(±)-Pindolol Acts as a Partial Agonist at Atypical β-Adrenoceptors in the Guinea Pig Duodenum

Takahiro Horinouchi and Katsuo Koike*

Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

Received July 19, 2000 Accepted September 29, 2000

ABSTRACT—The agonistic and antagonistic effects of (±)-pindolol (1-(1H-indol-4-yl)-3-[(1-methylethyl)amino]-2-propanol) were estimated to clarify whether (±)-pindolol acts as a partial agonist on atypical β-adrenoceptors in the guinea pig duodenum. (±)-Pindolol induced concentration-dependent relaxation with a pD2 value of 5.10 ± 0.03 and an intrinsic activity of 0.83 ± 0.03. However, the relaxations to (±)-pindolol were not antagonized by the non-selective β1- and β2-adrenoceptor antagonist (±)-propranolol (1 µM). In the presence of (±)-propranolol (1 µM), the non-selective β1-, β2- and β-adrenoceptor antagonist (±)-bupranolol (30 µM) induced a rightward shift of the concentration-response curves for (±)-pindolol (apparent pA2 = 5.41 ± 0.06). In the presence of (±)-propranolol, (±)-pindolol (10 µM) weakly but significantly antagonized the relaxant effects to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline), a selective β2-adrenoceptor agonist BRL37344 (R*,R*)-(±)-4-[2-(3-chlorophenyl)-2-hydroxyethylamino]propyl]phenoxyacetic acid sodium salt) and a non-conventional partial β-adrenoceptor agonist (±)-CGP12177A([4-[(1,1-dimethylethyl)amino]-2-hydroxyprooxy]-1,3-dihydro-2H-benzimidazol-2-one hydrochloride). These results demonstrate that (±)-pindolol possesses both agonistic and antagonistic effects on atypical β-adrenoceptors in the guinea pig duodenum.

Keywords: (±)-Pindolol, Partial agonist, Atypical β-adrenoceptor, β1-Adrenoceptor, Guinea pig duodenum

β-Adrenoceptors were initially subdivided by Lands and co-worker (1) into β1- and β2-adrenoceptors based on the selectivity of action to different β-adrenoceptor agonists as cardiac stimulant and bronchodilators, respectively. In addition, many lines of evidence for the existence of atypical β-adrenoceptors, distinct from β1- and β2-adrenoceptors, are accumulating. Thus, atypical β-adrenoceptors including β2-adrenoceptors have been identified in a variety of tissues including gastrointestinal smooth muscle (for review, see Arch and Kaufmann (2)), and it is suggested that this novel receptor subtype can be a suitable target for the development of new drugs active in the treatment of gastrointestinal tract disorders and non-insulin-dependent diabetes (3–5). For an effect to be mediated through β2-adrenoceptors, the following criteria need to be fulfilled: i) stimulation by selective β2-adrenoceptor agonists (e.g., BRL37344), ii) stimulation by non-conventional partial agonists (e.g., CGP12177A), iii) low sensitivity to antagonists with affinity for β1- or β2-adrenoceptors (e.g., propranolol) and iv) sensitivity to selective β2-adrenoceptor antagonists (e.g., SR59230A) (2, 6). Evidence for the existence of further atypical β-adrenoceptor subtypes is accumulating: e.g., putative β2-adrenoceptors in cardiac and adipose tissue that fulfill criteria (ii) and (iii) but not (i) and (iv) (6–8). Since our previous study fulfilled three criteria, (i), (ii) and (iii), but not (iv) in the guinea pig duodenum (9), we called β-adrenoceptors which share criteria (i), (ii) and (iii) by the term ‘atypical β-adrenoceptors’ but not ‘β2-adrenoceptors’.

Recently, we have reported that functional atypical β-adrenoceptors exist in the guinea pig duodenum and that the relaxations to β-adrenoceptor agonists in this tissue are mediated via atypical β-adrenoceptors (9).

