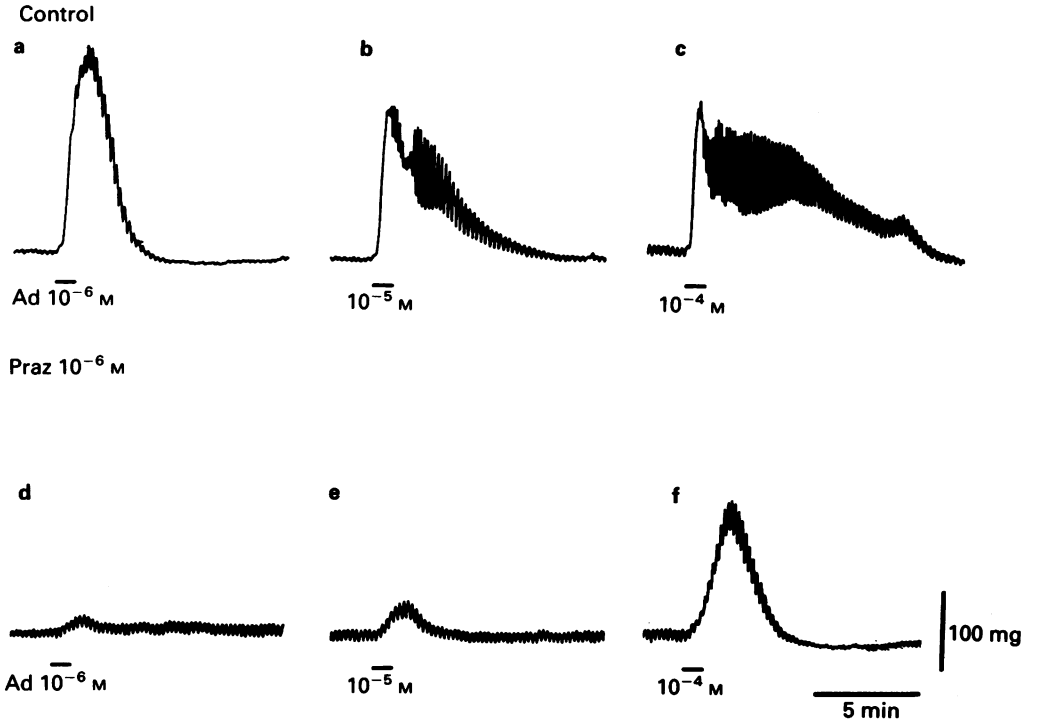

Figure 7 Effects of prazosin (Praz) on contractions induced by different concentrations of adrenaline (Ad, 10^{-6}, 10^{-5}, and 10^{-4} M) in the presence of propranolol (10^{-6} M).

approximately 50 times greater than that of yohimbine.

Discussion

Adrenaline may release transmitters, such as vasoactive intestinal polypeptide, from nerve varicosities by acting on dopamine receptors, as suggested for the opossum oesophageal muscle (Daniel *et al.*, 1987). Although this possibility was not properly investigated in the present experiments, contribution of dopamine receptors is unlikely, because the observations that phenylephrine mimicked the effect of adrenaline and that prazosin had a clear antagonizing effect are different from those obtained in the opossum oesophagus.

In the present study, the mechanical response to adrenaline varied, depending on the concentration and also from preparation to preparation. At low concentrations ($<10^{-6}$ M), the response was monophasic and was not accompanied by significant changes in membrane potential. As the concentration of adrenaline was increased, the response usually became biphasic, with an early transient and a late slow component. The early phase coincided with membrane hyperpolarization and often with inhibition of slow waves. During the late phase, the amplitude of phasic contractions was increased in association with an increase in the amplitude and frequency of electrical slow waves. The larger amplitude of the slow waves and the appearance of a small spike component on top of each wave are likely to be responsible for this increased mechanical activity. However, it is difficult to decide whether or not the spike component is essential for generation of phasic contractions, because of a possible difference in slow wave configuration within the tissue and the graded nature of spike amplitude.

Essentially similar changes in the slow wave have been reported in the antral circular muscle of the dog stomach in response to noradrenaline ($1-2.5\times10^{-5}$ M) (El-Sharkawy & Szurszewski, 1978). It is interesting in the present experiments that the early phase of contraction coincided with membrane hyperpolarization and inhibition of the slow wave, whilst the late increase in slow wave amplitude was not associated with membrane hyperpolarization. However, the mechanisms underlying these responses were not investigated in the present experiments.

