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Characterization of rat liver β-adrenoceptors during perinatal development as determined by [^{125}I]-iodopindolol radioligand binding assays

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1 The subtype specificity of β-adrenoceptors in foetal (20 days *post coitum*) rat liver membrane preparations has been determined by use of [^{125}I]-iodopindolol binding assays and the characteristics of radioligand binding have been resolved.

2 The kinetics of radioligand association and dissociation (in the presence of 5×10^{-4} M isoprenaline) showed an association rate constant of $1.5 \times 10^{7}\,M^{-1}s^{-1}$ and dissociation rate constant of $9.1 \times 10^{-4}\,s^{-1}$, corresponding to a dissociation constant for [^{125}I]-iodopindolol of 60.7 pM. A similar dissociation constant (75 pM) was determined by saturation binding assays.

3 The rank order of potency for displacement of [^{125}I]-iodopindolol binding was consistent with binding to a predominantly β_2-adrenoceptor population (i.e. ICI 118551 > isoprenaline > adrenaline > noradrenaline > atenolol). Computer analysis of displacement curves in the presence of a β_1-subtype selective agent (atenolol) or a β_2-subtype selective agent (ICI 118551) revealed the presence of β_2- and β_1-adrenoceptor subtypes in a ratio of about 80 : 20%.

4 Saturation binding assays by use of [^{125}I]-iodopindolol were carried out at different perinatal ages to determine total β-adrenoceptor concentrations and β_2-subtype (in the presence of 5×10^{-7} M atenolol) adrenoceptor concentrations. Competition binding assays with atenolol confirmed that at all ages apparent β_2-adrenoceptor binding accounted for 84–95% of the total β-adrenoceptor binding. The total β- and β_2-adrenoceptor binding capacity increased by 2.3 fold from 20 days *post coitum* to birth, and then decreased postnatally at 1 and 2 days *post partum*. The dissociation constant for [^{125}I]-iodopindolol binding did not show any change with age.

5 The change in β_2-adrenoceptor concentration with age is discussed in relation to the changing β-adrenoceptor-mediated responsiveness of glucose production by rat liver during perinatal development.

Introduction

Catecholamines play a critical role in a number of essential developmental adaptations that occur at the time of birth. For example, foetal lung maturation (Davis *et al.*, 1987) and perinatal hepatic glucose production (Sperling *et al.*, 1984) are processes under the control of endogenous catecholamine concentrations in the blood. In the rat there are high concentrations of plasma adrenaline and noradrenaline at birth, which rapidly decrease to low levels before showing significant secondary increases at a time (2 h *post partum*) which coincides temporally with the mobilization of liver glycogen for increased hepatic glucose production (Cuezva *et al.*, 1982). At this time there is a refractoriness in the liver to the stimulating effects of glucagon on glycogenolysis (Blazquez *et al.*, 1976; Snell & Walker, 1978), which emphasizes the important role of catecholamines in this metabolic process at birth.

The physiological responses of target tissues to catecholamines are mediated by binding to α- and β-adrenoceptors. In foetal and neonatal rat liver, in sharp contrast to adult male rat liver, metabolic responses to adrenoceptor agonists appear to be mediated by β-adrenoceptors (Sherline *et al.*, 1974; Moncany & Plas, 1980; Hühn *et al.*, 1983). However, caution is needed in the interpretation of some of these findings which have used hepatocytes in culture, in view of the recent demonstrations of a

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rapid shift from α- to β-adrenoceptors when adult rat hepatocytes are cultured *in vitro* (Nakamura *et al.*, 1983; Kunos *et al.*, 1984). The characterization of hepatic adrenoceptors by radioligand binding studies in the perinatal rat has received little attention, and the single study described in the literature (McMillian *et al.*, 1983) was limited in the extent of measurements made during the perinatal period. The aim of the present study was to characterize the nature of the β-adrenoceptor subtype in perinatal rat liver and to investigate possible changes in β-adrenoceptor number and ligand affinity during late foetal and early neonatal life by use of [^{125}I]-iodopindolol as a radioligand for the binding studies.

Methods

Cell membrane preparation

AHA strain rats (200–300 g body wt) from the breeding unit of Glaxo Research Ltd were mated overnight and certified pregnant on recovery of vaginal plugs on day 1 *post coitum*. Foetal rats, at the gestational ages indicated in Results, were rapidly delivered by caesarian section of unanaesthetized pregnant dams killed by cervical dislocation. Parturition occurred naturally between days 21 and 22 *post coitum*, and newborn rats were used immediately after the birth of the complete litter, or 24 and 48 h later, for receptor binding studies. Foetuses and neonates were decapitated, the livers were rinsed in ice-cold homogenization medium (145 mM NaCl, 2 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.5), and then homogenized in about 40 vol of homogenization medium by a Silverson Vortmix homogenizer followed by a Dounce homogenizer. Cell debris and unbroken cells were removed by centrifugation at 2000 *g* for 10 min, and the supernatant was centrifuged at 40000 *g* for 15 min. This membrane preparation was resuspended in fresh homogenizing medium by the Dounce homogenizer and recentrifuged at 40000 *g* for 15 min. The washed membrane preparation was then resuspended in storage medium (250 mM sucrose, 5 mM $MgCl_2$, 50 mM Tris-HCl, pH 7.5). Aliquots of the preparation were used freshly prepared or were snap-frozen in liquid N_2 and used after storage at −60°C. Stored frozen preparations produced results identical to fresh preparations.

