0026-895X/92/040649-07$03.00/0

MOLECULAR PHARMACOLOGY, 42:649–655

Influence of Age on the β_1- and β_2-Adrenergic Receptors in Rat Liver

A. VAN ERMEN,[1] E. VAN DE VELDE, P. VANSCHEEUWIJCK, and N. FRAEYMAN
Heymans Institute of Pharmacology, De Pintelaan 185, B-9000 Gent, Belgium
Received March 12, 1992; Accepted June 24, 1992

SUMMARY

The influence of maturation and aging on β receptors in rat liver was studied. Competition binding experiments with the nonselective β-antagonist propranolol and the subtype selective antagonists ICI 118,551 (β_2), ICI 89,406 (β_1), and CGP 20,712A (β_1) revealed the presence of a mixed β_1 and β_2 receptor population in crude plasma membrane preparations from livers of newborn, mature, and senescent rats. The percentage of β_1 receptors was lowest in livers from newborn rats and was increased in livers from mature and senescent rats. This increase is caused by a decrease in β_2 receptor density on maturation, although the β_1 receptor density is nearly constant throughout the life span of the rat. Isoproterenol-stimulated adenylate cyclase activity was inhibited in livers from senescent rats by propranolol and ICI 118,551 and to a lesser extent by ICI 89,406 and CGP 20,712A. The isoproterenol-stimulated glucose output in hepatocytes from senescent rats was inhibited concentration dependently by propranolol, ICI 118,551, ICi 89,406, and CGP 20,712A. From these results we conclude that β_1 and β_2 receptors are present in livers from rats of the three age groups and that the β_1 to β_2 receptor ratio is increased in livers from mature and senescent rats compared with newborn rats. Both β receptor subtypes are linked to the cAMP second messenger system in newborn and senescent rats; β_1 and β_2 receptors are equally involved in the regulation of glycogenolysis in hepatocytes from senescent rats.

The influence of age on the adrenergic control of glycogenolysis in rat liver is complex. At birth, glycogenolysis is mediated mainly by β receptors, whereas in the liver of mature rats the adrenergic control of glucose output is α_1-receptor mediated (1–5). In senescent rats, the β component in the glycogenolytic response becomes predominant (5). These age-dependent changes in the β receptor-mediated glycogenolysis are related to changes in β receptor density, which is high in livers from newborn rats, low in livers from mature rats, and slightly increased in livers from senescent rats (1, 6–10).[2]

Based on the rank order of potency of agonists and antagonists in competition binding experiments, it is generally accepted that the β_2 receptor is the predominant β receptor subtype in livers from newborn (1) and mature (6, 11–13) rats. Using the highly selective β_2 antagonist ICI 118,551 in competition binding experiments, Snell and Evans (14) confirmed that, in livers from newborn rats, 80–90% of the β receptors are of the β_2 subtype. No additional data on the influence of age on the presence of β receptor subtypes and on their corresponding transduction system are available.

The present study was performed in order to answer three questions as follows: (a) what β receptor subtypes can be identified with ligand binding experiments in rat liver and what is the influence of maturation (2–3-month- versus 1–4-day-old rats) and aging (24–26-month- versus 2–3-month-old rats) thereupon, (b) are the β receptor subtypes present linked to adenylate cyclase, and (c) are these receptor subtypes involved in the regulation of glycogenolysis?

Firstly, pharmacological characterization of the β receptor was done in competition binding experiments with the nonselective β-antagonist propranolol, the β_1-selective antagonists ICI 89,406 (15) and CGP 20,712A (16), and the β_2-selective antagonist ICI 118,551 (17). Secondly, we investigated the inhibitory effects of propranolol and of the β_1- and β_2-selective antagonists on isoproterenol-stimulated adenylate cyclase activity. Finally, we examined the inhibitory effects of propranolol and of the β_1- and β_2-selective antagonists on isoproterenol-stimulated glycogenolysis.

The results of our investigations indicate that β_1 as well as β_2 receptors are present in the liver throughout the life-span of the rat. Both β receptor subtypes are functionally coupled to adenylate cyclase in livers from newborn and senescent rats and are equally involved in the regulation of glycogenolysis in hepatocytes prepared from livers of senescent rats.

The financial support of the Belgian National Fund for Scientific Research (NFWO), Grant 3.9006.87, is acknowledged.

[1] Bursary of the Belgian I.W.O.N.L.

[2] A. Van Ermen, E. Van de Velde, and N. Fraeyman. Effect of maturation and aging on the β-adrenergic transduction chain in the liver of the rat. Submitted for publication.

ABBREVIATIONS: ICYP, (−)-^{125}I-cyanopindolol; KBB, Krebs bicarbonate buffer; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

Experimental Procedures

Materials. ICYP and [8-^{3}H]cAMP were obtained from Amersham (Amersham, UK). (−)-Propranolol, (−)-isoproterenol, collagenase (type IV), glucose oxidase, and peroxidase were obtained from Sigma (Poole, UK). Guanylylimidobisphosphate and GTP were purchased from Boehringer-Mannheim (Brussels, Belgium). CGP 20,712A was a gift from Ciba Geigy (Groot-Bijgaarden, Belgium). ICI 118,551 and ICI 89,406 were gifts from ICI (Destelbergen, Belgium). Glucagon was obtained from Novo Industrie (Brussels, Belgium).

Animals. Male Wistar rats of either 2–3 months (average weight, 259 ± 11 g; range, 174 to 340 g; n = 15; hereafter called "mature" rats) or 24–26 months of age (average weight, 611 ± 15 g; range, 500 to 769 g; n = 21; "senescent" rats) were obtained from the Proefdierencentrum of the University of Leuven. Rats were housed individually and had free access to food and acidified water. Wistar rats of 1–4 days of age (average weight, 12 ± 1 g; range, 7 to 20 g; n = 40; "newborn" rats) were bred in our own laboratory. For each experiment, livers of four to nine newborn animals were pooled in order to obtain approximately 1 g of wet weight liver tissue.

