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## Effects of beta-adrenergic blocking agents on specific binding of [3H]D-Ala<sup>2</sup>-Met<sup>5</sup>-enkephalinamide and [3H]naloxone.

Haruhiko Takayama\*

Norio Ogawa<sup>†</sup>

Masato Asanuma<sup>‡</sup>

Hiroshi Hirata\*\*

Toshio Ogura<sup>††</sup>

Zensuke Ota<sup>‡‡</sup>

\*Okayama University,

<sup>†</sup>Okayama University,

<sup>‡</sup>Okayama University,

\*\*Okayama University,

<sup>††</sup>Okayama University,

<sup>‡‡</sup>Okayama University,

# Effects of beta-adrenergic blocking agents on specific binding of [3H]D-Ala2-Met5-enkephalinamide and [3H]naloxone.\*

Haruhiko Takayama, Norio Ogawa, Masato Asanuma, Hiroshi Hirata, Toshio Ogura, and Zensuke Ota

## Abstract

To gain further insight into the central nervous system (CNS)-action of beta-adrenergic blocking agents (beta-blockers), we examined the effects of various kinds of beta-blockers on opioid receptors (Op-Rs) using radiolabeled receptor assay (RRA). We demonstrated that beta-blockers are competitively bound to Op-Rs in the CNS. Sodium index of beta-blockers in [3H]naloxone binding study indicated that beta-blockers had the mixed agonist-antagonist activity of opiates. The relative potency of beta-blockers in opioid RRA was negatively correlated with their membrane stabilizing activity. Neither beta-blocking activity nor intrinsic sympathomimetic activity was correlated with IC<sub>50</sub> values of beta-blockers in opioid RRA. While it is widely accepted that beta-blockers have a tranquilizing activity, a part of the tranquilizing action of beta-blockers may be mediated through Op-Rs in the CNS. Although beta-blockers may have effects on their own receptors (beta-receptors) in the CNS, the more precise mechanisms of central action of these drugs must be further investigated.

**KEYWORDS:**  $\beta$ -blocker, opioid receptor, membrane stabilizing activity, sodium index

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## Effects of $\beta$ -Adrenergic Blocking Agents on Specific Binding of [ $^3\text{H}$ ]D-Ala<sup>2</sup>-Met<sup>5</sup>-Enkephalinamide and [ $^3\text{H}$ ]Naloxone

Haruhiko Takayama<sup>a</sup>, Norio Ogawa<sup>b\*</sup>, Masato Asanuma<sup>b</sup>, Hiroshi Hirata<sup>a,b</sup>, Toshio Ogura<sup>a</sup> and Zensuke Ota<sup>a</sup>

<sup>a</sup>Third Department of Internal Medicine and <sup>b</sup>Department of Neurochemistry, Institute for Neurobiology, Okayama University Medical School, Okayama 700, Japan

To gain further insight into the central nervous system (CNS)-action of  $\beta$ -adrenergic blocking agents ( $\beta$ -blockers), we examined the effects of various kinds of  $\beta$ -blockers on opioid receptors (Op-Rs) using radiolabeled receptor assay (RRA). We demonstrated that  $\beta$ -blockers are competitively bound to Op-Rs in the CNS. Sodium index of  $\beta$ -blockers in [ $^3\text{H}$ ]naloxone binding study indicated that  $\beta$ -blockers had the mixed agonist-antagonist activity of opiates. The relative potency of  $\beta$ -blockers in opioid RRA was negatively correlated with their membrane stabilizing activity. Neither  $\beta$ -blocking activity nor intrinsic sympathomimetic activity was correlated with  $\text{IC}_{50}$  values of  $\beta$ -blockers in opioid RRA. While it is widely accepted that  $\beta$ -blockers have a tranquilizing activity, a part of the tranquilizing action of  $\beta$ -blockers may be mediated through Op-Rs in the CNS. Although  $\beta$ -blockers may have effects on their own receptors ( $\beta$ -receptors) in the CNS, the more precise mechanisms of central action of these drugs must be further investigated.

