Br. J. Pharmacol. (1990), **101**, 809–814

Receptors involved in mechanical responses to catecholamines in the circular muscle of guinea-pig stomach treated with meclofenamate

Mohsin Md. Syed, *Anant B. Parekh & [1]Tadao Tomita

Department of Physiology, School of Medicine, Nagoya University, Nagoya 466, Japan and *Department of Pharmacology, Oxford University, Oxford OX1 3QT

1 In circular muscle strips of the fundus and corpus of guinea-pig stomach, mechanical responses to catecholamines were studied mainly in the presence of a prostaglandin biosynthesis inhibitor, meclofenamate.

2 Normal preparations developed considerable muscle tone, and adrenaline (10–100 μM) in the presence of 3–5 μM propranolol produced a multiphasic response, generally consisting of transient relaxation and contraction, followed by slow relaxation and then contraction. Responses to phenylephrine were similar to those of adrenaline.

3 Meclofenamate (0.3 μM) nearly abolished the muscle tone and under this condition, both adrenaline and phenylephrine produced a simple contraction. This response was strongly inhibited by prazosin, but only weakly by yohimbine.

4 When muscle tone was maintained by prostaglandin E_2 (10 nM) in the presence of meclofenamate, phenylephrine (30 μM) produced transient relaxation followed by slow contraction in most preparations. These were strongly inhibited by prazosin. Adrenaline produced a similar response, but the relaxation was only partially reduced by prazosin. The remaining relaxation was more dominant in the middle fundic region and this was considered to be mediated through β-adrenoceptors.

5 It is concluded that in the circular muscle of the fundic region of guinea-pig stomach, endogenous prostaglandins are involved in maintaining muscle tone and in modifying the response to catecholamines and that both contraction and relaxation are mediated by α_1-adrenoceptors.

Introduction

In the circular muscle of the guinea-pig stomach, catecholamines produce multiphasic mechanical responses through activation of α-adrenoceptors. The relative contribution of contraction and relaxation varies in different regions of the stomach wall and with endogenous muscle tone (Guimaraes, 1969; Bailey, 1971; Haffner, 1971; 1972; Yamaguchi & Tomita, 1974). It has been shown that in the corpus, noradrenaline produces contraction at low concentrations through activation of α_2-adrenoceptors, but relaxation at high concentrations through α_1-adrenoceptors (Sahyoun *et al.*, 1982a,b). On the other hand, activation of α_1-receptors is also considered to be responsible for the contractile response in the guinea-pig stomach (Chihara & Tomita, 1987).

Since the response to α-adrenoceptor activation is composed of contraction and relaxation and their pattern is affected by muscle tone, analysis of receptor types involved in the response is difficult. In some tissues, spontaneous development of muscle tone may be due to endogenous prostaglandins, because it is reduced by an inhibitor of prostaglandin synthesis (meclofenamate or indomethacin; Parekh *et al.*, 1989). Examples include the canine (Milenov & Golenhofen, 1982) and rat stomach fundus (Frankhuizen & Bonta, 1975). In guinea-pig stomach muscles, phospholipase A_2, purified from snake venom, produces mechanical responses very similar to those caused by α-adrenoceptor activation (unpublished observations). It is possible that stimulation of α-adrenoceptors increases endogenous production of prostaglandins, as found in the rabbit vas deferens (Trachte, 1987) and this modifies the direct mechanical response to catecholamines. Thus, in the present experiments, the effects of receptor blocking agents on catecholamine-induced responses were studied in the presence of meclofenamate.

Methods

Hartley guinea-pigs (250–350 g) of either sex were killed by stunning and bleeding. The stomach was removed and opened by cutting the wall along the greater curvature, and the mucosa was completely removed under a binocular microscope. We defined the stomach wall as fundus, corpus and antrum by dividing it into nearly equal parts from the rostral to caudal direction. Four muscle strips (approximately 1 mm width, 7 mm length) were dissected in the direction of the circular muscle fibres between the middle fundus and the rostral side of corpus of the ventral wall of stomach.

Preparations were suspended vertically in a small tube (1 ml in capacity) and superfused with a physiological solution at a rate of 2.5 ml min^{-1} at 35°C. Mechanical responses were measured with an isometric strain gauge and recorded on a potentiometric pen recorder. The experiments were started after the preparation had been equilibrated for at least 1 h to allow full development of muscle tone. Drugs were applied to the superfusing solution. The physiological solution contained (mM): NaCl 129, $KHCO_3$ 6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, glucose 12, Tris-HCl 7.5, the pH being adjusted to 7.4 at 35°C with HCl (ungassed). Experiments were carried out in the presence of propranolol (5 μM) to inhibit β-adrenoceptors, except for β-receptor analysis. When the contribution of β-adrenoceptors was studied, preparations were treated with phenoxybenzamine (50 μM for 30 min followed by a 20 min period of washing) to block α-adrenoceptors, neuronal and extraneuronal catecholamine uptakes (O'Donnell & Wanstall, 1976).

