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CONCENTRATION OF (±)-PROPRANOLOL IN ISOLATED, PERFUSED LUNGS OF RAT

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1 The metabolism and the accumulation of (±)-propranolol have been studied in isolated lungs of the rat, perfused with an artificial medium.

2 Little or no metabolism took place during the perfusion periods (up to 10 minutes).

3 Accumulation was observed with high tissue/medium ratios for substrate concentrations of 0.2 μM to 1 mM; there was evidence for saturability, but no real plateau could be seen. The presence of two binding sites with different affinities was established.

4 Cold greatly inhibited the accumulation process at low substrate concentrations, but had no effect at 1 mM propranolol.

5 Inhibition of accumulation was measured in the presence of imipramine, desmethylimipramine, nortryptiline, chlorpromazine and of Na^+-free medium. Cocaine, 5-hydroxytryptamine and noradrenaline had no effect. Lidocaine enhanced the accumulation process. Release of previously bound propranolol was accelerated in the presence of propranolol and imipramine, unaffected by a Na^+-free medium and decreased by cold and by lidocaine.

6 Experiments on lung tissue slices yielded qualitatively similar results to those obtained with perfused lungs. Ouabain and KCN had no or little effect on propranolol accumulation.

Introduction

Previous studies on the distribution of (±)-propranolol in tissues have shown that the lungs were able to concentrate (±)-propranolol to a remarkable extent since a tissue/medium ratio of up to 250 was observed (Black, Duncan & Shanks, 1965). Further analysis (Hayes & Cooper, 1971) showed that in rats, dogs and monkeys, large amounts of metabolites of propranolol could also be detected in the lungs. It was not known if the presence of these metabolites was the result of the local pulmonary metabolism of propranolol or of their accumulation after production elsewhere in the body.

The present studies were therefore undertaken to investigate the mechanism of concentration and the possible metabolism of (±)-propranolol in isolated perfused lungs of the rat. In view of their similar physicochemical properties, it was also interesting to compare the mode of accumulation of propranolol with that of imipramine and other basic amines, whose concentration in the lungs has already been the object of recent studies (Junod, 1972a, Andersen, Orton, Pickett & Eling, 1974). The possibility of a type of uptake similar to the amine pump had to be examined in view of the effect of propranolol on noradrenaline uptake by the heart as reported by Foo, Jowett & Strafford (1968), although, according to Iwasawa & Gillis (1974), propranolol had no effect on noradrenaline uptake by the lung.

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Methods

Medium and materials

The perfusion medium consisted of Krebs-Ringer bicarbonate buffer, containing 5 mM glucose and 4.5% bovine serum albumin; to obtain a Na^+-free medium, sucrose was substituted for NaCl and Tris HCl for $NaHCO_3$, while the albumin was made Na^+-free according to the procedure already described (Junod, 1972b). [^{14}C]-propranolol hydrochloride (14.66 μCi/mg or 4.377 mCi/mmol), (±)-propranolol, 4-OH propranolol and desisopropylpropranolol were all given by ICI. Imipramine and desmethylimipramine were given by Geigy Pharmaceuticals, iproniazid phosphate by Roche Products Ltd., nortryptiline by E. Lilly & Co. Ltd., chlorpromazine hydrochloride by May and Baker Ltd. and lidocaine hydrochloride by Astra Chemicals Ltd. Ouabain,

noradrenaline bitartrate and 5-hydroxytryptamine creatinine sulphate were purchased from BDH. Bovine serum albumin fraction V was obtained from Sigma Chemicals.

Unless otherwise specified, drugs were added to the perfusion medium and were therefore present during the entire perfusion. In the experiments where the effects of drugs on the efflux of propranolol were studied, drugs were added to the perfusion medium only during the period when efflux of ^{14}C was measured.

