Receptor for catecholamines responding to catechol which potentiates voltage-dependent calcium current in single cells from guinea-pig taenia caeci

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- 1 Single isolated cells were obtained from the taenia of the guinea-pig's caecum by enzymic digestion and held under voltage clamp. The effects of various catecholamines, sympathomimetics and related compounds were tested for their ability to potentiate the voltage-dependent calcium current (I_{Ca}) evoked in these cells by a depolarizing step.
- 2 I_{Ca} was potentiated by up to 60% by isoprenaline, adrenaline, and noradrenaline which were equipotent. The EC_{50} for isoprenaline was about 40 nM.
- 3 The racemic mixtures of the optical isomers of isoprenaline, adrenaline, and noradrenaline, and (+)-isoprenaline, were equipotent with the (–)-isomers of these drugs. Dopamine, L-dopa, and catechol were equipotent with these catecholamines.
- 4 Removal or substitution of one or more of the hydroxy groups of the catechol moiety, as in phenylephrine, salbutamol, procaterol, methoxamine, terbutaline, BRL 37344, ICI 215001 or tyramine substantially reduced efficacy and/or potency.
- 5 The adrenoceptor blockers propranolol, phentolamine, dihydroergotamine, atenolol, CGP 20712A and ICI 118551, or the dopamine receptor blockers, haloperidol or flupenthixol, did not block the potentiating action of catechol or the catecholamines.
- 6 The receptor activated by catecholamines to increase I_{Ca} we suggest should be called a C-receptor in view of its sensitivity to catechol. It may arise by enzymic modification of a conventional adrenoceptor but its transduction also involves a novel mechanism which might indicate that it is present in the muscle cells before enzyme treatment.

Keywords: Ca channel; smooth muscle; catecholamines; adrenoceptor; C-receptor; catechol; taenia caeci

Introduction

The structure of the voltage-dependent calcium channel of smooth muscles shows considerable similarities to that of the cardiac channel (Biel *et al.*, 1990; Koch *et al.*, 1990). However, β_1 -adrenoceptor activation in heart increases the force of cardiac contraction and increases the calcium current (I_{Ca}) by activation of an adenosine 3':5'-cyclic monophosphate (cyclic AMP)-dependent kinase and phosphorylation of the channel (Kameyama *et al.*, 1985; 1986) whereas in smooth muscle β_2 -adrenoceptor activation causes relaxation and generally hyperpolarization (see Bülbring & Tomita, 1987). Despite this, β -adrenoceptor activation in tracheal smooth muscle (Welling *et al.*, 1991; 1992) and in smooth muscle cells from pig coronary artery (Fukumitsu *et al.*, 1990) has been reported to increase I_{Ca} as β_1 -receptor activation does in heart.

Although cyclic AMP-dependent phosphorylation of calcium channels is believed to be responsible for the increase in I_{Ca} in heart, we have found activators of adenylyl cyclase, cyclic AMP, or its analogues, did not mimic the potentiation of I_{Ca} produced by isoprenaline in smooth muscle cells from the guinea-pig taenia caeci (Muraki *et al.*, 1993). This result is similar to that of Ohya *et al.* (1987) who found that cyclic AMP did not potentiate I_{Ca} in cells from the longitudinal smooth muscle of rabbit jejunums in contrast to results in pig coronary artery smooth muscle where forskolin potentiated I_{Ca} (Fukumitsu *et al.*, 1990). We have also found that potentiation of I_{Ca} in guinea-pig taenia cells did not appear to be mediated by a G-protein, contrary to results obtained in tracheal smooth muscle by Welling *et al.* (1991; 1992) or to be mediated by activation of protein kinase C which has been implicated in the increase in I_{Ca} produced by muscarinic and other receptor activation in amphibian gastric smooth muscle cells (Vivaudou *et al.*, 1988).