Recently, the pharmacological effects of (±)-pindolol (1-(1H-indol-4-yl)-3-[(1-methylethyl)amino]-2-propanol), cyanopindolol and analogues of cyanopindolol were tested on β2-adrenoceptors in the rat ileum (10, 11). Cyanopindolol and their analogues act as partial agonists with intrinsic activities ranging from 0.39 to 0.84; however, the intrinsic activity of (±)-pindolol was 0 (11). Thus, (±)-pindolol has been described to act as an antagonist at β2-adrenoceptors with the pKb value of 6.68 against effects of (−)-isoprenaline in the rat ileum (10). However, Blin et al.

*Corresponding author. FAX: +81-47-472-1419
E-mail: ktikoike@phar.toho-u.ac.jp
(12) showed that (±)-pindolol possessed both a \( \beta_3 \)-adrenoceptor agonistic property with an intrinsic activity of 0.55 and a potent \( \beta_2 \) and \( \beta_3 \)-adrenoceptor antagonistic effect in Chinese hamster ovary cells expressing the human \( \beta_1 \), \( \beta_2 \), and \( \beta_3 \)-adrenoceptors, respectively. It is possible that there is a species difference in \( \beta_3 \)-adrenoceptors or atypical \( \beta \)-adrenoceptors. Furthermore, it has been shown that cyclic AMP production is potently stimulated by BRL37344 (\((R^*, R^*)\)-(±)-4-[2-[(3-chlorophenyl)-2-hydroxyethyl]aminopro]py|phenoxyacetic acid sodium salt) in the human cloned \( \beta_3 \)-adrenoceptors (13). However, this same drug has been shown to lack an intrinsic activity on atypical \( \beta \)-adrenoceptors in human colon (14) and adipocytes (15).

Effects of drugs may differ between cloned atypical \( \beta \)-adrenoceptors and native atypical \( \beta \)-adrenoceptors. Therefore, we performed the present study to confirm whether (±)-pindolol possesses both agonistic and antagonistic effects at native atypical \( \beta \)-adrenoceptors in the guinea pig duodenum and to obtain further evidence to determine if there is a species difference between the gastrointestinal smooth muscle of the guinea pig and the rat in atypical \( \beta \)-adrenoceptors or \( \beta \)-adrenoceptors. To define the effects of (±)-pindolol under experimental conditions similar to those used by Hoey et al. (10, 11), we employed functional experiments that were established by Horinouchi and Koike (9) for atypical \( \beta \)-adrenoceptors in guinea pig duodenum.

**MATERIALS AND METHODS**

**Tissue preparation**

Male Hartley guinea pigs weighing 300 – 500 g were killed by cervical dislocation and the entire duodenum was removed and placed in oxygenated (a mixture of 95% O\(_2\) and 5% CO\(_2\)) Ringer-Locke solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl\(_2\), 2.1 mM MgCl\(_2\), 5.9 mM NaHCO\(_3\) and 2.8 mM glucose. 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 kept 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. Desmethylinipramine (1 \( \mu \)M, a neuronal uptake inhibitor), normetanephrine (10 \( \mu \)M, a catecholaminergic uptake inhibitor) and phentolamine (10 \( \mu \)M, an \( \alpha \)-adrenoceptor antagonist) were present in the medium throughout all experiments. Preparations were equilibrated for 60 min before experimental protocols were begun.

**Experimental protocol**

After two histamine concentration-response curves had been obtained, the preparations were contracted with histamine (10 \( \mu \)M), which induced the submaximal contraction. The relaxant responses to \( \beta \)-adrenoceptor agonists were determined by measuring inhibition of histamine-induced contraction. The concentration-response curves for catecholamines were obtained cumulatively and the relaxation induced by these drugs was expressed as a percentage of the maximal relaxation produced by (–)-isoprenaline (3 \( \mu \)M), the reference drug, in the absence of (±)-propranolol. To test the antagonism of (±)-propranolol (1 \( \mu \)M) against (±)-pindolol, (±)-propranolol was added to the bath 30 min before the addition of (±)-pindolol. To test the antagonism of (±)-pindolol (10 \( \mu \)M) in the presence of (±)-propranolol (1 \( \mu \)M), however, one of the agonists was added to the bath after a maximal relaxant response to (±)-pindolol (10 \( \mu \)M) was established. The concentration-response curves for the agonists were then obtained in the presence of antagonists. The time interval between two consecutive curves was usually set at 30 min. In our previous experiments, after the control concentration-response curves were determined, four or five successive cumulative concentration-response curves for catecholamines were determined. 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 effects of (±)-pindolol, BRL37344 and (±)-CGP12177A faded during the course of the same experiment in previous studies (data not shown); therefore, only a single concentration-response curve to each agonist was constructed per tissue. When studying the effects of antagonists for relaxation induced by (±)-pindolol, BRL37344 and (±)-CGP12177A, two untreated preparations always served as controls.

