Biochemical Pharmacology, Vol. 31, No. 9, pp. 1743–1747, 1982
Printed in Great Britain.

0006-2952/82/091743-05 $03.00/0

HOMOGENEOUS CLASS OF BETA-1 ADRENERGIC RECEPTORS IN RAT KIDNEY

IDENTIFICATION BY (±)-^{125}IODOCYANOPINDOLOL BINDING

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(*Received* 23 *September* 1981; *accepted* 29 *December* 1981)

Abstract—The highly specific β-adrenergic radio-ligand (±)-^{125}iodocyanopindolol (ICYP) was used to characterize the β-adrenergic receptor subtype present in rat kidney. Binding of ICYP to membranes from rat kidney was of high affinity (K_D = 68.9 pM) and saturable with 1.06 pmoles ICYP bound/g tissue wet wt at maximal occupancy of the sites. Analysis of inhibition of ICYP binding by β_1- and β_2-selective adrenergic drugs via pseudo-Scatchard ('Hofstee') plots resulted in linear plots indicating the existence of a homogeneous population of β-adrenergic receptors. From the resulting K_D-values for practolol (2.2 μM), metoprolol (0.21 μM), zinterol (0.4 μM) and IPS 339 (0·046 μM) it is concluded that the β-adrenergic receptor in rat kidney is of the β_1-subtype. This subclassification is further supported by the fact that (−)-noradrenaline and (−)-adrenaline were equipotent in inhibiting ICYP binding. The β-adrenergic agonists (−)-isoprenaline and zinterol bind to two distinct states of this β_1-receptor, a high and low affinity state. GTP (10^{-4} M) converts this heterogeneous binding into a homogeneous low affinity binding.

Three important mechanisms are involved in the secretion of renin from the kidney (for review see [1]). The first, the renal vascular receptor (baroreceptor) mechanism responds to changes in wall tension in the afferent arteriole. The second, the macula densa mechanism is dependent on changes in the rate of sodium and/or chloride excretion in the distale tubule. The third mechanism is mediated by stimulation of β-adrenergic receptors on the juxtaglomerular cells.

β-Adrenergic receptors can be subdivided into β_1- and β_2-subtypes with β_1-receptors mediating positive inotropic responses in the heart and β_2-receptors subserving broncho- and vasodilation [2, 3]. In order to identify the β-adrenergic receptor subtype controlling renin release, in the present study the properties of the intrarenal β-adrenergic receptor were characterized in membranes from rat kidney by binding studies with the highly specific β-adrenergic radio-ligand (±)-^{125}iodocyanopindolol (ICYP) [4, 5]. For this purpose inhibition of binding by β_1-selective (practolol, metoprolol) and β_2-selective (zinterol, IPS 339) adrenergic drugs was analyzed via pseudo-Scatchard ('Hofstee') plots [6, 7] in order to find out whether the β-adrenergic receptor present in rat kidney is of the β_1- and/or β_2-subtype.

MATERIALS AND METHODS

Membrane preparation. Male Wistar rats (300–350 g) were killed by decapitation. The kidneys were rapidly excised, cleaned of connective tissue and homogenized in 20 vol. ice-cold 0.25 M sucrose containing 5 mM Tris–HCl, 1 mM $MgCl_2$ pH 7.5 by the use of an Ultra-Turrax (Jahnke and Kunkel, Staufen, West Germany) for 30 sec. The homogenate was centrifuged at 45,000 g × 30 min at 4°. The resulting pellets were washed twice with ice-cold incubation buffer (50 mM Tris–HCl, 10 mM $MgCl_2$ pH 7.5) by resuspension and recentrifugation and finally resuspended in 60 vol. incubation buffer.

Binding assay. (±)-Cyanopindolol was iodinated with ^{125}I and ICYP was purified as recently described [4] to the theoretical activity of 2175 Ci/mmole. ICYP and all drugs investigated in this study were prepared in 50 mM Tris–HCl, 10 mM $MgCl_2$ buffer pH 7.5. An aliquot of the membrane suspension (150 μl) was incubated with ICYP in a final volume of 250 μl. Incubations were carried out for 60 min at 37° and terminated by adding 10 ml of incubation buffer (37°) to the entire incubation mixture followed by rapid filtration over Gelman AE glass fiber filters. Each filter was washed with additional 10 ml of incubation buffer (37°). The radioactivity of the wet filters was determined in a Gamma counter (Beckman Gamma 4000) at an efficiency of 80%. 'Non-specific' binding of ICYP was defined as radioactivity bound to membranes which is not displaced by a high concentration of (−)-propranolol (1 μM). 'Specific' binding of ICYP is defined as total radioactivity minus non-specific binding and amounted to 80% (at 10–80 pM) and 75% (at 150 pM) of ICYP.

