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Effects of metabolic inhibitors and incubation temperature on the saturable uptake of propranolol by isolated rat lung tissue

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Abstract—The effects of several metabolic inhibitors (50 μM) on the initial uptake rate of propranolol (0·5 to 500 μg mL^{-1}) by the minced lungs (0·4 g) isolated from 7-week-old rats has been investigated in oxygenated, pH 7·4 Krebs-Ringer bicarbonate buffer solution (20 mL) containing 3% BSA at 37°C for 5 min. The effect of the incubation temperature was also examined. Metabolism of propranolol was almost insignificant (i.e. less than 1·3% of the initial load). The overall initial uptake rate was considered to be a combination of apparent linear transport and saturable processes. For the control uptake rate, the linear transport rate constant was $1·26 \pm 0·16$ g^{-1} mL^{-1} min^{-1}, while V_{max} and K_m' of the capacity limited uptake process were estimated as $0·727 \pm 0·074$ mg g^{-1} min^{-1} and $24·8 \pm 2·71$ μg mL^{-1}, respectively. No metabolic inhibitor tested had an effect on the linear transport rate of propranolol but 2,4-dinitrophenol and potassium cyanide inhibited saturable uptake rate (i.e. V_{max}) of propranolol significantly ($P < 0·01$) while ouabain, phloridine and iodoacetic acid did not do so significantly. Reduction of the incubation temperature to 15°C decreased and at 25°C tended to decrease, both linear transport and saturable uptake rates.

Propranolol has been shown to be extensively extracted by rat lungs after its intravenous administration (Hayes & Cooper 1971; Schneck et al 1977; Rikihisa et al 1981; Iwamoto et al 1987, 1988a) or its in-vitro perfusion (Dollery & Junod 1976; Iwamoto et al 1988b, c). Dollery & Junod (1976) predicted saturability for the pulmonary accumulation of propranolol in isolated, perfused rat lungs by proposing two binding sites with different affinities. Our recent paper (Iwamoto et al 1988b) has also suggested a trend toward saturation kinetics in the in-vitro pulmonary clearance of propranolol by the perfused rat lungs at the perfusate drug level between 10 and 100 μg mL^{-1}. However, neither the saturation kinetic parameters nor its mechanism has yet been clarified in any animal species.

The present study for characterizing the saturable kinetics, therefore, was designed to examine the effects of several metabolic inhibitors and the incubation temperature on the initial uptake rate of propranolol at various initial drug concentrations by the minced lung tissue isolated from 7-week-old rats.

Materials and methods

Materials. Propranolol hydrochloride,(±)-racemate, was donated by ICI-Pharma Ltd. (Osaka, Japan). Other chemicals and reagents of analytical grade were purchased from the following sources: bovine serum albumin (BSA, fraction V, essentially fatty acid free) from Sigma Chemical Co. (St. Louis, USA); 2,4-dinitrophenol (DNP) from Tokyo Kasei Co. (Tokyo, Japan); iodoacetic acid, ouabain and phloridine from Wako Pure Chemicals Ltd. (Nagoya, Japan); potassium cyanide (KCN) from Katayam Chemicals Ltd. (Nagoya, Japan). All other chemicals including n-heptane and iso-amylalcohol used for extracting unchanged propranolol from its metabolites were also of analytical grade.

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Animals. 7-week-old male Wistar rats (205–225 g), were purchased from the Shizuoka Laboratory Animal Farm (Hamamatsu, Japan) and housed in a specific pathogen-free room where the relative humidity and temperature were kept between 50–60% and 22–24°C, respectively. Animals were fasted overnight before the experiments.

Incubation of minced lung tissue with propranolol. Each rat was anaesthetized and treated for anticoagulation as reported by Iwamoto et al (1988b, c). Lungs were then exposed by a midline thoracotomy. Immediately after cannulations into the pulmonary artery and vein with PE-205 tubings, a single-pass lung perfusion was initiated with 5 mL of warmed (37°C), pH 7·4 Krebs-Ringer bicarbonate buffer solution containing 3% BSA, oxygenated with 95% O_2 – 5% CO_2 to displace the intravascular blood. The lungs were carefully isolated from the rat and were then rinsed with chilled buffer solution to remove adherent blood. The tissue was blotted thoroughly with filter paper and then minced into small (approximately 0·5 mm) cubic pieces with scissors.