**Data analyses**

Agonistic potency was expressed as the pD\(_2\) value (16). The intrinsic activity of each agonist was expressed as the ratio between its maximal relaxation and the maximal response of the full agonist (–)-isoprenaline. The competitive antagonistic potency is expressed as the apparent pA\(_2\) value. Apparent pA\(_2\) values for (±)-bupranolol and (±)-pindolol were calculated according to the method of Van Rossum (16) from the equation:

\[
\text{apparent pA}_2 = \log \left( \frac{\text{agonist concentration ratio} - 1}{\text{agonist concentration ratio} + 1} \right) - \log [\text{antagonist}]
\]

Numerical results are expressed as means ± S.E.M. of 8 to 12 experiments. Statistical analyses were performed with the Newman-Keuls test when appropriate. A \( P \) value of less than 0.05 was considered statistically significant.
**Drugs**

The drugs used were obtained from the following sources: (-)-isoprenaline hydrochloride, (-)-noradrenaline bitartrate, (-)-adrenaline bitartrate, (±)-propranolol hydrochloride, histamine dihydrochloride, desmethylimipramine hydrochloride, normetanephrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); (±)-pindolol, BRL37344 (Nacalai Tesque, Kyoto); (±)-CGP12177A ([4-[3-[(1,1-dimethylethyl) amino]-2-hydroxypropoxy]-1,3-dihydro-2H-benzimidazol-2-one] hydrochloride) (Research Biochemicals International, Natick, MA, USA); phentolamine mesylate (Novartis, Basel, Switzerland) and (±)-bupranolol hydrochloride (Kaken Pharmaceutical Co., Ltd., Tokyo). (±)-Pindolol was dissolved (0.2 M) in 1 N HCl and further diluted in distilled water. All other drugs were dissolved in distilled water. All other chemicals used were of analytical grade.

**RESULTS**

**Agonistic effect of (±)-pindolol**

In the absence of (±)-propranolol (1 μM), (±)-pindolol induced a concentration-dependent relaxation of the guinea pig duodenum (Figs. 1 and 2). The pD$_{25}$ value and the intrinsic activity were 5.10 ± 0.03 and 0.83 ± 0.03, respectively. The concentration-response curve for (±)-pindolol was not shifted significantly by (±)-propranolol (1 μM) (Fig. 2). The pD$_{25}$ value and the intrinsic activity was 5.00 ± 0.03 and 0.82 ± 0.03, respectively.

**Effect of (±)-bupranolol on relaxation responses induced by (±)-pindolol**

In the presence of (±)-propranolol (1 μM), the relaxant responses to (±)-pindolol were antagonized by (±)-bupranolol (30 μM) (Fig. 3). The data gave the apparent pA$_{2}$.
value of 5.41 ± 0.06.

Antagonistic effect of (±)-pindolol

In the presence of (±)-propranolol (1 μM), catecholamines ((-)-isoprenaline, (-)-noradrenaline and (-)-adrenaline) and β₁-adrenoceptor agonists (BRL37344 and (±)-CGP12177A) induced concentration-dependent relaxations of the guinea pig duodenum and the relaxations to these agonists were weakly but significantly antagonized by (±)-pindolol (10 μM) (Fig. 4: a – e). The pD₂ values of catecholamines and β₁-adrenoceptor agonists and the apparent pA₂ values of (±)-pindolol against these five agonists are shown in Table 1.