For determination of the number of β-adrenergic receptors in membranes from rat kidney the amount of specific ICYP bound was determined at 8 different concentrations of ICYP ranging from 10–200 pM. In order to determine the potency of β-adrenergic drugs in inhibiting binding ICYP (40,000–60,000 cpm; 40–60 pM) was incubated with 9–10 different concentrations of the competing agent and specific binding was determined as described above.

Statistical evaluations. The experimental data given in text and figures are means ± S.E.M. of n experiments. The regression lines were calculated by the least squares method. The equilibrium dissociation constant (K_D) and the maximal number of binding sites (B_{max}) were calculated from plots according to Scatchard [8].

Analysis of inhibition of ICYP binding by β_1- and β_2-selective adrenergic drugs was performed as previously described [6, 7]. For this purpose the concentration–inhibition curves were transformed into Hofstee plots, i.e. plotting % inhibition of binding vs % inhibition divided by the concentration of the competing agent. Under these conditions non-linear Hofstee plots are obtained, if both β_1- and β_2-receptors coexist in the organ investigated, while linear plots are obtained in organs with a homogeneous population of β-adrenoceptors [6, 7, 9, 10]. From the Hofstee plots EC_{50}-values were calculated, which were finally transformed into K_D-values according to the equation of Cheng and Prusoff [11]:

$$K_D = \frac{EC_{50}}{S/K_m + 1}$$

where EC_{50} is the concentration of the competing agent which inhibits ICYP binding by 50%, S is the concentration of ICYP present in the assay and $K_m = K_D$ for ICYP binding determined from saturation experiments (68.9 pM; cf. Fig. 1).

(±)-Cyanopindolol was synthesized in the chemical laboratories of Sandoz-Ltd. (Basle, Switzerland) as described by Berthold [12]. All other drugs used in this study were from sources recently described [5].

RESULTS

Number of β-adrenergic receptors in rat kidney membranes

Specific binding of ICYP to membranes from rat kidney increased with increasing ICYP concentrations ranging from 10 to 200 pM (Fig. 1). Complete saturation was obtained between 100 and 150 pM. Scatchard analysis of these binding data (Fig. 1, inset) resulted in a single line suggesting the existence of one class of binding sites. From these plots a K_D-value of 68.9 ± 3.1 pM (n = 6) and a maximal number of binding sites of 1.06 pmoles ICYP bound/g tissue wet wt were calculated.

Kinetics of ICYP binding to rat kidney membranes

In order to determine optimal conditions for the binding assay, the time-dependent association and dissociation from rat kidney β-adrenergic receptors of ICYP was studied (Fig. 2). Binding of ICYP at 37° reached equilibrium between 30 and 60 min with a $t_{1/2}$ of about 10 min and remained stable for a further two hours. When equilibrium was reached, addition of a high concentration of (−)-propranolol (10 μM) led to a dissociation of ICYP from the binding sites. The dissociation reaction, however, was biphasic as already shown for ICYP binding to guinea-pig lung [4] and left ventricle membranes [13].

Characterization of the β-adrenergic receptor in rat kidney membranes

For characterization of the β-adrenergic receptor subtype present in rat kidney inhibition of specific ICYP binding by nonselective (propranolol, iso-

