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Partial agonist activity of carteolol on atypical β-adrenoceptors in the guinea pig duodenum

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Abstract

The partial agonist activities of carteolol were investigated on atypical β-adrenoceptors of duodenum on the guinea pig. Carteolol produced a concentration-dependent relaxation of the guinea pig duodenum ($pD_2 = 4.85$), which was not significantly affected by propranolol (1 μM). In the presence of propranolol (1 μM), however, the non-selective β_1-, β_2- and β_3-adrenoceptor antagonist, bupranolol (30 μM), caused a rightward shift of the concentration–response curves for carteolol (apparent $pA_2 = 5.31$). Moreover, carteolol (10 μM) weakly, but significantly, antagonized the relaxations in response to catecholamines (isoprenaline, noradrenaline and adrenaline), to a selective β_3-adrenoceptor agonist, (R^*,R^*)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt (BRL37344), and to a non-conventional partial β_3-adrenoceptor agonist, [4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one] hydrochloride (CGP12177A), also in the guinea pig duodenum (apparent $pA_2 = 5.77$, 5.92, 6.05, 6.56 and 5.58, respectively). These results suggest that the partial agonist effects of carteolol are mediated by atypical β-adrenoceptors in the guinea pig duodenum.

Keywords: β-Adrenoceptor, atypical; β_3-Adrenoceptor; Duodenum, guinea pig; Carteolol; Partial agonist; Bupranolol

1. Introduction

Carteolol induces relaxation and behaves as a β-adrenoceptor partial agonist in the guinea pig taenia caecum (Takayanagi and Koike, 1983). Carteolol is generally accepted as a partial β_1-/β_2-adrenoceptor agonist and exhibits both stimulatory and inhibitory effects (Lipworth and Grove, 1997). Moreover, we showed that β_2- and β_3-adrenoceptors were involved in the β-adrenoceptor-mediated relaxation of the guinea pig taenia caecum (Koike et al., 1994, 1995a,b) and that carteolol did not act as a β_3-adrenoceptor agonist in the guinea pig taenia caecum (Koike et al., 1996).

However, Zhao et al. (1998) demonstrated that carteolol, described earlier as a partial agonist on β_1- and β_2-adrenoceptors, also possesses β_3-adrenoceptor partial agonist properties on brown fat cells from mouse, rat and hamster. These facts suggest that carteolol-stimulated thermogenesis is mediated by β_3-adrenoceptors in brown adipose tissue, but that carteolol-induced relaxation is mediated by β_2-adrenoceptors in the taenia caecum. However, it remains possible that there are species differences in β_3-adrenoceptors that β_3-adrenoceptors of the guinea pig taenia caecum cannot recognize carteolol.

Recently, we have suggested that atypical β-adrenoceptors were involved in mediating the relaxant response of the guinea pig duodenum (Horinouchi and Koike, 1999a). We also showed that the relaxations in response to catecholamines (isoprenaline, noradrenaline and adrenaline), to a selective β_3-adrenoceptor agonist, (R^*,R^*)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt (BRL37344), and to a non-conventional partial β_3-adrenoceptor agonist, [4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one] hydrochloride (CGP12177A), were competitively antagonized by a non-selective β-adrenoceptor antagonist, bupranolol (Horinouchi and Koike, 1999a), although at a concentration much higher than that necessary for the blockade of β_1- or β_2-adrenoceptors (Kaumann, 1989).

Therefore, we attempted to clarify whether carteolol acts as a β_3-adrenoceptor partial agonist at atypical β-

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adrenoceptors in the guinea pig duodenum by using catecholamines (isoprenaline, noradrenaline and adrenaline), BRL37344, CGP12177A and bupranolol.

2. Methods

2.1. Mechanical responses

Male Hartley guinea pigs weighing 300–500 g were killed by cervical dislocation and the entire duodenum was rapidly isolated and placed in oxygenated (a mixture of 95% O_2 and 5% CO_2) Ringer–Locke solution of the following composition (in mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9; and glucose, 2.8. The intraluminal contents were flushed out with Ringer–Locke solution and the connective tissue was dissected away. The outer layer of duodenum containing longitudinal smooth muscle was carefully removed with a cotton swab. Strips of 10-mm length were mounted on tissue hooks and suspended in jacketed 20-ml organ baths containing Ringer–Locke solution maintained at 32°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The mechanical responses of strips were recorded isometrically under a load of 0.5 g. Tissue strips were equilibrated for 30 min in the presence of desmethylimipramine (1 μM) to block neuronal uptake, normetanephrine (10 μM) to block extraneuronal uptake and phentolamine (10 μM) to block α-adrenoceptors.