DISCUSSION

We previously have confirmed the existence of functional atypical β₂-adrenoceptors in the guinea pig duodenum (9). In the present study, we have assessed partial agonistic activities of (±)-pindolol in this system. The relaxant response to (±)-pindolol was resistant to blockade by (±)-propranolol (1 μM), a concentration that blocked the response

![Fig. 4. Antagonistic effects of (±)-pindolol on concentration-response curves for (–)-isoprenaline (a), (–)-noradrenaline (b), (–)-adrenaline (c), BRL37344 (d) and (±)-CGP12177A (e) after incubation with (±)-propranolol (1 μM) in histamine-precontracted guinea pig duodenum. Control, no (±)-pindolol (○); (±)-pindolol, 10 μM (●). Ordinate: relaxation (%), expressed as a percentage relative to the maximal relaxation induced by (–)-isoprenaline (3 μM) in the absence of (±)-propranolol and abscissa: concentration (M) of the test drugs. The number above the arrow indicates the agonist concentration ratio. The dotted lines indicate the points used to perform the analysis. The upper and lower solid lines indicate the maximal relaxation induced by 10 μM (±)-pindolol and the test drugs, respectively. Each point represents the mean ± S.E.M. of 8 – 12 experiments.](image-url)
through \(\beta_1\)- and \(\beta_2\)-adrenoceptors and that did not interact with atypical \(\beta\)-adrenoceptors, implying that conventional \(\beta_1\)- and \(\beta_2\)-adrenoceptors play no role in the relaxations induced by (+)-pindolol in the guinea pig duodenum. However, (+)-pindolol binds with relatively high affinity (K_d < 0.1 \(\mu\)M) to cerebral 5-HT_3 receptors (17–20). In addition, 5-hydroxytryptamine (5-HT) can cause positive chronotropic effects mediated directly through sinoatrial 5-HT receptors (21). Both 5-HT and (+)-pindolol are unrelated to atypical \(\beta\)-adrenoceptors in guinea pig duodenum. Because of this structural resemblance, (+)-pindolol may induce relaxant responses through 5-HT receptors in the guinea pig duodenum. We therefore used a non-selective \(\beta_1\)-, \(\beta_2\)- and \(\beta_3\)-adrenoceptor antagonist, (+)-bupranolol, to clarify whether the relaxant responses to (+)-pindolol were solely mediated through atypical \(\beta\)-adrenoceptors in guinea pig duodenum. Antagonism of the relaxant responses to (+)-pindolol by (+)-bupranolol (30 \(\mu\)M) has been observed in guinea pig duodenum, indicating a competitive form of antagonism at atypical \(\beta\)-adrenoceptors, but not 5-HT receptors in guinea pig duodenum. Furthermore, we have confirmed that 5-HT itself caused contraction of the guinea pig duodenum preparation rather than relaxation (data not shown). Thus, the relaxations to (+)-pindolol are unrelated to 5-HT receptors in the guinea pig duodenum. The pA_2 value for (+)-bupranolol of 5.41 ± 0.06 is lower than that reported by Kaumann (22) for \(\beta_1\)- and \(\beta_2\)-adrenoceptors. These results suggest that the relaxant responses to (+)-pindolol are mediated through atypical \(\beta\)-adrenoceptors in the guinea pig duodenum. Since a partial agonist is generally described as a compound with an intrinsic activity ranging from 0 of a competitive antagonist to 1 of a full agonist (23, 24), the partial agonist possesses both agonistic and antagonistic effects (24). In the presence of (+)-propranolol (1 \(\mu\)M), the intrinsic activity of (+)-pindolol was 0.82 ± 0.03 in the guinea pig duodenum, indicating that (+)-pindolol is a partial agonist because this value was significantly lower than 1. These results support the hypothesis that (+)-pindolol acts as a partial agonist (a weak atypical \(\beta\)-adrenoceptor antagonist with a partial atypical \(\beta\)-adrenoceptor agonist action) at atypical \(\beta\)-adrenoceptors in guinea pig duodenum. However, the failure of agonistic activity to (+)-pindolol has been observed in rat ileum (10, 11), while (+)-pindolol acts as a partial agonist (a potent \(\beta_1\)- and \(\beta_2\)-adrenoceptor antagonist with a partial \(\beta_1\)-adrenoceptor agonist effect) with a pD_2 value of 6.0 and 6.8 in cloned mouse and human \(\beta_2\)-adrenoceptors, respectively (25). A similar phenomenon has been reported that (+)-CGP12177A acts as a partial agonist for human \(\beta_2\)-adrenoceptors, while (+)-CGP12177A was full agonist for canine \(\beta_2\)-adrenoceptors (26). This discrepancy may be due to species differences between atypical \(\beta\)-adrenoceptors or \(\beta_2\)-adrenoceptors in the guinea pig, rat, mouse, human and dog. It is also possible that the receptor density and/or the receptor expression increases and the receptor tightly couples to effector at cloned atypical \(\beta\)-adrenoceptors or \(\beta_2\)-adrenoceptors in Chinese hamster ovary cells (26, 27).

