Nipradilol Displays a Unique Pharmacological Profile of Affinities for the Different $\alpha_1$-Adrenoceptor Subtypes

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ABSTRACT—The selectivity of antagonistic effects of nipradilol, its four isomers and denitronipradilol, a major metabolite of nipradilol, on $\alpha_1$-adrenoceptor subtypes in rat heart, brain and spleen were examined by radioligand binding assay with $[^3\text{H}]$-prazosin. Pharmacological characteristics of these compounds were determined in isolated aortae from rats and guinea pigs. The order of the $pK_i$ values for $\alpha_{1\text{High}}$-affinity sites in the heart, spleen and brain was SR > nipradilol > RR > SS ≈ RS > denitronipradilol, but the order of the $pK_i$ values for the $\alpha_{1\text{Low}}$-affinity sites was different in the heart and brain. There were good correlations between the $pK_i$ values of these compounds for the $\alpha_{1\text{High}}$-affinity sites and the $pA_2$ values for the contractile inhibition of the phenylephrine-induced response in rat aorta. There was no correlation between the $pK_i$ values of these compounds for the $\alpha_{1\text{Low}}$-affinity sites and the $pA_2$ values. These results indicate that: 1) $\alpha_{1\text{High}}$-Affinity sites are related to vasoconstriction mediated by $\alpha_1$-adrenoceptors; 2) Nipradilol and its isomers possess low affinity to $\alpha_1$-adrenoceptors; and 3) The nitroxy group in nipradilol is important for its $\alpha_1$-blocking activity.

Keywords: $[^3\text{H}]$-Prazosin, Nipradilol, $\alpha_1$-Adrenoceptor subtype, $\alpha_1$- and $\beta$-Blocking activity, Optical isomer

Our recent studies on the binding characteristics of $[^3\text{H}]$-prazosin for the $\alpha_1$-adrenoceptor subtypes revealed that there are two binding sites having different affinities, high- and low-affinity sites, for prazosin in the rat ventricular muscle, brain and spleen membranes; and these sites are designated as $\alpha_{1\text{High}}$- and $\alpha_{1\text{Low}}$-affinity sites (1, 2). We also found that rat ventricular muscle and brain possess both $\alpha_{1\text{High}}$- and $\alpha_{1\text{Low}}$-affinity sites, whereas rat spleen had only $\alpha_{1\text{High}}$-affinity sites (1, 2). Most $\alpha_1$-adrenergic antagonists had different affinities for each site in these tissues, suggesting that there may exist five different $\alpha_1$-adrenoceptors ($\alpha_{1\text{High}}$-sites in the ventricular muscle, brain and spleen and $\alpha_{1\text{Low}}$-sites in the ventricular muscle and brain).

Recently, $\alpha_1$, $\beta$-blockers possessing antagonistic potency for both $\alpha_1$- and $\beta$-adrenoceptors have been shown to be useful for the clinical treatment of patients with hypertension and angina. Nipradilol, one of these $\alpha_1$, $\beta$-blockers, possesses two asymmetric carbon atoms in its chemical structure and is a mixture of four isomers, as shown in Fig. 1 (3, 4). We previously reported the binding characteristics of nipradilol for $\beta_1$-, $\beta_2$-adrenergic and $5\text{HT}_{1\text{B}}$-serotonergic binding sites by using $[^{125}\text{I}]$-iodo-cyanopindolol (ICYP) and $[^3\text{H}]$-CGP12177, respectively; and the results showed that nipradilol had a higher affinity for $\beta$-adrenoceptors (with no selectivity for $\beta_1$- and $\beta_2$-adrenoceptors) than for $5\text{HT}_{1\text{B}}$-receptors (5). The rank orders of potency of the isomers for $\beta$-adrenoceptors and $5\text{HT}_{1\text{B}}$-receptors were SR > nipradilol > SS > RR > RS and SS > SR > nipradilol > RS > RR, respectively (5).