Radioligand binding assays

Incubations (150 μl) in triplicate were carried out at 25°C in polypropylene tubes containing 145 mM NaCl, 20 mM Tris-HCl buffer (pH 7.5), 2 mM $MgCl_2$, 1 mM ascorbic acid, and various concentrations of [^{125}I]-iodopindolol and of competing drugs where appropriate. The reaction was started by addition of liver membrane preparation (0.20–0.40 mg of protein) and was continued for 45 min for saturation binding assays, for varying times up to 45 min when studying association kinetics, or for 45 min followed by varying times up to 20 min after addition of displacing drug (5 μM, final concentration) when studying dissociation kinetics. The reaction was terminated by the addition of 2 ml of ice-cold wash buffer (145 mM NaCl, 10 mM Tris-HCl, pH 7.5), followed by immediate and rapid filtration through Whatman GF/C glass fibre filters (Whatman Lab Sales Ltd., Maidstone, Kent) using a Millipore 1225 vacuum filtration manifold (Millipore (UK) Ltd., Harrow, Middlesex). The incubation tubes were rinsed with 2 ml of ice-cold wash buffer and the filters were washed with this and with 2 × 5 ml of ice-cold wash buffer. Membrane-bound [^{125}I]-iodopindolol trapped on the filters was counted in a Searle Analytic Inc. 1185R gamma counter. Specific non-displaceable binding was defined as the difference between binding in the absence (total binding) and in the presence of 2×10^{-4} M (−)-isoprenaline (Nahorski & Richardson, 1979). In all saturation binding assays Scatchard analyses were based on data derived from measurements at 10–12 concentrations of radioligand from 10–600 pM. In competition displacement studies K_i values were determined from IC_{50} values derived from Hill plots as described by Cheng & Prusoff (1973). In these studies the radioligand was added at 70 pM, corresponding to the determined K_D for this ligand (see Results). In the same studies computer-assisted iterative curve-fitting procedures based on models with one, two or three non-interacting binding sites were carried out to determine the relative proportions of β-adrenoceptor subtypes as described by Hancock *et al.* (1979).

Materials

[^{125}I]-iodopindolol was prepared and purified by the method of Barovsky & Brooker (1980) by iodination of (−)-pindolol and was a gift from N. Cook and S.R. Nahorski, Department of Pharmacology and Therapeutics, University of Leicester. The radioligand was stored at −60°C and was used within 6 weeks of preparation. (−)-*l*-[Propyl-2,3-^{3}H]-dihydroalprenolol and (−)-[^{125}I]-iodocyanopindolol were obtained from Amersham International plc (Amersham, Bucks.). Atenolol, ICI 118551(D,L-erythro-3-isopropylamino-1-[7-methyl-4-indanyloxy]-2-butanol hydrochloride), ketanserin, atropine sulphate, spiperone, mepyramine, ranitidine, and phentolamine mesylate were gifts from

Glaxo Group Research Ltd. (Ware, Herts.). (−)-Isoprenaline bitartrate, (±)-adrenaline, (±)-noradrenaline-HCl, (±)-propranolol-HCl, Tris and all other reagents were of highest purity available from Sigma Chemical Co. (Poole, Dorset).

Results

Kinetics of [^{125}I]-*iodopindolol binding in foetal rat liver*

The characteristics of binding of [^{125}I]-iodopindolol were determined in liver membrane preparations from foetal rats at 20 days *post coitum*. These characteristics have not previously been described for foetal liver and are an essential validation of the saturation binding assay subsequently developed. A time course of association showed a hyperbolic relationship with time for total and specific binding, reaching a plateau at 25 min (Figure 1). Since less than 10% of the total radioligand present was specifically bound at equilibrium, the association reaction shows pseudo-first order kinetics and an association rate constant of $1.5 \times 10^{7}\,M^{-1}s^{-1}$ was determined from the linear plot of log [Be/Be]-[B_t] versus time (where Be is the concentration of ligand specifically bound at equilibrium and [B_t] is the concentration specifically bound at time t in s).