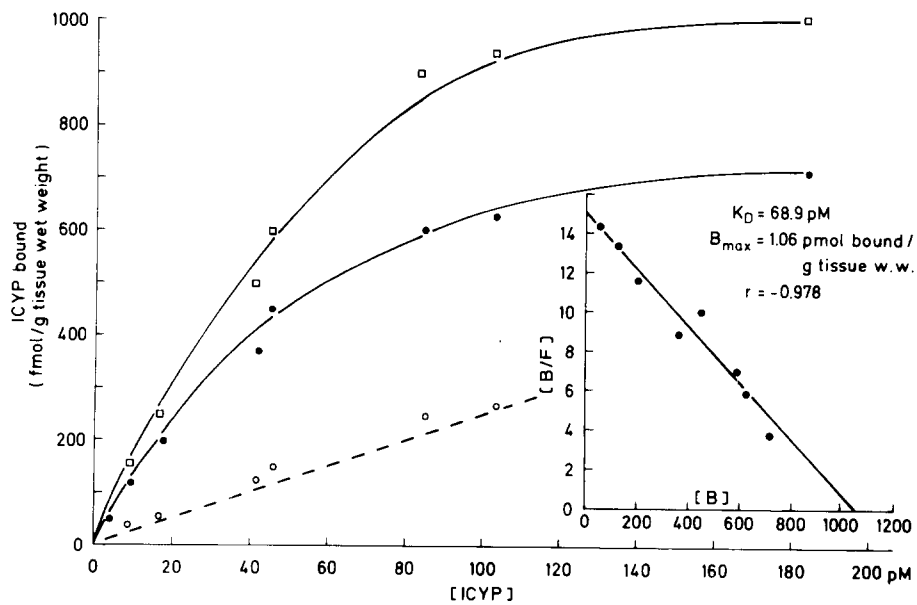

Fig. 1. Binding of ICYP to membranes from rat kidney as function of increasing ICYP concentrations. Binding was carried out as described in Materials and Methods at various concentrations of ICYP ranging from 10 to 200 pM in the absence (□——□) and presence (○- - -○) of 1 μM (−)-propranolol to determine specific binding (●——●). Ordinate: ICYP bound (fmoles/g tissue wet wt); abscissa: free ICYP concentration (pM). Inset: Scatchard plot of specific ICYP binding. The ratio B/F of specifically bound ICYP (fmoles/g tissue wet wt) to free ICYP (pM) is plotted as function of B = specifically bound ICYP. Each value is the mean of 6 experiments with S.E.M. < 4%.

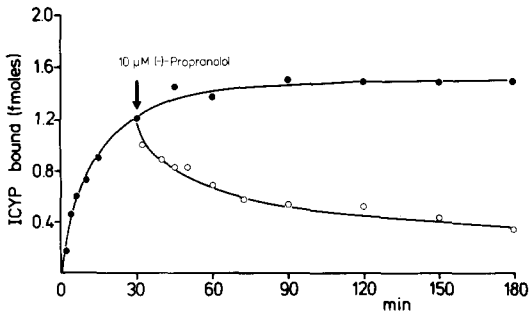

Fig. 2. Forward and reverse kinetics of ICYP binding to rat kidney membranes. Membranes (1.4 mg/ml) and ICYP (62 pM) were incubated in the presence or absence of 10^{-6} M (−)-propranolol at 37° (●——●). At the indicated times 250 μl in triplicates were removed and specific binding was determined as described in Materials and Methods. After 30 min of incubation (−)-propranolol was added to an aliquot of the original incubation solution to a final concn of 7×10^{-6} M and specific binding was determined at subsequent time intervals after the addition of (−)-propranolol (○——○). Each value is the mean of 3 experiments with S.E.M. < 3%.

prenaline), β_1-selective (practolol, metoprolol) and β_2-selective (zinterol, IPS 339) drugs was determined. The concentration–inhibition curves are shown in Fig. 3. Binding of ICYP to rat kidney membranes was stereospecific as indicated by the 100-times greater potency of (−)-isoprenaline and (−)-propranolol in inhibiting binding than their respective (+)-isomers. In addition, phentolamine (up to 10^{-4} M) and serotonin (up to 10^{-5} M) had no effects on ICYP binding, while both agents have been shown to drastically reduce specific binding of ^{125}iodohydroxybenzylpindolol [4, 6].

β-Adrenergic agonists inhibited binding with an order of potency: (−)-isoprenaline > (−)-noradrenaline = (−)-adrenaline ⩾ (+)-isoprenaline (Fig. 3(B)), which is typical for β_1-adrenergic receptors [2, 3].