![Fig. 1. Chemical structure of nipradilol and its isomers.](image-url)
In this study, we examined the selectivity of nipradilol, its isomers and its major metabolite denitronipradilol (6) for \(\alpha_1\)-adrenoceptor subtypes and compared them with those of other \(\alpha_1/\beta\)-blockers, labetalol, amosulalol and arotinolol.

MATERIALS AND METHODS

Materials

\([^{3}H]\)-Prazosin (76.6 Ci/mmmole) was purchased from New England Nuclear/Dupont, Ltd., Boston, MA, U.S.A. Nipradilol, 3,4-dihydro-8-(2-hydroxy-3-isopropylamino)propoxy-3-nitroxy-2H-1-benzopyran, its isomers (Fig. 1) and denitronipradilol were kindly donated by Kowa Co., Ltd.

Animals

Male Wistar rats weighing 200–350 g were used.

Preparation of membrane-enriched fractions

The membrane-enriched fractions from rat heart, brain and spleen were prepared as described previously (1, 2). Protein was determined by the method of Lowry et al. (7).

Binding assay

Displacement analysis for \(\alpha_1\)-adrenoceptor subtypes was performed in duplicate with \([^{3}H]\)-prazosin as described previously (1, 2). In brief, the membrane suspension (0.1 mg of heart and brain and 0.25 mg of spleen membrane proteins) was incubated for 45 min at 23°C in a total volume of 0.5 ml containing 60 mM Tris-HCl (pH 7.4) with an appropriate concentration of \([^{3}H]\)-prazosin in the presence or absence of unlabelled ligand. The concentrations of \([^{3}H]\)-prazosin were 0.04, 0.1 and 0.2 nM for assessing \(\alpha_1^{\text{High}}\)-affinity sites in the brain, heart and spleen membrane, respectively. The affinity of the \(\alpha_1^{\text{Low}}\)-affinity sites for unlabelled ligand were determined with 0.5 nM \([^{3}H]\)-prazosin in the presence of 0.1 \(\mu\)M phenoxybenzamine in the brain membrane, or with 0.6 nM \([^{3}H]\)-prazosin in the presence of 1 \(\mu\)M phenoxybenzamine in the heart membrane. Phenoxybenzamine inhibited the \(\alpha_1^{\text{High}}\)-affinity sites completely (1, 2). After the incubation period of 45 min, the medium was immediately filtered through a GF/C glass fiber filter and washed with the incubation buffer according to previously described methods (8). The radioactivity on the filter was counted by a Packard 2200 Tri-Carb Scintillation Analyzer. The specific binding was determined by subtracting the non-specific binding in the presence of 10 \(\mu\)M of phentolamine from the total binding.

Kinetic analysis

All kinetic analyses were performed on an NEC PC-9801 computer system with an iterative non-linear regression program (9–12). The data were fitted to models having only one or two receptor binding sites (9–12). To quantitate the displacement characteristics, the slope factor (\(n_H\)) for the displacement curves was determined as described previously (9–12). Most \(K_i\) values of various ligands are expressed as \(pK_i\left( = -\log K_i\right)\) in this report.

Pharmacological observations

The contractile tension of the rat and guinea pig aorta was determined as described previously (13, 14). Briefly, aortae were cut into rings and freed of excess tissues.