Concentration–response curves for histamine were made to determine the concentration producing submaximal contraction. Histamine (10 μM) was chosen to precontract longitudinal smooth muscle strips in subsequent experiments. The relaxations caused by β-adrenoceptor agonists were evaluated by measuring the inhibition of the histamine (10 μM)-induced contraction. Firstly, concentration–response curves for isoprenaline (up to 3 μM) were generated as controls (100%). Histamine (10 μM) was added to the bath 30 min after washing out the drug, then β-adrenoceptor agonists were added cumulatively until a maximal relaxant response was observed, and the relaxation induced by these drugs was expressed as a percentage of the maximal relaxation produced by isoprenaline (3 μM), the reference drug.

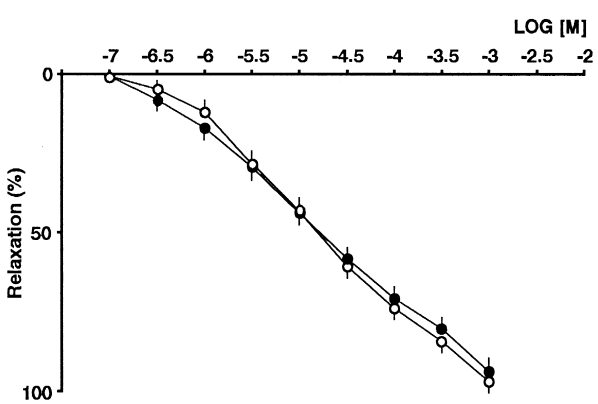

Fig. 1. Relaxant responses to carteolol on the guinea pig duodenum in the absence of propranolol (○) and in the presence of propranolol (1 μM) (●). Ordinate: relaxation (%), expressed as a percentage relative to the maximum relaxation induced by isoprenaline (3 μM), abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of 8–12 experiments.

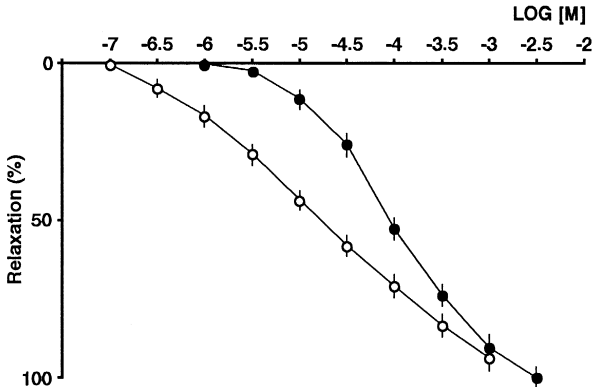

Fig. 2. Effect of bupranolol on concentration–response curves for carteolol after incubation with propranolol (1 μM) in the guinea pig duodenum. Control, no bupranolol (○); bupranolol 30 μM (●). Ordinate: relaxation (%), expressed as a percentage relative to the maximum relaxation induced by isoprenaline (3 μM), abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of 8–12 experiments.

To assess the antagonistic effects of propranolol and bupranolol, each antagonist was added to the bath 30 min before the cumulative addition of the agonist. To test the antagonism of carteolol, however, agonist administration started after the relaxant response to carteolol (3 or 10 μM) had been established. In preliminary experiments, after the control concentration–response curves were made, four or five successive cumulative concentration–response curves for catecholamines were made. The curves were nearly superimposable and changes in sensitivity (sensitization or desensitization) were slight (data not shown). Seven or more concentration–response curves could be made in succession. However, the tissue sensitivity and the maximum response to carteolol, BRL37344 and CGP12177A decreased when two consecutive concentration–response curve for these agonists were performed with the same segment (data not shown); therefore, a single cumulative concentration–response curve to each agonist was made for each strip. Agonistic potency was expressed as the pD_2 value (Van Rossum, 1963). The intrinsic activity of each agonist was calculated as the ratio of the maximal relaxation induced by each agonist to the maximal relaxation induced by the full agonist, isoprenaline (3 μM). The competitive antagonistic potency is expressed as the apparent pA_2 value. Apparent pA_2 values for bupranolol and carteolol were calculated according to the method of Van Rossum (1963) from the equation:

$$\text{apparent } pA_2 = \log(\text{agonist concentration ratio} - 1) - \log[\text{antagonist}].$$

2.2. Data analysis

All results are expressed as means ± S.E. of 8–12 experiments. Statistical analyses were performed with the Newman–Keuls test when appropriate. A probability level of $P < 0.05$ was considered statistically significant.