The kinetics of radioligand dissociation were studied, after equilibrium binding was attained (45 min), by the addition of 5×10^{-4} M isoprenaline. [^{125}I]-iodopindolol binding dissociated in a monophasic manner with a half-life of 12.7 min and with a dissociation rate constant of $9.1 \times 10^{-4}\,s^{-1}$ as deter-

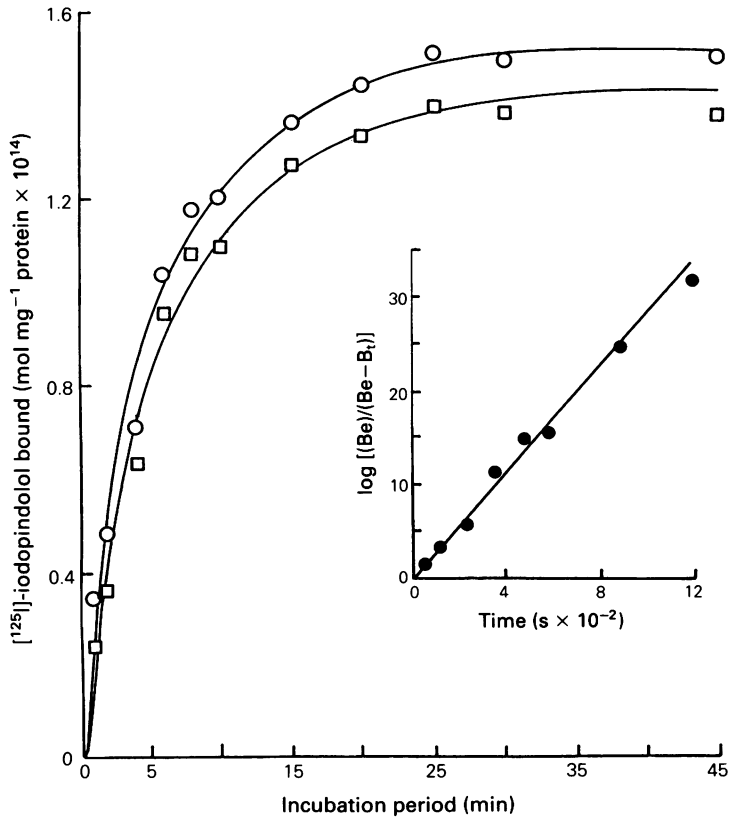

Figure 1 Time-course of association of [^{125}I]-iodopindolol with a foetal rat liver membrane preparation. Foetal (20 days *post coitum*) rat liver membranes were prepared as indicated in Methods, and incubated for the times indicated with 70 pM [^{125}I]-iodopindolol. Specific radioligand binding (□) was calculated as the difference between total binding (○) in the absence of 2×10^{-4} M (−)-isoprenaline and binding in the presence of the β-agonist and is expressed as mol mg^{-1} membrane protein $\times 10^{14}$. Each point is the mean of triplicate determinations. Inset shows the secondary plot of log (Be/Be − B_t) against incubation period in s, where Be is the concentration of radioligand specifically bound at equilibrium and B_t is the concentration specifically bound at time t in s.

