Does treatment with beta-adrenergic blocking agents cause a decrease in beta₂-adrenoceptor affinity?

W. M. Blankestijn¹, S. J. Graafsma¹, M. P. C. Hectors¹, E. A. M. Olde Riekerink¹, J. F. Rodrigues de Miranda², and Th. Thien¹

¹ Department of General Internal Medicine, St Radboud University Hospital and ² Department of Pharmacology, University of Nijmegen, Nijmegen, The Netherlands

Received: July 22, 1991/Accepted in revised form: December 23, 1991

Summary. The effect of β-adrenoceptor antagonists (BAAs) differing in lipophilicity and partial agonist activity (PAA), and a full agonist, on the dissociation constant for [125I](-)-iodocyanopindolol binding to β₂-adrenoceptors (K_D) has been investigated.

Twelve healthy, normotensive male volunteers (mean age 22.3 y) were treated with different BAAs according to a cross-over design. The drugs used were propranolol (highly lipophilic BAA, no PAA), pindolol (moderately lipophilic BAA, strong PAA), dilevalol (highly lipophilic BAA, weak PAA) and salbutamol (full agonist). Before and after a single dose and an 8 day course of one of the drugs, blood pressure and the β₂-adrenoceptor characteristics of mononuclear leukocytes (MNL) were determined. Between the treatment periods, there was a wash-out interval of 14 days.

All BAAs decreased the blood pressure, but only propranolol lowered heart rate. Treatment with salbutamol decreased the diastolic and increased the systolic blood pressure and heart rate. Three hours after the single dose of any of the BAAs, a more than 2-fold increase in K_D was observed, and the increase became larger after 8 days of administration (up to 3.7-fold increase). In contrast, no effect on K_D was observed after treatment with salbutamol. BAAs with PAA and salbutamol induced a 30 % decrease in β₂-adrenoceptor density.

It is concluded that treatment with BAAs, irrespective of their lipophilicity or PAA, induces a decrease in the affinity of MNL β₂-adrenoceptors for antagonists. This phenomenon may help to explain the contradictory relationship between the kinetics and dynamics of BAAs.

Key words: Beta-adrenoceptor blocking agents; salbutamol, propranolol, pindolol, dilevalol, mononuclear leukocytes, β₂-adrenoceptor affinity, β₂-adrenoceptor density
ceceptor characteristics were studied. PRO was used as a highly lipophilic drug without PAA, whereas the moderately lipophilic PIN was used as a compound with strong PAA [1, 9]. Dilevalol (DIL), the R,R isomer of labetalol, was used as a highly lipophilic BAA with a weak PAA for β2-adrenoceptors [10-12]. Salbutamol (SAL) was also tested, in order to investigate the effect of a full agonist on β2-adrenoceptor characteristics. The effect of trapping of BAA in intact MNL [4], as a contributory factor to the changes in KD, was studied by comparing the β2-adrenoceptor characteristics of intact and broken MNL after one week of PIN treatment.

Materials and methods

Materials

[125I]-(−)-Iodocyanopindolol ([125I]-CYP) was purchased from Amersham International (± 1850 Ci/mmol). CGP-12177 was generously supplied by Dr. M. Staehelin, Ciba Geigy. All other reagents were of analytical grade.

Subjects and treatments

Twelve male, non-smoking normotensive volunteers (age 22.3 (2.3) y) were studied. All subjects gave their written informed consent and the study was approved by the hospital Ethics Committee. The subjects were asked to abstain from beverages containing caffeine or alcohol for 12 h before each test. The part of the study involving the BAAs was performed double blind, whereas the effect of the β2-adrenoceptor agonist salbutamol (SAL, 4 mg t.d.s.) was monitored at the end of the study in an open design. The BAAs were administered orally in equipotent doses [13, 14, 15]: propranolol sustained release (PRO) 160 mg o.d., pindolol sustained release (PIN) 20 mg o.d. and dilevalol (DIL) 400 mg o.d. The volunteers were randomised into 3 groups according to a Latin square method. Since all subjects were tested before and after each of the 4 treatment periods, they acted as their own controls and a placebo treatment period was not necessary.

On Day 1, volunteers visited the outpatient clinic in the morning. After cannulating an antecubital vein in the left arm, the subject rested supine for at least 15 min, followed by measurement of the blood pressure with a Hawksley Random Zero sphygmomanometer and heart rate registration, both in triplicate. Thereafter, a 40 ml blood sample was collected in heparinised tubes for determination of BAA in the morning. On Day 8, before intake of the BAA, the part of the study was approved by the hospital Ethics Committee. Twelve male, non-smoking normotensive volunteers (age 22.3 (2.3) y) were studied. All subjects gave their written informed consent and the study was approved by the hospital Ethics Committee. The subjects were asked to abstain from beverages containing caffeine or alcohol for 12 h before each test. The part of the study involving the BAAs was performed double blind, whereas the effect of the β2-adrenoceptor agonist salbutamol (SAL, 4 mg t.d.s.) was monitored at the end of the study in an open design. The BAAs were administered orally in equipotent doses [13, 14, 15]: propranolol sustained release (PRO) 160 mg o.d., pindolol sustained release (PIN) 20 mg o.d. and dilevalol (DIL) 400 mg o.d. The volunteers were randomised into 3 groups according to a Latin square method. Since all subjects were tested before and after each of the 4 treatment periods, they acted as their own controls and a placebo treatment period was not necessary.

