J. Pharm. Pharmacol. 1989, 41: 398–401
Received September 26, 1988

Specific Age-dependence in Capacity-limited Uptake of Propranolol by Isolated Rat Lung

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Abstract—To investigate the effect of age on the pulmonary uptake of propranolol, minced tissue (0·4 g) of lungs isolated from 3- to 104-week-old rats was incubated with the drug (1 to 500 $\mu g\ mL^{-1}$) prepared in oxygenated, pH 7·4 Krebs-Ringer bicarbonate buffer solution (20 mL) containing 3% BSA for 60 min at 37°C. In any age-group the metabolism of propranolol was not significant (i.e. less than 0·6% of any initial load) under the present in-vitro conditions. The extent of uptake after the incubation with 2·5 $\mu g\ mL^{-1}$ of the drug was largest in the 7 weeks (i.e. 82% of the initial load) and relatively small in the 3(64%), 24(61%), 52(51%) and 104(48%) week old rats. Similar, specific age-dependence was observed in the tissue-to-medium concentration ratio of the drug. In any age-group, the initial uptake rate obtained in the first 5 min of the incubation was found to be a combination of apparently linear transport and saturable (capacity-limited) processes. There was a marked, specific age-dependence in the capacity-limited uptake rate. Although K_m' value was almost equivalent in any age-group (i.e. 24·4 to 25·4 $\mu g\ mL^{-1}$), V_{max} exhibited a specific age-dependence by yielding the highest value in 7 weeks ($0·726 \pm 0·101\ mg\ g^{-1}\ min^{-1}$) and relatively low values in 3 ($0·501 \pm 0·082\ mg\ g^{-1}\ min^{-1}$), 52 ($0·410 \pm 0·088\ mg^{-1}\ g^{-1}\ min^{-1}$) and 104 ($0·397 \pm 0·074\ mg\ g^{-1}\ min^{-1}$) weeks.

It has been reported that propranolol is extensively extracted by rat lungs after the intravenous dosage (Hayes & Cooper 1971; Schneck et al 1977; Rikihisa et al 1981; Iwamoto et al 1987). Recent reports have also shown that the pulmonary extraction of this drug exhibits a specific age-related change, namely relatively high and low extraction in 7-week-old and in immature or senescent rats, respectively (Iwamoto et al 1988a, b). However, no major kinetic factor has so far been clarified for this specific age-related difference in propranolol handling by the lung. A few in-vitro perfusion experiments have suggested a trend towards saturable extraction of this drug by rat lungs (Dollery & Junod 1976; Iwamoto et al 1988b). Well-designed in-vitro kinetic studies by incubating the lung tissue pieces or the homogenate with propranolol may enable us to characterize the age-dependent pulmonary uptake kinetics of this drug.

The present work, therefore, was designed to investigate the effect of age on the extent and initial rate of propranolol uptake at various initial substrate concentrations by the minced lung tissue prepared from 3- to 104-week-old rats.

Materials and Methods

Incubation of lung tissue with propranolol

Each male Wistar rat, 3(65–85 g), 5(110–135 g), 7(205–225 g), 11 (345–375 g), 15(380–415 g), 24(440–505 g), 52(655–735 g) or 104 (765–830 g) weeks old, housed in a well-controlled, specific pathogen-free room and fasted overnight, was anaesthetized with urethane (800 $mg\ kg^{-1}$ i.p.) and thoracotomized on the midline to expose the lungs. Immediately after cannulations into pulmonary artery and vein with PE-50 or PE-205 tubing, a single-pass perfusion was started with 2 to 8 mL of warmed (37°C), pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% bovine serum albumin (BSA), oxygenated with $95\%O_2 - 5\%CO_2$ from the artery to vein. The lungs were then isolated from the rat and placed in the ice-cold buffer solution until use. Minced lung tissue (0·4 g, approximately 2500 pieces of about 1 mm cube) was suspended to incubate with propranolol hydrochloride (ICI-Pharma, Osaka, Japan) prepared at 1, 2·5, 5, 10, 25, 50, 75, 100, 250 or 500 μg (as base) mL^{-1} in the same, warmed buffer solution (20 mL) as described above. An instantaneous mixing of the medium with tissue did not cause either a dilution or diffusion effect on the initial drug level in the uniform suspension. Under the aeration with $95\%O_2 - 5\%CO_2$, incubation was carried out at 37°C by means of mechanical agitation. An aliquot (0·1 mL) of the incubation medium was withdrawn periodically over 60 min and used for the analysis of drug concentration. These periodic samplings did not affect the kinetics of drug disappearance from the incubation medium.