Membrane preparation. A crude plasma membrane preparation was prepared as described before (18).[2] Briefly, livers were homogenized in homogenization buffer (250 mM sucrose, 50 mM Tris, 2 mM EGTA, pH 7.4) at 1/20 dilution (g wet weight/ml), with an Ultra Turrax, three times for 15 sec, with 1-min intervals; the homogenate was centrifuged at low speed (500 × g for 10 min), and the supernatant was centrifuged at high speed (27,000 × g for 30 min). The pellet was washed three times by gentle homogenization (Potter-Elvehjem, four strokes) and centrifugation (27,000 × g for 30 min) in homogenization buffer. The final pellet was resuspended at 2–4 mg of protein/ml in assay buffer (50 mM Tris, 20 mM $MgCl_2$, 2 mM EGTA, pH 7.4). Ligand binding experiments with antagonists were performed either immediately after

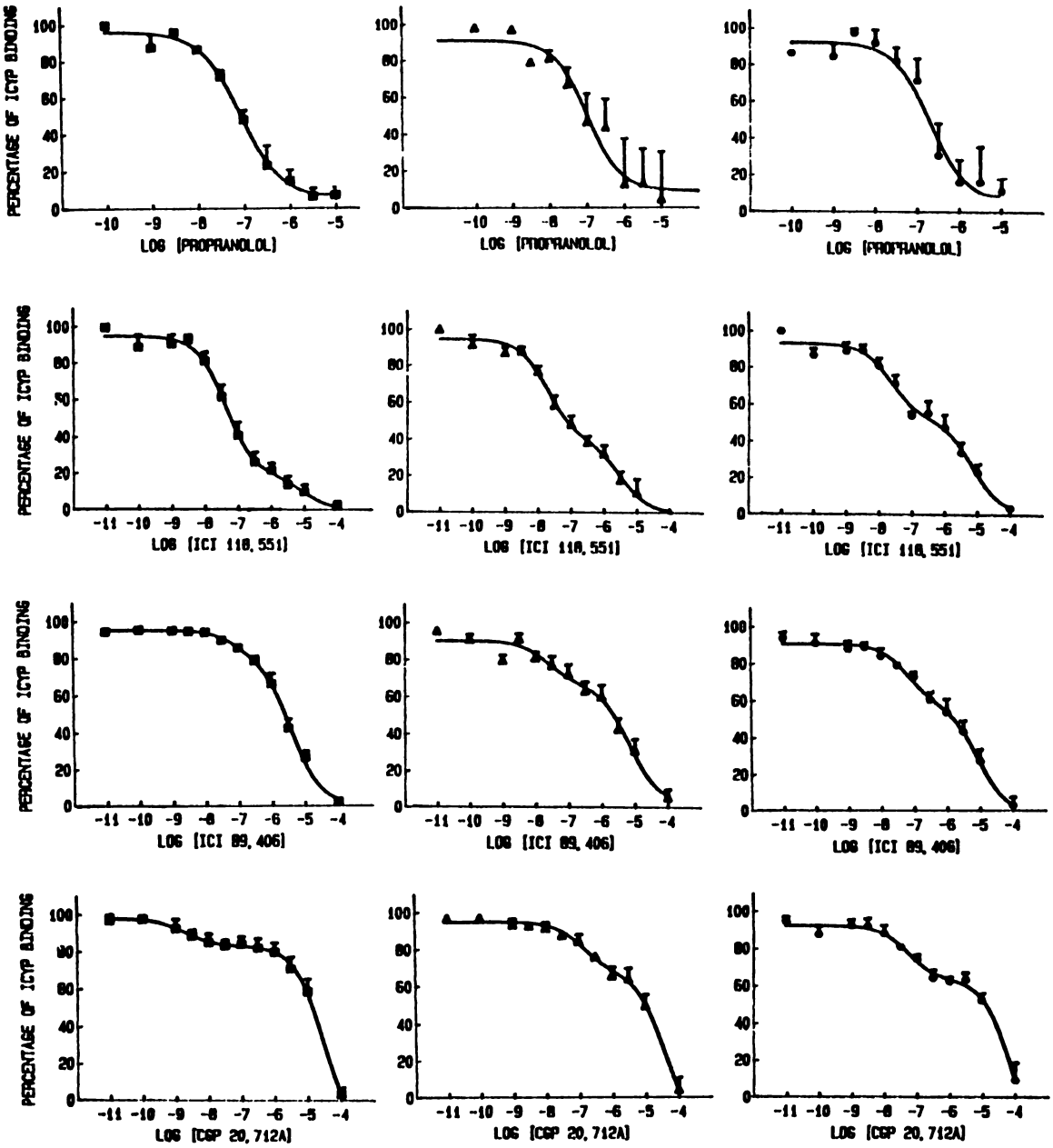

Fig. 1. Mean competition binding curves with ICYP as ligand and the nonselective β antagonist propranolol, the β_2-selective antagonist ICI 118,551, and the β_1-selective antagonists ICI 89,406 and CGP 20,712A as displacers. The binding at the lowest and highest concentration of competitor is set as 100 and 0%, respectively. *Curves* are the mean of four experiments. ■, Newborn rats; ▲, mature rats; ●, senescent rats.

tissue preparation or after storage of the sample at −70° for at most 1 week; adenylate cyclase assays were performed immediately after the membrane preparation. The protein content was determined with the dye-binding method of Bradford, as modified by Macart and Gerbaut (19), using bovine serum albumin as standard.