Experimental procedures

Details of the procedure used have already been reported (Junod, 1972a). Male Wistar rats, weighing 200–300 g, were used. After anaesthesia with 50 mg/kg Na pentobarbitone injected intraperitoneally and tracheostomy, the animal was artificially ventilated with a gas mixture of 95% O_2 and 5% CO_2. The lungs were then isolated, perfused at the rate of 10 ml/min and placed in a closed chamber. After a 5 min equilibration period, the lungs were perfused with medium containing various concentrations of [^{14}C]-propranolol for various periods of time. They were then dissected, blotted dry, weighed and homogenized in 6 ml 0.4 N $HClO_4$. When the efflux of ^{14}C was measured, the effluent was collected during the 8 min following the perfusion with [^{14}C]-propranolol and its radioactive content was measured and expressed as % of the initial uptake calculated from the inflow-effluent difference during the 5 min perfusion with [^{14}C]-propranolol.

When rat lung slices were used, the lungs were perfused *in situ* with 0.9% w/v NaCl solution (saline) at 4°C to get rid of the blood and subsequently sliced. Slices were then incubated for 60 min in 2.5 ml standard medium (without albumin) containing 1 μM [^{14}C]-propranolol and another drug when necessary, at 37°C, in 5% CO_2 and 95% O_2. At the end of the incubation period, slices were blotted dry, weighed, dissolved in Soluene (Packard) and their radioactive content measured.

Analytical procedures

The radioactive content of samples of inflow, effluent and lung homogenate was measured in a scintillation spectrometer after addition of 10 ml of Instagel (Packard). An external standard was used to monitor counting efficiency and radioactivity measurements were corrected accordingly. The tissue/medium ratio was calculated from the ratio

$$\frac{\text{d min}^{-1}\ \text{g}^{-1}\ \text{lung tissue}}{\text{d min}^{-1}\ \text{ml}^{-1}\ \text{inflow}}$$

In the studies on [^{14}C]-propranolol metabolism by perfused rat lungs, the lungs were homogenized in 10 ml 10% Na_2CO_3 at pH 10. An aliquot of lung homogenate was extracted with 17 ml toluene. Another aliquot was acidified to pH 1–2 and extracted with ethyl acetate. The radioactivity in the solvent phase was measured and compared with that of the lung homogenate. The toluene extract was then concentrated under a stream of N_2 and an aliquot of the concentrate was analyzed by thin-layer chromatography on glass plates coated with Silica Gel F-254. The solvent system was : isopropanol: ammonia: water (20:1:3) and good separation was obtained between propranolol, 4-OH propranolol and desisopropylpropranolol after a 6 h development.

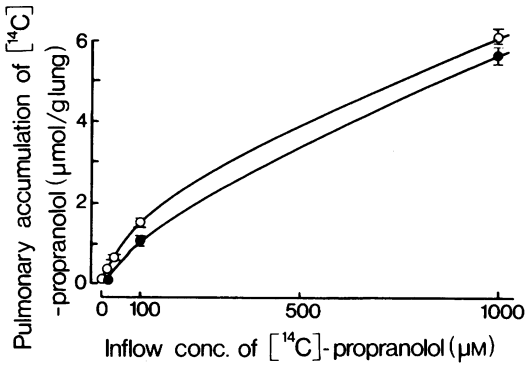

Figure 1 Relationship between the inflow concentration of [^{14}C]-(±)-propranolol and its accumulation in the lung after a 5 min perfusion period, at 37°C (○) and at 4°C (●). Each point represents the mean of 3 to 11 experiments. Vertical lines show s.e. mean.

Results

The percentage of ^{14}C extracted in toluene at pH 10 was 81.2 ± 2.6% (mean of 13 determinations ± s.e.) for propranolol concentrations in perfusion medium ranging from 1 to 34 μM. After a second extraction of the aqueous residue, 9.3 ± 1.2% of the ^{14}C initially present in the lung homogenate was present in the solvent phase. Ninety per cent of the radioactivity was therefore extractable in toluene at alkaline pH, and chromatographic analysis of the toluene extracts established that all of that radioactivity could be attributed to propranolol. Extraction of lung homogenate in ethyl acetate at pH 1–2 resulted in the presence of 10.6 ± 1.3% of the ^{14}C present in the lung homogenate in the solvent phase. No chromatographic analysis of that fraction was made. The radioactive content of the lung was taken as a measure of the [^{14}C]-propranolol accumulation in the lung, since, to the extent of at least 90%, it corresponded to [^{14}C]-propranolol, and no correction factor was applied.

