(±)-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-(1*H*-indol-4-yloxy)-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 pD_2 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 β_1 - and β_2 -adrenoceptor antagonist (±)-propranolol (1 μ M). In the presence of (±)-propranolol (1 μ M), the non-selective β_1 -, β_2 - and β_3 -adrenoceptor antagonist (±)-bupranolol (30 μ M) induced a rightward shift of the concentration-response curves for (±)-pindolol (apparent $pA_2 = 5.41 \pm 0.06$). In the presence of (±)-propranolol, (±)-pindolol (10 μ M) weakly but significantly antagonized the relaxant effects to catecholamines ((–)-isoprenaline, (–)-noradrenaline and (–)-adrenaline), a selective β_3 -adrenoceptor agonist BRL37344 ((*R**,*R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt) and a non-conventional partial β_3 -adrenoceptor agonist (±)-CGP12177A([4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-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, β_3 -Adrenoceptor, Guinea pig duodenum

β -Adrenoceptors were initially subdivided by Lands and co-worker (1) into β_1 - and β_2 -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 β_1 - and β_2 -adrenoceptors, are accumulating. Thus, atypical β -adrenoceptors including β_3 -adrenoceptors have been identified in a variety of tissues including gastrointestinal smooth muscle (for review, see Arch and Kaumann (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 β_3 -adrenoceptors, the following criteria need to be fulfilled: i) stimulation by selective β_3 -adrenoceptor agonists (e.g., BRL37344), ii) stimulation by non-conventional partial agonists (e.g., CGP12177A), iii) low sensitivity to antagonists with affinity for β_1 - or β_2 -adrenoceptors (e.g., propranolol) and iv) sensitivity to selective β_3 -adrenoceptor an-

tagonists (e.g., SR59230A) (2, 6). Evidence for the existence of further atypical β -adrenoceptor subtypes is accumulating: e.g., putative β_4 -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 ‘ β_3 -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-(1*H*-indol-4-yloxy)-3-[(1-methylethyl)amino]-2-propanol), cyanopindolol and analogues of cyanopindolol were tested on β_3 -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 β_3 -adrenoceptors with the pK_b 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: ktkoike@phar.toho-u.ac.jp

(12) showed that ($\pm$)-pindolol possessed both a β_3 -adrenoceptor agonistic property with an intrinsic activity of 0.55 and a potent β_1 - and β_2 -adrenoceptor antagonistic effect in Chinese hamster ovary cells expressing the human β_1 -, β_2 - and β_3 -adrenoceptors, respectively. It is possible that there is a species difference in β_3 -adrenoceptors or atypical β -adrenoceptors. Furthermore, it has been shown that cyclic AMP production is potently stimulated by BRL37344 ((R^* , R^*)-($\pm$)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt) in the human cloned β_3 -adrenoceptors (13). However, this same drug has been shown to lack an intrinsic activity on atypical β -adrenoceptors in human colon (14) and adipocytes (15). Effects of drugs may differ between cloned atypical β -adrenoceptors and native atypical β -adrenoceptors.

Therefore, we performed the present study to confirm whether ($\pm$)-pindolol possesses both agonistic and antagonistic effects at native atypical β -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 β -adrenoceptors or β_3 -adrenoceptors. To define the effects of ($\pm$)-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 β -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% O₂ and 5% CO₂) Ringer-Locke solution of the following composition: 154 mM NaCl, 5.6 mM KCl, 2.2 mM CaCl₂, 2.1 mM MgCl₂, 5.9 mM NaHCO₃ 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% O₂ and 5% CO₂. The mechanical responses of strips were recorded isometrically under a load of 0.5 g. Desmethylinipramine (1 μ M, a neuronal uptake inhibitor), normetanephrine (10 μ M, an extraneuronal uptake inhibitor) and phentolamine (10 μ M, an α -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 μ M), which induced the submaximal contraction. The relaxant responses to β -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 μ M), the reference drug, in the absence of ($\pm$)-propranolol. To test the antagonism of ($\pm$)-propranolol (1 μ M) against ($\pm$)-pindolol, ($\pm$)-propranolol was added to the bath 30 min before the addition of ($\pm$)-pindolol. To test the antagonism of ($\pm$)-pindolol (10 μ M) in the presence of ($\pm$)-propranolol (1 μ M), however, one of the agonists was added to the bath after a maximal relaxant response to ($\pm$)-pindolol (10 μ 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 ($\pm$)-pindolol, BRL37344 and ($\pm$)-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 ($\pm$)-pindolol, BRL37344 and ($\pm$)-CGP12177A, two untreated preparations always served as controls.