Preparation of hepatocytes and glucose assay. Hepatocytes were isolated from livers of fed mature and senescent rats by the method of Berry and Friend (20), as modified by Seglen (21). Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (6 mg/100 g of rat). Livers were perfused at 37° with 150 ml of Ca^{2+}-free KBB, pH 7.4, containing 20 mM glucose, followed by perfusion in a recirculation system with 100 ml of KBB containing 20 mM glucose, 1.2 mM Ca^{2+}, and 9200 units of collagenase (type IV), for 20 min. The medium was vigorously bubbled with O_2/CO_2 (95:5). After 20 min, the liver was transferred to a Petri dish containing KBB with 20 mM glucose, and the cells were gently dispersed with a spatula. After filtration of the crude cell suspension through nylon mesh (100 mesh), the cells were washed twice in KBB with 20 mM glucose and once in KBB with 5 mM glucose, by centrifugation for 30 sec at 50 × *g*. Hepatocytes were resuspended in KBB with 5 mM glucose and were preincubated for 30 min at 37° in KBB with 5 mM glucose, under an atmosphere of O_2/CO_2 (95:5). Hepatocytes were centrifuged again and resuspended in glucose-free KBB. Viability of cells was determined by trypan blue exclusion. Attempts to isolate hepatocytes from newborn animals were unsuccessful; viability was <50%, and the number of cells recovered per gram of tissue was very small.

Viable cells (1–2 × 10^6) were incubated for 20 min at 37° with seven different concentrations of isoproterenol (10^{-10} to 10^{-4} M) or with seven different concentrations of glucagon (10^{-12} to 10^{-6} M), in a total volume of 800 μl. In inhibition experiments, propranolol, ICI 118,551, ICI 89,406, or CGP 20,712A (10^{-11} to 10^{-4} M) was incubated together with 10^{-5} M isoproterenol; agonists and antagonists were dissolved in glucose-free KBB. The reaction was stopped by immersion of the incubation mixtures in ice and addition of 200 μl of $HClO_4$ (final concentration, 10%), after which the denaturated proteins were pelleted (1000 × *g* for 10 min). Glucose was measured in the supernatant by using the glucose oxidase method (22). Basal glucose output was measured in each experiment using four to seven individual cell samples, incubated without agonist or antagonists. Preliminary experiments demonstrated that for all experimental conditions the glucose output was linear over at least 30 min. Basal glucose output was set as 100%, and agonist stimulation was calculated as percentage increase in glucose output above basal outflow. In experiments with antagonists, inhibition was expressed as percentage decrease of net isoproterenol-stimulated glucose output (i.e., glucose output in the presence of 10^{-5} M isoproterenol minus basal glucose output). The method for the measurement of glycogenolysis was evaluated as follows. (a) At least five identical incubation mixtures were carried through the whole procedure and the glucose concentration was measured. The coefficients of variation for seven different experiments were 1.9, 5.5, 2.1, 2.4, 2.4, 3.2, and 2.1. (b) The between-day variation was measured by the estimation of a weighed amount of glucose (100 μg). An average of 96.2 ± 2.4 μg (n = 21) was found.

Ligand binding experiments. Ligand binding experiments were done using a manual and semiautomatic cell harvester filtration method, as described before (18). Saturation binding experiments were performed as described before.[2] Briefly, nine concentrations of the nonselective β ligand ICYP, ranging from 5 to 300 pM, were incubated for 90 min at 37° with 20–30 μg of membrane proteins for the livers of newborn rats or with 50–70 μg of membrane proteins for the livers from mature and senescent rats. Nonspecific binding was measured using 10 μM (−)-propranolol or 100 μM (−)-isoproterenol in the presence of 0.1% ascorbic acid. In competition binding experiments, ICYP (100–150 pM) was incubated with 20–30 μg of proteins (newborn rats) or 50–70 μg of proteins (mature and senescent rats), for 90 min at 37°, with 10 different concentrations of propranolol (10^{-10} to 10^{-5} M) or with 12 different concentrations of ICI 118,551, ICI 89,406, or CGP 20,712A (10^{-11} to 10^{-4} M). In preliminary experiments, we ascertained that the difference in protein content had no influence on the results[2] and that the incubation time was adequate for complete equilibrium (23).

Adenylate cyclase activity. The activity was assayed as described before (18), and cAMP was determined according to the method of Tovey *et al.* (24). We reported earlier that isoproterenol caused a concentration-dependent increase in adenylate cyclase activity in livers from rats of the three age groups and that the maximal effect was elicited by 5 × 10^{-5} M isoproterenol.[2] In the inhibition experiments, 90–100 μg of proteins were incubated with isoproterenol (5 × 10^{-5} M) in the presence of 5 × 10^{-5} M GTP, for 20 min at 30°, in the absence or in the presence of the following antagonists (10^{-8} to 10^{-4} M): propranolol, ICI 118,551, CGP 20,712A, or ICI 89,406. Percentage inhibition was calculated relative to the net increase in adenylate cyclase activity obtained with 5 × 10^{-5} M isoproterenol (increase in activity above the activity in the presence of GTP alone). In livers of mature rats, only a limited stimulation of the adenylate cyclase activity was observed, as reported before.[2] Under these experimental conditions, it was impossible to evaluate the effect of antagonists on isoproterenol-induced activation of adenylate cyclase.

Calculations. The data from saturation binding experiments were analyzed using the method of Scatchard. Curve fitting of the data from competition binding experiments was done using the program GraphPAD (25). Curves were routinely fit according to one- and two-site models, and the best model was chosen on the basis of the F test (26). K_i values were calculated from IC_{50} values using the formula of Cheng and Prusoff (27). The β_1 and β_2 receptor density was calculated in each individual experiment by multiplying total β receptor density, derived from Scatchard plot analysis, by the percentage of high affinity binding sites for CGP 20,712A or for ICI 118,551, for the estimation of β_1 or β_2 receptor number, respectively. All values are given as means ± standard errors. Differences between the three age groups and between dose-response curves were evaluated using one-way analysis of variance. When significance was reached, differences between two groups were evaluated using the Student t test, with Bonferroni correction for repetitive measurements (28). Differences between two independent groups were evaluated using the two-tailed Student t test. Statistical significance was accepted at the $p < 0.05$ level.