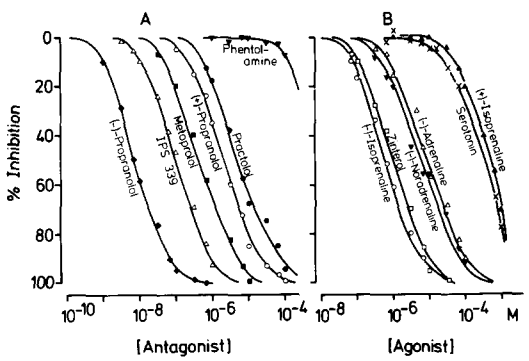

Fig. 3. Inhibition of specific ICYP binding by β-adrenergic antagonists (A) and agonists (B). Rat kidney membranes were incubated with ICYP (40–60 pM) in the presence or absence of 9–10 concentrations of the indicated agents and specific binding was determined as described in Materials and Methods. For inhibition of binding by the agonists isoprenaline, (−)-adrenaline, (−)-noradrenaline and zinterol GTP (10^{-4} M) was included into the assay. '100%' inhibition refers to inhibition of specific binding by 1 μM (−)-propranolol. Each value is the mean of 4 experiments with S.E.M. < 3%.

Transformation of the competition curves into Hofstee plots (Fig. 4) resulted for all β-adrenergic drugs investigated in linear plots suggesting the existence of a homogeneous population of β-adrenergic receptors in rat kidney. From these plots K_D-values for the β_1- and β_2-selective drugs practolol (2.2 μM), metoprolol (0.21 μM), zinterol (0.4 μM) and IPS 339 (0.046 μM) were calculated, which strongly support the view that rat kidneys contain a homogeneous class of β_1-adrenergic receptors.

Influence of GTP (10^{-4} M) on agonist binding in rat kidney membranes

It has been recently reported [14, 15] that in the absence of guanyl nucleotides β-adrenergic agonists display 'shallow' displacement curves at β_1- and β_2-adrenergic receptors with apparent negative cooperativity resulting in non-linear Hofstee plots. Similar effects were observed for the β_1-adrenergic receptor in rat kidney. In the absence of GTP inhibition of ICYP binding by the β-receptor agonists (−)-isoprenaline and zinterol resulted in 'shallow' displacement curves with non-linear Hofstee plots (Fig. 5). After addition of GTP (10^{-4} M) to the incubation medium the curves were shifted to the right and became steeper. Now linear Hofstee plots were obtained. From the non-linear Hofstee plots in the absence of GTP the affinity constants for high (K_H) and low (K_L) affinity state of the receptor were roughly estimated. They amounted for isoprenaline to: K_H = 24 nM, K_L = 500 nM and for zinterol to: K_H = 19.2 nM, K_L = 340 nM. The K_L-values were in good agreement with the K_D-values determined in the presence of GTP (isoprenaline 306 nM, zinterol 395 nM) indicating that GTP mediates the transition from the high to the low affinity state.

DISCUSSION

It has been recently demonstrated in a variety of tissues that inhibition of binding of a non-selective β-adrenergic radioligand (^{125}iodohydroxybenzylpindolol or (−)-[^{3}H]dihydroalprenolol) by β_1- or β_2-selective adrenergic drugs resulted in linear Hofstee plots if only one β-receptor subtype exists in the organ investigated, while in organs where both β_1- and β_2-adrenergic receptors coexist always non-linear Hofstee plots were obtained [4–7, 9, 10, 16–18]. In the present study inhibition of binding of the non-selective β-adrenergic radioligand ICYP [4] by practolol, metoprolol (β_1-selective), zinterol (in the presence of 10^{-4} M GTP), IPS 339 (β_2-selective) resulted in linear Hofstee plots. These results strongly support the view that in rat kidney a homogeneous population of β-adrenergic receptors exists. The affinities of practolol, metoprolol, zinterol and IPS 339 (expressed as K_D-values, cf. Fig. 4) for the β-adrenergic receptor in rat kidney are in close agreement with those recently determined for the same drugs for β_1-adrenergic receptors either in organs with a homogeneous population of β_1-receptors (left ventricle of guinea-pig and cat [18]) or in tissues with a heterogeneous β-receptor population [6, 17]. Furthermore, (−)-noradrenaline and (−)-adrenaline were equipotent in inhibiting ICYP binding (cf. Fig. 3(B)), which is a typical characteristic of β_1-