Assay of propranolol in the medium, lung tissue and homogenate of the tissue suspension

After the incubation for 60 min, the incubation mixture was divided into two portions. One portion was filtered to separate the tissue pieces, which were immediately washed, followed by homogenization with 5 mL of drug free fresh buffer solution. The other portion was directly homogenized using a Potter-type Teflon homogenizer. The extent of propranolol bound to 3% BSA in the control buffer solution was determined by the equilibrium dialysis method (Iwamoto et al 1988b), where the equilibration was attained within 4 h.

The propranolol concentration in the homogenate of the separated tissue pieces, in the directly homogenized incubation mixture, and inner or outer medium after the equilibrium dialysis was then determined in the periodic sample withdrawn from the incubation mixture. Extraction and re-extraction of intact propranolol, and flurometric determina-

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tion of the resultant sample were as reported by Iwamoto & Watanabe (1985). The cumulative amount of metabolites in both medium and tissue was determined by the difference of the total amount of intact drug withdrawn at each time and that remaining in the homogenate after 60 min, from the initial load (i.e. 0·02 to 10 mg) at time zero.

Statistical analysis

The results were analysed statistically with the Student's *t*-test. *P* value of 0·05 or less was considered to be significant.

Results and Discussion

Time-course of propranolol uptake by rat lungs

In any age-group, the extent of metabolism after incubation with 2·5 μg mL^{-1} of propranolol for 60 min was less than 0·6% of the initial load, i.e. only 0·3 μg. The largest cumulative amount of metabolites after the incubation of the lungs from any age-group with 1 to 500 μg mL^{-1} of the drug was less than about 0·5 μg, i.e. only 0·005% of the highest initial load. The results were confirmed by analysis using an HPLC method which detected only trace amount of 4-hydroxy propranolol and its conjugates as the metabolites. Lack of significant pulmonary metabolism of propranolol in the present in-vitro incubation experiments was consistent with the previous in-vivo (Iwamoto et al 1987, 1988a) and in-vitro results (Dollery & Junod 1976; Iwamoto et al 1988b). Therefore, the amount of drug that disappeared from the medium was regarded as that taken up (or accumulated) by the tissue. Binding of propranolol to 3% BSA, ranging from about 85 to 60% at 1 to 500 μg mL^{-1} of the drug, did not seem to affect the uptake time-course, as suggested previously (Iwamoto et al 1988b). Fig. 1 shows typical time-courses for propranolol uptake at the initial drug concentration of 2·5 μg mL^{-1} by the lung tissue isolated from 3-, 7- and 52-week-old rats. Similar time-courses were obtained in other age-groups. In each age-group, uptake was relatively rapid in the first 5 min and was almost completed after 30 min. However, there was a marked age-related difference in these uptake profiles, namely the cumulative extent and the initial rate.

The extent of uptake by rat lungs after 60 min

Fig. 2 summarizes the effect of age on the extent of propranolol uptake by rat lung tissue after the incubation with 2·5 μg mL^{-1} of the drug. The uptake percent was highest in 7-week-old rats, i.e. 82% of the initial load, compared with that in either immature (3 weeks, 64%) or aged (52 to 104

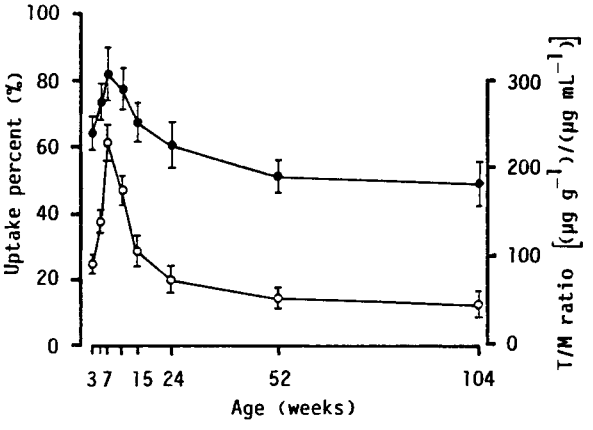

FIG. 2. Effect of age on the extent of propranolol uptake by isolated rat lung tissue in 60 min expressed as percent of the initial load (●, left ordinate) or tissue to medium concentration ratio (T/M ratio ○, right ordinate). The initial drug concentration was 2·5 μg mL^{-1} and the incubation was carried out in the same manner as described in Fig. 1. Each point is the mean ± s.d. of four rats. Uptake percentage of the initial load in 7 weeks was significantly higher than those in 3, 24, 52 or 104 weeks ($P < 0.01$ or 0·05). T/M ratio in 7 weeks was significantly higher than those in all other age-groups ($P < 0.001$ to 0·05).