**Key words :**  $\beta$ -blocker, opioid receptor, membrane stabilizing activity, sodium index

$\beta$ -adrenergic blocking agents ( $\beta$ -blockers) were originally used as cardiovascular-acting agents, and a variety of these drugs are commercially available. However,  $\beta$ -blockers have recently been used as anti-tremor drugs in patients with essential tremor or Parkinson's disease (1-5). Because of the different pharmacological effects, each  $\beta$ -blocker has its own characteristic properties, which allow precise selection of the most suitable drug for each patient. In general, drugs acting on the central nervous system (CNS) do not have a single, but

plural actions. We also have demonstrated that CNS-acting drugs interact with several kinds of receptors in the CNS (6-9). In the present study, to gain further insight into the CNS-action of  $\beta$ -blockers, we examined the effects of various kinds of  $\beta$ -blockers on opioid receptors (Op-Rs) using radiolabeled receptor assay (RRA).

### Materials and Methods

Male Sprague-Dawley (SD) rats were obtained from Charles River Japan Inc. [ $^3\text{H}$ ]D-Ala<sup>2</sup>-Met<sup>5</sup>-enkephalinamide (ENK, specific activity, 36 Ci/mmol)

\*To whom correspondence should be addressed.

and [ $^3\text{H}$ ]naloxone (NAL, specific activity 20 Ci/mmol) were purchased from New England Nuclear (Boston, MA, USA) and Amersham (Buckinghamshire, UK), respectively. The following five types of  $\beta$ -blockers were used in this study: propranolol, bufetolol, indenolol, oxprenolol, pindolol. Table 1 summarizes the pharmacological properties and relative potency of the five  $\beta$ -blockers tested in this study (4).

**Crude synaptic membranes.** Adult male SD rats (200–250 g) were decapitated, and the brain tissues (except cerebellum) were immediately removed and homogenized in 10 volumes of ice-cold 0.32 M sucrose with a Brinkman PT-10 homogenizer (dial setting 7, duration 10 sec, repeated once). The homogenate was centrifuged at 900 x G for 10 min, and the resulting supernatant was centrifuged at 11,500 x G for 20 min (10). The pellet (synaptosomal fraction) was homogenized in 10 volumes of Tris-HCl buffer (50 mM, pH 7.6) and centrifuged at 11,500 x G for 20 min. The pellet was resuspended in 10 volumes of the same buffer and stored at  $-70^\circ\text{C}$  until assay. This preparation provided the crude synaptic membranes ( $P_2$  preparation) used in the experiments. Before use, the  $P_2$  preparation was thawed and recentrifuged at 11,500 x G for 20 min at  $4^\circ\text{C}$ . The pellet was resuspended in the original volume of Tris-HCl buffer.

**Radiolabeled Receptor Assay (RRA).** Assay of specific ENK and NAL binding were measured as previously described (6, 7). Briefly, the binding assays were done in glass tubes with 100  $\mu\text{l}$  of standard or sample, 100  $\mu\text{l}$  of [ $^3\text{H}$ ]ENK (final 5 nM) or [ $^3\text{H}$ ]NAL (final 2 nM), 300  $\mu\text{l}$  of Tris-HCl buffer and 500  $\mu\text{l}$  of membrane receptor preparation ( $P_2$  preparation). After incubation for 2 h at  $4^\circ\text{C}$ , membrane bound and free [ $^3\text{H}$ ]ENK or [ $^3\text{H}$ ]NAL were separated by a filtration under vacuum through Whatman GF/C glass-fiber filters. The filter was washed three times under reduced

pressure with 3 ml of ice-cold Tris-HCl buffer and placed in scintillation fluor. The radioactivity of the filter was counted with an automatic beta counter. Specific binding was the difference between radioactivity bound to receptors in the presence of excess ENK (final 10  $\mu\text{M}$ ) or NAL (final 2  $\mu\text{M}$ ) and that bound in the absence of these agents.

**Competition studies.** Binding assays were conducted as described above, except that, in addition to assay of total (no inhibitor) and nonspecific binding, incubations contained various concentrations of  $\beta$ -blockers (seven concentrations ranging from 100 nM to 10 mM for each).  $\text{IC}_{50}$  was defined as the concentration which displaced specific binding by 50 % and determined by log-probit analysis.

## Results

The effect of  $\beta$ -blockers on the specific [ $^3\text{H}$ ]ENK or [ $^3\text{H}$ ]NAL binding to Op-Rs are presented in Table 2. Similar  $\text{IC}_{50}$  values were obtained in ENK-RRA and NAL-RRA with the five  $\beta$ -blockers tested. Among the tested  $\beta$ -blockers, propranolol and indenolol were most potent in competing for ENK-RRA and NAL-RRA. In the presence of 100  $\mu\text{M}$   $\text{Na}^+$ , all  $\beta$ -blockers showed an increase in  $\text{IC}_{50}$  for [ $^3\text{H}$ ]NAL binding. Sodium index of  $\beta$ -blockers for [ $^3\text{H}$ ]NAL binding were similar (from 2.7–10.0).