Data analyses

Agonistic potency was expressed as the pD₂ 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 pA₂ value. Apparent pA₂ values for ($\pm$)-bupranolol and ($\pm$)-pindolol were calculated according to the method of Van Rossum (16) from the equation:

$$\text{apparent pA}_2 = \log(\text{agonist concentration ratio} - 1) - \log[\text{antagonist}]$$

Numerical results are expressed as means $\pm$ 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, ($\pm$)-propranolol hydrochloride, histamine dihydrochloride, desmethylinipramine hydrochloride, normetanephrine hydrochloride (Sigma Chemical Co., St. Louis, MO, USA); ($\pm$)-pindolol, BRL37344 (Nacalai Tesque, Kyoto); ($\pm$)-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 ($\pm$)-bupranolol hydrochloride (Kaken Pharmaceutical Co., Ltd., Tokyo). ($\pm$)-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 ($\pm$)-pindolol

In the absence of ($\pm$)-propranolol (1 μ M), ($\pm$)-pindolol induced a concentration-dependent relaxation of the guinea pig duodenum (Figs. 1 and 2). The pD_2 value and the intrinsic activity were 5.10 ± 0.03 and 0.83 ± 0.03 , respectively. The concentration-response curve for ($\pm$)-pindolol was not shifted significantly by ($\pm$)-propranolol (1 μ M) (Fig. 2). The pD_2 value and the intrinsic activity was 5.00 ± 0.03 and

Fig. 1. Representative original trace showing the relaxant effect by ($\pm$)-pindolol in the absence of ($\pm$)-propranolol on histamine-precontracted guinea pig duodenum. Arrows indicate administration of drugs. Half log unit increments in ($\pm$)-pindolol concentration were added.

0.82 ± 0.03 , respectively.

Effect of ($\pm$)-bupranolol on relaxation responses induced by ($\pm$)-pindolol

In the presence of ($\pm$)-propranolol (1 μ M), the relaxant responses to ($\pm$)-pindolol were antagonized by ($\pm$)-bupranolol (30 μ M) (Fig. 3). The data gave the apparent pA_2

Fig. 2. Responses of histamine-precontracted guinea pig duodenum to cumulative addition of ($\pm$)-pindolol in the absence of ($\pm$)-propranolol (○) and in the presence of ($\pm$)-propranolol (1 μ M) (●). Ordinate: relaxation (%), expressed as a percentage relative to the maximal relaxation induced by (–)-isoprenaline (3 μ M) in the absence of ($\pm$)-propranolol, abscissa: concentration (M) of the test drugs. Each point represents the mean $\pm$ S.E.M. of 8–12 experiments.

Fig. 3. Effect of ($\pm$)-bupranolol on concentration-response curves for ($\pm$)-pindolol in histamine-precontracted guinea pig duodenum. The relaxant responses to ($\pm$)-pindolol after 30-min incubation with ($\pm$)-propranolol (1 μ M); Control, no ($\pm$)-bupranolol (○); ($\pm$)-bupranolol, 30 μ M (●). Ordinate: relaxation (%), expressed as a percentage relative to the maximal relaxation induced by (–)-isoprenaline in the absence of ($\pm$)-propranolol and abscissa: concentration (M) of the test drugs. Each point represents the mean $\pm$ S.E.M. of 8–12 experiments.

value of 5.41 ± 0.06 .

Antagonistic effect of ($\pm$)-pindolol

In the presence of ($\pm$)-propranolol ($1 \mu\text{M}$), catecholamines ((-)-isoprenaline, (-)-noradrenaline and (-)-adrenaline) and β_3 -adrenoceptor agonists (BRL37344 and ($\pm$)-CGP12177A) induced concentration-dependent relaxations of the guinea pig duodenum and the relaxations to these agonists were weakly but significantly antagonized by ($\pm$)-pindolol ($10 \mu\text{M}$) (Fig. 4: a – e). The pD_2 values of catecholamines and β_3 -adrenoceptor agonists and the apparent pA_2

values of ($\pm$)-pindolol against these five agonists are shown in Table 1.