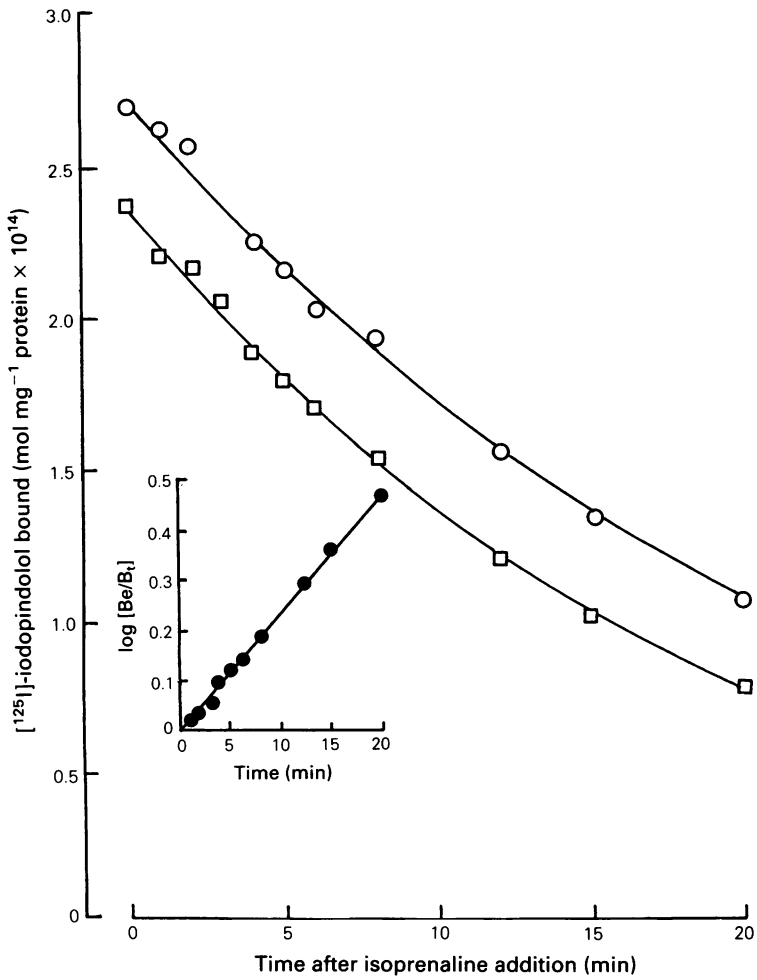

Figure 2 Time-course of dissociation of [^{125}I]-iodopindolol with a foetal (20 days *post coitum*) rat liver membrane preparation. Total (○) and specific (■) radioligand binding was as defined in Figure 1. Dissociation of radioligand binding was determined for different time periods after equilibrium binding of 70 pM [^{125}I]-iodopindolol was achieved (45 min), by the addition of excess (−)-isoprenaline (5×10^{-4} M). Each point is the mean of triplicate determinations. Inset shows the secondary plot of log (Be/B_t) against time, where Be and B_t are as defined in the legend to Figure 1.

mined from the linear plot of log [Be/B_t] versus time (Figure 2). From the rate constants of association and dissociation, a dissociation constant (K_D) for [^{125}I]-iodopindolol of 60.7 pM was determined.

Compounds with known affinity for β-adrenoceptors displaced equilibrium-bound [^{125}I]-iodopindolol from foetal liver membrane preparations in a concentration-dependent manner (Figure 3). The rank order of displacing potency (with K_i values in parentheses) for these compounds was ICI 118551 (5.6×10^{-9} M) > (−)-isoprenaline (2.1×10^{-7} M) > (±)-adrenaline (3.5×10^{-7} M) > (±)-noradrenaline (5.1×10^{-6} M) > atenolol (1.4×10^{-5} M) which is consistent with the binding of iodopindolol to a predominantly β_2-adrenoceptor population (see Williams & Lefkowitz, 1978). Various compounds with known pharmacological activity other than for β-adrenoceptors were also tested for their ability to displace radioligand and had no significant effect when present at 10^{-5}–10^{-6} M: phentolamine (α-adrenoceptor), ketanserin (5-hydroxytryptamine receptor), atropine (muscarinic

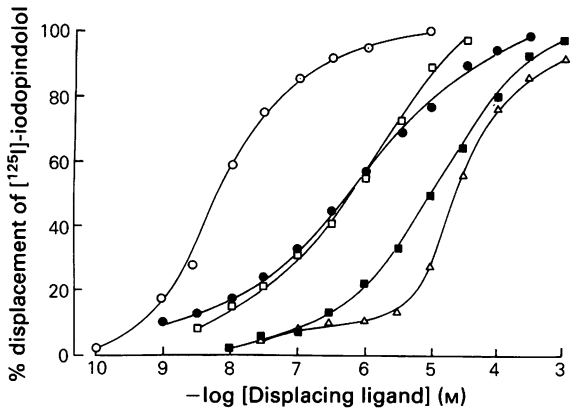

Figure 3 Competition displacement of [^{125}I]-iodopindolol binding to foetal (20 days *post coitum*) rat liver membrane preparations. [^{125}I]-iodopindolol was present at a concentration of 70 pM and displacing agents used were: ICI 118551 (○), (−)-isoprenaline (●), (±)-adrenaline (□), (±)-noradrenaline (■), and atenolol (△). Binding is expressed as the percentage of [^{125}I]-iodopindolol displaced by the competing agent compared to the binding in the absence of competing agent. The isotherms shown are those representative of two or three separate experiments; each point is the mean of triplicate determinations.

cholinoceptor), spiperone (D_2-dopamine receptor), mepyramine (H_1-histamine receptor), ranitidine (H_2-histamine receptor).

The appearance of the atenolol displacement curve shown in Figure 3 suggests that displacement of radioligand is not from a single class of specific binding site within the tissue preparation. This was confirmed by analysis of the data by use of the Eadie-Hofstee transformation (Figure 4). It can be seen that, whereas the non-subtype selective agonist isoprenaline showed a linear relationship, the β_1-subtype selective antagonist (atenolol) showed a curvilinear relationship. This suggests that the foetal liver membrane preparation does not contain a single homogeneous population of receptor corresponding to one subtype or the other. Analysis of the Hofstee plots by the method of Rugg *et al.* (1978) for the data with atenolol revealed that about 20% of the binding sites present had affinity for this β_1-selective antagonist. Although this procedure has been suggested to lead to overestimation of the proportion of the high-affinity component (Hancock *et al.*, 1979), it has been shown to provide an adequate assessment when antagonists of adequate (> 30 fold) subtype selectivity are used (Rugg *et al.*, 1978; Dickinson *et al.*, 1981). Nevertheless, further characterization of β-adrenoceptor subtype proportions was carried out by computer-assisted curve-fitting of the