2.3. Drugs

The following drugs were used: isoprenaline hydrochloride, noradrenaline bitartrate, adrenaline bitartrate, propranolol hydrochloride, histamine dihydrochloride, desmethylimipramine hydrochloride, normetanephrine

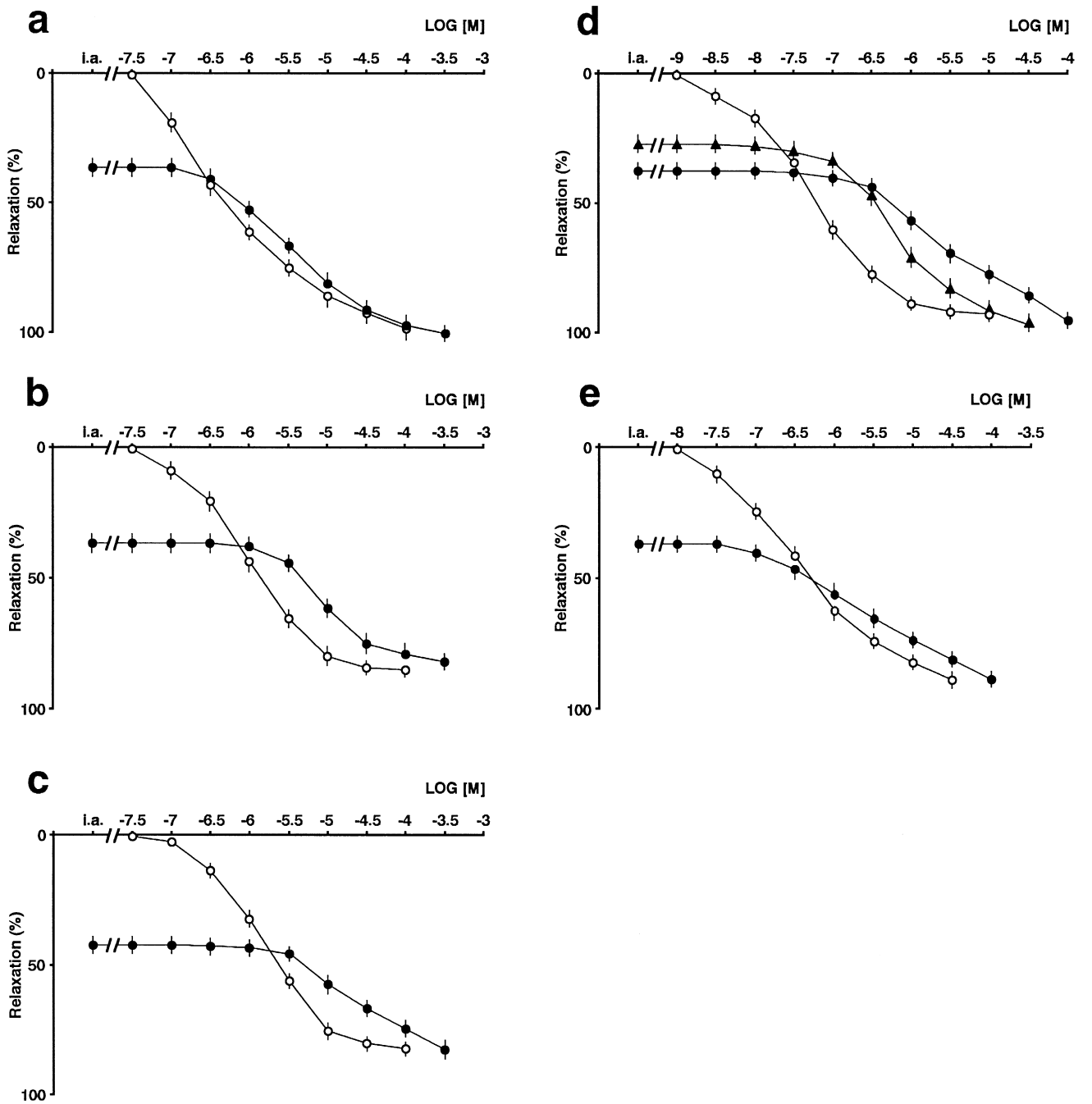

Fig. 3. Effect of carteolol on concentration–response curves for isoprenaline (a), noradrenaline (b), adrenaline (c), BRL37344 (d) and CGP12177A (e) after incubation with propranolol (1 μM) in the guinea pig duodenum. Control, no carteolol (○); carteolol 3 μM (▲); carteolol 10 μM (●). Ordinate: relaxation (%), expressed as percentage of maximum relaxation induced by isoprenaline (3 μM), abscissa: concentration (M) of the test drugs, i.a.: intrinsic activity (relaxation by carteolol (3 or 10 μM) in the presence of propranolol with respect to the maximum relaxation induced by the full agonist, isoprenaline (3 μM), in the absence of propranolol). Each point represents the mean ± S.E. of 8–12 experiments.