DISCUSSION

We previously have confirmed the existence of functional atypical β -adrenoceptors in the guinea pig duodenum (9). In the present study, we have assessed partial agonistic activities of ($\pm$)-pindolol in this system. The relaxant response to ($\pm$)-pindolol was resistant to blockade by ($\pm$)-propranolol ($1 \mu\text{M}$), a concentration that blocked the response

Fig. 4. Antagonistic effects of ($\pm$)-pindolol on concentration-response curves for (-)-isoprenaline (a), (-)-noradrenaline (b), (-)-adrenaline (c), BRL37344 (d) and ($\pm$)-CGP12177A (e) after incubation with ($\pm$)-propranolol ($1 \mu\text{M}$) in histamine-precontracted guinea pig duodenum. Control, no ($\pm$)-pindolol ($\circ$); ($\pm$)-pindolol, $10 \mu\text{M}$ ($\bullet$). Ordinate: relaxation (%), expressed as a percentage relative to the maximal relaxation induced by (-)-isoprenaline ($3 \mu\text{M}$) in the absence of ($\pm$)-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 $10 \mu\text{M}$ ($\pm$)-pindolol and the test drugs, respectively. Each point represents the mean $\pm$ S.E.M. of 8–12 experiments.

Table 1. Effects of ($\pm$)-pindolol (10 μ M) on the relaxant responses to catecholamines and β_3 -adrenoceptor agonists in the presence of ($\pm$)-propranolol (1 μ M) on the guinea pig duodenum

Agonist	pD ₂ value		Apparent pA ₂ value ^a
	absence of ($\pm$)-pindolol	presence of ($\pm$)-pindolol	
(-)-Isoprenaline	6.60 $\pm$ 0.02	5.81 $\pm$ 0.06	5.70 $\pm$ 0.06
(-)-Noradrenaline	6.04 $\pm$ 0.06	5.21 $\pm$ 0.08	5.77 $\pm$ 0.06
(-)-Adrenaline	5.78 $\pm$ 0.03	4.99 $\pm$ 0.09	5.72 $\pm$ 0.09
BRL37344	7.18 $\pm$ 0.02	6.02 $\pm$ 0.07	6.14 $\pm$ 0.07
($\pm$)-CGP12177A	6.42 $\pm$ 0.02	5.71 $\pm$ 0.06	5.52 $\pm$ 0.08

Values are means $\pm$ S.E.M. from 8 – 12 experiments. ^aDetermined from Fig. 4.

through β_1 - and β_2 -adrenoceptors and that did not interact with atypical β -adrenoceptors, implying that conventional β_1 - and β_2 -adrenoceptors play no role in the relaxations induced by ($\pm$)-pindolol in the guinea pig duodenum. However, ($\pm$)-pindolol binds with relatively high affinity ($K_D < 0.1 \mu$ M) to cerebral 5-HT₁ 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 ($\pm$)-pindolol are indolamines. Because of this structural resemblance, ($\pm$)-pindolol may induce relaxant responses through 5-HT receptors in the guinea pig duodenum. We therefore used a non-selective β_1 -, β_2 - and β_3 -adrenoceptor antagonist, ($\pm$)-bupranolol, to clarify whether the relaxant responses to ($\pm$)-pindolol were solely mediated through atypical β -adrenoceptors in guinea pig duodenum. Antagonism of the relaxant responses to ($\pm$)-pindolol by ($\pm$)-bupranolol (30 μ M) has been observed in guinea pig duodenum, indicating a competitive form of antagonism at atypical β -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 ($\pm$)-pindolol are unrelated to 5-HT receptors in the guinea pig duodenum. The pA₂ value for ($\pm$)-bupranolol of 5.41 $\pm$ 0.06 is lower than that reported by Kaumann (22) for β_1 - and β_2 -adrenoceptors. These results suggest that the relaxant responses to ($\pm$)-pindolol are mediated through atypical β -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 ($\pm$)-propranolol (1 μ M), the intrinsic activity of ($\pm$)-pindolol was 0.82 $\pm$ 0.03 in the guinea pig duodenum, indicating that ($\pm$)-pindolol is a partial agonist because this value was significantly lower than 1. These results support the hypothesis that ($\pm$)-pindolol acts as a partial agonist (a weak atypical β -adrenoceptor antagonist with a partial atypical β -adrenoceptor agonist action) at atypical β -adrenoceptors in guinea pig

duodenum. However, the failure of agonistic activity to ($\pm$)-pindolol has been observed in rat ileum (10, 11), while ($\pm$)-pindolol acts as a partial agonist (a potent β_1 - and β_2 -adrenoceptor antagonist with a partial β_3 -adrenoceptor agonist effect) with a pD₂ value of 6.0 and 6.8 in cloned mouse and human β_3 -adrenoceptors, respectively (25). A similar phenomenon has been reported that ($\pm$)-CGP12177A acts as a partial agonist for human β_3 -adrenoceptors, while ($\pm$)-CGP12177A was full agonist for canine β_3 -adrenoceptors (26). This discrepancy may be due to species differences between atypical β -adrenoceptors or β_3 -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 β -adrenoceptors or β_3 -adrenoceptors in Chinese hamster ovary cells (26, 27).