| Table 1. \(pK_i\) values of nipradilol and its optical isomers for the \(\alpha_1\)-adrenoceptor subtypes |
|--------------------------------------------|
|                | \(\alpha_1^{\text{High}}\) | \(\alpha_1^{\text{Low}}\) |
| Rat spleen     | Rat heart | Rat brain     | Rat heart | Rat brain |
| Nipradilol     | 5.41±0.10 (6) | 5.61±0.03 (3) | 5.54±0.11 (3) | 5.23±0.03 (3) | 5.43±0.27 (4) |
| SR             | 5.69±0.15 (6) | 5.96±0.01 (3) | 6.10±0.15 (3) | 5.47±0.14 (3) | 6.18±0.20 (3) |
| RR             | 4.95±0.07 (6) | 5.62±0.03 (3) | 5.37±0.18 (4) | 5.60±0.19 (3) | 6.01±0.23 (3) |
| SS             | 4.72±0.19 (6) | 5.06±0.16 (3) | 4.90±0.13 (3) | 6.56±0.11 (3) | 5.65±0.23 (3) |
| RS             | 4.68±0.17 (6) | 5.17±0.08 (3) | 4.98±0.18 (3) | 5.20±0.12 (3) | 5.60±0.19 (3) |
| Denitronipradil | 4.15±0.11 (6) | 4.75±0.22 (3) | 4.06±0.10 (3) | 5.71±0.21 (3) | 3.69±0.14 (3) |
| Prazosin*      | 11.14±0.04 (4) | 10.60±0.13 (3) | 10.29±0.14 (10) | 8.87±0.25 (4) | 8.04±0.23 (3) |
| Amosulalol*    | 7.80±0.24 (5) | 7.85±0.17 (3) | 8.23±0.21 (4) | 6.46±0.16 (3) | 5.78±0.20 (3) |
| Labetalol*     | 6.68±0.09 (5) | 6.89±0.05 (3) | 7.30±0.11 (3) | 5.85±0.09 (3) | 6.24±0.03 (4) |
| Arotinolol*    | 6.25±0.07 (4) | 6.04±0.23 (3) | 6.49±0.20 (3) | 5.97±0.27 (4) | 5.58±0.06 (4) |

Values in parentheses are the numbers of experiments. Data are the mean values ±S.E. The slope factors (\(n_H\)) of the displacement curves of all ligands used in the present study were equal to one. *Some of these data were reported previously (11).
These rings, about 2 mm in width, were mounted in 12 ml organ baths. The Krebs-Henseleit solution had the following composition: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 25 mM NaHCO₃, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, and 11 mM glucose. The temperature of the solution was maintained at 37 ± 1°C and aerated with a mixture of 95% O₂ and 5% CO₂. The contractile tension of these preparations was recorded on a potentiometric recorder (Hitachi APD-74) with a strain gage transducer and a carrier amplifier (Nihon Kohden RP-3 or San'ei Instrument Co., Ltd. 6M52). The aorta was always stretched to 0.5-1 g to obtain the optimum response. Concentration–response curves were determined for phenylephrine before and after addition of each compound, and their pA₂ values were calculated by the previously described equation (15).

RESULTS

Table 1 summarizes the pKi values of nipradilol, its isomers and denitronipradilol for a1-adrenoceptor subtypes in [³H]-prazosin binding to brain, heart and spleen membranes. Both a₁High and a₁Low-affinity sites were found in the brain and heart membranes, while only a₁High-affinity sites existed in the spleen membranes (1, 2). These five a₁-adrenoceptors had different affinities for various antagonists (1, 2). The binding affinity of labetalol for these subtypes were distinguishable among the compounds used in the present study: brain a₁High > heart a₁High ≈ spleen a₁High > brain a₁Low > heart a₁Low. The pKi values of the other a₁-blockers, arotinolol and amosulalol, for a₁High-affinity sites were higher than those for a₁Low-affinity sites. On the other hand, the pKi values of nipradilol for various subtypes were similar except for heart a₁High > heart a₁Low. The pKi values of nipradilol and related compounds for the a₁High-affinity site were significantly lower than those of prazosin, amosulalol, labetalol and arotinolol. The order of potency of nipradilol, its isomers and denitronipradilol for a₁High-affinity sites was SR ≈ nipradilol ≈ RR > SS = RS > denitronipradilol in the heart, brain and spleen. Thus, the orders of potency were similar for the a₁High-affinity sites in these three tissues. On the other hand, the orders of potency for the a₁Low-affinity sites were not consistent in the heart and brain. The rank order of the pKi values of denitronipradilol was heart a₁Low > heart a₁High > spleen a₁High ≈ brain a₁High > brain a₁Low. The rank order of the pKi values of the optical isomers of nipradilol for these subtypes was different for each compound: SR, brain a₁Low ≈ brain a₁High ≥ heart a₁High ≥ spleen a₁High ≥ heart a₁High, SS, heart a₁Low > brain a₁Low ≥ heart a₁High ≈ brain a₁High ≥ spleen a₁High; RR, brain a₁Low > heart a₁High ≥ heart a₁Low ≥ brain a₁High > spleen a₁High; RS, brain a₁Low.