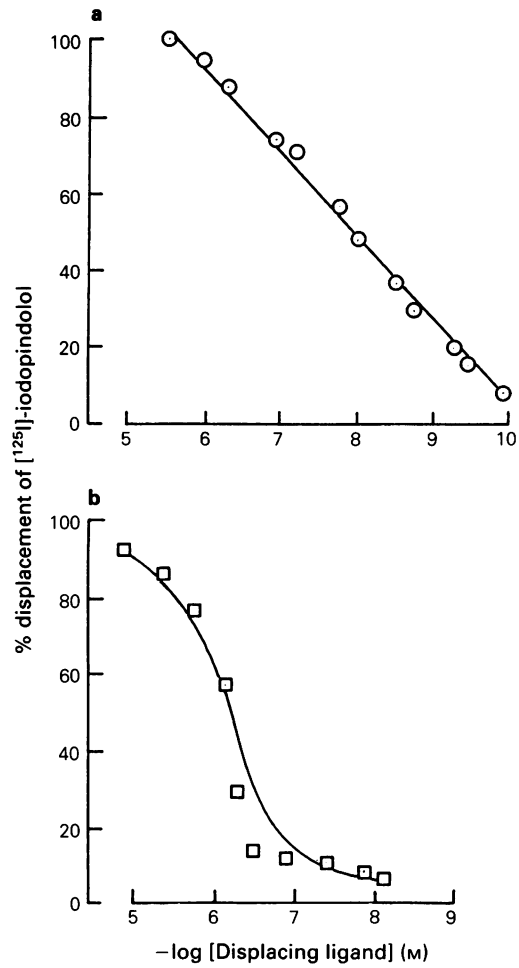

Figure 4 Eadie-Hofstee transformation of competition of [^{125}I]-iodopindolol binding from foetal (20 days *post coitum*) rat liver membrane preparations by (a) (−)-isoprenaline and (b) atenolol. The displacement of binding by the competing agents is expressed as a percentage of binding in the absence of competing agent and is plotted against the ratio of % displacement to the concentration of the competing agent added in M units. [^{125}I]-iodopindolol was present at 70 pM. Each point is the mean of triplicate determinations in each experiment, and the plots shown are representative of three separate experiments.

data from the displacement curves in the presence of the β_1-subtype and β_2-subtype selective agents, by the methods described by Hancock *et al.* (1979). This analysis revealed that in both cases the experimental data were best described by theoretical curves corresponding to a two-site binding model. Assuming these to be the β_1- and β_2-subtype receptors, the cal-

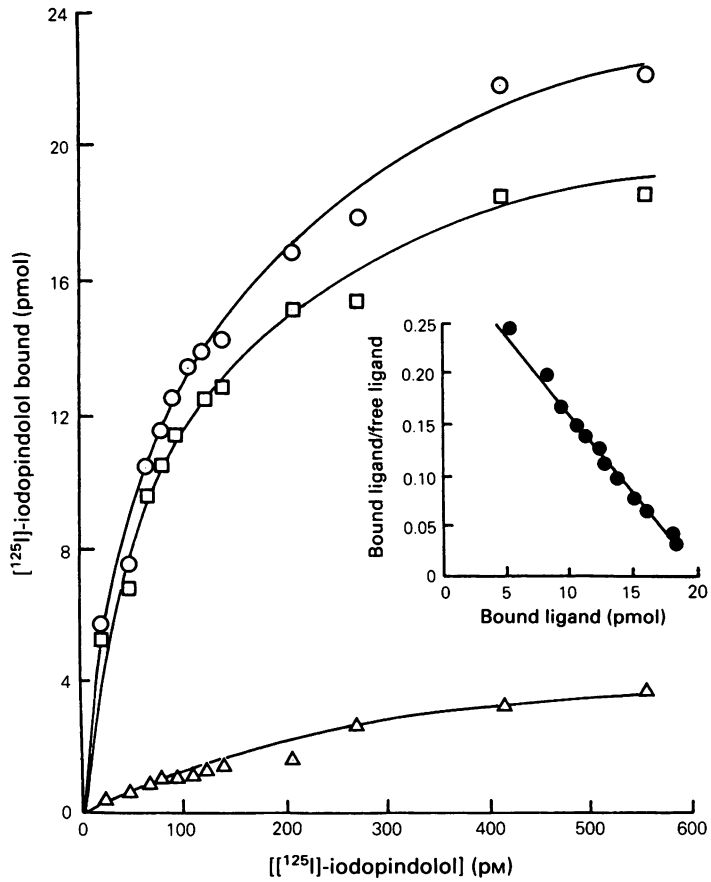

Figure 5 Saturation binding isotherm of increasing concentrations of [^{125}I]-iodopindolol to foetal (20 days *post coitum*) rat liver membrane preparation. Specific radioligand binding (□) was calculated as the difference between total binding (○) in the absence of 2×10^{-4} M (−)-isoprenaline and non-specific binding (△) in the presence of the β-agonist and is expressed in pmol. The inset shows the derived Scatchard plot for specific binding. Each point is the mean of triplicate determinations, and the experiment is representative of 4 separate experiments.