The actions of noradrenaline (NA), adrenaline (Ad) and other sympathomimetics on smooth and cardiac muscles have been intensively investigated since the pioneer work of Barger & Dale (1910). Around the time of identification of NA as the main, or as an important, transmitter at sympathetic nerve endings (von Euler, 1946) and Ad as the main hormone released from the adrenal medulla (Bülbring, 1949; Bülbring & Burn, 1949) the subdivision of adrenoceptors into α - and β -subtypes on the basis of the relative potencies of some sympathomimetic amines was proposed (Ahlquist, 1948). The characteristics of the molecule which confer ability to activate presently known adrenoceptors are the 3:4 dihydroxybenzene moiety and the aliphatic side chain of two carbon atoms (β and α) which terminates in an amino group (Barlow, 1964). β -Hydroxylation further intensifies activity but catechol itself is without sympathomimetic activity (Barger & Dale, 1910). Since Ahlquist's division into two groups, adrenoceptors have been further subdivided into α_1 and α_2 (Dubocovitch & Langer, 1974; Drew & Whiting, 1979) and β_1 , β_2 and 'atypical' β_3 varieties (Lands *et al.*, 1967; Wilson *et al.*, 1984; Bond & Clarke, 1987; Hollenga & Zaagsma, 1989).

We noticed that NA, Ad and isoprenaline (Iso) were all able to increase I_{Ca} in guinea-pig taenia single cells under voltage clamp (Muraki *et al.*, 1993). NA and Ad also increased I_{Ca} in smooth muscle cells from rabbit ear artery (Benham & Tsien, 1988). In cultured aortic smooth muscle (Serebryakov & Takeda, 1992) NA or Ad decreased I_{Ca} . In both these last two tissues the receptors through which NA and Ad produced these effects were not blocked by α - or by β -adrenoceptor blockers. It was therefore decided to characterize the adrenoceptors which mediated the increase in I_{Ca} in cells of the taenia caeci of the guinea-pig. In this tissue, Ad or NA inhibit contraction and relax the muscle (Burnstock, 1958; Bülbring & Tomita, 1969) through actions on both α -

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(Bülbring & Tomita, 1969) and β -adrenoceptors (Bülbring & den Hertog, 1980). Single cells were used because we wished to examine the possibility that activation of adrenoceptors modulated I_{Ca} in these cells, and because when adrenoceptor stimulants are applied to a whole tissue, actions on one type of mechanism may be obscured by other actions on other mechanisms especially if only tension is recorded.

Methods

Preparation of cells

Guinea-pigs of either sex, weighing 200–400 g were killed by stunning and exsanguination and small pieces of taenia from the caecum were dissected. After fat and connective tissues were removed under a binocular microscope, 10–20 mm $\times$ 0.5–1.0 mm pieces of taenia were immersed in Ca^{2+} and Mg^{2+} -free physiological salt solution (Ca^{2+} - Mg^{2+} free solution for 10 min. They were then incubated for 15 min in the Ca^{2+} - Mg^{2+} free solution which contained 1 mg ml⁻¹ collagenase and 0.5 mg ml⁻¹ pronase using a similar method to that previously described (Inoue & Isenberg, 1990; Pacaud & Bolton, 1991). After that, the solution was changed to enzyme-free fresh Ca^{2+} - Mg^{2+} free solution and the pieces agitated gently. Then cells were spun down at 1000 r.p.m. for 2 min, resuspended in low- Ca^{2+} physiological salt solution which contained 0.55 mM Ca^{2+} , and stored at 4°C until use on the same day.

Membrane current measurements

Membrane currents were recorded with standard whole-cell voltage-clamp methods (Hamill *et al.*, 1981) using a List-EPC 5 patch clamp amplifier using pipettes which had 3 to 5 M Ω resistance. No correction for leakage currents was made. Voltage steps were produced by a command generated by a BBC microcomputer linked through a CED 1401 interface (Cambridge Electronic Devices, Cambridge, U.K.). Current signals were stored on FM tape (Racal) and later captured on a BBC micro computer using the CED 1401 and our own capturing program for precise analysis. All experiments were carried out at 22 $\pm$ 2°C.