[1] Author for correspondence.

To obtain dose-response curves, responses to agonists were calculated as a percentage of the maximum contraction obtained with 100 μM adrenaline or phenylephrine, or of the maximum relaxation caused by 10 μM isoprenaline, and were plotted against log concentration of agonist, applied cumulatively with a contact time of 4 min. The concentration producing 50% of the maximum response, EC_{50}, was then interpolated. The dissociation constant (K_D) of antagonists was obtained by a Schild plot (Arunlakshana & Schild, 1959). Numerical values were expressed as mean ± s.d. with the number of preparations in parentheses.

Drugs used were (±)-adrenaline, (±)-isoprenaline, (±)-propranolol, prazosin, yohimbine (all HCl salts from Sigma), indomethacin (Sigma), clonidine and BHT-920 (2-amino-6-allyl-5,6,7,8-tetrahydro-4H-thiazolo-(4,5-d)-azepine) (a gift from Boehringer), sodium meclofenamate monohydrate (a gift from Parke-Davis) and prostaglandin E_2 (a gift from Ono Pharmaceutical Co.). Adrenaline (10 mM) was dissolved in diluted HCl solution (pH about 3) as stock solution and renewed every week.

Results

Circular muscle strips gradually developed muscle tone, after they had been mounted in the chamber, which reached a more or less steady state in about 1 h. Figure 1 shows examples of the adrenaline response in two preparations before and after treatment with meclofenamate (0.1 μM). Adrenaline produced a complicated response depending on the concentration and the prevalent muscle tone, as previously found (Yamaguchi & Tomita, 1974). Furthermore, the response changed during the course of repeated applications of adrenaline at 20–30 min intervals. When the adrenaline concentration was low (about 1 μM), only a slow contraction usually appeared. However, when the concentration was increased, the pattern varied greatly from preparation to preparation. The general pattern was an early transient relaxation, a transient contraction followed by a slow relaxation and a late slow contraction lasting after washout. In 22% of the preparations, the response to the third application of 30 μM adrenaline was mainly contraction (as shown in Figure 1b,c), in 38% it was mainly relaxation (as in Figure 1h,i) and in 40% an intermediate pattern (a complicated mixture of contraction and relaxation) was seen (n = 104), although clear discrimination of the pattern was sometimes difficult. There was a tendency for the transient contraction to increase when adrenaline was applied repeatedly at 20 min intervals.

Meclofenamate (0.1–0.3 μM) strongly reduced the muscle tone and converted the adrenaline response to a simple contraction, independent of the pattern before meclofenamate. The tone decreased to a minimum within 20 min and remained at this level in most of the preparations, but the rate of decrease varied in different preparations. When the inhibition of muscle tone was incomplete, as judged by Ca^{2+} removal, the small early transient relaxation to adrenaline remained (24 in 280 preparations).

Carbachol produced a phasic rhythmic activity on top of a slow contraction (Figure 2). In contrast to the adrenaline response, the pattern of contractions produced by carbachol (50–100 nM) was not much affected by meclofenamate (0.1–0.3 μM) or indomethacin (0.5–1 μM). The small response caused by a low carbachol concentration (10–50 nM) was slightly reduced (86 ± 7% of the control, n = 8) by 0.3 μM meclofenamate.

The responses to phenylephrine, an agonist relatively specific to α_1-adrenoceptors, were similar to those to adrenaline, both before and after meclofenamate application (compare Figure 3a,d with c,f). Small differences were that phenylephrine produced less relaxation in the absence of meclofenamate and faster recovery from contraction in the presence of meclofenamate compared with adrenaline. On the other hand, clonidine, an α_2-adrenoceptor specific agonist, produced a very small and prolonged contraction at the high concentration of 30 μM, compared with phenylephrine and adrenaline at the same concentration (Figure 3b,e). The responses to BHT-920, another α_2-adrenoceptor agonist, were slightly larger and faster than those to clonidine at the same concentration (30 μM). For both BHT-920 (Flavahan et al., 1984) and clonidine (Agrawall et al., 1984) this high concentration gave a maximum contraction in vascular muscles. In the present