**Table 2** Effects of various kinds of  $\beta$ -blockers on receptor binding of [ $^3\text{H}$ ]D-Ala $^2$ -Met $^5$ enkephalinamide (ENK) and [ $^3\text{H}$ ]naloxone (NAL)

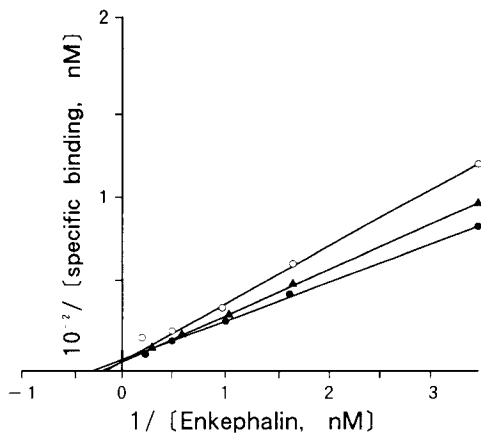
	[ $^3\text{H}$ ]ENK $\text{IC}_{50}$ ( $\mu\text{M}$ )	[ $^3\text{H}$ ]NAL		Sodium Index
		$\text{IC}_{50}$ ( $\mu\text{M}$ ) –Na	+Na	
Propranolol	74	46	125	2.7
Bufetolol	650	160	900	5.6
Indenolol	80	40	300	7.5
Oxprenolol	350	100	1000	10.0
Pindolol	700	200	1200	6.0

$\text{IC}_{50}$  values were calculated as the concentrations required to displace 50 % of specific binding, determined by log-probit analysis. Sodium index is the ratio of the  $\text{IC}_{50}$  with  $\text{Na}^+$  (100  $\mu\text{M}$ ) to that without  $\text{Na}^+$  ( $+\text{Na}^+/-\text{Na}^+$ ). Each value represents the mean for four experiments.

**Table 1** Pharmacological properties and relative potency of the various kinds of  $\beta$ -blockers

$\beta$ -blockers	$\beta$ -blocking activity <sup>a</sup>	ISA <sup>b</sup>	MSA <sup>a</sup>
Propranolol	1.0	0	1.0
Bufetolol	2.0	0	0.2
Indenolol	1.0	0.11	1.2
Oxprenolol	0.5	0.29	0.4
Pindolol	4.0	0.56	0.1

These values are obtained compared with a: propranolol=1, b: Isoproterenol = 1. Abbreviations; ISA: intrinsic sympathomimetic activity, MSA: membrane stabilizing activity.

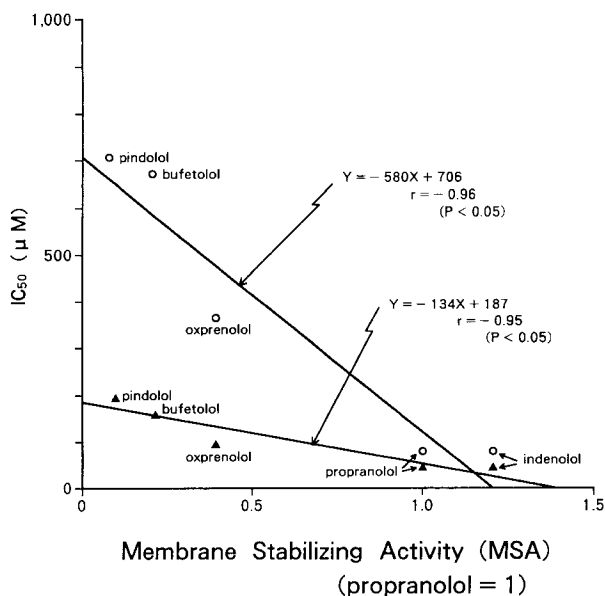


**Fig. 1** Double reciprocal plots (Lineweaver-Burk plot) of the  $[^3\text{H}]\text{D-Ala}^2\text{-Met}^5\text{-enkephalinamide}$  (ENK)-RRA. Each value represents the mean of triplicate determinations from one representative experiment, which was repeated three times (total = four experiments). ENK-RRA ( $\bullet$ ) and ENK-RRA in the presence of oxprenolol ( $100\ \mu\text{M}$ ) ( $\blacktriangle$ ) or bufetolol ( $200\ \mu\text{M}$ ) ( $\circ$ ).