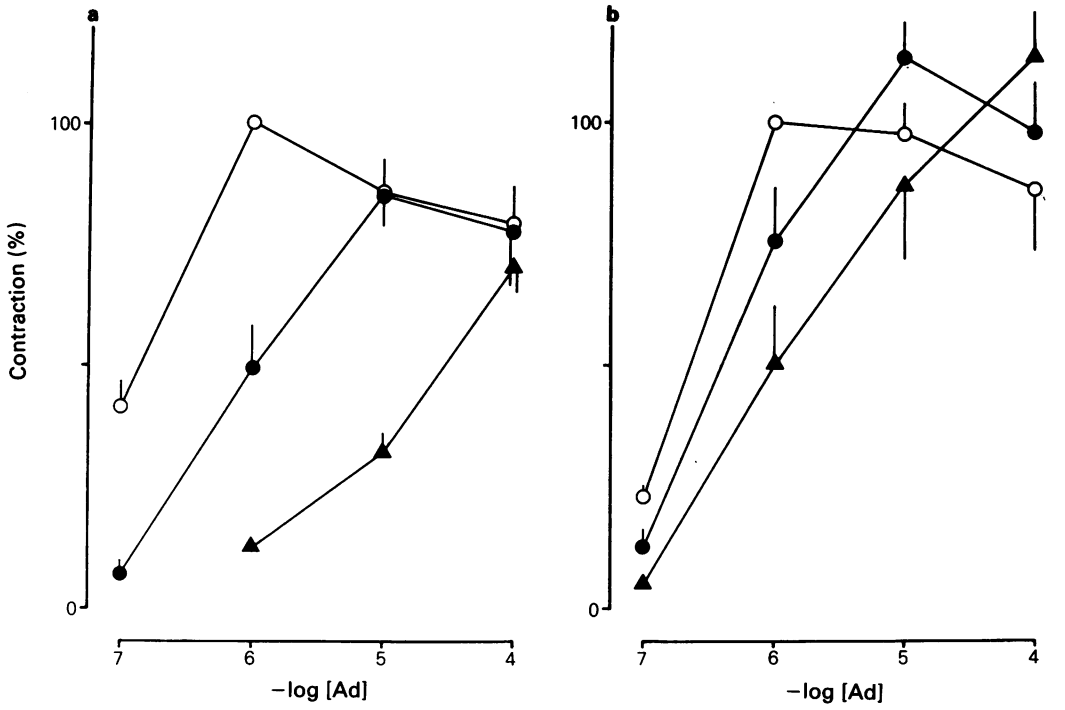

Figure 8 Effects of prazosin (a) and yohimbine (b) on concentration-tension relationship for adrenaline (Ad). The maximum tension of each response was expressed as a percentage of the control response to 10^{-6} M adrenaline. In (a), (○) control; (●) prazosin 5×10^{-8} M; (▲) prazosin 10^{-6} M; in (b) (○) control; (●) yohimbine 5×10^{-8} M; (▲) yohimbine 10^{-6} M. Each point and bar indicate the mean and s.e.mean ($n = 8$).

In a previous study on the guinea-pig stomach (Yamaguchi & Tomita, 1974) it was shown that the monophasic contraction to adrenaline became biphasic, showing an initial relaxation followed by a slow contraction, at adrenaline concentrations $> 5 \times 10^{-6}$ M. In the present experiments, however, initial inhibition was weak or not clearly observed, whereas the contractile response consisted of two phases. Such differences may partly be due to the low muscle tone in the present experiments, because the initial relaxation has been shown to be strongly dependent on basal muscle tone (Yamaguchi & Tomita, 1974). Also a difference in response pattern may result from regional differences in the stomach wall preparations. In previous studies, preparations were obtained closer to the fundus region where the muscle produces a higher basal tone compared with that from the corpus region.

Using the circular muscle of the guinea-pig corpus region, Sahyoun *et al.* (1982a,b) obtained only relaxations following exposure to the α_1-adrenoceptor-selective agonist, phenylephrine (2×10^{-7}–6×10^{-5} M). During β-adrenoceptor blockade, these workers also observed that noradrenaline ($< 10^{-6}$ M) produced a contraction whilst relaxation was seen at higher concentrations. Furthermore, the contraction was inhibited by yohimbine, while the relaxation was inhibited by prazosin. They concluded that relaxation was mediated through α_1-adrenoceptors and that contraction was an α_2-adrenoceptor-mediated response.

In the present experiments, phenylephrine produced contractions and changes in electrical activity similar to those of adrenaline, whereas clonidine (an agonist showing some selectivity for α_2-adrenoceptors) produced only weak responses. Therefore, α_1-adrenoceptors seem mainly responsible for the observed mechanical and electrical changes. This conclusion is supported by the observation that prazosin, not yohimbine, strongly antagonized the effects of adrenaline. In the presence of yohimbine, the early inhibition of mechanical activity caused by adrenaline or phenylephrine was clearly observed. It is likely that the early inhibition is also mediated by α_1-adrenoceptor activation and that this is partially masked by an α_2-adrenoceptor-mediated component.

It is concluded that α_1-adrenoceptors are predominantly involved in the contraction and also in the early

transient relaxation. Activation of α_2-adrenoceptors may cause a weak slow contraction. However, the selectivity of clonidine for α_2-adrenoceptors is only relative and it is a partial agonist at α_1-adrenoceptors (Langer & Hicks, 1984). Thus, α_2-adrenoceptors may play little part in the observed responses. Such conclusions are difficult to reconcile with the results presented by Sahyoun *et al.* (1982a,b).

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