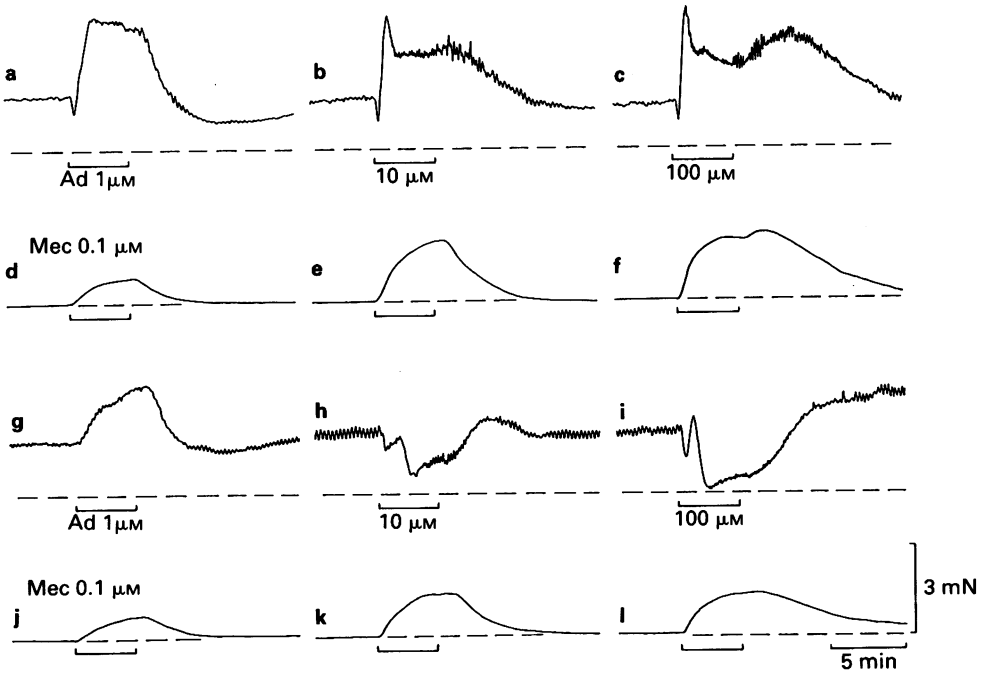

Figure 1 Mechanical responses to adrenaline before and after meclofenamate application in circular muscle strips of guinea-pig stomach fundus. Three different concentrations (1, 10, 100 μM) of adrenaline (Ad) were applied for 4 min, as indicated by horizontal bars, at intervals of 20 min. After recording (c) and (i), meclofenamate (Mec, 0.1 μM) was applied continuously and 30 min later adrenaline application was started. Propranolol (3 μM) was present throughout the experiments. Dotted lines indicate the lowest level of muscle tone. Records, (a–f) and (g–l), are from different preparations. See text for further explanation.

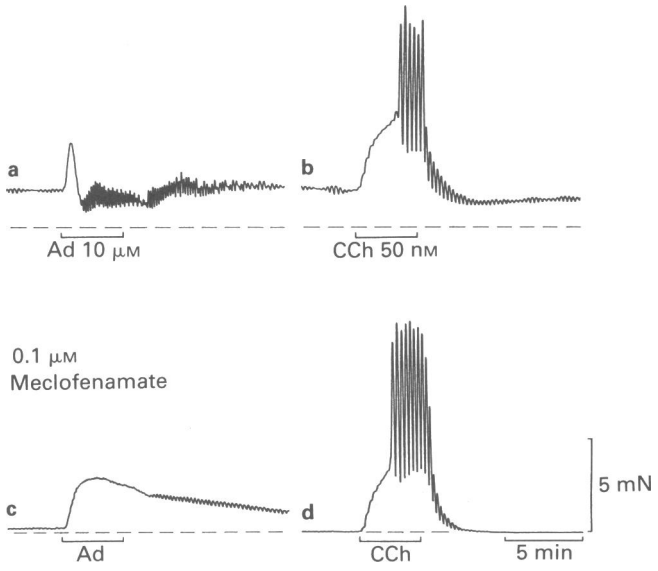

Figure 2 Comparison of the effects of meclofenamate on adrenaline- and carbachol-induced responses. (a and b) Control responses to adrenaline (Ad, 10 μM) and carbachol (CCh, 50 nM). The interval between drug applications was 20 min. (c and d) Responses to the same concentration of the drugs in the presence of meclofenamate (0.1 μM) which was applied after recording (b). Propranolol (3 μM) was present throughout.

experiments, neither produced relaxation between 1 and 30 μM ($n = 8$).

Figure 3 also shows the strong inhibitory effects of prazosin, a specific α_1-adrenoceptor blocker, on responses to three different agonists (30 μM) (g–i). Prazosin (1 μM) almost completely blocked not only the contraction produced by phenylephrine (g) but also that by clonidine (h). The adrenaline response was slightly less susceptible to prazosin (i).

Figure 4 shows the effects of prazosin on the dose-response curve to adrenaline in the presence of meclofenamate (0.3 μM). Prazosin (0.001–0.1 μM) strongly inhibited the response. The slope of the Schild plot was 0.84 ± 0.11 ($n = 6$) which was significantly different ($P < 0.01$) from unity. The apparent pA_2 of prazosin was 9.94 ± 0.12. Yohimbine (0.1–1 μM) had only a weak effect on the size of contraction. The contraction produced by 30 μM adrenaline was reduced to $97 \pm 7\%$ with 0.1 μM and $84 \pm 9\%$ of the control with 1 μM yohimbine ($n = 6$). On the other hand, yohimbine increased the rate of relaxation of contractions produced by adrenaline. The duration of contraction produced by a 4 min application of 30 μM adrenaline, measured at 50% amplitude was 13.5 ± 3.7 min