TABLE 1

Quantitative data from the competition binding experiments with ICYP as ligand and the nonselective β antagonist propranolol, the β_2-selective antagonist ICI 118,551, and the β_1-selective antagonists ICI 89,406 and CGP 20,712A as displacers.

K_h, HA and K_l, LA, inhibition constants of the high and low affinity binding sites, respectively. HA (%), percentage of high affinity binding sites. n, number of experiments. Values are mean ± standard error.

	Newborn	Mature	Senescent
Propranolol			
n	4	4	5
K_i (10^{-9} M)	20.2 ± 8.8	38.2 ± 13.2	33.4 ± 12.1
ICI 118,551			
n	6	5	5
HA (%)	76.1 ± 4.9[a]	43.9 ± 7.5	45.4 ± 3.6
K_i, HA (10^{-9} M)	6.9 ± 2.5	7.1 ± 4.4	5.2 ± 1.9
K_i, LA (10^{-7} M)	36.3 ± 18.5	9.3 ± 4.6	20.1 ± 7.5
ICI 89,406			
n	4	4	3
HA (%)	11.1 ± 0.6[a]	26.8 ± 2.5[b]	44.9 ± 6.2
K_i, HA (10^{-9} M)	1.3 ± 0.5	9.4 ± 6.3	27.9 ± 8.1
K_i, LA (10^{-7} M)	6.7 ± 0.9	22.3 ± 9.9	34.7 ± 3.5
CGP 20,712A			
n	4	4	3
HA (%)	18.3 ± 8.3	21.3 ± 3.1	25.1 ± 2.9
K_i, HA (10^{-9} M)	1.2 ± 0.8	22.6 ± 9.8	7.9 ± 2.7
K_i, LA (10^{-7} M)	76.3 ± 26.8	129.4 ± 63.3	94.0 ± 25.8

[a] Significantly different from mature and senescent rats.
[b] Significantly different from senescent rats.

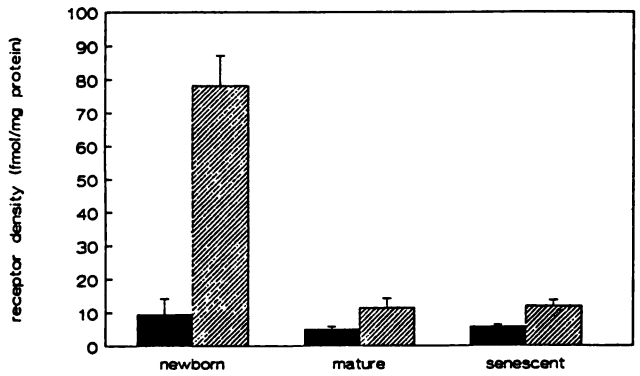

Fig. 2. Calculated β_1 and β_2 receptor density in plasma membranes prepared from livers of newborn, mature, and senescent rats. Receptor density is expressed in fmol/mg of protein. Calculations are described in Experimental Procedures. ■, β_1 receptor density; ▨, β_2 receptor density.

Results

Ligand binding experiments. The results of the competition binding experiments with the different antagonists are shown in Fig. 1; the quantitative data are summarized in Table 1.

Competition curves with the nonselective β antagonist propranolol were monophasic in livers from rats of the three age groups ($p < 0.05$). Competition curves obtained with the β_2-selective antagonist ICI 118,551 and with the β_1-selective antagonists ICI 89,406 and CGP 20,712A were biphasic in each age group ($p < 0.05$). Upon maturation, the percentage of high affinity binding sites for ICI 118,551 decreased and for ICI 89,406 increased significantly ($p < 0.05$). No further changes in the percentage of high affinity binding sites for ICI 118,551 occurred upon aging, whereas for ICI 89,406 this percentage increased further upon aging ($p < 0.05$). For CGP 20,712A there was a tendency for an increase in the percentage of high affinity binding sites (from 18% in livers from newborn rats to 25% in livers from senescent rats), but statistical significance was not reached.

In Fig. 2, the calculated β_1 and β_2 receptor densities are shown. The β_2 receptor density was significantly lower in livers from mature and senescent rats than in livers from newborn rats ($p < 0.05$). The β_1 receptor density did not change upon maturation or aging. The β_1/β_2 receptor ratio, calculated with CGP 20,712A and ICI 118,551, increased from 0.12 in livers from newborn rats to 0.44 in livers from mature rats and 0.48 in livers from senescent rats.

Adenylate cyclase activity. The results on the inhibition of isoproterenol-stimulated adenylate cyclase by the different antagonists in livers from newborn and senescent rats are shown in Fig. 3.

In plasma membranes prepared from livers of newborn and senescent rats, propranolol, ICI 118,551, and ICI 89,406 caused a significant decrease in isoproterenol-stimulated adenylate cyclase activity (analysis of variance). Inhibition with propranolol and ICI 118,551 was greater than with ICI 89,406. For CGP 20,712A there was a tendency for inhibition of isoproterenol-stimulated adenylate cyclase in livers from senescent rats, whereas in livers from newborn rats no inhibition was found.

Glycogenolysis. Viability of hepatocytes from mature rats (76.3 ± 1.4%, $n = 16$) and senescent rats (78.0 ± 1.6%, $n = 13$) was similar. Basal glucose output in hepatocytes from mature rats was significantly higher than that in senescent rats [554.0 ± 78.7 ($n = 6$) versus 390.3 ± 31.7 μg of glucose/5 × 10^6 cells · 20 min ($n = 13$), respectively ($p < 0.05$)].