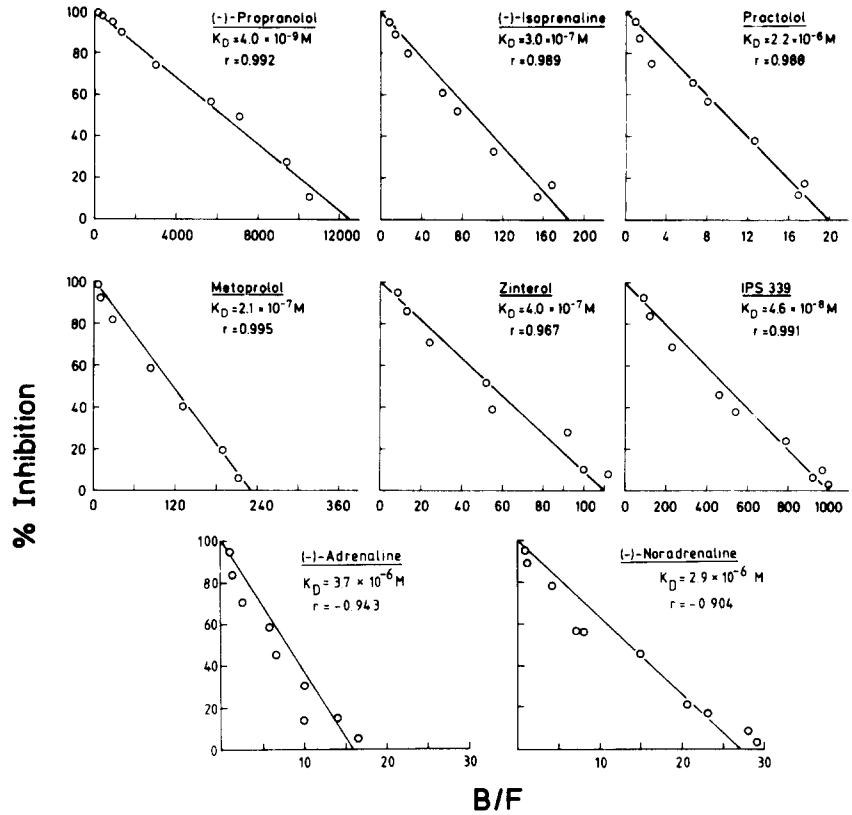

Fig. 4. Hofstee plots for inhibition of specific ICYP binding by β-adrenergic drugs in membranes from rat kidney. Effects of (−)-propranolol, (−)-isoprenaline, practolol, metoprolol, zinterol, IPS 339, (−)-adrenaline and (−)-noradrenaline are shown. Ordinates: inhibition of specific ICYP binding in %; abscissae: B/F = inhibition of binding (%) divided by the concentrations of the drugs (micromolar). Each value is the mean of 4 experiments with S.E.M. < 3%.