At time zero, a given amount (0·4 g, about 2500 pieces) of the minced lung tissue was mixed with propranolol prepared at 0·5, 1, 2·5, 5, 10, 25, 50, 75, 100, 250 or 500 μg mL^{-1} in the warmed buffer solution (20 mL). The resultant mixture, which was oxygenated with 95% O_2 – 5% CO_2 and kept at 37°C, was mechanically agitated at about 2 strokes s^{-1} throughout the incubation. An aliquot (0·1 mL) of the medium was withdrawn periodically in the first 5 min and used for the analysis of drug concentration to estimate the initial uptake rate.

The effects of the metabolic inhibitors, 2, 4-dinitrophenol, iodoacetic acid, ouabain, phloridine and KCN at 50 μM, prepared in the same incubation buffer solution as above, and the effect of lowering the incubation temperature to 15°C or 25°C on the initial uptake rate, were also examined.

Determination of propranolol in the medium, lung tissue and homogenate of the tissue suspension. After 5 min incubation, the residual, uniform suspension of tissue pieces was divided into two portions. One portion was filtered to separate the tissue pieces, which were immediately washed and homogenized with 5 mL of drug free fresh buffer solution, and the other portion was directly homogenized using a Potter-type Teflon homogenizer. Propranolol concentration in the samples withdrawn from the incubation medium or in the two kinds of homogenate prepared were determined by extraction and re-extraction of the unchanged propranolol and fluorometric determination of the resultant sample as reported previously (Iwamoto & Watanabe 1985). Cumulative amounts of metabolites in both medium and tissue were estimated from the balance of intact drug, which was withdrawn and that remaining in the homogenate after 5 min, and the initial load (i.e. 0·01 to 10 mg) at time zero.

Results and discussion

Initial uptake rate of propranolol by rat lungs. The extent of metabolism after incubation for 5 min was found to be almost negligible at all initial propranolol concentrations, and ranged

from about 0·001 to 1·3% of the initial load with 10 to 0·01 mg of the drug (i.e. 20 mL of 500 to 0·5 μg mL^{-1}). This evidence was consistent with previous results (Dollery & Junod 1976; Iwamoto et al 1988b, c) also from which we have confirmed that the incubation medium containing BSA may be a prerequisite and adequate for the in-vitro lung perfusion with propranolol in rats. In addition, there was no adsorption of the drug to the experimental glassware. Therefore, the amount of the drug that disappeared from the medium could be thought to represent the amount taken up by the tissue. This was verified by the direct determination of the amount of drug that accumulated in the tissue after 5 min.

At all initial drug concentrations tested, the uptake seemed to take place rapidly in the first 5 min. To estimate the initial uptake rate, the time-course of propranolol (amount) taken up per g of the tissue was examined in the first 5 min at various initial drug concentrations (0·5 to 500 μg mL^{-1}). The regression analysis of each time-course showed the linear increase of propranolol uptake with time. Apparent, overall initial uptake rate at each drug concentration was then estimated from the slope. When the apparent initial uptake was plotted against the initial drug concentration, (closed symbols in Fig. 1), a curvilinear saturation profile (curve a) was obtained. The overall initial uptake

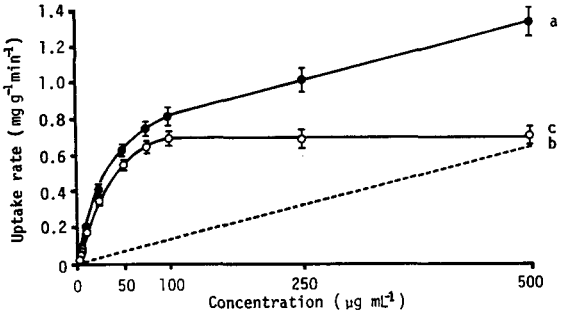

FIG. 1. Effect of initial substrate concentration (0·5 to 500 μg mL^{-1}) on the overall initial uptake rate of propranolol by isolated rat lung tissue (0·4 g) prepared from 7-week-old male Wistar rats in pH 7·4 Krebs-Ringer bicarbonate buffer solution (20 mL) containing 3% BSA, oxygenated with 95%O_2 – 5%CO_2 at 37°C. The overall uptake rate (curve a, ●) shows a curvilinear profile. The linear regression line of curve a in the concentration range of 100–500 μg mL^{-1} was extrapolated to zero concentration and was then drawn to coincide with the origin as shown with the broken line (b), Y = 0·00126X, where Y and X are the uptake rate and initial concentration, respectively. Saturable uptake rate of propranolol was then calculated by subtracting the individual linear transport rate (b) from the individual overall uptake rate (a) and expressed as the mean value (hyperbolic curve c, ○). Each point is the mean ± s.d. of four rats.

process for propranolol was, therefore, considered to be a combination of a linear transport process, which seemed to be completely independent of wide initial drug concentrations, and a saturable process. The former linear transport rate constant (K_{LT}) was estimated to be 1·26 ± 0·16 g^{-1} mL^{-1} min^{-1} from the slope of the line (broken line, line b, in Fig. 1). This linear transport rate was then subtracted from the overall initial uptake rate at each initial concentration to obtain the saturable uptake rate as represented with open symbols (curve c, Fig. 1).