culated relative proportions of these were 18%: 82% by use of the atenolol data, and 20% : 80% by use of the ICI 118551 data (in good agreement with the proportions derived from the Hofstee analysis). In liver preparations from newborn and one day-old rats the corresponding relative proportions were 7 : 93% (atenolol) and 10 : 90% (ICI 118551) at both ages by use of the curve-fitting approach.

Saturation binding assays of receptors in perinatal rat liver preparations

For saturation binding assays, a minimum of 10 concentrations between 10 and 600 pM [^{125}I]-iodopindolol was used in each assay, the top concentration representing an approximate 10 fold value of the K_D (as determined above) in order to approach complete saturation of binding sites with radioligand. Equilibrium binding was attained in each case by incubating with radioligand for 45 min as determined above (see Figure 1). Total and specific binding in the assays was shown to be directly proportional to the amount of protein in the assay tube up to 0.4 mg. Saturation binding assays were carried out on rat liver preparations from animals of different foetal and postnatal ages. A typical binding isotherm with a liver preparation from 20 day-old foetal rats is shown in Figure 5, together with the Scatchard analysis of the data. At all ages studied, non-specific binding ranged from 5–15% of the total binding observed. Scatchard analysis of the data always resulted in linear plots (mean $r \pm$ s.e. mean of 0.99 ± 0.01, $P < 0.001$), and Hill coefficients close to

Table 1 [^{125}I]-iodopindolol binding capacities in rat liver membrane preparations during perinatal development

	B_{max} (fmol mg^{-1} protein)				
Adrenoceptor type	*Day 20 p.c.*	*Day 21 p.c.*	*Newborn*	*Day 1 p.p.*	*Day 2 p.p.*
Total β-receptors	***13.0 ± 2.6	**18.3 ± 1.3	30.4 ± 2.4	*21.6 ± 2.4	**19.9 ± 1.2
K_D (pM)	66.4 ± 16.0	85.8 ± 4.7	81.2 ± 9.7	72.9 ± 10.6	69.9 ± 5.2
β_2-receptors	***12.3 ± 2.3	**15.3 ± 2.0	28.5 ± 1.6	**19.8 ± 1.7	*18.5 ± 3.7
K_D(pM)	94.7 ± 9.2	81.8 ± 5.5	86.2 ± 20.9	101.7 ± 19.4	76.4 ± 6.1

Maximal binding capacities (B_{max}) were determined by saturation binding assay at 25°C with 40000 *g* particulate preparations of liver homogenates from rats of different perinatal ages. Total β-adrenoceptor binding capacity and that attributable to apparent β_2-adrenoceptor binding were determined in the absence and presence of 5×10^{-7} M atenolol, respectively. Results are given as mean values ± s.e. mean for 3 or 4 different experiments (each involving triplicate determinations). Statistical analysis was by Student's *t*-test and values different from those in newborn rats are indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. *p.c.*, foetal age *post coitum*; *pp.*, postnatal age *post partum*.

unity (mean ± s.e. mean of 1.00 ± 0.10) indicating an absence of co-operative interactions. No significant change in K_D (75 ± 7 pM, mean ± s.e. mean, $n = 16$) for [^{125}I]-iodopindolol was observed with age. In contrast, B_{max} values for binding capacity showed a clear age dependence (Table 1). Total β-adrenoceptor binding capacity increased during late foetal life by 2.3 fold from 20 days *post coitum* to birth, falling in the first postnatal days of life to 71% and 65% at 1 and 2 days *post partum* compared to the peak value found at birth.