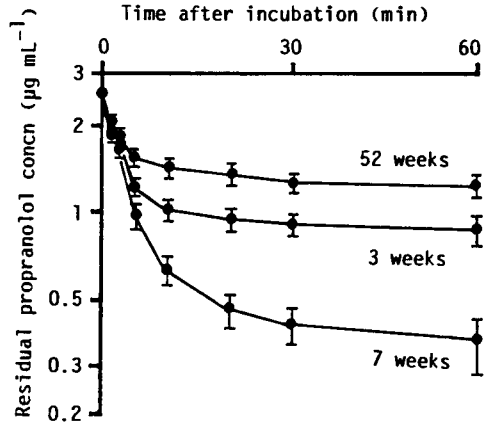

FIG. 1. Typical uptake time-course of propranolol by lung tissue isolated from 3, 7- and 52-week-old rats expressed as the residual drug concentration in the incubation medium. Minced lung tissue (0·4 g) was incubated with 2·5 μg mL^{-1} of propranolol in pH 7·4 Krebs-Ringer bicarbonate buffer solution (20 mL) containing 3% BSA and oxygenated with 95%O_2 − 5%CO_2 at 37°C. Each point is the mean ± s.d. of four rats. Any significant amount of metabolites was not detected in either medium or tissue after 60 min.

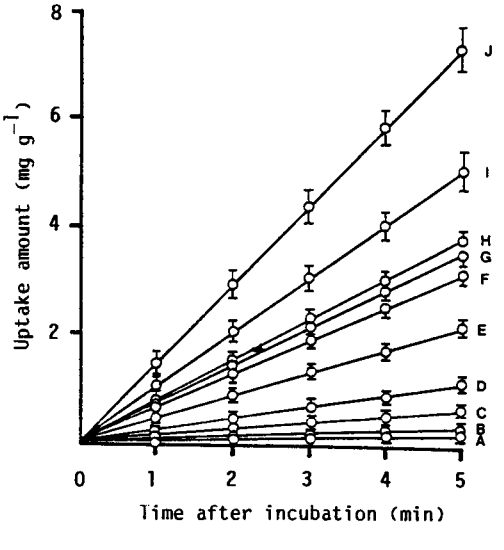

FIG. 3. Typical time-course for the initial uptake of propranolol by lung tissue isolated from 7-week-old rats at various initial concentrations. Incubation condition was the same as that in Fig. 1. The initial drug concentrations (μg mL^{-1}) were as follows: 1(A); 2·5(B); 5(C); 10(D); 25(E); 50(F); 75(G); 100(H); 250(I) and 500(J). Ordinate represents the amount of uptake per g wet tissue. Each point is the mean ± s.d. of four rats. The initial uptake rate was calculated from each slope of the linearity obtained in the individual rat.

weeks, approximately 50%) rats. A similar, but more distinct, age-related difference was found in the T/M ratio, yielding 89, 228 and 52 ($\mu g\ g^{-1}$)/($\mu g\ mL^{-1}$) in 3, 7, 52 week-old rats, respectively. These results suggest incomplete and reduced capacity of the lung to extract the drug due to immaturity and senescence, respectively. Specific age-dependence in the extent of propranolol uptake (Fig. 2) was similar to that reported in the in-vivo first-pass pulmonary clearance of this drug (Iwamoto et al 1988a). However, the effect of age on the pulmonary uptake kinetics of propranolol was not characterized in any animal species.

Initial uptake rate of propranolol by rat lungs

To estimate the initial uptake rate, the time-course of propranolol uptake (expressed as amount) per g of the tissue was examined in the first 5 min of the incubation at various initial drug concentrations in 3- to 104-week-old rats. Fig. 3 represents typical initial uptake time-courses for propranolol at 1 to 500 $\mu g\ mL^{-1}$ in 7-week-old rats. At any initial concentration, uptake was linear with time for 5 min. Similar time-courses were obtained in other age-groups. The apparent (overall) initial uptake rate at each drug concentration was then estimated from the slope of each linearity. The slope of the linearity at each initial concentration was always found to be largest in 7 week ($P < 0.05$) and relatively small in 3 or 52 to 104 week-old rats. The apparent initial uptake rates were then plotted against the initial substrate concentrations to characterize the uptake kinetics.