Fig. 2. represents single-reciprocal plots for the saturable uptake rate (i.e. the initial concentration divided by the rate) against the initial drug concentration. From these results, kinetic parameters were determined as V_{max} = 0·727 ± 0·074 mg g^{-1} min^{-1} and K_m' = 24·8 ± 2·71 μg mL^{-1}. Both of these parameters appeared to be large compared with those obtained from uptake of the drug by rat hepatocytes on the tissue or cellular protein

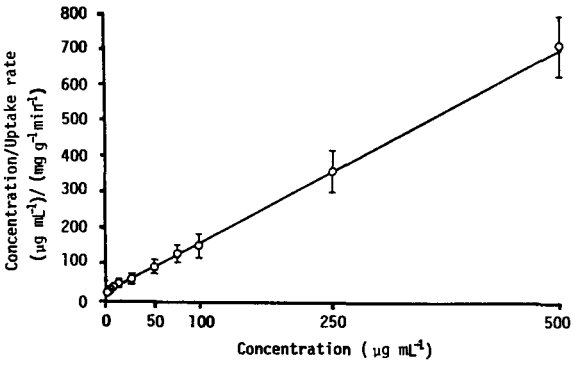

FIG. 2. Single-reciprocal plots for the mean saturable uptake rate of propranolol (Y, concentration/uptake rate) against the initial substrate concentration (X). Each point is the mean ± s.d. of four rats. The linear regression analysis of the individual experiment yielded the relationship (r = 0·999) expressed as the mean data points as Y = 1·375X + 34·1. Accordingly, the uptake kinetic parameters were obtained as follows: V_{max} = 0·727 ± 0·074 mg g^{-1} min^{-1} and K_m' = 24·8 ± 2·71 μg mL^{-1}.

Table 1. Effects of metabolic inhibitors and incubation temperature on the initial uptake kinetics of propranolol by isolated rat lungs.

Inhibitor or temperature	K_{LT} g^{-1} mL^{-1} min^{-1}	V_{max} mg g^{-1} min^{-1}	K_m' μg mL^{-1}
None (control)	1·26 ± 0·16[a]	0·727 ± 0·074	24·8 ± 2·71
2, 4-DNP	1·22 ± 0·11	0·370 ± 0·035[b]	25·5 ± 2·66
KCN	1·27 ± 0·19	0·366 ± 0·041[b]	24·1 ± 2·29
Ouabain	1·31 ± 0·21	0·662 ± 0·039	23·9 ± 2·45
Phloridine	1·28 ± 0·17	0·648 ± 0·065	24·3 ± 2·23
Iodoacetic acid	1·20 ± 0·21	0·644 ± 0·059	23·8 ± 2·11
15°C	1·01 ± 0·07[c]	0·545 ± 0·033[c]	24·2 ± 2·09
25°C	1·17 ± 0·11	0·647 ± 0·058	24·4 ± 2·02

[a] Mean ± s.d. of four rats.
[b] Significantly different from control at $P < 0·01$
[c] Significantly different from control at $P < 0·05$

content basis (Iwamoto et al 1986). This suggests that the rat lungs may possess some specific high-capacity site with relatively low affinity to extract propranolol from the incubation medium.

Effects of metabolic inhibitors and incubation temperature on the uptake rate of propranolol. Incubation was carried out with the same initial drug concentration and the kinetic analyses, as employed in Figs 1 and 2 were then applied to the relationships between overall initial uptake rate and the initial substrate concentration. Table 1 summarizes the effects of several metabolic inhibitors (50 μM) and the reduction of the incubation temperature on the initial uptake kinetic parameters for propranolol, i.e. K_{LT}, V_{max} and K_m'. There was no effect from any metabolic inhibitor tested or from lowering the incubation temperature on K_m'. Furthermore, none of the metabolic inhibitors showed any effect on K_{LT}, but 2,4-DNP and KCN did have a significant inhibitory effect on V_{max} by reducing it to about 50% of the control value ($P < 0·01$); the other inhibitors only tended towards inhibition. Reduction of the incubation temperature to 15°C decreased both K_{LT} and V_{max} by about 20 and 25% ($P < 0·05$), respectively, but at 25°C this was not significant. Only 2, 4-DNP and KCN inhibited the saturable uptake rate significantly. The effect of reduced incubation temperature was small. Such incomplete energy-dependence of V_{max} for propranolol by the lung tissue (Table 1) suggested that the saturable uptake capacity for this drug by rat lungs might be relatively large, as discussed above.