Solutions

Experiments were conducted in physiological salt solution (PSS) containing (mM): NaCl 137, KCl 6, MgCl₂ 1.2, CaCl₂ 2.2, glucose 10 and HEPES 10. Ca^{2+} and Mg^{2+} -free solution for dispersing cells contained (mM) NaCl 128, KCl 6, glucose 10 and HEPES 10. The pH of these solutions was adjusted to 7.4 using NaOH. The composition of standard pipette solution was (mM): CsCl 140, MgCl₂ 1, ATP-2Na 2, GTP-3Na 0.3, HEPES 10 and EGTA 4 (titrated to pH 7.4 with CsOH).

Drugs

($\pm$)- and (-)-adrenaline, catechol, L- β -3,4-dihydroxyphenylalanine (L-dopa), dihydroergotamine methane sulphonate, dopamine hydrochloride, isoetharine mesylate, haloperidol, ($\pm$)-isoprenaline hydrochloride, (-)- and (+)-isoprenaline bitartrate, methoxamine hydrochloride, (-)- and ($\pm$)-nora-drenaline hydrochloride, phentolamine hydrochloride, (-)-phenylephrine hydrochloride, procaterol hydrochloride, (-)-propranolol hydrochloride, salbutamol hemisulphate, terbutaline hemisulphate, tyramine hydrochloride (all from Sigma). 1-[2-((3-Carbomoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-propan-2-ol methane sulphonate (CGP 20712A, Ciba Geigy), 4-[2-(2-hydroxy-2-(3-chlorophenyl)ethyl)amino]propyl]-phenoxycetic acid (BRL 37344, SmithKline Beecham), erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol

hydrochloride (ICI 118551, Cambridge Research Biochemicals), ICI 215001 (M 215001, metabolite of D7114, ICI-Zeneca) (Holloway *et al.*, 1991), *cis*(z)-flupenthixol hydrochloride (Research Biochemicals).

Results

Cells were held at -50 mV and depolarized for 200 ms to 0 mV every 20 s. This evoked an inward current carried by calcium ions (I_{Ca}) which was not significantly contaminated by potassium currents due to the use of caesium chloride instead of potassium chloride in the pipette solution. The peak size of the evoked inward current was measured. Drugs were applied for short periods until any change in the peak size of the current had stabilized; the effect of a drug was sometimes expressed as a change in the size of the current relative to the size of the current immediately before drug application. Because current size often increased without drug application for several minutes after beginning recording in the whole-cell mode, the first response to drug application was sometimes less reliable.

It was found that NA, Ad or isoprenaline (Iso) increased the size of the voltage-dependent current and although this effect varied in size in different cells a 60% increase was not uncommon (Figure 1a,b). Because the potentiation could be produced by Iso it was suspected that the effect was mediated by β -receptors but an unexpected finding was that β -receptor antagonists such as propranolol up to 10 μ M had no inhibitory effect (Figure 3a). Therefore, further experiments were made in order to characterize the adrenoceptor involved.

It was observed that the racemates of NA, Ad and Iso were about equipotent (Figure 1a-c). This observation immediately suggested that the adrenoceptor involved was not either an α - or a β -subtype as NA is invariably much more potent at α -receptors than Iso and *vice-versa* at β -receptors (Barlow, 1964). The EC₅₀ for (-)-Iso was 41 nM ($n = 1-13$) (Figure 1e,f) and for ($\pm$)-Iso it was 42 nM; for NA and Ad it was 24 nM ($n = 1-5$) and 58 nM ($n = 1-7$) respectively.

Those catecholamines with a β -OH group can exist as two isomers (-) and (+). At α and at β -receptors the (-)-isomer of Ad and NA is usually 10–100 times more potent than the (+)-isomer and at β -receptors in the case of (-)- and (+)-isoprenaline, this ratio is even greater (Barlow, 1964). The importance of the β -OH group for activity suggests that it is involved in binding at α - and at β -receptors. However, (-), (+)- and ($\pm$)-isoprenaline (Figure 2a), (-)- and ($\pm$)-nora-drenaline, and (-)- and ($\pm$)-adrenaline were equipotent; in these experiments equal concentrations of the catecholamines were applied successively to the same cell and the ratio of the increases in Ca current calculated. No ratio was significantly different from unity (Table 1) (Figure 1c). This result suggested that the β -OH group was unimportant for binding at this receptor. This was confirmed when experiments showed that dopamine which lacks a β -hydroxy group was equipotent with the other catecholamines examined (Figure 1d) (Table 1).