Table 2. pA₂ values of nipradilol and its optical isomers for a₁-adrenoceptors in rat and guinea pig aorta

|                  | Rat        | Guinea pig |
|------------------|------------|------------|
| Nipradilol       | 6.49 ± 0.19 (6) | 6.62 ± 0.18 (6) |
| SR               | 6.53 ± 0.12 (5) | 6.94 ± 0.20 (5) |
| RR               | 6.43 ± 0.15 (5) | 6.41 ± 0.16 (5) |
| SS               | 5.42 ± 0.19 (5) | 6.05 ± 0.22 (5) |
| RS               | 5.26 ± 0.10 (5) | 5.46 ± 0.28 (5) |
| Denitronipradilol| 5.14 ± 0.12 (5) | 5.86 ± 0.14 (5) |
| Amosulalol       | 7.89 ± 0.08 (7) | 7.46 ± 0.40 (5) |
| Labetalol        | 6.89 ± 0.12 (5) | 6.59 ± 0.40 (4) |
| Arotinolol       | 6.07 ± 0.16 (5) | 5.95 ± 0.29 (5) |

Values in parentheses are the numbers of experiments. Data are the means ± S.E.

≈ heart a₁Low ≈ heart a₁High ≥ brain a₁High ≥ spleen a₁High.

Table 2 also summarizes the pA₂ values of the contractile tension of rat and guinea pig aorta. a₁-Adrenoceptor subtypes in the rat and guinea pig aorta were a₁High and a₁Low-affinity sites, respectively, as determined by the pA₂ values of prazosin (10.80 in rat aorta and 8.30 in guinea pig aorta) (16). All compounds antagonized the phenylephrine-induced contractile response in a competitive manner when analyzed by Schild plots (data are not shown). The pA₂ value of nipradilol in the rat aorta was lower than the value of amosulalol and labetalol, and it was higher than that of arotinolol. On the other hand, the pA₂ values of SS, RS and denitronipradilol were lower than the value of arotinolol. The order of pA₂ values of these compounds was SR > nipradilol > RR > SS > RS > denitronipradilol in the rat aorta. The pA₂ of nipradilol in the guinea pig aorta was lower than that of amosulalol and was higher than that of arotinolol. On the other hand, the pA₂ values of RS and denitronipradilol were lower than the value of arotinolol. The order of pA₂ values of these compounds was SR > nipradilol > RR > SS > denitronipradilol > RS in guinea pig aorta. The pA₂ values of these compounds in the guinea pig aorta were similar to those in the rat aorta except for those of denitronipradilol.

Figure 2 shows the relationship between the pKᵢ values of these compounds for a₁High-affinity sites in the three tissues and the pA₂ values for the antagonistic potency in the contractile response to phenylephrine of rat and guinea pig aorta. These results suggested a good correlation between the pKᵢ values of the a₁High-affinity sites and the pA₂ values in rat aorta (r = 0.90 – 0.94, P < 0.001). The pA₂ values in the guinea pig aorta showed less correlation (r = 0.74 – 0.79, P < 0.01) with the pKᵢ values than those obtained in the rat aorta. There was no correlation between the pKᵢ values of the a₁Low-affinity sites and the pA₂ values in both rat and guinea pig aorta (heart vs. rat aorta).
aorta, r=0.26; brain vs. rat aorta, r=0.56; heart vs. guinea pig aorta, r=0.28; brain vs. guinea pig aorta, r=0.43).

