A Beta-Adrenergic Partial Agonist (Befunolol) Discriminates Two Different Affinity Sites

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Abstract—Interactions of beta-adrenergic partial agonists with the beta-adrenoceptor were studied in isolated guinea-pig taenia caecum. The competitive inhibition curve for specific binding of a high concentration (50 nM) of [3H]-befunolol by befunolol showed a biphasic shape, although the curve for specific binding of a low concentration (1 nM) was monophasic. All the competitive inhibition curves for specific binding of [3H]-befunolol (1 nM and 50 nM) by isoprorenaline and propranolol showed monophasic shapes. These results suggest that befunolol may be able to discriminate two different binding sites of the beta-adrenoceptor: the high affinity site and the low affinity site.

Since a partial agonist has an affinity to its receptors and an intrinsic activity which lies somewhere between that of a full agonist (=1) and that of a competitive antagonist (=0), a partial agonist has both agonistic and antagonistic actions (1). Moreover, as a partial agonist generally has little receptor reserve (2, 3), its pD2-value must be equal to its pA2-value. However, in our previous papers (4–6), we reported that the pD2-values of the beta-adrenergic partial agonists were significantly different from their pA2-values, suggesting that the beta-adrenergic partial agonists interact with two different binding sites. Possible interactions of beta-adrenergic partial agonists with the beta-adrenoceptor were studied in this paper.

Pieces of taenia caecum were isolated from male guinea-pigs (300–500 g) and suspended in a 20 ml organ bath filled with Locke-Ringer solution (NaCl, 154; KCl, 5.6; CaCl2, 2.2; MgCl2, 2.1; NaHCO3, 5.9; and glucose, 2.8 mM) kept at 32°C and bubbled with air. The responses to drugs were recorded isotonically under a tension of 0.5 g. Concentration-action curves of drugs were obtained cumulatively. Agonistic activity was expressed as the pD2-value and competitive antagonistic activity as the pA2-value, which were calculated by the method of Van Rossum (7). The intrinsic activity was expressed as the ratio of the maximum response to a test drug and that to isoprenaline. All the results in this study were presented as a mean±S.E.M. of 8 experiments.

Microsomal fractions of guinea-pig taenia caecum were prepared according to the methods reported previously (8). Microsomal fractions were incubated with various concentrations of [3H]-befunolol (specific activity: 115.0 Ci/mmol, New England Nuclear) in a total volume of 150 μl of incubation buffer (50 mM Tris/HCl, pH 7.4, at 35°C) for 15 min. The incubation mixture was diluted with 1 ml of 50 mM Tris/HCl (pH 7.4) and rapidly filtered through Whatman GF/C glass fiber filters. Nonspecific binding was determined as radioactivity bound to microsomal fractions, which was not displaced by 5 μM propranolol. Protein concentrations were determined by the method of Lowry et al. (9) using bovine serum albumin.

It was reported that befunolol and carteolol were beta-adrenergic partial agonists in the taenia caecum and tracheal smooth muscle of the guinea-pig (6). The intrinsic activities and pD2-values estimated in the guinea-pig
isolated taenia caecum were 0.29±0.04 and 7.09±0.23 for befunolol and 0.63±0.07 and 6.37±0.10 for carteolol, respectively. The pA2-values of the test drugs against isoprenaline were 9.23±0.13 for befunolol and 9.97±0.14 for carteolol (Table 1). The pD2-values of some beta-adrenergic partial agonists were significantly different from their pA2-values. Similar results were reported for the guinea-pig trachea (10). However, the pA2-value (8.41±0.07) of propranolol against carteolol, whose intrinsic activity was 0.63, was equal to the pA2-value (8.65±0.40) against isoprenaline, suggesting that the three drugs interact at the same binding site. As the intrinsic activity of befunolol is too low for it to be used as an agonist, the pA2-value of propranolol against befunolol was not estimated. The pA2-value (9.23±0.13) of befunolol against isoprenaline was significantly different from its pA2-value (7.05±0.10) against carteolol. These facts indicate the possibility that the competitive site of action of isoprenaline with befunolol is different from that of carteolol.