The structure-activity relationship was more systematically investigated; (-)-phenylephrine, and ($\pm$)-methoxamine, normally agonists at α -adrenoceptors (Barlow, 1964) were of low efficacy and/or were more than a thousand times less potent than NA (Figure 1b,c). ($\pm$)-Salbutamol had a tenth to a hundredth of the potency, and procaterol about a thirtieth to a thousandth of the potency of NA or Iso (Figure 2e and Table 1), although relative potency was not reliable as these drugs were partial agonists (Figure 2b,e and Table 1). ($\pm$)-Salbutamol has about a sixth of the potency of ($\pm$)-Iso at β_2 -adrenoceptors but negligible activity at β_1 -adrenoceptors (Brittain *et al.*, 1973). ($\pm$)-Salbutamol is interesting because the 3-OH is replaced by 3-CH₂OH. However, ($\pm$)-procaterol is more potent than ($\pm$)-salbutamol at β_1 and β_2 receptors (Yabuuchi, 1977) whereas in the present experiments the opposite was found.

Figure 1 Effects of several sympathomimetic amines on I_{Ca} in single isolated smooth muscle cells. Voltage-clamped cells were depolarized every 20 s from -50 to 0 mV for 200 ms. ($\pm$)-Isoprenaline (Iso), ($\pm$)-adrenaline (Ad) and ($\pm$)-noradrenaline (NA) potentiated Ca current in the absence (a) and presence (b) of 10μ M phentolamine. (-)-Iso, (-)-Ad, (-)-NA, dopamine and ($\pm$)-isoetharine also potentiated Ca current with almost the same potency in the presence of 3μ M phentolamine (c, d). However, (-)-phenylephrine ((-)-Phe) and methoxamine (Metho) had no effect. (e) Increase of Ca current by cumulative application of (-)-Iso in the presence of 3μ M phentolamine. Insets show superimposed current traces before and at the peak of the effect of the drug in each panel. In 4th inset of Figure 1b, 3 traces which indicate control, (-)-phenylephrine and NA treated were superimposed. Currents traces in (e) are control and in the presence of 30 nM, 0.3 and 3μ M (-)-Iso. (f) Concentration-response of (-)-Iso on Ca current. The data were fitted to a curve drawn from $I_{Iso}/I_{cont} = 1 + [A/(1 + K_D/((-)-Iso))]$ where I_{cont} and I_{Iso} are relative current amplitudes before and during application of (-)-Iso, $[(-)-Iso]$ is the concentration of (-)-Iso and K_D its apparent dissociation constant. The Hill coefficient was assumed to be one and the maximum relative increase in I_{Ca} , $A = 0.615$. K_D was calculated to be 41 nM. Drugs were prepared immediately before use and were applied externally by replacement of external solution by drug-containing one.

Figure 2 Effects of some sympathomimetic amines, β_2 (salbutamol and procaterol) and β_3 agonists (BRL 37344 and ICI 215001) and catechol itself on I_{Ca} . (-)-Isoprenaline ((-)-Iso), (+)-Iso, L-Dopa and catechol (a, c, d) equally potentiated I_{Ca} . However, BRL 37344 (BRL) and ICI 215001 had a very small effect on I_{Ca} (b,e). Salbutamol and procaterol had some effects on I_{Ca} but the potentiation was smaller than that produced by Iso in the same cells (b,e). Insets show superimposed current traces before and during application of drugs. In first and second traces of (d), current traces before and during application of 3 μ M (-)-Iso, or 3 μ M catechol, and in the presence of both 3 μ M (-)-Iso and 3 μ M catechol, were superimposed; (e) Summarises results of application of salbutamol, procaterol, BRL 37344 and ICI 215001 to the indicated number of cells (open columns); in each case (-)-Iso was applied at the concentration indicated to the same cells (solid columns). (f) Summarised the results of experiments of this type on the indicated number of cells where 0.1 or 3 μ M concentrations of catechol (Cat) or (-)-Iso were used. Phentolamine (3 μ M) was present in (a), (b), (c) and (d).