DISCUSSION

Nipradilol is a potent β-adrenergic blocking agent that has direct vasodilating and α₁-blocking properties. It possesses two asymmetric carbon atoms and is a mixture of four isomers (3, 4). There is one asymmetric carbon atom at the 3 position of the benzopyran ring and another at the 2' position of the aryloxypropanolamine group. Denitronipradilol, which has weak α₁-adrenergic activity, is a major metabolite (6). We demonstrated that α₁-adrenoceptor subtypes exist in various tissues and that most of the α₁-adrenergic antagonists had various affinities for each subtype in these tissues (1, 2). The present study also showed that nipradilol, its isomers and denitronipradilol had different affinities for various α₁-adrenoceptor subtypes. The rank order of pKᵢ values of the isomers and denitronipradilol was also different for these subtypes. Denitronipradilol and SS were selective for α₁low-affinity sites in the heart, but RR was selective for α₁low-affinity sites in the brain. These results suggested that these subtypes have different affinities for various α₁-adrenoceptor antagonists and that the configuration of the 3 position of the benzopyran ring and the 2' position of the aryloxypropanolamine residue was important for the recognition of the α₁-adrenoceptor subtypes.

The order of potency of these compounds for the α₁High-affinity sites in the heart, brain and spleen was generally SR > nipradilol ≥ RR ≥ SS ≈ RS >> denitronipradilol. Therefore, the results suggest that the R configuration of the nitroxy group of the benzopyran ring and the S configuration of the 2' position at the aryloxypropanolamine residue were important for the α₁-antagonistic actions of these drugs. We previously reported that nipradilol and its isomers were not selective for β₁- and β₂-adrenoceptors and that the order of potency of these compounds for β-adrenoceptor subtypes and 5HT₁₅-receptors
was SR > nipradilol > SS > RR > RS and SS > SR ≥ nipradilol > RS > RR, respectively (5). Previous reports (14, 17–20) have also shown that β-blockers possessing the S configuration are more potent than those of the R configuration among aryloxypropanolamines. On the other hand, the R configuration of the nitroxy group of nipradilol played an important role in the α1-blocking activity. These results suggested that these three receptors, α1A, α1B, and 5HT1 receptors, had different characteristics in the recognition sites for the optical isomers of nipradilol.

Based on recently available data, an increasing number of complex classification schemes for the α-adrenoceptor subtypes are being published. In blood vessels, there are two distinct subtypes (α1H and α1L) of α1-adrenoceptors that are distinguished by their affinities for prazosin and yohimbine (21). Our results also show that there are two distinct receptor subtypes (α1H and α1L) that are distinguished by their affinities for [3H]-prazosin in the rat brain, spleen, and heart (1, 2). On the other hand, Morrow and Creese (22) reported that rat brain contains two subtypes (α1A & α1B) with similar affinities for prazosin but different affinities for phentolamine. Han et al. (23, 24) proposed a similar classification (α1A & α1B) in rat brain, spleen, heart and other tissues. The α1A-subtype has a high affinity for WB-4101, while the α1B-subtype has a low affinity for this drug and is susceptible to chloretethylclonidine (CEC); these subtypes have similar affinities for prazosin. The relationship between α1H and α1L subtypes and α1A and α1B-subtypes in the rat brain, spleen and heart has not yet been clarified (1, 2). Muramatsu et al. (25) recently proposed that the α1-adrenoceptors in blood vessels can be divided into three subtypes (α1H, α1L, & α1N) by their antagonist affinity and susceptibility to CEC. The α1H-subtype has a high affinity for prazosin, susceptible to CEC and includes α1B-subtypes in the dog carotid artery and atypical α1A-subtypes in the rat aorta (26). The α1L-subtype has a low affinity for prazosin in the guinea pig aorta, and the α1N-subtype has a higher affinity for HV-723 and WB-4101 than for prazosin. On the other hand, Han and Minneman (27) classified the subtype in the rat aorta as α1B. Four subtypes (α1A, α1B, α1C, and α1D) have been cloned (28–31), and the α1B, α1C and α1D-subtypes are susceptible to CEC. These classifications (cloned α1A, α1B, α1C & α1D, α1A & α1B, and α1A & α1C) do not include the subtypes with low affinities for prazosin.