In conclusion, the present in-vitro results, obtained from the

analysis of the initial uptake rate of propranolol by rat lung tissue, have confirmed our previous proposal for the saturation process as made from in-vivo and in-vitro investigations that there is a possible contribution from specific, high capacity sites with relatively low affinity, to the extensive pulmonary first-pass removal of the drug from the circulation.

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Influence of capsaicin-sensitive fibres on experimentally-induced colitis in rats

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Abstract—Systemic capsaicin pretreatment worsens trinitrobenzene sulphonic acid-induced colitis but has no effect on colitis induced by ethanol or acetic acid. The influence of capsaicin-sensitive fibres on experimentally-induced colitis seems to depend upon the type of ulcerogenic stimuli in relation to its chronic nature.

Capsaicin, when administered in high doses induces selective degeneration (Jancsò et al 1985) and functional impairment of certain primary sensory neurons (Nagy 1982) innervating various viscera, including the gastrointestinal tract (Sternini et al 1987; Mulderry et al 1988). Subcutaneous capsaicin, at desensitizing doses, increases the degree of gastric ulcers induced by pylorus ligation, acid distension (Szolcsànyi & Barthó 1981), indomethacin, cysteamine or ethanol (Holzer & Sametz 1986), as well as duodenal ulcers induced by dulcerozine or cysteamine (Maggi et al 1987) and small intestinal lesions induced by indomethacin (Evangelista et al 1987).

Since capsaicin-sensitive afferent fibres are present also in the large intestine (Barthó & Szolcsànyi 1981) it appeared worthwhile to determine their potential involvement in the pathogenetic mechanisms of some experimentally-induced colitis in rats.

Materials and methods

Male albino Sprague-Dawley Nossan strain, 180-210 g, were housed at constant room temperature (21 ± 1°) and relative humidity (60%) and with 12 h light-dark cycle (light on 6.00 am).

Trinitrobenzene sulphonic acid (TNB)-induced colitis. The rats were lightly anaesthetized with ether and a rubber cannula (8 cm long) was inserted into the colon via the anus (Wallace 1988). A solution of TNB (120 mg mL^{-1}) in 50% ethanol (v/v) was instilled into the lumen of the colon (total volume 0·25 mL). After 24 h or 1 week groups of rats were killed and colonic damage assessed by applying on the distal 8 cm of colon a transparent foil and tracing the borders of the necrotic area. The ulcer areas were weighed to quantitate differences between groups. The excised colonic samples were then blotted, dried overnight at 60°C in an oven and weighed. In control rats, samples of colonic tissue were taken from the same region (their mean ± s.e. weight was 114 ± 8 mg).

Ethanol-induced colitis. The rats were fasted overnight and 0·25 mL of 30% ethanol (v/v in distilled water) was administered intrarectally according to the method of Wallace et al (1985). Control rats were given 0·25 mL of saline in place of ethanol. Ten minutes after the ethanol challenge the rats were killed by CO_2 asphyxiation.

Ulcers developed in the terminal colon and were scored by an observer unaware of the treatment according to an arbitrary scale was: 0 = no visible damage, 1 = diffuse patches of superficial hyperaemia, 2 = patches of severe hyperaemia, 3 = extensive hyperaemia and haemorrhage (Wallace et al 1985).

Acetic acid induced colitis. The rats were fasted overnight and under light ether anaesthesia, the colon was exposed through a midline incision of the abdomen and the colon-cecum junction ligated. Two mL of a 5% acetic acid solution was injected into the lumen of the colon through a 25-gauge needle followed immediately by 3 mL of air which cleared the solution from the colon (Sharon & Stenson 1985). The incision was closed and 24 h later the animals were killed and their colon removed and the ulcers developing in the upper site of the colon scored as described for ethanol ulcers.

Capsaicin desensitization. Capsaicin was injected subcutaneously 50 mg kg^{-1} in a volume of 2 mL kg^{-1} two days after birth. Controls received the vehicle constituted by 10% ethanol, 10% Tween 80 in 0·9% NaCl in H_2O. The animals were used two months after this treatment.

Data analysis. Statistical analysis of the data related to pathologic score was performed by means of Smirnov's test for non-

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