Some other compounds tried had very low efficacy and/or low potency. ($\pm$)-Terbutaline which resembles Iso except that 4-OH is replaced by 5-OH was more than a 1000 times less potent than (–)-Iso, emphasising the importance of the 3-OH, 4-OH catechol nucleus for activity. BRL 37344 (12) and ICI 215001 (M 215001), the active acid metabolite of ICI D7114 (Growcott *et al.*, 1992), both β_3 -receptor agonists, were virtually inactive (Figure 2b and 2e).

The N-substitute on the molecule was clearly not important for activity at this receptor either since Iso, Ad and NA (– or $\pm$ forms) were equipotent (Table 1). Further, ($\pm$)-isoetharine which resembles Iso except for an α -ethyl group was also equipotent (Table 1) (Figure 1d). These results taken with those concerning the β -OH group suggested that the receptor was indifferent to the nature of the aliphatic tail. This was confirmed as L-Dopa, which has an α -COOH and

Figure 3 Effects of β -adrenoceptor and dopamine antagonists on potentiation of I_{Ca} by (–)-isoprenaline ((–)-Iso) and dopamine in the presence of α -adrenoceptor blocker phentolamine 3 μ M; 10 μ M β -adrenoceptor blocker (–)-propranolol, 5 μ M β_1 -adrenoceptor blocker CGP 20712A (CGP) with 5 μ M β_2 -adrenoceptor blocker ICI 118551 (ICI), 10 μ M ICI 118551, and dopamine receptor blockers haloperidol (1 μ M) and flupenthixol (1 μ M) had no effect on potentiation of I_{Ca} by (–)-Iso or dopamine when expressed as percentage or fractional increase in basal current. (f) Summarizes the effects on I_{Ca} of 0.1 μ M of various sympathomimetic amines mostly in 3 μ M phentolamine. Same set of cells (between gaps) were used to compare the potencies. (–)-Phe, phenylephrine; Metho, methoxamine; Isoeth, isoetharine.

Table 1 Relative potencies of various sympathomimetics and related compounds on voltage-dependent Ca current

Relative potency	Highest conc. used μM	No. of cells
OH	OH	β OH	α H	NH $\text{CH}(\text{CH}_3)_2$	(-) Isoprenaline β	1.00	3	> 30
					($\pm$) Isoprenaline β	1.00	3	5
					(+) Isoprenaline β	1.2 ± 0.11		5
OH	OH	OH	H	CH_3	(-) Adrenaline $\alpha\beta$	1.1 ± 0.11	0.3	3
					($\pm$) Adrenaline $\alpha\beta$	0.91 ± 0.06	3	5
OH	OH	OH	H	H	(-) Noradrenaline $\alpha\beta_1$	0.91 ± 0.13	1	3
					($\pm$) Noradrenaline $\alpha\beta_1$	0.88 ± 0.03	3	3
OH	OH	H	H	H	Dopamine α	1.0 ± 0.09	0.1	9
OH	OH	OH	CH_2CH_3	$\text{CH}(\text{CH}_3)_2$	($\pm$) Isoetharine β	1.0 ± 0.07	0.1	6
OH	OH	H	COOH	H	L-Dopa -	1.2 ± 0.18	0.1	4
OH	OH	-	-	-	Catechol -	0.96 ± 0.11	3	5
H	OH	OH	H	CH_3	(-) Phenylephrine α	$>>> 0.001$	3	4
5OCH_3	2OCH_3	OH	CH_3	H	($\pm$) Methoxamine α	$>>> 0.001$	3	4
5OH	OH	OH	H	$\text{C}(\text{CH}_3)_3$	($\pm$) Terbutaline β_2	$>>> 0.001$	3	3
OH	CH_2OH	OH	H	$\text{C}(\text{CH}_3)_3$	($\pm$) Salbutamol β_2	$0.1-0.01\%$	1	6
OH	Ring	OH	C_2H_5	$\text{CH}(\text{CH}_3)_2$	($\pm$) Procaterol β_2	$0.03-0.001\%$	10	4
H	Cl	OH	H	Complex	($\pm$) BRL 37344 β_3	0.0003	10	5
H	H	Complex			ICI 215001 β_3	0.0003	10	2
OH	H	H	H	H	Tyramine -	$>>> 0.001$	3	2