It was revealed that there was a good correlation between the pK_i values for the α1H-affinity sites and the pA_2 values in the rat aorta (r=0.90–0.94, P < 0.001). There was a significant but lower correlation (r=0.74–0.79, P < 0.01) between the pK_i values for the α1H-affinity sites and the pA_2 values in guinea pig aorta, as compared to that in the rat aorta. There was no correlation between the pK_i values for α1L-affinity sites and the pA_2 values in the rat and guinea pig aorta. Muramatsu et al. (25) demonstrated that the rat and guinea pig aorta contained α1H and α1L-adrenoceptor subtypes, respectively. The pA_2 values of prazosin for these α1H- and α1L-subtypes were 9.89 and 8.45, respectively. The α1H-subtype in the rat aorta has high affinity for WB-4101 and is sensitive to CEC (25, 26), and the α1L-subtype in the guinea pig aorta has low affinity for WB-4101 and is insensitive to CEC (25). The classification of α1H- and α1L-affinity sites is based on the different affinities for prazosin (1, 2), suggesting that the α1H- and α1L-subtypes may be identical to the α1H and α1L-subtypes. We also showed that the pA_2 values of prazosin for the subtypes of the rat and guinea pig aorta were 10.80 and 8.30, respectively (16). On the other hand, the present study showed that denitrornipradilol had a higher affinity for guinea pig aorta than rat aorta. Our results suggest that the α1H-affinity sites for [3H]-prazosin binding were similar to the vascular α1H (α1H)-subtypes, but that the α1L-affinity binding sites were different from the vascular α1L (α1L)-subtypes. On the other hand, for the α1H-affinity sites, the pK_i values of some compounds were not correlated with the pA_2 values in the rat aorta when the pK_i values of all compounds were compared with the pA_2 values. These observations suggest that α1-adrenoceptors in the rat and guinea pig aorta may be new types of α1H and α1L-affinity sites, respectively. Furthermore, the present findings imply that the α1H- and α1L-affinity sites are involved in vasoconstriction, while the α1L-affinity binding sites in the heart and brain were different from the α1L-subtypes in guinea pig aorta.

Recently, α1-β-blockers have been used for the treatment of hypertension or ischemic heart diseases. The affinities of α1-β-blockers, amosulalol, labetalol, arotinolol and pipradil for β-adrenoceptors were 0.6 to 170-, 3 to 90-, 1300 to 10000- and 1200 to 3000-fold higher than those for α1-adrenoceptor subtypes, respectively (5, 11). Amosulalol, labetalol and arotinolol had higher selectivity for α1H-affinity sites than α1L-affinity sites, while pipradil had similar affinities for α1H- and α1L-affinity sites. Thus pipradil has a unique pharmacological profile of selectivity towards the α1-adrenoceptor subtypes.

REFERENCES

1 Tsuchihashi, H., Maruyama, K., Baba, S., Mano, F., Kinami, J. and Nagatomo, T.: Comparison of α1-adrenoceptors between rat brain and spleen. Japan. J. Pharmacol. 56, 523–530 (1991)