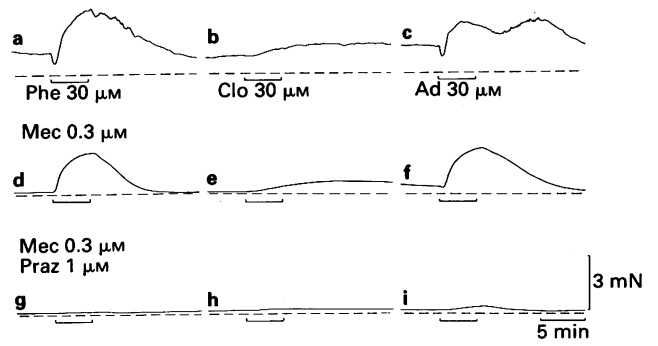

Figure 3 Effects of meclofenamate and prazosin on responses to phenylephrine (Phe, a,d,g), clonidine (Clo, b,e,h), and adrenaline (Ad, c,f,i), each at a concentration of 30 μM. (a–c) Control responses, (d–f) in the presence of meclofenamate (Mec, 0.3 μM), and (g–i) in the presence of meclofenamate (0.3 μM) and prazosin (Praz, 1 μM). These represent successive recordings. Meclofenamate and prazosin were applied 30 min before (d) and (g), respectively. Propranolol (3 μM) was present throughout.

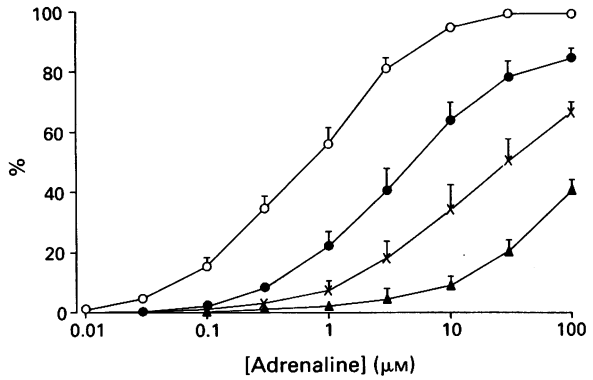

Figure 4 Effects of prazosin (0.001–0.1 μM) on dose-response curves to adrenaline in the presence of 0.3 μM meclofenamate and 3 μM propranolol. Adrenaline was cumulatively applied at each concentration for 4 min, 20 min after preincubation with prazosin and the maximum tension caused by 100 μM adrenaline in the absence of prazosin was taken as 100%. (○) Responses in absence of prazosin; responses in presence of (●) 0.001 μM, (×) 0.01 μM and (▲) 0.1 μM prazosin. Vertical lines show s.d. ($n = 6$).

($n = 14$) and this was reduced to 11.8 ± 3.5 and 7.4 ± 1.5 min by 0.1 and 1 μM yohimbine, respectively.

In order to study the relaxation caused by catecholamines, muscle tone was raised with prostaglandin E_2 (10 nM) to a level similar to that before meclofenamate treatment. As shown in Figure 5, BHT-920, an α_2-adrenoceptor agonist, phenylephrine and adrenaline all produced a simple contraction in the presence of meclofenamate, but the response to BHT-920 was smaller and slower, as with clonidine, compared to other catecholamines. When these catecholamines were applied during sustained prostaglandin application, phenylephrine and adrenaline produced a transient relaxation followed by a slow contraction (e,f), but BHT-920 (and clonidine) failed to produce any relaxation (d). Prazosin (3 μM) blocked the response to phenylephrine almost completely (h), but only partially blocked the adrenaline response (i).

In the preparation shown in Figure 6 which was obtained from the middle region of the fundus, relaxation was the dominant response to adrenaline in the presence of propranolol (3 μM) (a). This changed to a slow contraction after meclofenamate treatment (b). The adrenaline response in the presence of prostaglandin (10 nM) was also mainly relaxation (c). This response was not significantly affected by yohimbine even at 5 μM (d), but was clearly reduced by prazosin (e). The relaxation observed in the presence of prostaglandin is, therefore, unlikely to be mediated through α_2-adrenoceptors and is partly resistant to prazosin. A possibility that β-receptors might be involved in the relaxation was tested by comparing responses to adrenaline and isoprenaline.

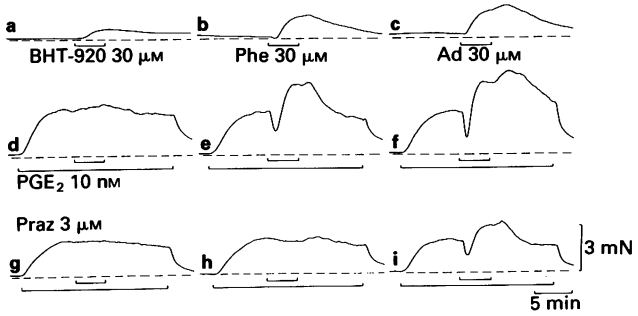

Figure 5 (a,b,c) Responses to three different agonists (30 μM) (BHT-920, phenylephrine, and adrenaline, respectively) in the presence of propranolol (3 μM) and meclofenamate (0.3 μM). (d,e,f) The same agonists were applied during application of prostaglandin E_2 (PGE_2, 10 nM), as indicated by the horizontal bars. (g,h,i) The same as (d,e,f) but in the presence of prazosin (Praz, 3 μM). All records are from the same preparation. See text for further explanation.