By including 5×10^{-7} M atenolol (a concentration calculated to displace 90–100% of β_1- adrenoceptor-bound [^{125}I]-iodopindolol and <10% of β_2-specific radioligand binding at 70 pM of the radioligand, see Figure 3), it was possible to conduct saturation binding assays which would define the apparent β_2-adrenoceptor binding. Although pindolol is not specific for β_2- adrenoceptors, and therefore at the highest concentrations might compete to some extent with the β_1-adrenoceptor binding of atenolol, Scatchard plots were linear and allowed calculation of binding affinities and maximal binding capacities at the different ages (Table 1). Again there was no significant change in apparent K_D (88 ± 12 pM, mean ± s.e. mean, $n = 16$) with age, but the increase in apparent β_2-adrenoceptor binding capacity in late foetal life, and its subsequent decline *post partum*, paralleled the observations for total β-adrenoceptor binding capacity described above. Because of the very high relative proportion of β_2- to β_1-adrenoceptors in perinatal rat liver (see above), it proved impossible to conduct competition saturation binding assays for β_1-adrenoceptors by using ICI 118551 (at 5×10^{-8} M, calculated to displace 90–100% of 70 pM β_2-adrenoceptor-bound [^{125}I]-iodopindolol); the very low and variable radioligand binding and very high apparent K_D values for [^{125}I]-iodopindolol contributed to the difficulties. By comparing the binding capacities (B_{max}) obtained in saturation binding assays performed with and without the inclusion of atenolol (Table 1); it is possible to calculate the proportion of the total β-adrenoceptor population which is apparently due to β_1- or β_2-adrenoceptor subtypes. For the different ages, the proportion of apparent β_2-adrenoceptors is 84–95% of total β-adrenoceptor binding. This agrees with the estimates of adrenoceptor subtype proportions derived from displacement curves (see above) and thus provides justification for the use of atenolol to mask β_1-sites during saturation binding assays.

Discussion

Use of [^{125}I]*-iodopindolol to assay β-adrenoceptors in perinatal rat liver*

In this study we have used the high affinity specific β-adrenoceptor antagonist (−)-[^{125}I]-iodopindolol to characterize and quantitate β-adrenoceptor binding and subtype specificity. The binding of [^{125}I]-iodopindolol to perinatal rat liver membranes was rapid, saturable, reversible and showed displacement characteristics typical of binding to β_2-adrenoceptors. The radioligand showed no selectivity for β_1- or β_2-adrenoceptors. The dissociation constant (K_D) of [^{125}I]-iodopindolol binding was defined both by saturation binding assay (75 pM) and by kinetic analysis of radioligand association and dissociation (61 pM). The values were in good agreement with each other and with other published values in liver (68 pM, McMillan *et al.*, 1983; 65 pM, Dax *et al.*, 1986). Computer analysis by curve-fitting procedures (Hancock *et al.*, 1979) and procedures based on graphical extrapolation of Hofstee plots (Rugg *et al.*, 1978) derived from radioligand displacement studies with β_1- and β_2-subtype selective drugs, provided evidence in both cases of binding of [^{125}I]-iodopindolol to both adrenoceptor subtypes. Using

these data, and data derived from competition saturation binding assays, we could show independently that the predominant β-adrenoceptor population of perinatal rat liver is of the β_2-subtype (80–95%). Nevertheless, the evidence for a small proportion of β_1-subtype binding suggests that in foetal rat liver, as in some other tissues (Dickinson *et al.*, 1981), both adrenoceptor subtypes can coexist simultaneously.

[^{125}I]-iodopindolol is a relatively recently introduced radiolabelled antagonist for studying β-adrenoceptors (Barovsky & Brooker, 1980). While other radioligands have proved useful for assessing β-adrenoceptor binding in tissues where this receptor subtype predominates and is in relatively high concentration, quantitation in rat liver and other tissues has proved difficult and sometimes impossible (Krawietz & Erdmann, 1979; Dax *et al.*, 1981; 1982). We attempted to use [^{3}H]-dihydroalprenolol to characterize β-adrenoceptors in perinatal rat liver. Despite using the β-adrenoceptor agonist (−)-isoprenaline to characterize specific binding (see Nahorski & Richardson, 1979), non-specific binding was both great (usually between 60 and 70% of total binding) and highly variable. The inclusion of the α-adrenoceptor antagonist phentolamine at 10^{-4} M, as suggested by Dax *et al.* (1981), while eliminating a small proportion of the non-stereospecifically displaceable binding, did not resolve the difficulties encountered with this radioligand. These problems were not apparent with cat ventricular membrane preparations and K_D (1.7 nM) and B_{max} values (28 fmol mg^{-1} protein) comparable to those in the literature were obtained (Minneman & Molinoff, 1980; Dax *et al.*, 1981; Munnich *et al.*, 1981; Sharma & Corr, 1983). We conclude that [^{3}H]-dihydroalprenolol is unsuitable for the determination of β-adrenoceptor binding in rat liver, even at foetal and perinatal ages. Similar attempts using [^{125}I]-iodocyanopindolol as a radioligand for characterizing β-adrenoceptor binding in perinatal rat liver were also unsuccessful. Despite an acceptably low level of non-specific (non-displaceable by (−)-isoprenaline) binding of the order of 15–30% of total binding, saturation binding assays gave unacceptably erratic Scatchard plots. In radioligand association binding studies we observed that equilibrium binding was not attained within 60 min, in agreement with others (Brodde *et al.*, 1981; Engel *et al.*, 1981). The large cyano grouping on the radioligand molecule may impede its access to the binding site of the β-adrenoceptor, thus increasing the time to reach equilibrium (cf. iodopindolol in the present study). Furthermore, Engel *et al.* (1981) have noted biphasic dissociation characteristics (with less than 50% total dissociation in 8 h) with this radioligand, in contrast to the monophasic dissociation observed in all cases with [^{125}I]-iodopindolol in the present study. We conclude that [^{125}I]-iodopindolol is the most suitable, indeed the only, radioligand currently available for determining β-adrenoceptor binding in rat liver, at least in the relatively crude membrane preparations necessarily employed in this work.

