Binding of ^{3}H-dihydroalprenolol to beta-adrenoceptors in adipocytes of spontaneously hypertensive rats and essentially hypertensive patients

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Summary. An increase in the maximal number of ^{3}H-dihydroalprenolol binding sites and a decrease in the affinity have been demonstrated in adipocyte membranes from rats with spontaneous hypertension and from essentially hypertensive patients.

It has been recently demonstrated that in spontaneously hypertensive adrenalectomized rats the lipolytic response of adipocytes to adrenaline was higher than that in normotensive adrenalectomized rats[3]. Since adrenaline acts through adrenergic receptors localized in the plasma membrane, one may assume that the difference in the response is likely to be due to an alteration of the adrenaline-receptor interaction.

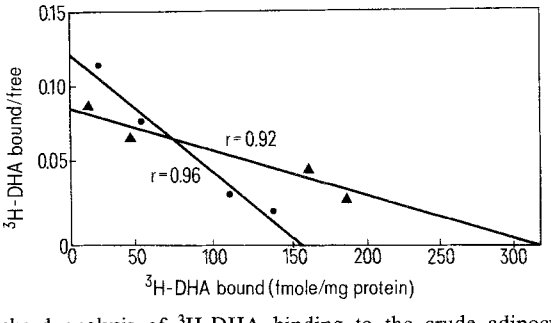
In the present study we investigated the binding between β-adrenergic antagonist ^{3}H-dihydroalprenolol (^{3}H-DHA) and adipocyte plasma membranes from spontaneously hypertensive rats and essentially hypertensive patients.

Material and methods. 1. 10–12-week-old spontaneously hypertensive male rats (SHR, Kyoto Wistar) 140 g, with a blood pressure of 180±10 mm Hg were used in the experiment. Inbred male Wistar rats of the same age, with a blood pressure of 80±10 mm Hg served as a control group. Systolic blood pressure was measured without anesthesia by tail plethysmography.

2. Pieces of adipose tissue from the omentum major weighing not more than 15 g were obtained during surgery in patients subjected to cholecystectomy because of chronic calculous cholecystitis. Some of the patients suffered from essential hypertension as well as the disease which had necessitated the surgical intervention (6 females of 30–60 years, blood pressure of 200–150/130–90 mm Hg, duration of disease 10–15 years), others had a normal arterial pressure (6 females of the same age). None of the patients exhibited cardiovascular or renal insufficiency, and no antihypertensive drugs had been systematically administered. The same intratracheal narcosis (N_2O+O_2) was used in all operations.

The epididymal fat pads of rats starved for 24 h prior to sacrifice with free access to water were removed after decapitation, washed in a saline solution and weighed. Adipose tissue of a human omentum major was also washed, weighed and cut into small pieces (300–500 mg).

Fat cells were obtained by treating the adipose tissue with collagenase (3 mg/ml) (Worthington Bioch. Corp., USA), according to Rodbell[4], in Krebs-Ringer phosphate buffer, pH 7.4, with the addition of 2% bovine serum albumin (fraction V, Serva, FRG), 3 ml of the solution per 1 g of tissue. The crude membrane fraction was prepared according to Williams[5]. Isolated fat cells were washed twice in Krebs-Ringer phosphate buffer with the addition of 2% bovine serum albumin, pH 7.4, and twice in a homogenization medium of the following composition: sucrose, 0.25 M; EDTA, 1 mM, Tris HCl, 10 mM, pH 7.5 (medium 1). Fat cells were homogenized in medium 1 (1:2) at room temperature in a glass-teflon homogenizer with 12 motions of a pestle. After centrifugation at 15,000×g for 15 min at 4 °C, the pellet was resuspended in the homogenization medium and nuclei were removed by centrifuging at 1500×g for 10 min. The supernatant was again centrifuged at 15,000×g for 15 min. The pellet was twice washed in medium 2 (Tris-HCl, 50 mM; pH 8.0, and $MgCl_2$, 10 mM). The plasma membrane fraction was resuspended in medium 3 (Tris-HCl, 50 mM; pH 7.4, $MgCl_2$, 10 mM). Protein measured by the Lowry et al. method[6] was 1–2 mg/ml. (−) ^{3}H-dihydroalprenolol (^{3}H-DHA, sp. act. 59 Ci/mmole, Amersham, England) was bound with plasma membranes in 0.6 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 10 mM of $MgCl_2$ and 0.4–25 nM of ^{3}H-DHA. The reaction was initiated by the addition of 100–200 µg of plasma membrane protein. After incubation for 10 min at 37 °C, 0.5 ml of the reacting mixture was rapidly filtered in vacuo through Whatman GF/C filters. The filters were washed with 4×4 ml of cold incubation buffer, put into vials for counting and dried overnight at room temperature. Then 10 ml of Triton X-100-toluene (1:2) mixture containing PPO and POPOP was added, and radioactivity was determined in a liquid scintillation counter (SL 4202 – Intertechnique, France). Nonspecific binding was determined by measuring the radioactivity retained on filters when incubations were carried out in the presence of 10 µM (±) propranolol (Sigma, USA). Specific binding averaged 60–70% of the total. The results of the experiment are presented as mean values±SE. The significance of the