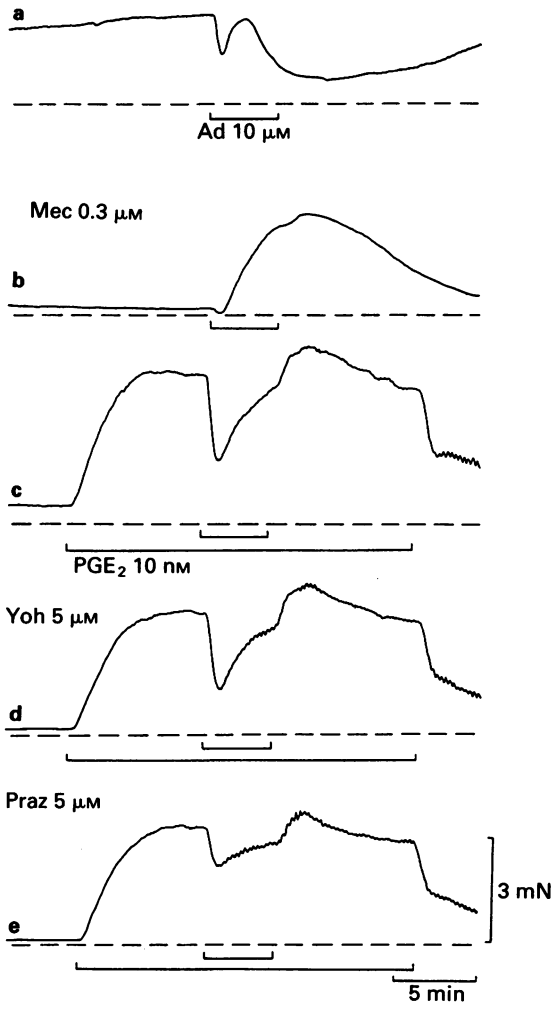

Figure 6 Effects of yohimbine (Yoh) and prazosin on relaxation produced by adrenaline in the presence of meclofenamate (Mec, 0.3 μM), prostaglandin E_2 (PGE_2, 10 nM) and propranolol (3 μM) in a preparation obtained from the middle fundic region. (a) Control response to adrenaline (Ad, 10 μM) before meclofenamate, (b) after meclofenamate and (c) during prostaglandin application. Yohimbine (5 μM) was applied after (c), and 30 min later adrenaline was applied during prostaglandin E_2 (PGE_2)-induced contraction (d). Similarly, prazosin (Praz, 5 μM) was applied between (d) and (e).

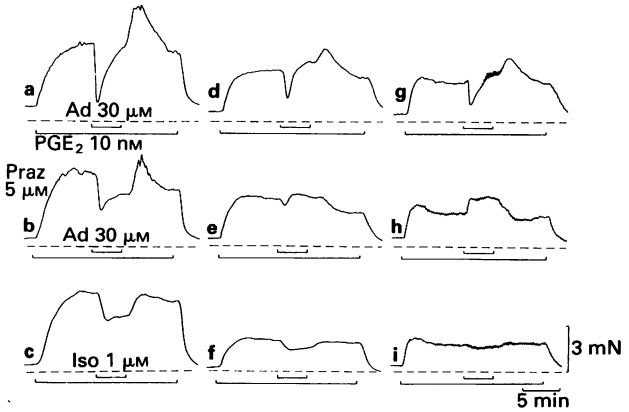

Figure 7 Responses to adrenaline and isoprenaline in the presence of propranolol (5 μM), meclofenamate (0.3 μM) and prostaglandin E_2 (PGE_2, 10 nM) in three different preparations from the same stomach wall; (a–c) middle fundus, (d–f) caudal fundus, and (g–i) rostral corpus. (a,d,g) Responses to adrenaline (Ad, 30 μM) before and (b,e,h) after prazosin (Praz, 5 μM) application. (c,f,i) Responses to isoprenaline (Iso, 1 μM) in the presence of prazosin. See text for further explanation.