($\pm$)-Pindolol is a β_3 -adrenoceptor antagonist with a pK_b value of 6.68 against the relaxant effects of (-)-isoprenaline in the rat ileum (10, 11). There, we performed studies to confirm whether ($\pm$)-pindolol possesses an antagonistic effect at atypical β -adrenoceptors in the guinea pig duodenum in which β_1 - and β_2 -adrenoceptors were blocked. In the presence of ($\pm$)-propranolol (1 μ M), the relaxant responses to three catecholamines ((-)-isoprenaline, (-)-noradrenaline and (-)-adrenaline) and β_3 -adrenoceptor agonists (BRL37344 and ($\pm$)-CGP12177A) were weakly but significantly blocked by ($\pm$)-pindolol (10 μ 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 ($\pm$)-pindolol acts as an atypical β -adrenoceptor partial agonist in this preparation. However, the apparent pA₂ value for ($\pm$)-pindolol of 5.70 against (-)-isoprenaline obtained in this study was lower than that for ($\pm$)-pindolol, 6.68, reported by Hoey et al. (10, 11). Horinouchi and Koike (9) also showed that the pA₂ value for ($\pm$)-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 pA₂ values of β_3 -adrenoceptor antagonists obtained in atypical β -adrenoceptors of the guinea pig were about one log unit less than those obtained in β_3 -

adrenoceptors of the rat, suggesting that there is a species difference in potency for β_3 -adrenoceptor antagonist. An explanation for those differences in potency for β_3 -adrenoceptor antagonist may be related to the possible heterogeneity and/or species differences due to variations in the atypical β -adrenoceptor or the β_3 -adrenoceptor amino acid sequence and/or gene code (25, 26, 29).

We conclude that ($\pm$)-pindolol acts as a partial agonist at atypical β -adrenoceptors in guinea pig duodenum. Furthermore, we characterized properties for β_3 -adrenoceptor antagonists including ($\pm$)-pindolol, in which antagonistic effects were less potent at atypical β -adrenoceptors of the guinea pig than at β_3 -adrenoceptors of the rat. Thus, it is possible that there is a species difference between the guinea pig and the rat in atypical β -adrenoceptors or β_3 -adrenoceptors.