Substances marked § were partial agonists (maximum response less than 25–30% of maximum response to Iso). Other compounds of low potency may also be partial agonists but high enough concentrations were not tested and their relative potencies were calculated by extrapolating the concentration-effect curve and assuming it was parallel with the curve for (–)-Iso or ($\pm$)-Iso. The potency of catechol or drugs containing a catechol nucleus was obtained by application of equal concentrations (usually 30 or 100 nM) to the same cell of the drug and (–)-Iso or ($\pm$)-Iso (which were assumed equipotent as EC_{50} s were 41.8 and 41.3 nM, see text). The relative sizes of the responses were then calculated for the indicated number of cells; none proved significantly different from unity at $P < 0.05$. The structure of the drug, and the adrenoceptor at which it has a major action are also shown.

no β -hydroxy group, was also equipotent with the catecholamines (Table 1, Figure 2a).

It was therefore predicted that catechol (3,4-dihydroxybenzene) should be active at this receptor. This was confirmed; freshly made solutions (catechol is somewhat unstable) indicated that catechol and catecholamines were equipotent (Figure 2c,d). Furthermore, when maximum potentiation of the current was produced by 3 μM (–)-Iso, 3 μM catechol had no effect; in the same way 3 μM (–)-Iso was without effect if 3 μM catechol had potentiated the current (Figure 2d,f).

Various adrenoceptor blocking agents were applied to test for their antagonism at this receptor. All of these were without effect if the potentiation by Iso or other catecholamine was expressed as a percentage or fractional increase in basal current size before application of the agonist. When expressed in this way, high concentrations of antagonists had no effect on the increase in size of the current produced by (–)-Iso but generally depressed basal calcium current size themselves. (–)-Propranolol up to 10 μM was without significant antagonism; this drug has a high affinity for type β_1 - and type β_2 -receptors, its pA_2 being in the range 8–9 (Arch *et al.*, 1984; Wilson *et al.*, 1984) although its affinity for β_3 -receptors is somewhat less, pA_2 about 7 (Arch *et al.*, 1984; Bond & Clarke, 1988). Atenolol, a β_1 -selective antagonist with a pA_2 of 6 to 7 (O'Donnell & Wanstall, 1983), the β_1 -selective antagonist CGP 20712A with a pA_2 greater than 9 (Kaumann, 1986), and the antagonist ICI 118551 with a pA_2 for β_2 -receptors in the range 8–10 (O'Donnell & Wanstall, 1983; 1985) in concentrations up to 10 μM did not significantly antagonize the effect of (–)-Iso or (–)-NA on I_{Ca} (Figure 3a,b,c). Phentolamine and dihydroergotamine are generally regarded as blockers of both α_1 and α_2 receptors with pA_2 values in the range 7–8 (Barlow, 1964; Borowski *et al.*, 1977). These agents showed some tendency at concentrations of 3–10 μM to potentiate the increase in the Ca current produced by catecholamines (Figure 3f) and responses were often obtained in their presence (Figures 1b–e, 2a–d, 3a–e).

The α -agonist, phenylephrine (10 μM), applied when I_{Ca} was already potentiated by 3 μM Iso, depressed I_{Ca} ; carbachol (10 μM) applied under similar conditions also depressed I_{Ca} (not shown).

The increase in Ca current produced by dopamine or by Iso was also unaffected by the dopamine receptor antagonists haloperidol (1 μM) and flupenthixol (1 μM) (Figure 3d,e). At D_1 and D_2 dopamine receptors their pA_2 is normally in the range 7–8 (Hilditch & Drew, 1985). At higher concentrations than 1 μM these two antagonists produced strong depression of the voltage-dependent Ca current themselves and 3 μM haloperidol virtually abolished it.