Scatchard analysis of ^{3}H-DHA binding to the crude adipocyte membranes from hypertensive (▲) and normotensive (●) rats. Each point is the mean of 12 determinations from 2 separate experiments.

Maximal number and affinity of ^{3}H-DHA binding sites in adipocyte membranes from hypertensive rats and patients with essential hypertension

Group	Maximal number of binding sites fmole/mg of protein	K_D (nM)
Rats with spontaneous hypertension	317±39	3.73±0.76
Normotensive rats	155±9	1.21±0.17
p	<0.001	<0.001
Patients with essential hypertension n=6	1780±160	8.98±1.1
Normotensive patients n=6	1080±240	4.72±1.44
p	<0.05	<0.05

n, number of patients.

difference between general mean values was assessed by Student's t-test. The difference was considered significant at $p < 0.05$.

Results and discussion. ^{3}H-DHA, a potent β-adrenergic antagonist, has been used to identify β-adrenergic receptors in adipocyte membrane preparations[5]. By using these direct binding methods, the number and affinity (K_D) of ^{3}H-DHA-binding sites in adipocyte membranes from SHR and essentially hypertensive patients and from controls were assessed by regression analysis of Scatchard plots[7]. As shown in the figure, the maximal number of ^{3}H-DHA binding sites in adipocyte membranes from SHR (317 fmoles/mg of protein) was 2-fold more than in membranes from control rats (155 fmoles/mg of protein) ($p < 0.001$). The dissociation constant of ^{3}H-DHA binding in membranes from SHR was 3-fold more than in control membranes. As shown in the table, the maximal number of ^{3}H-DHA binding sites in plasma membranes from hypertensive patients is 1780 fmoles/mg of protein; this exceeds the corresponding value in the normotensive control group (1080 fmoles/mg of protein) by 1.65-fold ($p < 0.05$). K_D in the case of hypertensive patients (8.98 nM) is 2-fold higher than the control dissociation constant (4.72 nM) ($p < 0.05$). Thus, an increase in the maximal binding sites and a decrease in the affinity of β-adrenoceptor to the β-adrenergic antagonist were observed both in hypertensive patients and in SHR. The decrease mentioned above in β-adrenoceptor affinity to ^{3}H-DHA is evidently due to membrane alterations in adipocytes from hypertensive rats and patients. This suggestion is confirmed by the finding of alterations in calcium binding with adipocyte plasma membranes from SHR and hypertensive patients[8]. Earlier it was reported that the erythrocyte and smooth muscle membranes are altered in these types of hypertension[9-11]. Therefore, the newly observed decrease in β-adrenoceptor affinity again confirms the hypothesis of a widespread membrane alteration in essential hypertension. It is difficult to explain at present the role of alteration in β-adrenergic receptor properties of adipocytes in the types of hypertension considered. Nevertheless, it evidently reflects some features of the interaction between catecholamines and β-adrenoceptors in hypertension.