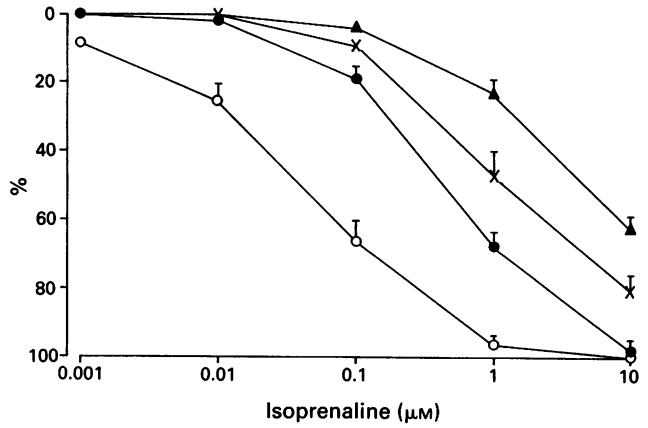

Figure 8 Effects of propranolol on the dose-response curve of isoprenaline-induced relaxation. Preparations (middle fundus) were treated with phenoxybenzamine for 30 min, and isoprenaline was cumulatively applied in the presence of prostaglandin E_2 (10 nM) and meclofenamate (0.3 μM). (○) Control responses in the absence of propranolol; responses in presence of (●) 0.1, (×) 1 and (▲) 5 μM propranolol. Each point represents the mean of 6 preparations with s.d. indicated by vertical bars.

Figure 7 shows responses to adrenaline (30 μM) and isoprenaline (1 μM) in three preparations obtained from the same stomach. When adrenaline was applied in the presence of propranolol (5 μM) and prostaglandin E_2 (10 nM), all preparations produced a transient relaxation (a,d,g). In the preparation obtained from the middle fundus (a–c), the relaxation became smaller but sustained in the presence of prazosin (b), whereas in the preparation from the corpus (g–i), the relaxation was converted to a contraction by prazosin (h). In the muscle strip of the caudal region of fundus (d–f), the adrenaline response was markedly reduced by prazosin (e). Isoprenaline still produced relaxation even in the presence of 5 μM propranolol, but the degree of relaxation decreased in muscle strips taken from the more caudal side of the stomach wall (c to i). This tendency was confirmed in two other experiments. These results suggest that the sustained relaxation caused by adrenaline in the presence of prazosin in the middle fundus (b) is due to activation of β-adrenoceptors.

In Figure 8, inhibition of the isoprenaline-induced relaxation with propranolol (0.1–5 μM) is shown. This result was obtained from 6 preparations of the middle fundic region in the presence of meclofenamate (0.3 μM) and prostaglandin E_2 (10 nM), following treatment with phenoxybenzamine (50 μM). The phenoxybenzamine treatment shifted the dose-response curve of isoprenaline to the left by approximately ten times. Under these conditions, the EC_{50} of isoprenaline was 40 ± 6 nM ($n = 6$). Propranolol, applied 20 min before, dose-dependently inhibited the relaxation by isoprenaline. The slope of the Schild plot was 0.81 ± 0.04 and the apparent dissociation constant of propranolol was 12 ± 5 nM ($n = 6$). Under similar conditions, adrenaline was less effective in producing relaxation, the EC_{50} being 1.6 ± 0.4 μM ($n = 4$). The slope of the Schild plot (0.83 ± 0.03) was similar to that for isoprenaline between 0.1 and 1 μM propranolol. However, increasing the concentration of propranolol from 1 to 5 μM produced only a very small further shift of the dose-response curve. The apparent dissociation constant of propranolol (0.1–1 μM) for adrenaline-induced relaxation was 32 ± 7 nM ($n = 4$).

Discussion

Indomethacin (Smith & Lands, 1971) and meclofenamate (Rome & Lands, 1975) are thought to interfere with prostaglandin biosynthesis mainly by inhibiting the cyclo-oxygenase enzyme, but also by reducing the activity of phospholipase A_2 (Kaplan *et al.*, 1978; Thakkar *et al.*, 1983). Therefore, the reduction of muscle tone and the alteration of the adrenaline

response with meclofenamate and indomethacin observed in the circular muscle of the guinea-pig stomach, suggest that prostaglandins are involved in maintaining the muscle tone and also in modifying the response to adrenaline. Meclofenamate may also act as a blocking agent of prostaglandin receptors (McLean & Gluckman, 1983). This effect was not studied in the present experiments, but since prostaglandin E_2 could induce a clear contraction at less than 10 nM in the presence of 0.3 μM meclofenamate, the receptor blocking action against prostaglandin E_2 in this tissue must be weak.

Since existing muscle tone affects the adrenaline-induced change in muscle tone (Yamaguchi & Tomita, 1974), the change of the adrenaline response into simple contraction in the presence of meclofenamate may result from the decreased muscle tone. However, full recovery of the response pattern was never achieved by raising the muscle tone to the control level with prostaglandin E_2 in the presence of meclofenamate, and the response pattern differed depending on the substance used to increase the muscle tone (e.g., prostaglandin E_2, $F_{2\alpha}$, carbachol) (unpublished observations). This suggests that the change in pattern of the response to catecholamines is not simply due to a fall in tone but to a decrease in production of endogenous prostaglandins or related substances. Catecholamines are known to stimulate prostaglandin synthesis in several tissues. This is probably caused by activation of phospholipase A_2 (Trachte, 1987; Ho & Klein, 1987) or by involvement of diglyceride lipase, following a process mediated by phospholipase C activation (Bell *et al.*, 1979; Irvine, 1982).

