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A response to isoprenaline unrelated to α- and β-adrenoceptor agonism

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1 The hypothesis that β-adrenoceptor agonism might explain a reported lack of competitive antagonism between α_2-adrenoceptor antagonists and agonists of the phenylethylamine class was tested in the electrically field stimulated ileum of the guinea-pig. The β-adrenoceptor agonist, isoprenaline, was used as the phenylethylamine and inhibition of 'twitch' response evoked by cholinergic stimulation was measured.

2 In the presence of idazoxan (3 μM), to block inhibitory α_2-adrenoceptors, propranolol (0.1 to 5.0 μM) failed to act competitively toward isoprenaline. Isoprenaline responses totally resistant to inhibition by propranolol were obtained.

3 As inhibitory α_1-adrenoceptors are absent from guinea-pig ileum, a recognition site distinct from the currently defined α- and β-adrenoceptors is postulated. Agonism by phenylethylamine based agonists at this site may explain their inability to act competitively with α- and β-adrenoceptor antagonists.

Introduction

α_2-Adrenoceptor agonists of the phenylethylamine class can be distinguished from agonists of the imidazol(id)ine (imidazoline and imidazolidine) groups by antagonists. For instance, in the field stimulated guinea-pig ileum and rat vas deferens, clonidine and UK-14,304-18 interact competitively with α_2-adrenoceptor antagonists whereas noradrenaline and α-methylnoradrenaline often interact in a manner inconsistent with competitive antagonism (Mottram 1983; Vizi *et al.*, 1983; Ruffolo, 1984; Hicks *et al.*, 1985; Bond *et al.*, 1986 a,b). In addition, the irreversible α-adrenoceptor antagonist, benextramine (Melchiorre *et al.*, 1978), at a concentration which totally abolishes responses to clonidine and UK-14,304-18, spares responses to noradrenaline, a difference which cannot be attributed to α_2-adrenoceptor reserve (Bond *et al.*, 1986a,b).

One hypothesis advanced to explain these discrepancies is the possibility that phenylethylamine agonists also evoke responses at a recognition site distinct from α_2-adrenoceptors (Bond *et al.*, 1986 a,b). However, although investigators have used propranolol (usually 1 μM) to block β-adrenoceptors, it remains possible that the affinity of the phenylethylamines for β-adrenoceptors could lead to a breakthrough of the propranolol-induced blockade. The high concentrations of noradrenaline and α-methylnoradrenaline required to elicit responses in the presence of α_2-adrenoceptor antagonists support this possibility.

Experiments were undertaken to examine the above hypothesis using the electrically evoked 'twitch' response in the field stimulated guinea-pig ileum. The 'twitch' response is mediated by cholinergic stimulation and is inhibited by atropine, α_2- and β-adrenoceptor agonism, but not by α_1-adrenoceptor agonism (Drew, 1978; Wikberg, 1978; Bond *et al.*, 1986 a,b). All experiments were done using isoprenaline: a phenylethylamine analogue which is a potent β-adrenoceptor agonist.

Methods

Guinea-pigs (300 to 500 g) were treated with reserpine (5 mg kg^{-1}, i.p. for 18 h) to deplete endogenous noradrenaline, and segments of ileum (about 3 cm in length) were set-up *in vitro* at 37°C in Krebs solution for transmural electrical stimulation (0.1 Hz, 1 ms pulse duration, supramaximal voltage). The Krebs solution was of the following composition (mM): NaCl 118, $CaCl_2$ 2.6, KCl 4.9, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1.2, glucose 11.7, choline 0.2 and ascorbic acid 0.11 to offset auto-oxidation of isopren-

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aline; it was bubbled continuously with 5% CO_2 and 95% O_2. In some experiments, idazoxan (3 μM) was included in the Krebs solution. Neuronal and extraneuronal uptake were inhibited competitively by cocaine (30 μM) and corticosterone (30 μM), respectively. The 30 μM concentration was selected to saturate the uptake processes so as to prevent high concentrations of agonist (which occur following antagonist induced dextral shifts in agonist response curves) from overcoming the inhibition. In previous studies (Bond *et al.*, 1986 a,b), agonists with a much greater affinity for neuronal uptake than isoprenaline were used. Therefore, the same cocaine concentration was retained to allow for direct comparison. Cocaine (30 μM) did not impair the 'twitch' response. Responses of 1.5 to 4 g in tension were recorded isometrically and a 45 min tissue equilibration time was allowed before starting experiments.

Concentration-effect curves to isoprenaline were constructed on a cumulative concentration schedule with the interval between successive additions of isoprenaline being adjusted to allow sufficient time for the response to develop fully. Two concentration-effect curves were constructed per segment of ileum. After completion of the first curve, segments were washed (10 times over 15 min) and allowed to equilibrate for 10 min. Propranolol or nadolol was then added to test segments and vehicle to control segments, and the second concentration-effect curve to isoprenaline was constructed 30 min later.