(+)-Pindolol is a \(\beta_2\)-adrenoceptor antagonist with a pK_a value of 6.68 against the relaxant effects of (–)-isoprenaline in the rat ileum (10, 11). There, we performed studies to confirm whether (+)-pindolol possesses an antagonistic effect at atypical \(\beta\)-adrenoceptors in the guinea pig duodenum in which \(\beta_1\)- and \(\beta_2\)-adrenoceptors were blocked. In the presence of (+)-propranolol (1 \(\mu\)M), the relaxant responses to three catecholamines (–)-isoprenaline, (–)-noradrenaline and (–)-adrenaline) and \(\beta_2\)-adrenoceptor agonists (BRL37344 and (+)-CGP12177A) were weakly but significantly blocked by (+)-pindolol (10 \(\mu\)M), which produced a 6-, 7-, 6-, 15- and 5-fold shift of the concentration-response curves to the right, respectively. These results suggest that (+)-pindolol acts as an atypical \(\beta\)-adrenoceptor partial agonist in this preparation. However, the apparent pA_2 value for (+)-pindolol of 5.70 against (–)-isoprenaline obtained in this study was lower than that for (+)-pindolol, 6.68, reported by Hoey et al. (10, 11). Horinouchi and Koike (9) also showed that the pA_2 value for (+)-bupranolol against BRL37344 was 6.51 in the guinea pig duodenum, while Langin et al. (28) reported that the value was 7.3 in the rat fat cells. The pA_2 values of \(\beta_2\)-adrenoceptor antagonists obtained in atypical \(\beta\)-adrenoceptors of the guinea pig were about one log unit less than those obtained in \(\beta_1\)-

### Table 1. Effects of (+)-pindolol (10 \(\mu\)M) on the relaxant responses to catecholamines and \(\beta_2\)-adrenoceptor agonists in the presence of (+)-propranolol (1 \(\mu\)M) on the guinea pig duodenum

| Agonist        | pD_2 value absence of (+)-pindolol | pD_2 value presence of (+)-pindolol | Apparent pA_2 value |
|----------------|------------------------------------|-------------------------------------|--------------------|
| (–)-Isoprenaline | 6.60 ± 0.02                        | 5.81 ± 0.06                         | 5.70 ± 0.06        |
| (–)-Noradrenaline | 6.04 ± 0.06                        | 5.21 ± 0.08                         | 5.77 ± 0.06        |
| (–)-Adrenaline  | 5.78 ± 0.03                        | 4.99 ± 0.09                         | 5.72 ± 0.09        |
| BRL37344       | 7.18 ± 0.02                        | 6.02 ± 0.07                         | 6.14 ± 0.07        |
| (+)-CGP12177A  | 6.42 ± 0.02                        | 5.71 ± 0.06                         | 5.52 ± 0.08        |

Values are means ± S.E.M. from 8 – 12 experiments. *Determined from Fig. 4.
adrenoceptors of the rat, suggesting that there is a species difference in potency for \( \beta_1 \)-adrenoceptor antagonist. An explanation for those differences in potency for \( \beta_1 \)-adrenoceptor antagonist may be related to the possible heterogeneity and/or species differences due to variations in the atypical \( \beta_1 \)-adrenoceptor or the \( \beta_1 \)-adrenoceptor amino acid sequence and/or gene code (25, 26, 29).