In the presence of meclofenamate, the contraction evoked by adrenaline and phenylephrine was strongly inhibited by prazosin, a blocking agent selective for α_1-adrenoceptors. The apparent pA_2 value of prazosin for adrenaline-induced contraction was 9.94, which is similar to that found in those smooth muscles which have predominantly α_1-adrenoceptors (Agrawal *et al.*, 1984). The contractile response to adrenaline was very weakly inhibited by yohimbine (0.1–1 μM). The low value of the slope of Schild plot (0.84) is probably due to inhibitory effects exerted by β-adrenoceptor stimulation at high concentrations of adrenaline, as will be discussed. The idea that α_1-adrenoceptors, rather than α_2-receptors, are responsible for the contraction is supported by the finding that the weak contraction produced by clonidine was blocked by prazosin. Adrenaline may stimulate α_2-receptors to prolong the slow contraction at a high concentration (30 μM), because yohimbine shortens the duration of contraction, but it may be concluded that α_1-receptors are mainly responsible for the contractile response to adrenaline and phenylephrine.

When muscle tone is high, either due to intrinsic generation of prostanoids or in the presence of exogenous prostaglandin, adrenaline and phenylephrine produce relaxation, in addition to the contractile response. Two different mechanisms seem to be involved in the relaxation caused by adrenaline, one involves activation of α_1-adrenoceptors and the other β-adrenoceptors. The contribution of α_1-adrenoceptors is suggested by the observation that phenylephrine, an agonist specific for α_1-adrenoceptors, produced relaxation and that this was blocked by prazosin. Adrenaline-induced relaxation was not affected by yohimbine. Furthermore, clonidine and BHT-920, which have relatively high selectivity for α_2-adrenoceptors, did not produce relaxation. Thus, α_1-adrenoceptors are mainly involved in the relaxation under conditions in which prostaglandin biosynthesis is blocked.

Activation of both α_1- and β-adrenoceptors seems to be responsible for the relaxation caused by adrenaline, particularly in the middle fundic region. The finding that the adrenaline-induced relaxation was more resistant to prazosin than that produced by phenylephrine may be explained if adrenaline at high concentrations (10–100 μM) stimulates β-receptors and that this relaxation is resistant to blockade by propranolol. In the circular muscle of guinea-pig stomach, the ED_{50} value for isoprenaline was 40 nM, and the apparent dissociation constant of propranolol was 12 nM. In other smooth muscles, the ED_{50} of isoprenaline for relaxation has been found to be in the range 6–27 nM, and the dissociation constant of propranolol for this relaxation in the range 0.7–43 nM (guinea-pig myometrium: O'Donnell *et al.*, 1978; guinea-pig trachea: Purdy *et al.*, 1988; rat stomach fundus: Lefebvre *et al.*, 1985; arterial smooth muscles: Purdy *et al.*, 1988; O'Donnell & Wanstall, 1985). Thus, β-adrenoceptors in the circular muscle of guinea-pig stomach fundus seem to be similar to those in other smooth muscles, although the type of β-adrenoceptor was not investigated in the present experiments. The failure of 5 μM propranolol to abolish the adrenaline-induced relaxation is probably due to the high concentration of adrenaline (30–100 μM) used in the present experiments, but the presence of some receptors resistant to propranolol blockade, as found in the guinea-pig ileum (Bond & Clarke, 1988), cannot be excluded.

The authors are indebted to Dr Alison F. Brading for her continued support throughout this study.

References

AGRAWAL, D.K., TRIGGLE, C.R. & DANIEL, E.E. (1984). Pharmacological characterization of the postsynaptic *alpha* adrenoceptors in vascular smooth muscle from canine and rat mesenteric vascular beds. *J. Pharmacol. Exp. Ther.*, **229**, 831–838.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BAILEY, D.M. (1971). Inhibitory and excitatory effects of sympathomimetic amines on muscle strips from the stomach of the guinea-pig. *Br. J. Pharmacol.*, **41**, 227–238.

BELL, R.L., KENNERLY, D.A., STANFORD, N. & MAJERUS, P.W. (1979). Diglyceride lipase: a pathway for arachidonate release from human platelets. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 3238–3241.

BOND, R.A. & CLARKE, D.E. (1988). Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β-subtypes. *Br. J. Pharmacol.*, **95**, 723–734.

CHIHARA, S. & TOMITA, T. (1987). Mechanical and electrical responses to α-adrenoceptor activation in the circular muscle of guinea-pig stomach. *Br. J. Pharmacol.*, **91**, 789–798.

FLAVAHAN, N.A., RIMELE, T.J., COOKE, J.P. & VANHOUTTE, P.M. (1984). Characterization of postjunctional *alpha*-1 and *alpha*-2 adrenoceptors activated by exogenous or nerve-released norepinephrine in the canine saphenous vein. *J. Pharmacol. Exp. Ther.*, **230**, 699–705.