Differences between mean values were analysed statistically by use of Student's *t* test for unpaired data. *P* values greater than 0.05 were considered to be non-significant.

Drugs were prepared freshly everyday in distilled water. Idazoxan was purchased from Reckitt & Colman Ltd, Hull, U.K. Cocaine hydrochloride, corticosterone, (−)-isoprenaline hydrochloride, (±)-propranolol hydrochloride, and reserpine were purchased from Sigma, St. Louis, MO, U.S.A. Nadolol was obtained as a gift from E.R. Squibb & Sons, Inc., Princeton, NJ, U.S.A.

Results

The effect of isoprenaline on the 'twitch' response induced by cholinergic stimulation is illustrated in Figure 1. In the absence of antagonists, isoprenaline produced a biphasic inhibition of the 'twitch' response (Figure 1a). The first phase caused about a 40% inhibition which was maximal at 0.1 μM isoprenaline. The second phase required much higher concentrations of isoprenaline, beyond 3 μM and was inhibited completely by 3 μM of the α_2-adrenoceptor antagonist, idazoxan (Figure 1b). Propranolol (5 μM), in the presence of idazoxan (3 μM), produced a rightward

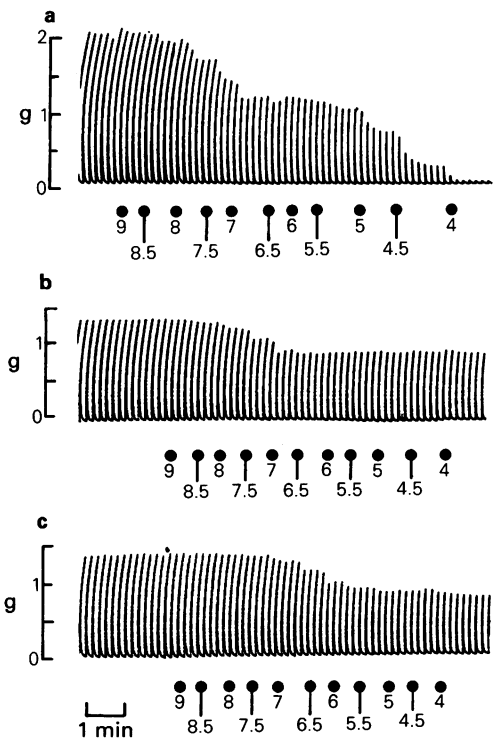

Figure 1 Inhibitory effect of isoprenaline on the 'twitch' response to transmural electrical stimulation of guinea-pig ileum: (a) isoprenaline alone, (b) isoprenaline in the presence of idazoxan (3 μM), (c) isoprenaline in the presence of idazoxan (3 μM) and propranolol (5 μM). Segments of ileum were stimulated continuously at 0.1 Hz, 1 ms pulse duration at supramaximal voltage. Shown are concentrations of isoprenaline (− log M).

shift in the first phase of the concentration-effect curve to isoprenaline (Figure 1c). Quantitative data for these effects are given in Figure 2.

Figure 2 shows that propranolol, 0.1, 1.0, and 5.0 μM produced parallel dextral shifts in the concentration-effect curves to isoprenaline with no marked change in the maximum responses. The mean concentration-ratios ± s.e.mean (measured at the 16% inhibition point on the concentration-effect curves) for isoprenaline were: 8.5 ± 3, 10.2 ± 3, and 14.7 ± 2 with propranolol 0.1, 1.0 and 5.0 μM, respectively. None of these concentration-ratios differed significantly from each other. Propranolol, in the concentrations shown, did not affect the 'twitch' response but a higher concentration (10 μM) depressed responses, precluding further quantitative studies.

Other experiments (not shown) were carried out using nadolol, a potent β-adrenoceptor antagonist devoid of local anaesthetic activity ($pA_2 = 7.7$ at β-