The effects of Iso, NA and catechol on tension in small strips of taenia in normal solution or when contracted by raising the potassium concentration to 20 or 40 mM were examined. Phentolamine (3 μM), propranolol (3 μM) and atropine (3 μM) were added to the bathing solution. Since these blockers in combination did not block C-receptors, any effects of Iso on tension might be mediated by these receptors. In normal solution, taenia strips showed spontaneous contractions of several g amplitude at a frequency of about 1 every 2 min, from time to time contractions spontaneously ceased. In the presence of blockers, Iso (10^{-7} M; four preparations, eight applications) or, NA ($1-3 \times 10^{-7}$ M; four preparations, five applications) did not alter the pattern of activity or increase the tension developed during spontaneous contractions. In preparations contracted in high-K solutions, Iso ($1-3 \times 10^{-7}$ M; seven preparations, 18 applications) NA ($0.3-1 \times 10^{-6}$ M; four preparations, six applications) or catechol ($0.3-1 \times 10^{-6}$ M; three preparations, four applications) did not increase tension and sometimes a small fall was observed, presumably due to incomplete blockade of α - and β -receptors. Thus, no increases in tension, indicative of increased entry of calcium through voltage-dependent channels were seen when any of the three catechols were added to preparations where α - and β -adrenoceptors and muscarinic receptors were blocked.

Discussion

The lack of effect of any of the antagonists indicate that the receptor which mediates the increase in the voltage-dependent calcium current in response to catecholamines is not an α - or a β -adrenoceptor nor is it a D_1 or D_2 dopamine receptor. This adrenoceptor showed unusual structure-activity relationships in that the presence of a catechol nucleus was essential for full activity and altering the position of one of the -OH groups on the benzene ring, or removing one or both of these, produced drastic reductions in potency and/or efficacy. However, this receptor was indifferent to the presence of the ethylamine side chain and so catechol was equipotent with L-dopa, dopamine, Ad, Iso and NA. The β -OH group was inessential and (-)- and (+)-isomers of Iso and (-) and ($\pm$)-isomers of NA and Ad were equipotent. An amino group was also without effect. In these latter two respects, this receptor differs from α - and β -adrenoceptors where the presence of a (-)- β -OH group substantially increases potency; at the β -receptor, N-substitution with isopropyl substantially increases potency. The sensitivity to catechol would suggest that it should be called a C-receptor.

The C-receptor shows several similarities to and some differences from the adrenoceptors, activation of which modulate I_{Ca} in various enzymatically dissociated smooth muscle cells. In smooth muscle cells of rabbit ear artery, NA and Ad were equipotent in increasing I_{Ca} although Iso was ineffective; propranolol, prazosin and phentolamine were ineffective as blockers as in taenia (Benham & Tsien, 1988). However, in smooth muscle cells from rabbit ear artery both NA and Iso have been reported by other investigators to reduce I_{Ca} ; the effects of NA and Iso were found to be unaffected by propranolol and phentolamine respectively (Droogmans *et al.*, 1987). In smooth muscle cells of pig coronary artery, NA and Iso were equipotent but the potentiation they produced was blocked by propranolol but not by phentolamine (Fukumitsu *et al.*, 1990). The reduction of I_{Ca} produced by NA in cultured aortic cells was unaffected by prazosin, phentolamine or propranolol (Serebryakov & Takeda, 1992). In rat portal vein single smooth muscle cells, the action of NA to increase I_{Ca} was blocked by phentolamine (Pacaud *et al.*, 1987) and in guinea-pig vas deferens the reduction of I_{Ca} produced by NA or phenylephrine was blocked by prazosin and less well by yohimbine (Imaizumi *et al.*, 1991). In bovine tracheal smooth muscle cells, Iso increased internal free calcium activity (measured by fura 2-AM) and this effect was blocked by propranolol (0.1 μ M) but not by prazosin or yohimbine (at 10 μ M) (Felbel *et al.*, 1988). The action of Iso was on calcium influx into the cell, which was blocked by nitrendipine or methoxyverapamil (D600); the calcium influx presumably corresponds to I_{Ca} studied in single cells by electrophysiological techniques (Welling *et al.*, 1991; 1992). However, the action of Iso in raising intracellular calcium activity is not confined to single, enzyme-digested, cells as it has been observed in strips of tracheal smooth muscle (Takuwa *et al.*, 1988).