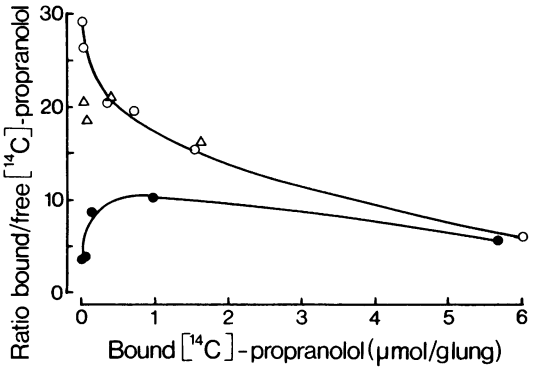

Figure 2 Relationship between the [^{14}C]-(±)-propranolol bound to the lung and the ratio of bound/free ([^{14}C]-(±)-propranolol during a 5 min perfusion period, at 37°C (○), 27°C (△) and 4°C (●). Each point represents the mean of 3 to 11 experiments.

The effect of the duration of perfusion with 1 μM propranolol on its pulmonary accumulation was slight, since the tissue/medium ratio per unit of time decreased from 6.1 ± 0.2 to 5.2 ± 0.5 and 4.6 ± 0.6 for periods of 2, 5 and 10 min (mean ± s.e. of four, eleven and four experiments, respectively). In the subsequent experiments 5 min perfusions with [^{14}C]-propranolol were used.

Increasing the substrate concentration from 0.2 μM to 1 mM resulted in a progressive decrease in the tissue/medium ratio but no real plateau could be obtained, when substrate concentration was related to the accumulation of propranolol in the lung (Figure 1).

The effect of temperatures from 4°C to 37°C on the pulmonary concentration of [^{14}C]-propranolol at three different concentrations of substrate was measured. The inhibitory effect of low temperature was found to be dependent on the substrate concentration, being very marked at 0.2 and 1 μM [^{14}C]-propranolol and nearly absent at higher concentrations (100 and 1000 μM).

From these data, a Scatchard plot relating the amount of bound propranolol to the ratio of bound/free propranolol was drawn (Figure 2); it was certainly made of two different curves, establishing the existence of two populations of binding sites with different affinities. Only the high affinity binding sites were apparently sensitive to the inhibitory effect of low temperature.

Replacement of Na^+ in the perfusion medium by sucrose or Li^+ resulted in a decrease in the accumulation of [^{14}C]-propranolol, that effect being particularly marked in the presence of sucrose (Table 1). The same table shows the inhibitory effect of high concentrations of drugs also known as lipophilic basic amines (imipramine, desmethylimipramine, chlorpromazine, and nortryptiline): its magnitude was in fact similar to that resulting from the effect of propranolol itself. Noradrenaline, 5-hydroxytryptamine, cocaine and 4-OH propranolol, on the other hand, did not affect the pulmonary accumulation of propranolol. The significant increase in tissue/medium ratio in presence of 0.1 mM lidocaine was unexpected. The experiments on the efflux of [^{14}C]-propranolol (Table 2) showed that propranolol itself and

Table 1 Effect of various experimental conditions on the accumulation of [^{14}C]-propranolol by isolated lungs of rat

Experimental condition	n	*T/M ± s.e.*
Control	11	26.0 ± 1.1
Control 4°C	7	3.4 ± 0.3*
Na ± free medium (Na^+ replaced by Li^+)	4	19.1 ± 1.4*
Na ± free medium (Na^+ replaced by sucrose)	4	13.7 ± 0.6*
Noradrenaline 0.1 mM	4	27.4 ± 1.2
5-hydroxytryptamine 0.05 mM	3	28.4 ± 2.8
4-OH propranolol 0.1 mM	4	23.1 ± 1.5*
Cocaine 0.1 mM	4	29.7 ± 2.0
Imipramine 0.01 mM	4	23.9 ± 2.8
Imipramine 0.1 mM	4	15.4 ± 0.9*
Desmethylimipramine 0.1 mM	4	14.7 ± 0.9*
Nortryptiline 0.1 mM	4	12.7 ± 1.1*
Chlorpromazine 0.1 mM	4	13.6 ± 0.8*
Lidocaine 0.1 mM	6	36.1 ± 1.2*