FRANKHUIJZEN, A.L. & BONTA, I.L. (1975). Role of prostaglandins in tone and effector reactivity of the isolated rat stomach preparation. *Eur. J. Pharmacol.*, **31**, 44–52.

GUIMARAES, S. (1969). *Alpha* excitatory, *alpha* inhibitory and *beta* inhibitory adrenergic receptors in the guinea-pig stomach. *Archs Int. Pharmacodyn.*, **179**, 188–201.

HAFFNER, J.F.W. (1971). The adrenergic receptors in isolated rabbit stomach muscle. *Acta Pharmacol. Toxicol.*, **29**, 327–338.

HAFFNER, J.F.W. (1972). α-Adrenergic receptor stimulation with phenylephrine in rabbit fundus muscle exposed to carbachol. *Acta Pharmacol. Toxicol.*, **31**, 121–128.

HO, A.K. & KLEIN, D.C. (1987). Activation of α_1-adrenoceptors, protein kinase C, or treatment with intracellular free Ca^{2+} elevating agents increases pineal phospholipase A_2 activity. *J. Biol. Chem.*, **262**, 11764–11770.

IRVINE, R. (1982). How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.*, **204**, 3–16.

LEFEBVRE, R.A., VERPLANKEN, P.A. & BOGAERT, M.G. (1985). Pharmacological characterization of the postjunctional β-adrenoceptors in the rat gastric fundus. *Eur. J. Pharmacol.*, **106**, 1–9.

KAPLAN, L., WEISS, J. & ELSBACH, P. (1978). Low concentrations of indomethacin inhibit phospholipase A_2 of rabbit polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 2955–2958.

McLEAN, J.R. & GLUCKMAN, M.I. (1983). On the mechanism of the

pharmacologic activity of meclofenamate sodium. *Arzneim. Forsch.*, **33**, 627–631.

MILENOV, K. & GOLENHOFEN, K. (1982). Contractile responses of longitudinal and circular smooth muscle of the canine stomach to prostaglandins E and $F_{2\alpha}$. *Prostaglandins Leukotrienes Med.*, **8**, 287–300.

O'DONNELL, S.T., PERSSON, C.G.A. & WANSTALL, J.C. (1978). An *in vitro* comparison of β-adrenoceptor stimulants of potassium-depolarized uterine preparations from guinea-pigs. *Br. J. Pharmacol.*, **62**, 227–233.

O'DONNELL, S.R. & WANSTALL, J.C. (1976). The contribution of external uptake to the trachea-blood vessel selectivity of β-adrenoceptor stimulants *in vitro* in guinea-pigs. *Br. J. Pharmacol.*, **57**, 369–373.

O'DONNELL, S.T. & WANSTALL, J.C. (1985). Responses to the β_2-selective agonist procaterol of vascular and atrial preparations with different functional β-adrenoceptor populations. *Br. J. Pharmacol.*, **84**, 227–235.

PAREKH, A., SYED, M.Md. & TOMITA, T. (1989). Modification of α-adrenergic response by prostaglandin synthesis inhibitors in isolated guinea-pig stomach muscles. *J. Physiol.*, **417**, 64P.

PURDY, R.E., STUPECKY, G.L. & COULOMBE, P.R. (1988). Further evidence for a homogeneous population of *beta*-1-adrenoceptors in bovine coronary artery. *J. Pharmacol. Exp. Ther.*, **245**, 67–71.

ROME, L.H. & LANDS, W.E.M. (1975). Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 4863–4865.

SAHYOUN, H.A., COSTALL, B. & NAYLOR, R.J. (1982a). Catecholamines act at α_2-adrenoceptors to cause contraction of circular smooth muscle of guinea-pig stomach. *J. Pharm. Pharmacol.*, **34**, 381–385.

SAHYOUN, H.A., COSTALL, B. & NAYLOR, R.J. (1982b). Benzamide action at α_2-adrenoceptors modifies catecholamine-induced contraction and relaxation of circular smooth muscle from guinea-pig stomach. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **319**, 8–11.

SMITH, W.L. & LANDS, W.E.M. (1971). Stimulation and blockade of prostaglandin biosynthesis. *J. Biol. Chem.*, **246**, 6700–6702.

THAKKAR, J.K., SPERELAKIS, N., PANG, D. & FRANSON, R.C. (1983). Characterization of phospholipase A_2 activity in rat aorta smooth muscle cells. *Biochem. Biophys. Acta*, **750**, 134–140.

TRACHTE, G.J. (1987). Adrenergic receptors mediating prostaglandin production in the rabbit vas deferens. *Prostaglandins*, **33**, 25–35.

YAMAGUCHI, T. & TOMITA, T. (1974). Mechanical responses to catecholamines in isolated strips of the guinea-pig stomach muscle. *Jpn. J. Pharmacol.*, **24**, 911–922.

(Received March 2, 1990
Revised July 20, 1990
Accepted July 23, 1990)