Acknowledgment

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

REFERENCES

- Lands AM, Arnold A, McAuliff JP, Luduena FP and Brown Jun TG: Differentiation of receptor systems activated by sympathomimetic amines. *Nature* **214**, 597–598 (1967)
- Arch JRS and Kaumann AJ: β_3 - And atypical β -adrenoceptors. *Med Res Rev* **13**, 663–729 (1993)
- Anthony A: β_3 -Adrenoceptor agonists: future anti-inflammatory drugs for the gastrointestinal tract? *Aliment Pharmacol Ther* **10**, 859–863 (1996)
- 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 β_3 -adrenoceptor in vitro with their gastro-protective effects in the conscious rat. *Br J Pharmacol* **117**, 580–586 (1996)
- Yamamoto H, Takakura S, Yamamoto T, Satoh H, Higaki M, Tomoi M and Shimomura K: FR149175, a β_3 -adrenoceptor-selective agonist, is a possible therapeutic agent for non-insulin-dependent diabetes mellitus. *Jpn J Pharmacol* **74**, 109–112 (1997)
- Kaumann AJ and Molenaar P: Differences between the third cardiac β -adrenoceptor and the colonic β_3 -adrenoceptor in the rat. *Br J Pharmacol* **118**, 2085–2098 (1996)
- Malinowska B and Schlicker E: Mediation of the positive chronotropic effect of CGP 12177 and cyanopindolol in the pithed rat by atypical β -adrenoceptors, different from β_3 -adrenoceptors. *Br J Pharmacol* **117**, 943–949 (1996)
- Galitzky J, Langin D, Verwaerde P, Montastruc JL, Lafontan M and Berlan M: Lipolytic effects of conventional β_3 -adrenoceptor agonists and of CGP 12,177 in rat and human fat cells: preliminary pharmacological evidence for a putative β_4 -adrenoceptor. *Br J Pharmacol* **122**, 1244–1250 (1997)
- Horinouchi T and Koike K: Characterization of atypical β -adrenoceptors in the guinea pig duodenum. *Eur J Pharmacol* **376**, 61–66 (1999)
- Hoey A, Jackson C, Pegg G and Sillence M: Atypical responses of rat ileum to pindolol, cyanopindolol and iodocyanopindolol. *Br J Pharmacol* **117**, 712–716 (1996)
- Hoey AJ, Jackson CM, Pegg GG and Sillence MN: Characteristics of cyanopindolol analogues active at the β_3 -adrenoceptor in rat ileum. *Br J Pharmacol* **119**, 564–568 (1996)
- Blin N, Camoin L, Maigret B and Strosberg AD: Structural and conformational features determining selective signal transduction in the β_3 -adrenergic receptor. *Mol Pharmacol* **44**, 1094–1104 (1993)
- Emorine LJ, Marullo S, Briand-Sutren M-M, Patey G, Tate K, Delavier-Klutchko C and Strosberg AD: Molecular characterization of the human β_3 -adrenergic receptor. *Science* **245**, 1118–1121 (1989)
- McLaughlin DP and MacDonald A: Characterization of catecholamine-mediated relaxations in rat isolated gastric fundus: evidence for an atypical β -adrenoceptor. *Br J Pharmacol* **103**, 1351–1356 (1991)
- Hollenga C, Haas M, Deinum JT and Zaagsma J: Discrepancies in lipolytic activities induced by β -adrenoceptor agonists in human and rat adipocytes. *Horm Metab Res* **22**, 17–21 (1990)
- Van Rossum JM: Cumulative dose-response curves, II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch Int Pharmacodyn* **143**, 299–330 (1963)
- Middlemiss DN, Buxton DA and Greenwood DT: Beta-adrenoceptor antagonists in psychiatry and neurology. *Pharmacol Ther* **12**, 419–437 (1981)
- Nahorski SR and Willcocks AL: Interactions of β -adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. *Br J Pharmacol* **78**, 107P (1983)
- Hamon M, Cossery JM, Spampinato U and Gozlan H: Are there selective ligands for 5-HT_{1A} and 5-HT_{1B} receptor binding sites in brain? *Trends Pharmacol Sci* **7**, 336–338 (1986)
- Langlois M, Brémont B, Rousselle D and Gaudy F: Structural analysis by the comparative molecular field analysis method of the affinity of β -adrenoceptor blocking agents for 5-HT_{1A} and 5-HT_{1B} receptors. *Eur J Pharmacol* **244**, 77–87 (1993)
- Kaumann AJ: A classification of heart serotonin receptors. *Naunyn Schmiedeberg's Arch Pharmacol* **332**, R42 (1983)
- Kaumann AJ: Is there a third heart β -adrenoceptor? *Trends Pharmacol Sci* **10**, 316–320 (1989)
- Stephenson RP: A modification of receptor theory. *Br J Pharmacol* **11**, 379–393 (1956)
- Ariëns EJ, van Rossum JM and Koopman PC: Receptor reserve and threshold phenomena. I. Theory and experiments with autonomic drugs tested on isolated organs. *Arch Int Pharmacodyn Ther* **127**, 459–478 (1960)
- Blin N, Nahmias C, Drumare MF and Strosberg AD: Mediation of most atypical effects by species homologues of the β_3 -adrenoceptor. *Br J Pharmacol* **112**, 911–919 (1994)
- Lenzen G, Pietri-Rouxel F, Drumare M-F, Amiard A, Guillot S, Archimbaud P and Strosberg AD: Genomic cloning and species-specific properties of the recombinant canine β_3 -adrenoceptor. *Eur J Pharmacol* **363**, 217–227 (1998)
- Cohen ML, Granneman JG, Chaudhry A, Schenck W, Cushing DJ and Palkowitz AD: Is there 'atypical' β -receptor in the rat stomach fundus the rat β_3 -receptor? *J Pharmacol Exp Ther* **272**, 446–451 (1995)
- Langin D, Portillo MP, Saulnier-Blache J-S and Lafontan M: Coexistence of three β -adrenoceptor subtypes in white fat cells of various mammalian species. *Eur J Pharmacol* **199**, 291–301 (1991)
- De Ponti F, Gibelli G, Crema F and Lecchini S: Functional evidence for the presence of β_3 -adrenoceptors in the guinea-pig common bile duct and colon. *Pharmacology* **51**, 288–297 (1995)