Glycogenolysis upon β receptor stimulation with 10^{-5} M isoproterenol in hepatocytes prepared from livers of mature rats was negligible (1.9 ± 1.9% above basal glucose output, $n = 6$). No further experiments were done with these cells. In hepatocytes prepared from livers of senescent rats, isoproterenol caused a concentration-dependent increase in glucose output, with an EC_{50} value of 7.6 10^{-8} M (Fig. 4). A maximal increase in glucose output of 42.3 ± 10.2% ($n = 11$) above basal value was obtained with 10^{-5} M isoproterenol. The effect of 10^{-5} M isoproterenol in hepatocytes from senescent rats was inhibited concentration-dependently by propranolol, ICI 118,551, ICI 89,406, and CGP 20,712A (Fig. 5).

Glucagon caused a concentration-dependent stimulation of glycogenolysis in hepatocytes from mature and senescent rats (Fig. 6), with EC_{50} values of 0.6 × 10^{-9} M and 0.3 × 10^{-9} M, respectively ($n = 11$). Glucagon, at a concentration of 10^{-7} M, caused a maximal increase in glucose output of 64.3 ± 12% and 76.8 ± 9.8% above basal values in hepatocytes from mature (n

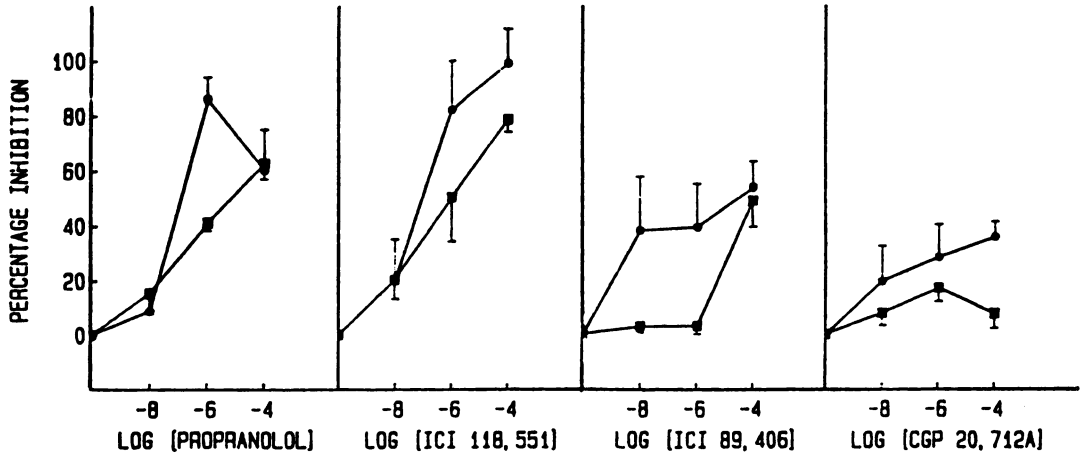

Fig. 3. Inhibition of isoproterenol-stimulated (5 × 10^{-5} M) adenylate cyclase activity, in plasma membranes prepared from livers of newborn (■) and senescent (●) rats, by the nonselective antagonist propranolol, the β_2-selective antagonist ICI 118,551, and the β_1-selective antagonists ICI 89,406 and CGP 20,712A. Percentage inhibition of the net isoproterenol-stimulated adenylate cyclase activity is plotted against the concentration of antagonist. *Curves* are the mean of four experiments.

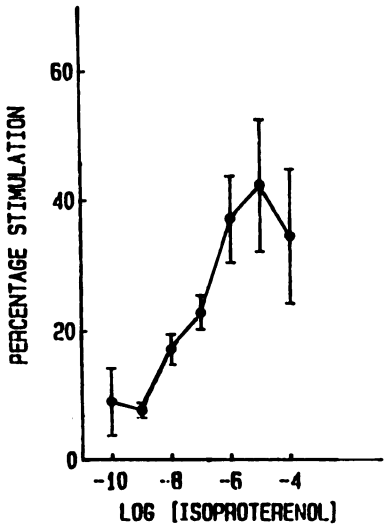

Fig. 4. Glucose output as a function of the concentration of isoproterenol in hepatocytes prepared from livers of senescent rats (n = 11). The results are expressed as percentage increase above basal glucose output.

= 11) and senescent (n = 11) rats, respectively. No significant differences in glucagon-stimulated glycogenolysis between the two age groups were found. Glucagon-stimulated glucose output was significantly higher than isoproterenol-stimulated glucose output in hepatocytes from mature and senescent rats.

Discussion

In this paper we describe our investigations on the effect of maturation and aging on β receptors in rat liver. We provide evidence that β_1 receptors are present throughout the life-span of the rat and that age affects the β_1/β_2 receptor ratio. The decrease in β receptor density upon maturation can be explained by a selective decrease in β_2 receptor density, with β_1 receptors remaining constant. Our results suggest, furthermore, that both receptor subtypes are linked to adenylate cyclase and that they are both involved in the regulation of glycogenolysis in hepatocytes. The evidence is based on results obtained with three types of experiments, i.e., ligand binding experiments, adenylate cyclase assays, and determination of glycogenolysis.

Competition curves with the subtype-selective antagonists displayed two binding sites in livers from rats of the three age groups, indicating the presence of β_1 and β_2 receptors. In livers from newborn rats, we found that the majority of the β receptors were of the β_2 subtype, which is in keeping with the results of Snell and Evans (14). The affinity of ICI 118,551 for the β_2 and β_1 receptors corresponds to what was found by Snell and Evans (14). To our knowledge, no data on the affinities of ICI 89,406 and CGP 20,712A for β receptors in rat liver are available, but the affinities calculated from our experiments are comparable to what was found for these compounds in other tissues (29–31). For ICI 89,406 and CGP 20,712A, K_i values for each binding site are increased in livers from mature and senescent rats, which indicates that the affinity for the binding sites decreases upon maturation and aging. Differences observed with the same competitor between the different age groups could be explained by changes in membrane composition occurring with increasing age. Sawada *et al.* (32) recently reported a decrease in liver membrane fluidity upon aging, which could influence the interaction between the receptor and the ligand or competitor.