On Day 1, volunteers visited the outpatient clinic in the morning. After cannulating an antecubital vein in the left arm, the subject rested supine for at least 15 min, followed by measurement of the blood pressure with a Hawksley Random Zero sphygmomanometer and heart rate registration, both in triplicate. Thereafter, a 40 ml blood sample was collected in heparinised tubes for determination of the baseline β2-adrenoceptor characteristics. Then, the first dose of BAA was given. The subjects were allowed to move freely for 3 h, after which the same protocol was followed, and a second blood sample was drawn.

On the following days (Day 2 to 7), the volunteers took the dose of BAA in the morning. On Day 8, before intake of the BAA, the volunteers again visited the outpatient clinic and blood samples were taken before and after drug administration, in the same sequence as on Day 1. Between the treatment periods, a wash-out interval of 2 weeks was maintained, resulting in a complete study period of 10 weeks.

Isolation of mononuclear leukocytes

For the measurement of β2-adrenoceptor characteristics, mononuclear leukocytes (MNL) were isolated from the blood samples [16]. Fresh whole blood was diluted with an equal volume of phosphate buffered saline (PBS, pH 7.4) containing 2.7 mM KCl, 7.7 mM NaH2PO4, 1.5 mM KH2PO4, and 150 mM NaCl, and layered on Ficoll Paque (Pharmacia Fine Chemicals, Sweden), in the proportion of 5:2, in Sarstedt tubes (25 ml). Tubes were centrifuged at 800 × g for 15 min at room temperature. The MNL were harvested in Falcon tubes and washed 3 times with PBS at 4°C. After the last washing step, the cells were suspended in incubation buffer, containing 10 mM Tris pH 7.2, 150 mM NaCl, 0.55 mM ascorbic acid and 0.1% bovine serum albumin to prevent cell adhesion. Cell density was determined with a Coulter Counter (Coulter Electronics, Holland) [17].

Comparison of intact and broken MNL

In a separate group of 7 healthy, normotensive subjects the effect of 8 days of treatment with PIN retard (20 mg o.d.) on the characteristics of the β2-adrenoceptors of intact and broken MNL were compared. The same administration protocol as for the double blind test was used. Blood was sampled before the first (Day 1, t = 0 h) and 24 h after the last (Day 8, t = 0 h) dose of PIN, and MNL were isolated as described above. For the preparation of broken cells, a modification of the method of Sandnes et al. [18] was used. Half of the MNL were lysed in an ice-cold hypotonic buffer (5 mM Tris, pH 7.4 with 0.4 mM MgCl2 and 3 mM KCl) for 5 min. The suspension was centrifuged in an MSE Hispin 21 centrifuge at 25,000 × g, for 15 min at 4°C. The supernatant was discarded and the remaining pellet was resuspended in a buffer containing 50 mM Tris, pH 7.4 with 4 mM MgCl2 and 30 mM KCl and again centrifuged. The supernatant was discarded and the pellet, resuspended in incubation buffer for the radioligand binding assay, was homogenised with 10 strokes of a tight fitting tissue grinder (Kontes, Vineland NJ, USA) on ice. The other half of the MNL was washed twice in PBS for 20 min, and harvested by centrifugation, bringing the total number of washings to 5. The additional washings were done to obtain comparable dissociation conditions for any possible retained BAA in the intact and broken MNL. The β2-adrenoceptor characteristics of the matching preparations of intact and broken MNL were always determined in the same radioligand binding experiment.

Radioligand binding assay

Receptor binding assays were performed as previously described [17]. Briefly, samples of approximately 106 cells were incubated with various concentrations of [125I]~-CYE ranging from 2-150 pM, in the presence or absence of 1 μM CGP-12177. After terminating the incubation by addition of 3 ml ice-cold buffer (10 mM Tris pH 7.4 and 150 mM NaCl), the samples were rapidly filtered over Whatman GF/C glass fibre filters. After rinsing the filters three-times, the bound activity was counted in a Philips PW 4800 automatic gamma counter (efficiency 66%). Non-specific binding was defined as radioactivity bound in the presence of 1 μM CGP-12177.

Analysis of data

Data are presented as mean with (SEM) unless indicated otherwise. Receptor density and equilibrium dissociation constant (Kd) were calculated by subjecting the binding data to analysis by the LIGAND nonlinear least square curve fitting program [19]. Statistical analysis of the results was performed using Student’s t-test for paired samples or Wilcoxon’s signed rank test, as appropriate, considering P < 0.05 (two-sided) as significant.

Results

Evaluation of the wash-out intervals

In this study, wash-out intervals of 14 days were maintained between each BAA treatment. Blood pressure (Table 1), heart rate (Table 2) and β2-adrenoceptor characteristics (Fig. 1 and 2) before each of the four treatment