2 Kinami, J., Tsuchihashi, H., Baba, S., Mano, F., Maruyama, K. and Nagatomo, T.: α1-Adrenoceptor subtypes in the rat ventricular muscle. J. Pharm. Pharmacol. 44, 97–100 (1992)
3 Shiratsuchi, M., Kawamura, K., Akashi, T., Ishihama, H. and Uchida, Y.: Synthesis and hypertensive and hypotensive activity of benzopyran derivatives. Chem. Pharm. Bull. (Tokyo) 35, 632–641 (1987)
4 Shiratsuchi, M., Kawamura, K., Akashi, T., Ishihama, H., Nakamura, M. and Takenaka, F.: Synthesis and activity of optical isomers of nipradilol. Chem. Pharm. Bull. (Tokyo) 35, 3691–3698 (1987)
5 Tsuchihashi, H., Nakashima, Y., Yokoyama, H., Kinami, J. and Nagatomo, T.: Assessments of nipradilol and its isomers by radioligand binding assay using [3H]-dihydroalprenolol and [3H]-CGP-12177 for $\beta_1$, $\beta_2$-adrenoceptors and 5HT$_{1B}$-serotonergic receptors. Asia Pacific J. Pharmacol. 5, 33–38 (1990)
6 Ebihara, A., Kondo, K. and Ohashi, K.: Pharmacodynamics and pharmacological effects of nipradilol (K-351) in healthy volunteers–Comparison with propranolol–. Rinryo Yakuri (Japan. J. Pharmacol. Ther.) 17, 391–401 (1986) (Abs. in English)
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
8 Tsuchihashi, H., Sasaki, M. and Nagatomo, T.: Binding characteristics of [3H]-dihydroalprenolol to $\beta$-adrenergic receptors of rat brain: Comparison with those of rat heart treated with neuraminidase. Chem. Pharm. Bull. (Tokyo) 33, 3972–3976 (1985)
9 Tsuchihashi, H. and Nagatomo, T.: Characterization of [3H]-dihydroalprenolol binding to $\beta$-adrenergic receptors of rat brain: Two binding sites of racemic propranolol in displacement experiments. Chem. Pharm. Bull. (Tokyo) 35, 2979–2984 (1987)
10 Tsuchihashi, H., Nagatomo, T. and Imai, S.: Three binding sites of [3H]-iodocyanopindolol, i.e. $\beta_1$, $\beta_2$-adrenergic and 5HT$_{1B}$-serotonergic receptors in rat brain determined by the displacement and Scatchard analysis. J. Pharmacobiodyn. 12, 509–516 (1989)
11 Tsuchihashi, H., Yokoyama, H. and Nagatomo, T.: Binding characteristics of [3H]-CGP12177 to $\beta$-adrenoceptors in rat myocardial membranes. Japan. J. Pharmacol. 49, 11–19 (1989)
12 Tsuchihashi, H., Nakashima, Y., Kinami, J. and Nagatomo, T.: Characteristics of [3H]-iodocyanopindolol to $\beta$-adrenergic and serotonin-1B receptors of rat brain: Selectivity of $\beta$-adrenergic agents. Japan. J. Pharmacol. 52, 195–200 (1990)
13 Nagatomo, T., Tsuchihashi, H., Sasaki, S., Nakagawa, Y., Nakahara, H. and Imai, S.: Displacement by $\alpha$-adrenergic agonists and antagonists of [3H]-prazosin bound to the $\alpha$-adrenoceptors of the dog aorta and the rat brain. Japan. J. Pharmacol. 37, 188–197 (1985)
14 Nakagawa, Y., Shimamoto, N., Nakazawa, M. and Imai, S.: Alpha- and beta-blocking activities of racemates of labetalol. Japan. J. Pharmacol. 30, 743–745 (1980)
15 Nagatomo, T., Tsuchihashi, H., Sasaki, M., Nakagawa, Y., Nakahara, H. and Imai, S.: Beta-receptor blocking potencies of the three newly synthesized $\beta$-adrenergic antagonists (S-596, K-351, N-696) as assessed with the radioligand binding assay method in cardiac muscle membrane treated with neuraminidase. Japan. J. Pharmacol. 34, 249–254 (1984)