Effect of age on the capacity-limited uptake rate of propranolol by rat lungs

Fig. 4 represents the typical results of the apparent initial uptake rates obtained in 3-, 7- and 52-week-old rats. When rate was plotted against initial drug concentration (closed symbols), a curvilinear saturation profile (curve a) was obtained. Similar results were obtained in other age-groups. the apparent, overall initial uptake process for propranolol by the lung tissue was, therefore, considered to be a combination of a saturable process and a linear transport process which seemed to be independent of the initial drug concentration. The latter concentration-independent transport rate was estimated from the linearity of the curve, a, over the concentration range from 100 to 500 $\mu g\ mL^{-1}$, which was followed by extrapolation to zero concentration as shown with the broken line (b) in Fig. 4. This linear transport rate was almost identical in any age-group, ranging from 0.125 ± 0.016 to 0.129 ± 0.018 mg g^{-1} min^{-1} at 100 $\mu g\ mL^{-1}$. The linear transport rate was then algebraically

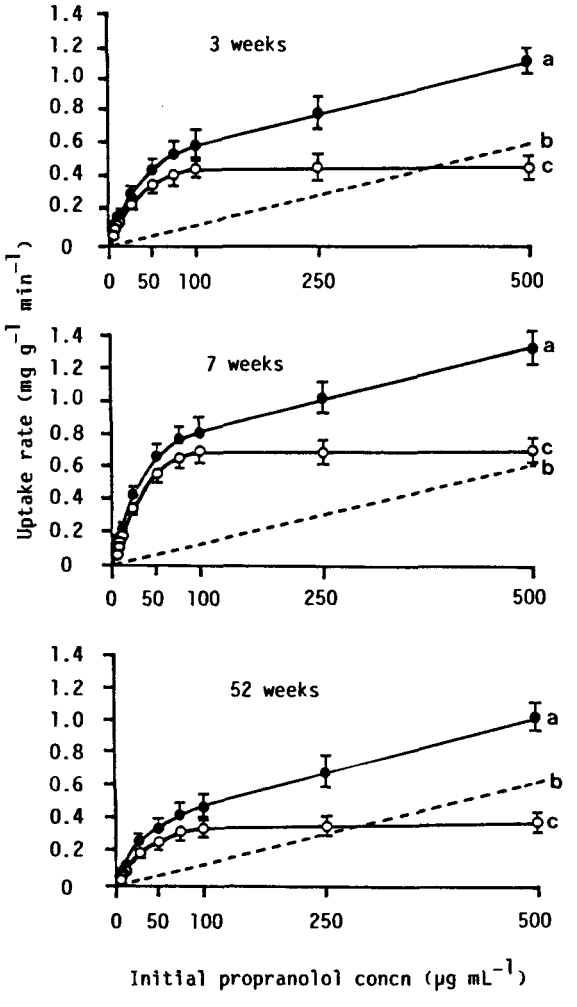

FIG. 4. Effect of initial propranolol concentration on the overall initial uptake rate (●, curve a) of the drug by lung tissue isolated from 3, 7, and 52-week-old rats. The initial uptake rate was expressed as mg g^{-1} min^{-1}. The linear regression line extrapolated from the overall initial uptake rate in the initial concentration range of 100–500 $\mu g\ mL^{-1}$ to zero was drawn to coincide with the origin as shown with the broken line (line b). These lines were expressed as $Y = 0.00129X$, $Y = 0.00125X$ and $Y = 0.00128X$ in 3-, 7- and 52-week-old rats, respectively. Mean saturable uptake rate of propranolol by the isolated rat lung tissue was calculated by subtracting the linear transport rate (b) from the overall uptake rate (a) as indicated with a hyperbolic curve (○, curve c). Each point is the mean ± s.d. of four rats.