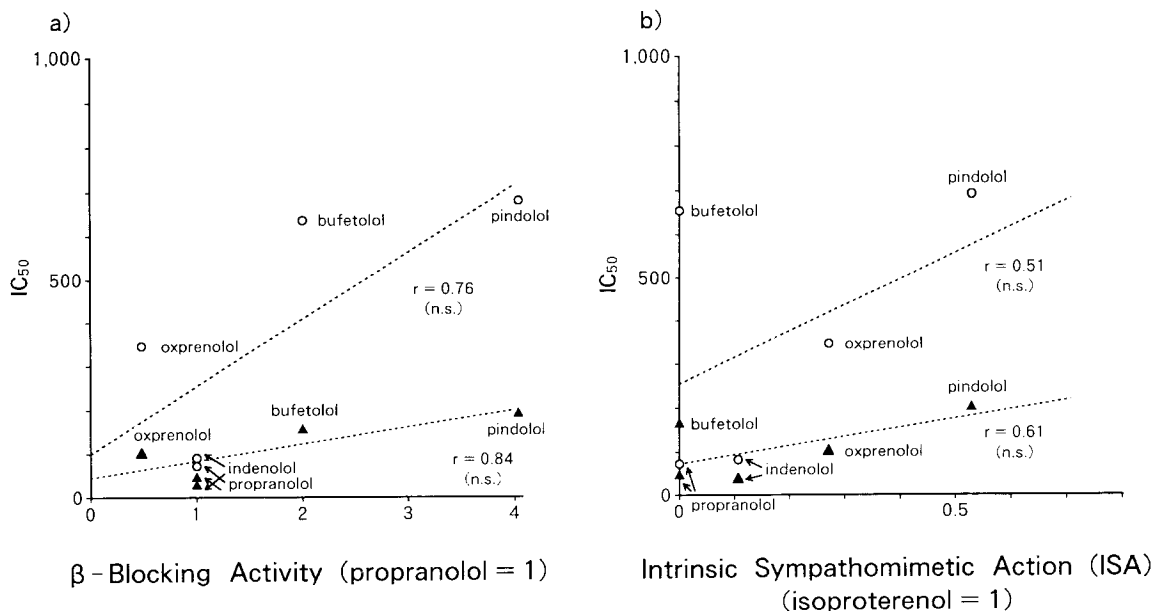
We examined whether these displacements were due to competitive inhibition of  $\beta$ -blockers to Op-R binding using double reciprocal plots (Lineweaver-Burk plot) (Fig.1). Bufetolol ( $200\ \mu\text{M}$ ) and oxprenolol ( $100\ \mu\text{M}$ ) were competitively bound to Op-R.

Correlations between the membrane-stabilizing activity (MSA) of  $\beta$ -blockers and their  $\text{IC}_{50}$  values in ENK- and NAL-RRA are presented in Fig.2. In ENK-RRA, the correlation coefficient was  $-0.96$ , and the level of significance was less than 5%. In NAL-RRA, the correlation coefficient was  $-0.95$ , and the level of significance was less than 5%. Therefore, it is clear that the  $\text{IC}_{50}$  values of  $\beta$ -blockers in opioid-RRA were significantly correlated negatively with the MSA of  $\beta$ -blockers.

The correlations between the  $\beta$ -blocking activity and  $\text{IC}_{50}$  values or these between the intrinsic sympathomimetic activity (ISA) and  $\text{IC}_{50}$  values are presented in Fig. 3. There was no significant correlation between these two components.



**Fig. 2** Correlation between the membrane stabilizing activity (MSA) of various kinds of  $\beta$ -blocker and  $\text{IC}_{50}$  values in  $[^3\text{H}]\text{-D-Ala}^2\text{-Met}^5\text{-enkephalinamide}$  (ENK) and  $[^3\text{H}]\text{naloxone}$  (NAL)-RRA. Each value represents the means of four experiments less than 10% coefficient of variations, each performed in triplicate determinations.  $\circ$ : ENK-RRA,  $\blacktriangle$ : NAL-RRA.