Recently many investigators have found that in studies of the radioligand binding to beta-adrenoceptor or other membrane receptors, guanine nucleotides cause a selective reduction in agonist binding, while having only a minimal effect in antagonist binding (11, 12). A Scatchard plot of [3H]-befunolol showed two affinity sites of the receptor in the absence of a guanosine 5'-triphosphate (GTP) analogue, guanyl-5'-yl imidodiphosphate, Gpp(NH)p, but the low affinity site was reduced and the high affinity site was not affected in the presence of Gpp(NH)p. The pKd-value of the high affinity site of befunolol was in agreement with its pA2-value against isoprenaline, and its pKd-value of the low affinity site agreed with its pD2-value and pA2-value against carteolol (Table 1). Competitive inhibition curves for specific binding of [3H]-befunolol (1 nM and 50 nM) by various concentrations of isoprenaline, propranolol and befunolol were obtained (Fig. 1). Only the high affinity site was supposed to be bound by 1 nM of [3H]-befunolol; however, both the high and low affinity sites might be bound by 50 nM of this substance. All the competitive inhibition curves for specific binding of [3H]-befunolol (1 nM and 50 nM) by isoprenaline and propranolol showed monophasic shapes. The competitive inhibition curve for specific binding of a high concentration (50 nM) of [3H]-befunolol by befunolol showed a biphasic shape, although the curve for specific binding of a low concentration (1 nM) of [3H]-befunolol by befunolol showed a monophasic shape, although the curve for specific binding of a high concentration (50 nM) of [3H]-befunolol by befunolol (0.66±0.03) was significantly (P<0.05) different from that of a low concentration (1 nM) of [3H]-befunolol by befunolol (0.93±0.06).

The most plausible explanation of our observations would seem to be as follows: The partial agonists such as befunolol and

| Table 1. The intrinsic activity, pD2-, pA2- and pKd-value of befunolol tested on guinea-pig taenia caecum |
|------------------|------------------|------------------|------------------|------------------|------------------|
| i.a. | From mechanical responses* (Befunolol) | From binding assay (3H)-Befunolol |
| pD2 | agonist (Iso.) | agonist (Car.) | -Gpp (NH)p | +Gpp(NH)p |
| pA2 | pKd (Low) | pKd (High) | pKd |
| 0.29±0.04 | 7.09±0.23 | 9.23±0.13 | 7.05±0.10 | 6.94±0.02 | 8.97±0.14 | 8.96±0.07 |

i.a.: intrinsic activity, Iso.: Isoprenaline (a full agonist), Car.: carteolol (a partial agonist). *n=8, b: n=3. The pKn-value was represented as the negative log of the dissociation constant (Kd) which was estimated from a Scatchard plot of the [3H]-befunolol binding to microsomal fractions in the presence or absence of Gpp(NH)p 100 µM.
carteolol are able to discriminate two different binding sites of the beta-adrenoceptor: the high affinity site and the low affinity site. When low concentrations of the beta-adrenergic partial agonist are used, the beta-adrenergic partial agonist interacts with only the high affinity site. The beta-adrenergic partial agonist in high concentrations is able to interact with both the low affinity and high affinity sites simultaneously. The interaction of isoprenaline with the high affinity site induces the beta-agonistic effect. The antagonistic effect of propranolol and partial agonists such as befunolol and carteolol is due to their ability to compete with isoprenaline for the high affinity site. On the other hand, a partial agonist such as carteolol interacts with the low affinity site to induce the beta-agonistic effect. The interaction of propranolol and other partial agonists such as befunolol with the low affinity site antagonize the agonistic action due to the partial agonist such as carteolol. Moreover, the interaction of isoprenaline with the low affinity site seems not to induce the beta-agonistic effect, although it is confirmed that isoprenaline interacts with the high affinity site.

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