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3 Yu.V. Postnov, S.N. Orlov, M.B. Reznikova and N.I. Pokudin, Pflügers Arch. *384*, 183 (1980).
4 M. Rodbell, J. biol. Chem. *239*, 375 (1964).
5 L.T. Williams, L. Jarett and R.J. Lefkowitz, J. biol. Chem. *251*, 3096 (1976).
6 O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.I. Randall, J. biol. Chem. *183*, 265 (1951).
7 G. Scatchard, Ann. N.Y. Acad. Sci. *51*, 660 (1949).
8 Yu.V. Postnov and S.N. Orlov, Pflügers Arch. *385*, 85 (1980).
9 A.W. Jones, Circulation Res. *5*, 563 (1973).
10 Yu.V. Postnov, S.N. Orlov and A.S. Shevchenko, Cardiology, Moscow *15*, 88 (1975).
11 G.M. Boriskina, P.V. Gulak and Yu.V. Postnov, Experientia *34*, 744 (1978).

Inhibition of food intake in the rat by cyproheptadine

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Summary. In a model of conditioned feeding behavior, oral administration of cyproheptadine (1–100 mg/kg), 30 min before presentation of food, produced a dose-dependent reduction of food intake in the rat ($ED_{50} \simeq 17$ mg/kg during the 1st h of testing). This anorexic effect persisted for at least 24 h. These results provide further evidence that under certain conditions cyproheptadine, which is used as an orectic agent in man, can produce anorexia.

Cyproheptadine (5-[1-methyl-piperidylidene-(4)]-5H-dibenzo-[a,d]-cycloheptane), a tricyclic antiserotonin-antihistaminic drug, stimulates appetite and produces an increase in body weight in man[2-7]. However, the evidence for such an action of cyproheptadine in rats is not so clear. For example, Lavenstein and coworkers[4] and Bergen[2] did not observe appetite-stimulating effects of this agent in rats, whereas Baxter and coworkers[8] and Oomura and colleagues[9] reported that it produced an increase in food intake in rats. Opitz and colleagues[10] found that cyproheptadine, given orally or i.p. (1–40 mg/kg), had no effect or could produce anorexia in the rat, but for the 1st 2 h after s.c. administration (12.5 mg/kg) it increased food intake in fasted animals. However, s.c.-administered cyproheptadine (7 or 20 mg/kg) did not have an appetite-stimulating effect in fasted rats that had been trained to bar-press for food, and at the higher dose the drug could produce an anorexigenic effect[11]. Due to these conflicting results, we decided to conduct further studies on the effect of cyproheptadine on feeding behavior in the rat.

Male Sprague-Dawley rats (Charles River), 180–215 g, were housed individually in plastic cages ($14 \times 20 \times 30$ cm) containing litter. They were allowed to acclimatize to their new surroundings for a week, with food and water ad libitum[12], and were subjected to a daily rhythm of 12 h of darkness (19.00–7.00 h) and 12 h of light (7.00–19.00 h). The animals were conditioned to consume food between 11.00 and 15.00 h[13], while having free access to water; they received their cups of food in plastic cages ($8.5 \times 13 \times 27$ cm). Food consisting of a mixture of 600 g of powdered standard rat chow (Union d'Alimentation Rationnelle) and 250 ml of soybean oil was given to the rats daily. The amount of food consumed was determined by weighing the cups before and at 1 h and 4 h after their presentation to the animals. The rats consumed 12.9 ± 0.5 g of food during the 1st h and 15.8 ± 0.4 g of food in the total 4-h test period (means $\pm$ SEM of 36 values from 6 rats in both cases). The criterion for stability of food intake was considered to have been met when the quantity consumed did not vary more than 5 g/day per animal. Determinations made on Tuesday and Wednesday were considered as control values, drugs were tested on Thursday, and determinations made on Friday permitted evaluation of sustained effects of the drug. Determinations made on Monday were not used since the animals had received 2 pellets of standard rat chow on Saturday and Sunday. Animals were tested in groups of 6. Cyproheptadine-HCl (Merck) was administered orally in a dose range of 1–100 mg/kg