The influence of age on the percentage of β_1 receptors and on the β_1/β_2 receptor ratio depends to some extent on the antagonist used; with both ICI 118,551 and ICI 89,406 the percentage of β_1 receptors increases upon maturation, and for ICI 89,406 it further increases upon aging. Using CGP 20,712A, the most selective β_1 antagonist available, β_1 receptors were also detected, but there was only a marginal increase in the percentage of β_1 receptors and there was no significant difference as a function of age. The discrepancy between the results among β_1-selective antagonists can possibly be explained by a different affinity of the β_1 receptors for the competitors or by differences in the physico-chemical properties of the antagonists. It has been shown before that the results of radioligand binding experiments are dependent on the liposolubility of the ligand in membranes (33). The previously reported decrease in β receptor density upon maturation (1, 6)[2] can be explained by a marked fall in β_2 receptor number, whereas the β_1 receptor density is unchanged during maturation or postmaturational aging. We must take into account the fact that the β_1 and β_2 receptor densities are calculated values; this could be avoided if β subtype-selective radioactive ligands were available.

These results indicate that β_1 as well as β_2 receptors are present in livers from newborn, mature, and senescent rats; the ratio of the β_1/β_2 receptor density increases upon maturation, due to a marked decrease in β_2 receptor number.

We reported earlier that there is an age-related difference in the synthesis of cAMP upon stimulation with isoproterenol, and the sequence newborn ≫ senescent > mature was ob-

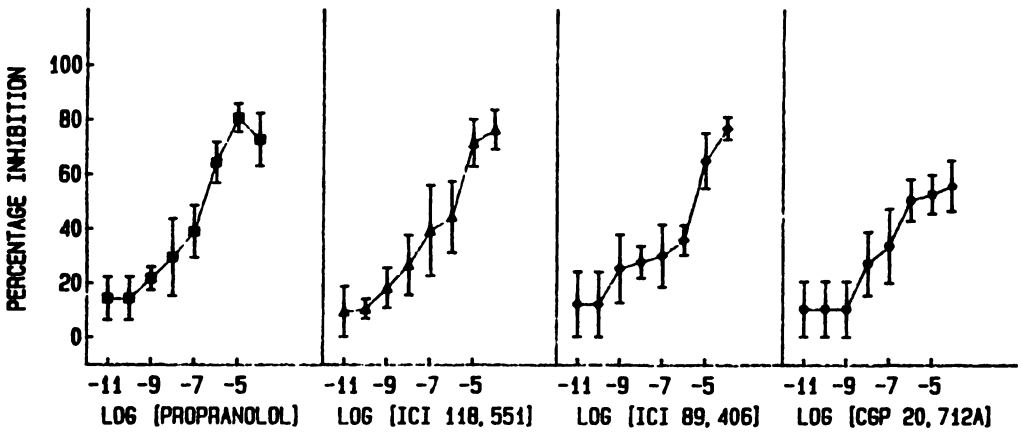

Fig. 5. Inhibition of isoproterenol-stimulated (10^{-5} M) glucose output by the nonselective β antagonist propranolol (■), the β_2-selective antagonist ICI 118,551 (▲), and the β_1-selective antagonists ICI 89,406 (◆) and CGP 20,712A (●), in hepatocytes prepared from livers of senescent rats. Percentage inhibition of the net isoproterenol-stimulated glucose output is plotted against the concentration of antagonist. *Curves* are the mean of five experiments.

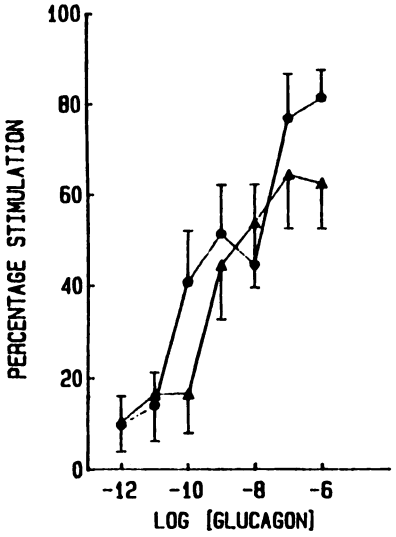

Fig. 6. Glucose output as a function of the concentration of glucagon in hepatocytes prepared from livers of mature (▲) and senescent (●) rats (n = 11). The results are expressed as percentage increase above basal glucose output.

tained.[2] In this series of experiments, these earlier results were confirmed. Due to the low basal adenylate cyclase activity and the low increase in activity upon β receptor stimulation in livers from mature rats,[2] inhibition with the antagonists was too small to obtain reliable results.

In livers from newborn and senescent rats, inhibition of the isoproterenol-stimulated adenylate cyclase activity was more pronounced with the nonselective antagonist propranolol and the β_2-selective antagonist ICI 118,551 than with the β_1-selective antagonists. These findings are in agreement with our results obtained from the ligand binding studies; despite age-related alterations in the β_1/β_2 receptor ratio, the calculated β_2 receptor density remains higher than the calculated β_1 receptor density.

These results suggest that β_1 as well as β_2 receptors are functionally coupled to the adenylate cyclase in livers from newborn and senescent rats.