hydrochloride (Sigma, St. Louis, MO, USA); phentolamine mesylate (Novartis, Basel, Switzerland); CGP12177A (Research Biochemicals International, Natick, MA, USA); BRL37344 (Nacalaitesque, Kyoto, Japan); bupranolol hydrochloride (Kaken Pharmaceutical, Tokyo, Japan) and carteolol hydrochloride (Otsuka Pharmaceutical, Tokyo, Japan). All drugs were dissolved in distilled water. All other chemicals used were of analytical grade.

3. Results

3.1. Agonistic effect of carteolol

In the absence of propranolol, carteolol relaxed the histamine-induced tone in the guinea pig duodenum, with a pD_2 value of 4.85 ± 0.03 and an intrinsic activity of 0.96 ± 0.03 (Fig. 1). The relaxant response to carteolol was insensitive to propranolol (1 μM; Fig. 1). The pD_2 value and the intrinsic activity in the presence of propranolol were 4.90 ± 0.01 and 0.93 ± 0.03, respectively (Fig. 1).

3.2. Effect of bupranolol on relaxation to carteolol

In the presence of propranolol (1 μM) to block typical β-adrenoceptors, bupranolol (30 μM) antagonized the relaxation to carteolol in the guinea pig duodenum with an apparent pA_2 value of 5.31 ± 0.07 (Fig. 2).

3.3. Antagonism by carteolol

In the presence of propranolol (1 μM), catecholamines (isoprenaline, noradrenaline and adrenaline) and β_3-adrenoceptor agonists (BRL37344 and CGP12177A) induced concentration-dependent relaxations of the guinea pig duodenum and carteolol (3 or 10 μM) weakly, but significantly, shifted the concentration-dependent curves for these five agonists to the right (Fig. 3a–e). The pD_2 values of catecholamines and β_3-adrenoceptor agonists and the apparent pA_2 values of carteolol are summarized in Table 1.

Table 1
Effects of carteolol on the concentration–response curves of catecholamines and β_3-adrenoceptor agonists in the presence of propranolol (1 μM) on the guinea pig duodenum

Agonist	pD_2 value		Apparent pA_2 value
	Absence of carteolol	Presence of carteolol	
Isoprenaline	6.35 ± 0.03	5.51 ± 0.04[a]	5.77 ± 0.03[a]
Noradrenaline	6.04 ± 0.04	5.05 ± 0.04[a]	5.92 ± 0.06[a]
Adrenaline	5.78 ± 0.03	4.72 ± 0.08[a]	6.05 ± 0.08[a]
BRL37344	7.28 ± 0.04	6.20 ± 0.06[b]	6.60 ± 0.07[b,c]
	7.26 ± 0.02	5.68 ± 0.05[a]	6.56 ± 0.05[a,c]
CGP12177A	6.43 ± 0.02	5.75 ± 0.05[a]	5.58 ± 0.06[a]

Values are means ± S.E. from 8–12 experiments.
[a] Determined from Fig. 3. Experiments were conducted in the presence of carteolol (10 μM).
[b] Determined from Fig. 3. Experiments were conducted in the presence of carteolol (3 μM).
[c] $P < 0.05$.

4. Discussion

In the present study, partial agonist effects of carteolol on β-adrenoceptors mediating relaxation in the guinea pig duodenum have been examined. Carteolol induced concentration-dependent relaxation of histamine-precontracted guinea pig duodenum. However, the classical β-adrenoceptor antagonist, propranolol (1 μM), failed to produce significant shifts of the carteolol concentration–response curve, indicating the possible involvement of atypical β-adrenoceptors. Strong evidence for atypical β-adrenoceptors mediating responses in the guinea pig duodenum was supplied by effects of a combination of the non-selective β_1-, β_2- and β_3-adrenoceptor antagonist bupranolol and propranolol. The same conditions were employed to detect atypical β-adrenoceptors in our previous study on the guinea pig duodenum (Horinouchi and Koike, 1999a). In the presence of propranolol (1 μM), bupranolol (30 μM) caused a rightward shift of the concentration–response curve to carteolol. These results suggested that carteolol behaved as an agonist to atypical β-adrenoceptors in the guinea pig duodenum. However, a clear steepening of the concentration–response curve to carteolol by bupranolol (30 μM) was obtained. This observation would suggest two possibilities: (i) dual stimulation of typical β-adrenoceptors and atypical β-adrenoceptors and (ii) a major atypical β-adrenoceptor-mediated response plus some non-specific relaxation at the higher concentrations of carteolol. Since propranolol (1 μM) did not have any effect on the control relaxation curve for carteolol (Fig. 1), whereas bupranolol (30 μM) markedly antagonized the lower but not the higher concentrations of carteolol, the first possibility can be excluded.