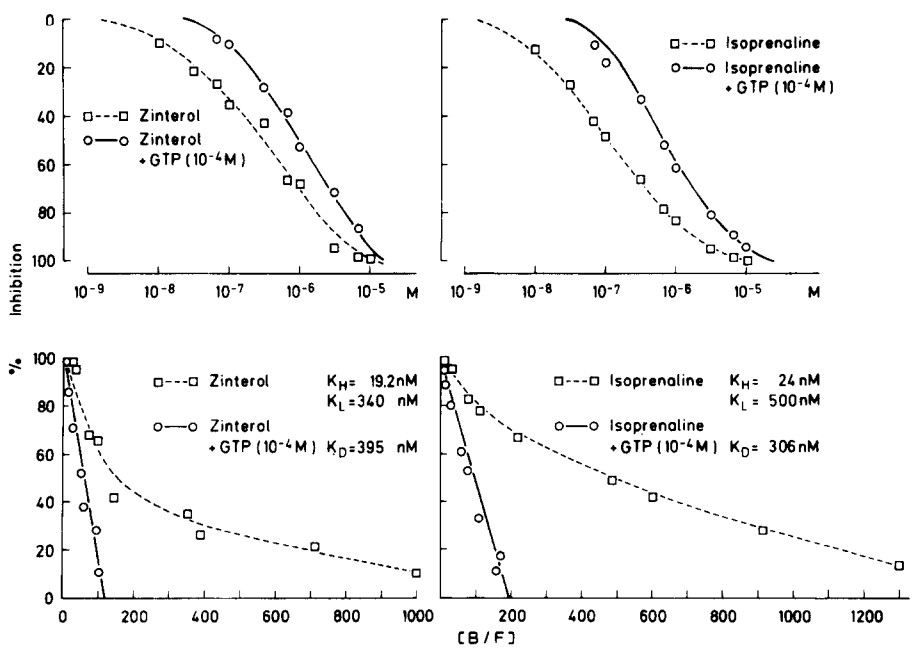

Fig. 5. Upper part: Influence of GTP (10^{-4} M) on inhibition of specific ICYP binding to membranes from rat kidney by zinterol (left) and (−)-isoprenaline (right). Inhibition of binding was performed in the absence (□----□) and presence (○——○) of 10^{-4} M GTP. For details see legend to Fig. 3. Lower part: Hofstee plots for inhibition of specific ICYP binding by zinterol (left) or (−)-isoprenaline (right) in the absence (□----□) or presence (○——○) of GTP (10^{-4} M). For details see legend to Fig. 4. Each value is the mean of 4 experiments with S.E.M. < 3%.

adrenergic receptors [2, 3]. Thus, it is concluded that the β-adrenergic receptor present in rat kidney is of the β_1-subtype.

In the present study the racemic radioligand (±)-ICYP was used to label the β-adrenergic receptor in rat kidney. It has been recently demonstrated that saturation binding curves with racemic radioligands like (±)-^{125}iodohydroxybenzylpindolol or (±)-[^{3}H]carazolol show deviation from a binding isotherm for a single ligand, which were more pronounced at high receptor concentrations [19]. However, this problem does not hold true for (±)-ICYP since it has been clearly shown that the contribution of the (+)-enantiomer in the binding of this racemic ligand could be neglected under low receptor concentrations [13].

The demonstration of a homogeneous class of β_1-adrenoceptors is consistent with the view that renin release from rat kidney may be controlled by β_1-adrenergic receptors [1]. Previous reports have shown that in anaesthetized rats as well as in cortical slices from rat kidney the isoprenaline induced renin release could be prevented by β_1-adrenergic antagonists like atenolol or metoprolol [20, 21], but not by β_2-adrenergic antagonists like IPS 339 [21] or butoxamine [20, 22]. Taking these results obtained by physiological studies and the present results obtained by biochemical studies into consideration it seems to be justified to conclude that the adrenergic nerve-stimulated release of renin in rat kidney is mediated by β_1-adrenergic receptors.

Finally, the present results show that in rat kidney β-adrenergic agonists bind to two distinct states of the β-receptor, a high affinity, guanyl nucleotides sensitive, and a low affinity, guanyl nucleotides insensitive, state, as recently demonstrated for the β-adrenoceptor in frog erythrocytes [15]. In the absence of GTP inhibition of ICYP binding by the nonselective β-receptor agonist isoprenaline and by the β_2-selective agonist zinterol resulted in 'shallow' displacement curves and non-linear Hofstee plots indicating the existence of a high and low affinity state of the receptor. In the presence of 10^{-4} M GTP, however, the concentration–inhibition curves were shifted to the right and became steeper. The K_D-values for isoprenaline (306 nM) and zinterol (395 nM) calculated from the resulting linear Hofstee plots were in the same range as the affinity constants for both agonists for the low affinity state (K_L isoprenaline 500 nM; K_L zinterol 340 nM). In agreement with recently reported data on frog erythrocytes [15], these results demonstrate that guanyl nucleotides can mediate the conversion of the high affinity into a homogeneous low affinity binding for agonists.

Acknowledgements—The author thanks Dr. G. Engel (Sandoz Ltd., Basle, Switzerland) for valuable information on the iodination procedure for CYP. The skilful technical assistance of Miss R. Morawietz, Mrs. B. Surbach and Mr. M. Krüger is gratefully acknowledged. This work was supported by the Landesamt für Forschung Nordrhein-Westfalen and the Sandoz-Stiftung für Therapeutische Forschung.

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