**Fig. 3** Correlation between  $\beta$ -blocking activity (a), intrinsic sympathomimetic activity (ISA) (b) of various kinds of  $\beta$ -blocker and  $IC_{50}$  values in [ $^3H$ ]D-Ala<sup>2</sup>-Met<sup>5</sup>-enkephalinamide (ENK) and [ $^3H$ ]naloxone (NAL)-RRA. Each value represents the means of four experiments less than 10% coefficient of variations, each performed in triplicate determinations. ○: ENK-RRA, ▲: NAL-RRA.

## Discussion

Generally drugs acting on the CNS rarely have a single effect, but are known to act on the receptors of several types of intrinsic agents (8, 11). We have previously reported that  $\beta$ -blockers might act on Op-Rs (12). The tranquilizing effect of these drugs might be mediated by Op-Rs.  $\beta$ -blockers are likely to have unidentified effects, in addition to the known effects, and these will require further study.

First, we studied the interaction of various kinds of  $\beta$ -blockers with Op-Rs.  $IC_{50}$  values of five  $\beta$ -blockers are micromolar range and similar values were obtained in ENK- and NAL-RRA. Among the  $\beta$ -blockers tested in this study, the rank order of potency to inhibit of [ $^3H$ ]ENK or [ $^3H$ ]NAL binding was as follows: propranolol = indenolol > oxprenolol > bufetolol > pindolol. These  $IC_{50}$  values are similar to the report from Tampier *et al.* (13). He reported that the degree of inhibition of opiate binding appears to parallel the potency of local anes-

thetics. In our experiment, propranolol which has a marked local anesthetic activity is the most potent inhibitor among the five  $\beta$ -blockers. The influence of sodium on unlabeled opiate binding can be investigated by determining the potency of the drug in inhibiting the binding of [ $^3H$ ]NAL to opiate receptors in the presence or absence of sodium (14). Usually opiate agonists become 9 to 60 times weaker in inhibiting [ $^3H$ ]NAL binding in the presence of sodium, whereas the potency of pure antagonists remains unchanged (or increases slightly, in some cases). A number of opiate compounds demonstrate both agonist and antagonist properties. In this study,  $IC_{50}$  values of  $\beta$ -blockers on NAL-RRA were increased in the presence of 100  $\mu$ M Na<sup>+</sup>. The sodium index of five  $\beta$ -blockers suggested that they act on Op-Rs with mixed agonist-antagonist properties. Charalampous and Askew (15) reported that, while propranolol caused a significant reduction in [ $^3H$ ]NAL binding, the presence of sodium did not significantly affect the binding. While it is difficult to give a clear explanation of the discrep-

ancies for the effect of sodium, it may be due to differences in species used and receptor preparations. In our study, Lineweaver-Burk plot was performed for ENK-RRA in the presence of cold ENK or bufetolol (200  $\mu$ M) or oxprenolol (100  $\mu$ M). We found that these two  $\beta$ -blockers were competitively bound to Op-Rs (Fig. 1).

Secondly, we compared the  $IC_{50}$  values of five different types of  $\beta$ -blockers and found that  $IC_{50}$  values were significantly correlated negatively with the MSA (Fig. 2). Interestingly, propranolol nonspecifically binds to lipid membranes (16), producing a "local anesthetic" or MSA similar to that produced by quinidine. The stronger the MSA is,  $IC_{50}$  values became decreased. It means that the MSA has a positive effect on inhibition of Op-R. There was no significant correlation between  $\beta$ -blocking activity and  $IC_{50}$  values or between ISA and  $IC_{50}$  values (Fig. 3).

In conclusion, we demonstrated that  $\beta$ -blockers are competitively bound to Op-Rs in the CNS. And the sodium index of  $\beta$ -blockers in NAL-RRA indicated that  $\beta$ -blockers had a mixed agonist-antagonist activity of opiates. The relative potency of  $\beta$ -blockers in opioid RRA was negatively correlated with their MSA. Neither  $\beta$ -blocking activity nor ISA was correlated with  $IC_{50}$  of  $\beta$ -blockers in opioid RRA. Since it is widely accepted that  $\beta$ -blockers have a tranquilizing activity, and we supposed that a part of the tranquilizing action of  $\beta$ -blockers may be mediated through Op-Rs in the CNS. Although these drugs may have effects on other receptors in the CNS, the central action of  $\beta$ -blockers must be investigated for understanding the more precise mechanism of  $\beta$ -blocking.

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