It has been reported that glucose output from hepatocytes prepared from fed animals is due to glycogenolysis from endogenous glycogen reserves (2, 34). Hepatocytes were isolated in the presence of glucose, in order to inhibit liver phosphorylase A and to prevent glycogen depletion during preparation (35). Hence, glucose output reflects glycogen breakdown. Due to the low yield of viable cells of livers from newborn rats, experiments were only performed on hepatocytes prepared from livers of mature and senescent rats. There was a significant decline, upon aging, in the ability of hepatocytes to produce glucose in the absence of stimulating agent. These findings are in agreement with the observations of Graham *et al.* (9), who claimed that the decreased basal glucose output reflects the continuous decline in the function of many physiological systems upon aging (36). Glucagon-stimulated glycogenolysis, which was used as an internal control in our experiments, was similar in hepatocytes from senescent rats and mature rats, as also found by others (5). We noticed that the response to β-adrenergic stimulation is smaller than the glycogenolytic response to glucagon, which is in contrast to the findings of Katz *et al.* (5). The results are possibly influenced by the use of different rat strains (F344 rats versus Wistar rats). Propranolol, as well as the subtype-selective antagonists ICI 118,551, ICI 89,406, and CGP 20,712A, caused a concentration-dependent inhibition of the isoproterenol-induced glucose output in hepatocytes from senescent rats. These results not only support the hypothesis that β_1 and β_2 receptors are present but also indicate that they are functional and involved in glucose homeostasis. Furthermore, the observation that the calculated β_2 receptor density is slightly higher than the calculated β_1 receptor density in livers from senescent rats is reflected by an almost equipotent inhibition of β receptor-mediated glycogenolysis by both the β_1-selective antagonists and the β_2-selective antagonist. It has to be stressed that, when intact hepatocytes are used, uptake and metabolism mechanisms remain intact, and drugs could be taken up and metabolized by the viable cells during the incubation, as reported for propranolol (37). Therefore, no correct IC_{50} values can be given and comparisons between antagonists are probably not useful.

From the results of our study, several conclusions can be drawn. (a) A mixed β_1/β_2 receptor population is present in plasma membranes prepared from livers of newborn, mature, and senescent rats, and there is an increase in the β_1/β_2 receptor ratio upon maturation and aging. The previously observed decrease in β receptor density is probably caused by a decrease in β_2 receptor density, whereas the β_1 receptor number is unaffected by the age of the rat. (b) The isoproterenol-stimulated increase in adenylate cyclase activity is blocked by propranolol and ICI 118,551 and, to a lesser extent, by ICI 89,406 and CGP 20,712A in livers from newborn and senescent rats. This indicates that both β receptor subtypes are linked to the cAMP second messenger system. (c) Both β_1 and β_2 antagonists block the β receptor-mediated glucose output in hepatocytes prepared from senescent rats, indicating that both β receptor subtypes are involved in the regulation of hepatic glycogenolysis in livers from senescent rats.

Acknowledgments

The authors thank Dr. M. G. Bogaert and Dr. R. A. Lefebvre for critical review of the text.

References

1. Bendeck, J. L., and A. Noguchi. Age-related changes in the adrenergic control of glycogenolysis in rat liver: the significance of changes in receptor density. *Pediatr. Res.* **19**:862–868 (1985).
2. Blair, J. B., M. E. James, and J. L. Foster. Adrenergic control of glucose output and adenosine 3′:5′-monophosphate levels in hepatocytes from juvenile and adult rats. *J. Biol. Chem.* **254**:7579–7584 (1979).
3. Agerbeck, M., G. Guellaen, and J. Hanoune. Adrenergic receptor of the α_1-subtype mediates the activation of the glycogen phosphorylase in the normal rat liver. *Biochem. Pharmacol.* **29**:643–645 (1980).
4. Studer, R. K., and A. B. Borle. Differences between male and female rats in the regulation of hepatic glycogenolysis. *J. Biol. Chem.* **257**:7987–7993 (1982).
5. Katz, M. S., C. M. Mc Nair, T. K. Hymer, and S. R. Boland. Emergence of *beta*-adrenergic responsive hepatic glycogenolysis in male rats during post-maturational aging. *Biochem. Biophys. Res. Commun.* **147**:724–730 (1987).
6. Mc Millian, M. K., S. M. Schanberg, and M. K. Cynthia. Ontogeny of rat hepatic adrenoceptors. *J. Pharmacol. Exp. Ther.* **227**:181–185 (1983).
7. Dax, E. M., D. K. Ingram, J. S. Partilla, and R. I. Gregerman. Food restriction prevents an age-associated increase in rat liver *beta*-adrenergic receptors. *J. Gerontol.* **44**:B72–B76 (1989).
8. Dax, E. M., J. S. Partilla, M. A. Pineyro, and R. I. Gregerman. β-Adrenergic receptors, glucagon receptors, and their relationship to adenylate cyclase in rat liver during aging. *Endocrinology* **120**:1534–1541 (1987).
9. Graham, S. M., P. A. Herring, and I. J. Arinze. Age-associated alterations in hepatic β-adrenergic receptor/adenylate cyclase complex. *Am. J. Physiol.* **253**:E277–E282 (1987).
10. Katz, M. S. Food restriction modulates β-adrenergic-sensitive adenylate cyclase in rat liver during aging. *Am. J. Physiol.* **254**:E54–E63 (1988).
11. Dax, E. M., J. S. Partilla, L. J. Karol, and R. I. Gregerman. (−)-[^{125}I] Iodopindolol binding to β-adrenergic receptors in tissues of the rat with particular reference to the characterization in liver. *J. Recept. Res.* **6**:165–182 (1986).