The accumulation of [^{14}C]-propranolol during a 5 min perfusion period with 1 μM [^{14}C]-propranolol was measured under various conditions and expressed as d min^{-1} g^{-1} lung/d min^{-1} ml^{-1} inflow (T/M).
*Statistically different from control value ($P < 0.05$).

Table 2 Effect of various drugs and medium compositions on the efflux of [^{14}C]-propranolol from isolated lungs of rat

Experimental condition	n	*Loss of ^{14}C in 8 min as % of ^{14}C uptake*
Standard medium	4	34.25 ± 2.54
+ Propranolol 10 μM	3	61.52 ± 1.32*
+ Imipramine 10 μM	3	52.74 ± 4.90*
+ Lidocaine 0.1 mM	3	24.75 ± 2.60*
Li Cl substituted for Na Cl	3	29.95 ± 2.91
Standard medium at 4°C	3	15.38 ± 2.12*

After a 5 min perfusion period with 1 μM propranolol and standard medium, the efflux of [^{14}C]-propranolol was measured during a 8 min perfusion period under various conditions (mean ± s.e.). The uptake of [^{14}C]-propranolol was taken as the inflow-outflow ^{14}C difference measured during the initial 5 min perfusion period.
*Statistically different from control values ($P < 0.05$).

imipramine had an accelerative effect; Na^+-free medium did not result in any significant change, whereas lidocaine decreased that efflux. A more important reduction of efflux resulted from perfusion at low temperature.

Finally, experiments were done with lung slices to study the effects of metabolic inhibitors and also to see if qualitative changes in the nature of the process of concentration could be observed under these experimental conditions (Table 3). Under control conditions, the tissue/medium ratio obtained was 7.68. This value was sufficiently high to make the correction for the radioactivity present in the extracellular space unnecessary. Lowering the temperature and adding unlabelled propranolol and chlorpromazine had the same effect as in the perfused lungs. Ouabain, at 1 mM, did not decrease the tissue/medium ratio, whereas KCN, also at 1 mM, had only a partial inhibitory effect.

Discussion

The analysis of the radioactivity found in lung homogenate clearly indicates that little or no metabolism of propranolol took place under these experimental conditions. In view of the results obtained by Hayes & Cooper (1971), it is therefore logical to assume that the lung can accumulate large amounts of metabolites produced elsewhere in the body, in the liver in particular. On the other hand, Walle, Ishizaki & Gaffney (1972) reported that, during perfusion of isolated lung of the dog, isopropylamine could be formed from propranolol, but no quantitative data were given.

The nature of the mechanism of concentration of propranolol presents several points of similarity with that of imipramine (Junod, 1972a): it shows the same trend towards saturation at the same range of substrate concentrations; it is affected by the same basic, lipophilic drugs, but not by noradrenaline or by 5-hydroxytryptamine. The efflux of ^{14}C is accelerated by propranolol itself as well as by compounds of similar physicochemical properties.

However, the observations that both cold and Na^+-free medium were inhibitory established a major difference. They strongly suggested the possibility of a Na^+-dependent, carrier-mediated transport, as has been shown to be the case for the uptake of 5-hydroxytryptamine and noradrenaline by pulmonary endothelial cells (Junod, 1972b; Iwasawa, Gillis & Aghajanian, 1973; Nicholas, Strum, Angelo & Junod, 1974). In the case of propranolol, however, the effect of Na^+-free medium was only partially inhibitory and varied depending on the nature of the substitute for Na^+, Li^+ or sucrose; the absence of effect of Na^+-free medium on the efflux of propranolol is a strong argument against such a type of transport, since reversal of Na^+ gradient should have resulted in an increased rate of release. The experiments done with lung slices in the presence of ouabain and KCN also support the view that propranolol accumulation does not depend on the existence of a normal Na^+ pump. It is interesting to note that Gillepsie & Towart (1973) found a similar effect of sucrose and Li^+ as substitutes for Na^+ in their studies on the extraneuronal uptake of noradrenaline; they could not explain that observation satisfactorily.