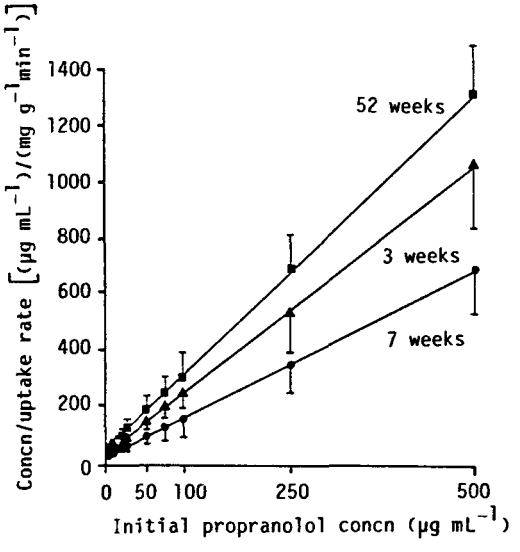

FIG. 5. Single-reciprocal plots for the mean saturable uptake rate of propranolol (Y, concentration/uptake rate) against the initial drug concentration (X) in 3(▲)-, 7(●)- and 52(■)-week-old rats. Each point is the mean ± s.d. of four rats. Linear regression analysis yielded the relationship expressed as $Y = 1.996X + 49.5$, $Y = 1.377X + 34.3$ and $Y = 2.439X + 60.6$ in 3 ($r = 0.998$), 7 ($r = 0.999$) and 52 ($r = 0.998$) weeks, respectively. Kinetic parameters, V_{max} and K_m', were estimated from the reciprocal of the slope and negative intercept on abscissa, respectively.

Table 1. Kinetic parameters for the saturable uptake process of propranolol by lung tissue isolated from 3- to 104-week-old rats.

Parameter[a]	Age (weeks)							
	3	5	7	11	15	24	52	104
V_{max}[b]	0·501	0·631	0·726	0·695	0·517	0·594	0·410	0·397
(mg g^{-1} min^{-1})	(0·082)[c]	(0·088)	(0·101)	(0·081)	(0·061)	(0·077)	(0·088)	(0·074)
K_m'[d]	24·8	24·5	24·9	24·4	25·4	25·2	24·8	24·6
(μg mL^{-1})	(4·5)	(5·2)	(3·8)	(5·1)	(3·9)	(4·4)	(4·0)	(4·6)

[a] The initial saturable uptake rate was determined in the first 5 min of the incubation of rat lung tissue with propranolol.
[b] V_{max} was estimated from the reciprocal of the slope of the linear regression line as exemplified in Fig. 5.
[c] s.d.
[d] K_m' was estimated from the negative intercept on abscissa of the regression line as exemplified in Fig. 5.

subtracted from the overall initial uptake rate to leave only saturable uptake rate (open symbols, curve c) in Fig. 4. The saturable uptake rate thus obtained at each initial drug concentration was always highest ($P<0·05$) in 7-week-old rats. These estimations were done for each preparation from individual rats.

Fig. 5 represents single-reciprocal plots for the mean values of the saturable uptake rate (i.e. the initial drug concentration divided by the rate) against the initial drug concentration in 3-, 7- and 52-week-old rats. Kinetic parameters for the saturable uptake process of propranolol by rat lungs, V_{max} and K_m', were estimated from the reciprocal of the slope and negative intercept on the abscissa, respectively as summarized in Table 1. Almost equivalent estimates were obtained for K_m' in all age-groups (about 25 μg mL^{-1}), whereas V_{max} took the largest value in 7 weeks ($0·726 \pm 0·101$ mg g^{-1} min^{-1}, $P<0·05$ vs 3, 52 and 104 weeks) and was relatively small in 3 ($0·501 \pm 0·082$ mg g^{-1} min^{-1}), 52 ($0·410 \pm 0·088$ mg g^{-1} min^{-1}) and 104 ($0·397 \pm 0·074$ mg g^{-1} min^{-1}) weeks. Both of these kinetic parameters seemed to be appreciably large compared with those obtained in the uptake of propranolol by rat hepatocytes, on the tissue or cellular protein contentration basis (Iwamoto et al 1986). These results suggest that the specific age-dependent difference in the pulmonary uptake of propranolol may exist in the saturable uptake process with relatively high capacity but with lower affinity. In addition, it is presumed that there may be a partial contribution of some tissue consistuents other than protein (e.g. lipid) to the initial saturable uptake mechanism by rat lungs.

In conclusion, the present in-vitro findings obtained by analysing the initial uptake rate of propranolol by rat lungs suggested a specific age-related difference in the capacity-limited uptake rate of this drug.

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Sciences and Culture of Japan.

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