It is tempting to suggest that as adrenoceptors of unusual pharmacology have been described in single enzymically dissociated cells, that the enzyme treatment has modified the adrenoceptors involved. This impression might be reinforced by our failure to detect any effects of Iso on tension in strips of taenia when α - and β -adrenoceptors were blocked. The actions of isoprenaline and other catecholamines have been studied on the electrical activity of the guinea-pig taenia by means of both microelectrode recording and sucrose-gap techniques. Catecholamines through an action on β -receptors (blocked by propranolol) inhibit the generation of the pacemaker potential (Bülbring & Tomita, 1969) and cause weak hyperpolarization (Bülbring & den Hertog, 1980). These effects are associated with inhibition of contractile activity, or relaxation, never contraction or increase in the force of contraction; α -receptor activation causes hyperpolarization and relaxation also (Bülbring & Tomita, 1987). However, Iso can

raise internal calcium activity (Ca_i) in tracheal smooth muscle strips as well as reducing the rise in Ca_i engendered by carbachol and this effect is mimicked by forskolin (Takuwa *et al.*, 1988). In the basilar artery of young rats, Iso elicits a depolarization instead of its usual hyperpolarization and this is a β -receptor effect; also NA and on occasions Iso can elicit a depolarization resistant to both α - and β -adrenoceptor blockers (Byrne *et al.*, 1985). Responses evoked by ' γ -receptors' in rat basilar artery segments required high (mM) concentrations of catecholamines, but like the responses evoked in our present experiments, NA, Ad, and dopamine had similar potency and isomers of NA and Ad were equiactive (Hirst *et al.*, 1982). The low concentrations of catecholamines which were effective (EC_{50} about 40 nM) in the present experiments are in contrast to those required to activate the γ -receptor of rat basilar artery which are four orders of magnitude higher (Hirst *et al.*, 1982). Thus receptors for catecholamines have been described on whole tissues which have unusual pharmacology and which may be related to the C-receptor described here. It is conceivable that the C-receptor exists in the whole tissue but its effects at the level of tension generation are for some reason not detectable.

If the C-receptor represented an α - or a β -adrenoceptor altered by collagenase and pronase treatment then it raises questions concerning the nature of the receptor binding site. The modification would represent presumably the removal of some enzyme-sensitive portion of the receptor molecule either directly or indirectly resulting in changes in the molecular architecture of the binding site. It would raise the possibility that receptors, like ion channels (e.g. Hescheler & Trautwein, 1988; Obejero-Pas *et al.*, 1991) might be deliberately modified by enzymic treatment which might allow investigation of the parts of the receptor involved in ligand binding, or perhaps even enzymic alteration of the structure-activity relationships of identified receptors with a view to making them susceptible to novel drugs or ligands.

It is curious, that, if the C-receptor should be a modified β -receptor, that the affinity for propranolol should be so severely reduced since it implies that binding of the naphthalene group to the catechol site is weak such that loss of the hydroxyethylamine binding site(s) results in very low affinity. Nevertheless, the catechol binding site alone was sufficient for retention of considerable affinity for Iso and other catechols since they were reasonably potent. An alternative possibility is that enzymic modification of the molecule causes a binding site for catechols to be revealed, or even to be created, by exposing some juxtaposition of charged groups previously inaccessible within the molecule. A surprising fact, however, is that the activated receptor retains (or perhaps gains) the ability to alter calcium channel function. Unlike a variety of other receptors activation of which alters I_{Ca} in smooth muscles (see Muraki *et al.*, 1993 for references) C-receptor activation was linked to changes in calcium channel activity by a mechanism which did not seem to involve a G-protein, cyclic AMP/adenylyl cyclase, or protein kinase C (Muraki *et al.*, 1993) i.e. not only is the C-receptor novel but its transduction mechanism is also. It may be that the C-receptor is an integral part of the voltage-dependent calcium channel but, if so, it seems a separate site from that at which Bay K 8644, a dihydropyridine, binds to increase I_{Ca} because the latter increased I_{Ca} in the occasional cell which was unresponsive to catecholamine (Muraki *et al.*, 1993).

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