Other studies (Huunan-Seppälä, 1972) have suggested the existence of a binding process to phospholipids, similar to that reported by Balzer, Makinose, Fiehn & Hasselbach (1968) for various lipophilic amines: chlorpromazine, prenylamine, reserpine. The same hypothesis was invoked in the case of imipramine concentration in the lung (Junod, 1972a), and the interrelations between drugs of similar nature are consistent with that concept.

That process appears to depend on the presence of sites with two different affinities, as can be seen from the Scatchard plot. A similar observation was reported by Huunan-Seppälä (1972) for binding of propranolol to mitochondria, by Kwant & Seeman (1969) for binding of chlorpromazine to red cells, by Balzer *et al.* (1968) for the binding of various amines to vesicles isolated from sarcoplasmic reticulum and by Weinstein, Varon & Roberts (1971) for binding of imipramine to brain synaptosomes.

Orton, Anderson, Pickett, Eling & Fouts (1973) studied the accumulation of several basic amines in isolated lungs of the rabbit, during steady state conditions. Their findings on the saturability of the concentration process correlate well with the results obtained with imipramine and propranolol in rat isolated lungs. Anderson *et al.* (1974) extended their studies on the nature of the concentration process of imipramine, amphetamine, chlorcyclizine and

Table 3 Effect of various drugs and conditions on the accumulation of [^{14}C]-propranolol in lung slices of rat

Experimental condition	n	*% T/M of control condition*
Standard medium	8	100
Standard medium at 4°C	4	48.7 ± 4.1
+ Propranolol 0.1 mM	4	34.4 ± 1.0
+ Chlorpromazine 0.1 mM	4	33.0 ± 2.0
+ Ouabain 1 mM	4	101.0 ± 3.7
+ KCN 1 mM	4	77.2 ± 4.8

Lung slices were incubated for 60 min in presence of 1 μM [^{14}C]-propranolol under various conditions. The tissue/medium ratio (T/M), measured as d min^{-1} g^{-1} lung/d min^{-1} ml^{-1} medium, is expressed as % of the tissue medium ratio obtained under standard conditions (mean ± s.e.).

methadone. They were able to find evidence for the presence of two components of accumulation: a linear one and a saturable one, for high and low substrate concentrations respectively. The results obtained in this study are compatible with these findings (cf. Figure 1); it is suggested that the temperature-sensitive component and the linear component might be the expression of the same phenomenon, whose nature has yet to be defined. It is conceivable that, for the low affinity sites, hydrophobic bonds might exist, since the tissue/medium ratio does not exhibit temperature dependence.

All these results point to the existence of two different processes, defined by either their temperature-sensitivity or their kinetic characteristics. More work is needed before the exact pharmacological nature of these two processes and the location of these sites are known.

The direct implications of these concentration phenomena have already been discussed (Junod, 1972a, Anderson *et al.*, 1974), and are concerned with the relationship between binding and release of these drugs and their plasma concentration. It is clear that the lung can act as a capacitor, storing large amounts of these compounds and releasing them slowly. The magnitude of this role can be assessed if the degree of concentration of the drug in the lungs and its volume of distribution are known. The mode of administration has also to be considered, since the effect of pulmonary binding will be more apparent after intravenous injection than after oral ingestion. Except for methadone (Orton *et al.*, 1973), binding is not accompanied by metabolism. Since the binding is a reversible process, administration of another drug of similar physicochemical properties can result in the release of the previously stored agent and an unwanted increase in its plasma concentration. It is therefore necessary to study more closely the possible interrelationships between these products. The liver remains the most important organ for the long term disposal of these drugs, but the role of the lung in the short-term fate of drugs should not be neglected.

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