12. Munnich, A., P. Geynet, P. H. Schmelck, and J. Hanoune. Rat liver β-adrenergic receptors: identification and characterization with (−)-[^{3}H]dihydroalprenolol. *Horm. Metab. Res.* **13**:18–21 (1981).
13. Minneman, K. P., A. Hedberg, and P. B. Molinoff. Comparison of *beta* adrenergic receptor subtypes in mammalian tissues. *J. Pharmacol. Exp. Ther.* **211**:502–508 (1979).
14. Snell, K., and C. A. Evans. Characterization of rat liver β-adrenoceptors during perinatal development as determined by [^{125}I]-iodopindolol radioligand binding assays. *Br. J. Pharmacol.* **93**:817–826 (1988).
15. Neve, K. A., D. A. Barrett, and P. B. Molinoff. Selective regulation of *beta*-1 and *beta*-2 adrenergic receptors by atypical agonists. *J. Pharmacol. Exp. Ther.* **235**:657–664 (1985).
16. Dooley, D. J., H. Bittiger, and N. C. Reymann. CGP 20,712A: a useful tool for quantitating β_1- and β_2-adrenoceptors. *Eur. J. Pharmacol.* **130**:137–139 (1986).
17. Bilski, A. J., S. E. Halliday, J. D. Fitzgerald, and J. L. Wale. The pharmacology of a β_2-selective adrenoceptor antagonist. *J. Cardiovasc. Pharmacol.* **5**:430–437 (1983).
18. Vanscheeuwijck, P., E. Van de Velde, and N. Fraeyman. The β-adrenergic transduction system in kidneys from young and senescent rats. *Eur. J. Pharmacol.* **188**:129–137 (1990).
19. Macart, M., and L. Gerbaut. An improvement of the Coomassie blue dye binding method allowing an equal sensitivity to various proteins: application to cerebrospinal fluid. *Clin. Chim. Acta* **122**:93–99 (1982).
20. Berry, M. N., and D. S. Friend. High yield preparation of isolated rat liver parenchymal cells. *J. Cell. Biol.* **43**:506–520 (1969).
21. Seglen, P. O. Preparation of rat liver cells. *Exp. Cell. Res.* **74**:450–454 (1972).
22. Bergmeyer, H. U., and E. Bernt. Determination of D-glucose with glucose oxidase and peroxidase, in *Methods of Enzymatic Analysis* (H. Bergmeyer, ed.), Vol. 3. Academic Press, New York, 1205–1215, (1965).
23. Fraeyman, N., and P. Vanscheeuwijck. Effect of age on the kinetics of the binding of iodocyanopindolol to a membrane preparation of rat lungs. *J. Recept. Res.* **11**:985–999 (1991).
24. Tovey, K. C., K. G. Oldham, and J. A. M. Whelan. A single direct assay for cyclic AMP in plasma and other biological samples using an improved competitive protein binding technique. *Clin. Chim. Acta* **56**:221–229 (1974).
25. Motulsky, H. J. GraphPAD: Plot, Analyze Data and Digitize Graphs. ISI Software, San Diego (1987).
26. Snedecor, G. W., and W. G. Cochran. Curvilinear regression, in *Statistical Methods* (G. W. Snedecor and W. G. Cochran, eds.), Iowa State University Press, Iowa City, IA, 447–456 (1973).
27. Cheng, Y. C., and W. H. Prusoff. Relation between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3109 (1973).
28. Wallenstein, S., C. L. Zucker, and J. L. Fleiss. Some statistical methods useful in circulation research. *Circ. Res.* **47**:1–9 (1980).
29. Hedberg, A., F. Kempf, M. E. Josephson, and P. B. Molinoff. Coexistence of *beta*-1 and *beta*-2 adrenergic receptors in the human heart: effects of treatment with receptor antagonists or calcium entry blockers. *J. Pharmacol. Exp. Ther.* **234**:561–568 (1985).
30. Molenaar, P., and R. J. Summers. Characterization of *beta*-1 and *beta*-2 adrenoceptors in guinea pig atrium: functional and receptor binding studies. *J. Pharmacol. Exp. Ther.* **241**:1041–1047 (1987).
31. Kim, Y. S., R. D. Sainz, P. Molenaar, and R. J. Summers. Characterization of β_1- and β_2-adrenoceptors in rat skeletal muscles. *Biochem. Pharmacol.* **42**:1783–1789 (1991).
32. Sawada, M., U. Sester, and J. C. Carlson. Superoxide radical formation and associated biochemical alterations in the plasma membrane of brain, heart, and liver during the lifetime of the rat. *J. Cell. Biol.* **48**:296–307 (1992).
33. Dax, E. M., and J. S. Partilla. Adrenergic ligand liposolubility in membranes: direct assessment in a *beta*-adrenergic binding system. *Mol. Pharmacol.* **22**:5–7 (1982).
34. Hutson, N. J., F. T. Brumley, F. D. Assimacopoulos, S. C. Harper, and J. H. Exton. Studies on the α-adrenergic activation of hepatic glucose output: studies on the α-adrenergic activation of phosphorylase and gluconeogenesis and inactivation of glycogen synthase in isolated rat liver parenchymal cells. *J. Biol. Chem.* **251**:5200–5208 (1976).
35. Stalmans, W., H. De Wulf, L. Hue, and H.-G. Hers. The sequential inactivation of glycogen phosphorylase and activation of glycogen synthetase in liver after the administration of glucose to mice and rats: the mechanism of the hepatic threshold to glucose. *Eur. J. Biochem.* **41**:127–134 (1974).
36. Scarpace, P. J., N. Tumer, and L. Mader. β-Adrenergic function in aging: basic mechanisms and clinical implications. *Drugs Aging* **1**:116–129 (1991).
37. Iwamoto, K., J. Watanabe, and M. Satoh. Age-dependence in capacity-limited uptake kinetics of propranolol by isolated rat hepatocytes. *Biochem. Pharmacol.* **35**:2677–2681 (1986).

Send reprint requests to: N. Fraeyman, Heymans Institute of Pharmacology, De Pintelaan 185, B-9000 Gent, Belgium.