16 Tsuchihashi, H., Aono, J., Nagatomo, T., Kawada, T., Ohta, H. and Imai, S.: Effects of bunitrolol on adrenergic and serotonergic receptors. Japan. J. Pharmacol. 45, 349–356 (1987)
17 Howe, R. and Shanks, R.G.: Optical isomers of propranolol. Nature 210, 1336–1338 (1966)
18 Weinstock, L.M., Mulvery, D.M. and Tull, R.: Synthesis of the $\beta$-adrenergic blocking agent timolol from optically active precursors. J. Org. Chem. 41, 3121–3125 (1976)
19 Dukes, M. and Smith, L.H.: $\beta$-Adrenergic blocking agents. 9. Absolute configuration of propranolol and a number of related aryloxypropanolamines and arylethanolamines. J. Med. Chem. 14, 326–328 (1971)
20 Danilewicz, J.C. and Kemp, J.E.G.: Absolute configuration by asymmetric synthesis of (+)-1-(4-acetamidophenoxy)-3-(isopropylamino)-propan-2-ol (practolol). J. Med. Chem. 16, 168–171 (1973)
21 Flavahan, N.A. and Vanhoutte, P.M.: $\alpha_1$-Adrenoceptor subclassification of vascular smooth muscle. Trends Pharmacol. Sci. 7, 347–349 (1986)
22 Morrow, A.L. and Creese, I.: Characterization of $\alpha_1$-adrenergic receptor subtypes in rat brain: A reevaluation of [3H]WB4101 and [3H]prazosin binding. Mol. Pharmacol. 29, 321–330 (1986)
23 Han, C., Abel, P.W. and Minneman, K.P.: $\alpha_1$-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca$^{2+}$ in smooth muscle. Nature 329, 333–335 (1987)
24 Han, C., Abel, P.W. and Minneman, K.P.: Heterogeneity of $\alpha_1$-adrenergic receptors revealed by chlorehylclonidine. Mol. Pharmacol. 32, 505–510 (1987)
25 Muramatsu, I., Ohmura, T., Kigoshi, S., Hashimoto, S. and Oshita, M.: Pharmacological subclassification of $\alpha_1$-adrenoceptors in vascular smooth muscle. Br. J. Pharmacol. 99, 197–201 (1990)
26 Muramatsu, I., Kigoshi, S. and Ohmura, T.: Subtypes of $\alpha_1$-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery. Japan. J. Pharmacol. 57, 535–544 (1991)
27 Han, C., Li, J. and Minneman, K.P.: Subtypes of $\alpha_1$-adrenoceptors in the rat blood vessels. Eur. J. Pharmacol. 190, 97–104 (1990)
28 Lomasney, J.W., Cotecchia, S., Lorenz, W., Leung, W.Y., Schwinn, D.A., Yang-Feng, T.L., Brownstein, M., Lefkowitz, R.J. and Caron, M.G.: Molecular cloning and expression of the cDNA for the $\alpha_1$-adrenergic receptor. The gene for which is located on human chromosome 5. J. Biol. Chem. 266, 6365–6369 (1991)
29 Cotecchia, S., Schwinn, D.A., Randall, R.H., Lefkowitz, R.J., Caron, M.G. and Kolbika, B.: Molecular cloning and expression of the cDNA for the hamster $\alpha_1$-adrenergic receptor. Proc. Natl. Acad. Sci. U.S.A. 85, 7159–7163 (1988)
30 Schwinn, D.A., Lomasney, J.W., Lorenz, W., Szkut, P.J., Fremeau, R.T., Yang-Feng, T.L., Caron, M.G., Lefkowitz, R.J. and Cotecchia, S.: Molecular cloning and expression of the cDNA for a novel $\alpha_1$-adrenergic receptor subtype. J. Biol. Chem. 265, 8183–8189 (1990)
31 Perez, D.M., Plascik, M.T. and Graham, R.M.: Solution-phase library screening for the identification of rare clones: Isolation of an $\alpha_1$-adrenergic receptor cDNA. Mol. Pharmacol. 40, 876–883 (1991)