We conclude that \((\pm)\)-pindolol acts as a partial agonist at atypical \( \beta_1 \)-adrenoceptors in guinea pig duodenum. Furthermore, we characterized properties for \( \beta_1 \)-adrenoceptor antagonists including \((\pm)\)-pindolol, in which antagonistic effects were less potent at atypical \( \beta_1 \)-adrenoceptors of the guinea pig than at \( \beta_1 \)-adrenoceptors of the rat. Thus, it is possible that there is a species difference between the guinea pig and the rat at atypical \( \beta_1 \)-adrenoceptors or \( \beta_1 \)-adrenoceptors.

Acknowledgment
We thank Kaken Pharmaceutical Co., Ltd. (Tokyo) for the generous gift of \((\pm)\)-bupranolol hydrochloride.

REFERENCES
1. Lands AM, Arnold A, McAuliffe JP, Luduena FP and Brown Jun T.G: Differentiation of receptor systems activated by sympathomimetic amines. Nature 214, 597 – 598 (1967)
2. Arch JRS and Kaumann AJ: \( \beta_1 \)- And atypical \( \beta_1 \)-adrenoceptors. Med Res Rev 13, 663 – 729 (1993)
3. Anthony A: \( \beta_1 \)-Adrenoceptor agonists: future anti-inflammatory drugs for the gastrointestinal tract? Aliment Pharmacol Ther 10, 859 – 863 (1996)
4. Bahl AK, Clayton NM, Coates J, Martin DP, Oakley IG, Strong P and Trevethick MA: Comparison of the profiles of agonists as stimulants of the \( \beta_1 \)-adrenoceptor in vitro with their gastroprotective effects in the conscious rat. Br J Pharmacol 117, 580 – 586 (1996)
5. Yamamoto H, Takakura S, Yamamoto T, Satoh H, Higaki M, Tomoi M and Shimomura K: FR149175, a \( \beta_{2A} \)-adrenoceptor- selective agonist, is a possible therapeutic agent for non-insulin-dependent diabetes mellitus. Jpn J Pharmacol 74, 109 – 112 (1997)
6. Kaumann AJ and Molenaar P: Differences between the third cardiac \( \beta_2 \)-adrenoceptor and the colonic \( \beta_1 \)-adrenoceptor in the rat. Br J Pharmacol 118, 2085 – 2098 (1996)
7. Malinowska B and Schlicker E: Mediation of the chro- notropic effect of CGP 12177 and cyanopindolol in the pithed rat by atypical \( \beta_1 \)-adrenoceptors, different from \( \beta_1 \)-adrenoceptors. Br J Pharmacol 117, 943 – 949 (1996)
8. Galitzky J, Langin D, Vervaerde P, Montastruct JL, Lafontan M and Berlan M: Lipolytic effects of conventional \( \beta_1 \)-adrenoceptor agonists and of CGP 12,177 in rat and human fat cells: preliminary pharmacological evidence for a putative \( \beta_1 \)-adrenoceptor. Br J Pharmacol 122, 1244 – 1250 (1997)
9. Horinouchi T and Koike K: Characterization of atypical \( \beta_1 \)-adrenoceptors in the guinea pig duodenum. Eur J Pharmacol 376, 61 – 66 (1999)
10. Hoey A, Jackson C, Pegg G and Silence M: Atypical responses of rat ileum to pindolol, cyanoopindolol and isocyanopindolol. Br J Pharmacol 117, 712 – 716 (1996)
11. Hoey AJ, Jackson CM, Pegg GG and Silence MN: Characteris-