Perinatal development of rat hepatic β-adrenoceptors

We have shown that the β_2-subtype is the predominant (>80%) β-adrenoceptor in perinatal rat liver at all ages studied and that the β_2-adrenoceptor concentration increases in late foetal liver, more than doubling between 20 days *post coitum* and birth, and then declines postnatally. The measurements were carried out on whole rat liver membrane preparations and it is important in a longitudinal comparison with age that the method of preparation is consistent and quantitative. To this end a relatively simple procedure was employed for membrane preparation which would avoid the inevitable losses of recovery associated with more extensive purification. The recovery of membrane protein was not significantly different between the different ages studied. The use of the high affinity, highly specific radioligand [^{125}I]-iodopindolol undoubtedly contributed to the accurate quantitation of β-adrenoceptors attainable with these relatively crude membrane preparations, as noted by others (Dax *et al.*, 1986). By using whole liver, although purification losses were minimized, the cellular heterogeneity of the tissue was retained. This is a significant point for foetal rat liver, in which at 20 days *post coitum* 30% of the volume fraction of the liver is attributable to haematopoietic cells (Greengard *et al.*, 1972). Rat reticulocytes and erythrocytes have a high concentration of β-adrenoceptors (Nahorski, 1981; Dickinson *et al.*, 1981) which will contribute to the binding observed in whole liver membrane preparations. However, since the volume fraction of rat liver occupied by haematopoietic cells falls from 30% to 10% between 20 days *post coitum* and birth, at the time when whole liver β-adrenoceptor concentration doubles, the increase in β-adrenoceptor concentration in membranes from whole liver must be attributable to differentiation of liver parenchymal cells. Indeed, for this reason, the magnitude of the late foetal increase in parenchymal liver cell β-adrenoceptor concentration may well be an underestimate.

The findings in the present study are apparently in contradiction to the only other published study of the perinatal development of rat liver β-adrenoceptors, by McMillian *et al.* (1983). By use of [^{125}I]-iodopindolol binding assays, they demonstrated an apparent fall in β-adrenoceptor concentration (assumed by them to represent solely β_2-subtype adrenoceptors) between 20.5 days *post*

coitum and 0.5 days *post partum*. However, because of the ages selected for study, they would not have observed the increase in β-adrenoceptor concentration in the last two days of gestation demonstrated in the present study. There is agreement on the postnatal decline of β-adrenoceptor concentration in rat liver and McMillian *et al.* (1983) show that this decline continues into adult life, and has been documented for β-adrenoceptor-mediated responses in rat liver (Blair *et al.*, 1979; Morgan *et al.*, 1983).

It is generally accepted that in the adult male rat liver adrenergic responses are mediated by α_1-adrenoceptors (see Exton, 1979; Kunos, 1984). Although, α-adrenoceptors have been identified in perinatal rat liver by radioligand binding studies, their concentration decreases between late-foetal and early postnatal life (Butlen *et al.*, 1980; McMillian *et al.*, 1983). Indeed, according to McMillian *et al.* (1983) the predominant subtype in late foetal rat liver is the α_2-adrenoceptor, although this point is still controversial (Butlen *et al.*, 1980). In fact, the evidence in juvenile and neonatal rats indicates that adrenergic responses are predominantly mediated by β-adrenoceptors (Exton, 1979; Blair *et al.*, 1979; Aggerbeck *et al.*, 1980; Morgan *et al.*, 1983). In particular it appears that glycogenolysis in perinatal rat liver is mediated by β-adrenoceptors (Sherline *et al.*, 1974; Moncany & Plas, 1980; Hühn *et al.*, 1983). We have found that the sensitivity of hepatic glycogenolysis to β-adrenoceptor activation increases in late foetal life to reach a peak at birth (unpublished observations) in a manner analogous to that described here for β_2-adrenoceptor concentration in liver membranes. This strongly suggests that the present observations have a physiological relevance for the development of hepatic adrenergic responsiveness, but does not exclude the possibility that signal transduction and post-receptor mechanisms might also contribute to this developmental adaptation.

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