In a previous study we had characterized atypical β-adrenoceptors mediating relaxation in response to catecholamines (isoprenaline, noradrenaline and adrenaline) and β_3-adrenoceptor agonists (BRL37344 and CGP12177A) in the guinea pig duodenum (Horinouchi and Koike, 1999a). The present study shows that in the presence of propranolol (1 μM), carteolol produces rightward shifts of the concentration–response curve to these agonists. A single concentration of carteolol (10 μM) gave apparent pA_2 values of 5.77–6.05 (catecholamines), 6.56 (BRL37344) and 5.58 (CGP12177A). The order of atypical β-adrenoceptor antagonistic potencies is consistent with the order of bupranolol for atypical β-adrenoceptors of both the guinea pig duodenum and the guinea pig gastric fundus where the pA_2 values of bupranolol were significantly higher against BRL37344 (6.5) than against the other agonists (5.70–6.08)

(Horinouchi and Koike, 1999a,b). This phenomenon is not easily explained and has been a matter for debate, although it is presumably related to the differences in affinities of these agonists that have been noted for cultured cells transfected with genes coding for human or rodent β_3-adrenoceptors (Liggett, 1992; Granneman et al., 1993; Blin et al., 1994; De Ponti, 1997; Nisoli and Carruba, 1997; Strosberg and Piétri-Rouxel, 1997). It is possible that catecholamines and CGP12177A possess high affinity for atypical β-adrenoceptors in the guinea pig duodenum, while BRL37344 possesses a low affinity. Furthermore, these results suggested the possibility that, in the guinea pig duodenum, the atypical β-adrenoceptors mediating relaxant responses to catecholamines (isoprenaline, noradrenaline and adrenaline) and to β_3-adrenoceptor agonists (BRL37344 and CGP12177A) are a mixed rather than a single receptor population. In other words, it is possible that carteolol, as an antagonist for atypical β-adrenoceptors, recognizes at least two atypical β-adrenoceptor subtypes, one of which is stimulated preferentially by BRL37344, and the other, by the other agonists. Therefore, we also studied the antagonistic effects of carteolol at 3 μM, in addition to the 10 μM concentration, against BRL37344. Carteolol (3 μM) weakly but significantly antagonized the relaxations in response to BRL37344 as well. The apparent pA_2 value (6.60) of carteolol, 3 μM, was not significantly different from the apparent pA_2 value (6.56) at the 10 μM concentration, indicating that the relaxant responses of the guinea pig duodenum to BRL37344 were mediated through a single atypical β-adrenoceptor.

In addition, the apparent pA_2 values for carteolol against the five agonists (5.58–6.56) were lower than the values of 9–11 obtained with β_1-/β_2-adrenoceptors (Kuwahara et al., 1987; Takayanagi et al., 1989; Koike et al., 1996). These results confirm that carteolol also has antagonist activity at atypical β-adrenoceptors in the guinea pig duodenum.

We have previously reported that carteolol is a partial agonist in the guinea pig taenia caecum (Koike and Takayanagi, 1983; Takayanagi and Koike, 1983). In addition, Koike et al. (1996) demonstrated that the relaxation response of this preparation to carteolol is mediated by a low affinity site of β_2-adrenoceptors and not by β_3-adrenoceptors. However, Zhao et al. (1998) showed that carteolol acts as a β_3-adrenoceptor partial agonist in hamster, rat and mouse brown adipocytes. In the present study, we showed that carteolol had both agonist and antagonist activity at atypical β-adrenoceptors in the guinea pig duodenum. These results suggest that there is no species difference in the ability of atypical β-adrenoceptors to recognize carteolol. Predominant β-adrenoceptor subtypes mediating smooth muscle relaxation are atypical β-adrenoceptors in the guinea pig duodenum and β_2-adrenoceptors in the guinea pig taenia caecum (Koike et al., 1994, 1995a,b; Horinouchi and Koike, 1999a).

The aim of the study was to characterize the agonistic effects of carteolol on atypical β-adrenoceptors in the guinea pig duodenum. The study presented in this paper shows that carteolol possesses a partial agonist and antagonist activity at atypical β-adrenoceptors in the guinea pig duodenum.

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