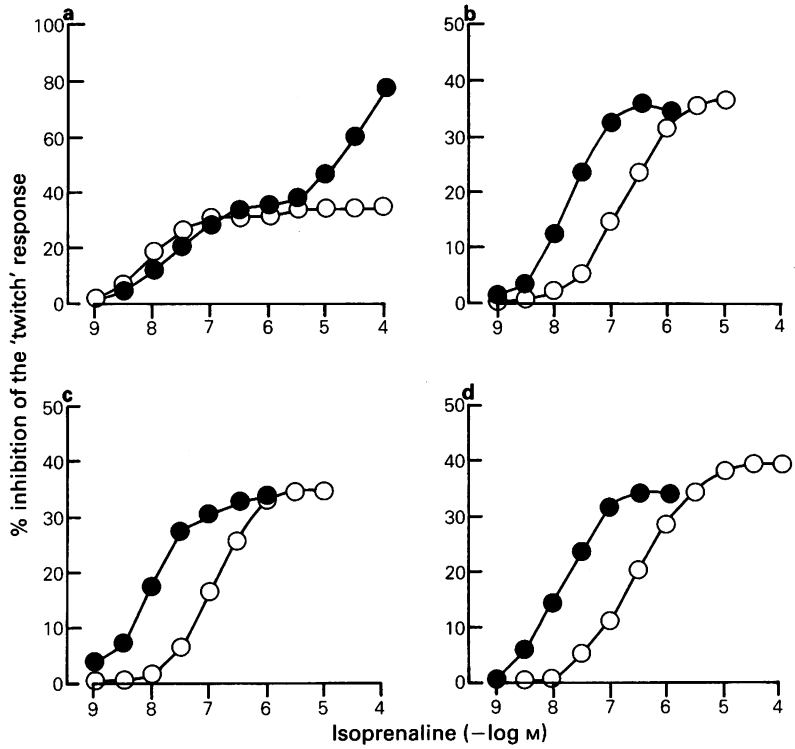

Figure 2 Effect of idazoxan (a) and propranolol (b, c and d) on isoprenaline-induced inhibition of the electrically evoked 'twitch' response in segments of guinea-pig ileum. In (b, c and d) ileal segments were preincubated with idazoxan (3 μM) for 45 min before starting the experiments. In (a), (●) control (n = 6); (○) effect of idazoxan 3 μM (n = 6). In (b, c and d), (●) control (n = 5, except in (d) where n = 6); (○) effect of propranolol (b) 0.1 μM (n = 5), (c) 1.0 μM (n = 5), (d) 5.0 μM (n = 6). Each experimental value is the mean percentage inhibition obtained using (n) guinea-pigs. (Standard errors were less than 5% of mean values and are not illustrated.) Note the different scale on the ordinate axis in (a) versus that in (b, c and d).

adrenoceptors; Lee *et al.*, 1975). In the presence of idazoxan (3 μM), nadolol caused parallel dextral shifts in the concentration-effect curves to isoprenaline with no change in the maximum response. The mean concentration-ratio ± s.e.mean for isoprenaline was 8.0 ± 3 (n = 4).

Discussion

The results reveal a lack of competitive antagonism toward isoprenaline by propranolol when this β-adrenoceptor antagonist was used over a 50 fold concentration range.

Propranolol (0.1 μM) produced a concentration-ratio with isoprenaline of 8.5. For competitive antagonism, 1 and 5 μM propranolol should have produced concentration-ratios of 76 and 376, respectively (Arunlakshana & Schild, 1959). Instead, no significant change from 8.5 was obtained. Furthermore, nadolol, used at 10 μM (500 times its equilibrium dissociation constant for β-adrenoceptors; Lee *et al.*, 1975), only shifted the concentration-effect curve to isoprenaline by 8 fold. It appears, therefore, that propranolol and nadolol, in the concentrations used, fully saturated β-adrenoceptors and that a breakthrough of the antagonism cannot explain the remaining responses to isoprenaline. Furthermore, the resistant responses to isoprenaline cannot be attributed to α_1-adrenoceptor agonism (see Introduction) or to overcoming α_2-adrenoceptor antagonism with idazoxan (Figures 1 and 2). Therefore the responses to isoprenaline which are resistant to a combination of propranolol and idazoxan, or nadolol and idazoxan, appear to result from a recognition site unrelated to currently defined α- and β-adrenoceptors, as postulated previously with noradrenaline as the agonist (Bond *et al.*, 1986 a,b). An action on dopamine receptors can be ruled out as dopamine receptors are not present in the guinea-pig ileum and dopamine-

induced inhibition of the 'twitch' response is mediated via α_2-adrenoceptors (Gorich *et al.*, 1981; Bond *et al.*, 1986a). Experiments using histamine to contract the ileum, instead of electrical stimulation, show isoprenaline to be an effective inhibitor of histamine-induced contractions (unpublished observations). These experiments were carried out in the presence of the α_1 and α_2-adrenoceptor antagonist phentolamine (3 μM), propranolol (5 μM) and the muscarinic receptor antagonist atropine (1 μM), and suggest an extraneuronal locus for the postulated site.

The characteristics and functional role of the proposed site have not been elucidated but its presence in tissues may explain deviations from competition or resistant responses to phenylethylamines when interacted with α- (for references, see Introduction) and β-adrenoceptor (Bristow *et al.*, 1970; Morris *et al.*, 1981; Drew & Hilditch, 1984; Broadley *et al.*, 1985) antagonists. Compliance with competitive kinetics at ileal α- and β-adrenoceptors would be restricted to low antagonist concentrations that produce limited dextral shifts in the agonist concentration-effect curve.

Finally, the presently proposed site may be related to the 'atypical' β-adrenoceptor found on adipocytes (Arch *et al.*, 1984) and other tissues (Bentley & Starr, 1986). However, in most studies these receptors interact competitively with propranolol and other β-adrenoceptor antagonists but derived pA_2 values are abnormally low (Arch *et al.*, 1984; Wilson *et al.*, 1984; Bojanic *et al.*, 1985).

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