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Uptake of zimelidine and inhibition of uptake of 5-hydroxytryptamine in the isolated, ventilated and perfused rat lung

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The non-respiratory functions of the lung include, among other things, uptake of both endogenous and exogenous substances from the pulmonary circulation (for review see [1–3]). The consequence of such uptake, which also can be followed by metabolism, is that the arterial circulation will be reached by a less amount of the chemical. The lung can therefore function as a buffer against potentially toxic xenobiotics and endogenous substances. Several drugs, mainly tricyclic antidepressants, have been shown to inhibit the pulmonary uptake of both noradrenaline and 5-hydroxytryptamine (5-HT) [4–7]. This could be the mechanism for the cardiac toxicity of these drugs, as noradrenaline will reach the heart in higher than normal concentrations, especially in situations with the combination of reduced lung clearance and increased catecholamine concentrations in the blood. Several inhibitors of uptake mechanisms for 5-HT in neurons have been developed in recent years, aiming at a more specific drug for the treatment of CNS diseases (for review see [8]). These drugs may, however, also inhibit the lung uptake of 5-HT from the circulation, since the uptake in pulmonary endothelial cells has characteristics similar to that of neurons [4]. Such effects were recently reported by Fjalland [9]. The aim of the present study was to investigate lung uptake of zimelidine as well as the interactions between zimelidine and lidocaine, and the influence of zimelidine on lung uptake of 5-HT.

The preparation of the rat lungs was essentially the same as that reported previously [14]. In brief, Sprague–Dawley rats of either sex weighing 250–350 g were anesthetized with pentobarbital (Mebumal vet®, ACO Läkemedel, Sweden) (40 mg/kg body wt, given i.p.). A dose of 500 I.U. heparin (KABI AB, Sweden) was administered intravenously to prevent thrombosis. At anesthesia, the rats were tracheotomized, and the pulmonary artery and veins were cannulated. The lungs were then carefully excised and placed in a humidified perfusion chamber at 37°. The pulmonary circulation was perfused with a modified Krebs buffer containing polymerized gelatin (Haemaccel Behringwerk AG, F.R.G.) added as an osmotic substituent. [^{3}H]Zimelidine (sp. act. 13 mCi/mmole) (Astra Läkemedel AB, Södertälje, Sweden) was added together with unlabelled zimelidine to the desired concentrations. In the experiments where lung uptake of 5-HT was studied, the buffer also contained 10^{-7} M [^{14}C]-5-HT (sp. act. 12 Ci/mmole; Radiochemical Centre, Amersham, U.K.) and iproniazide (0.5×10^{-5} M) to prevent metabolism of 5-HT. The respiratory rate was 70 cycles/min, the respiratory volume, 2 ml, and the perfusion rate, 10 ml/min. The lungs were ventilated with room air, and the buffers were bubbled continuously during the experiments with a gas mixture of 95% O_2, 5% CO_2.

Uptake of 5-HT and zimelidine was calculated using the formula:

$$\text{Extraction} = 100 \times \left(1 - \frac{\text{dpm/sample}}{\text{dpm/total}}\right)$$

The dpm/sample is dpm/l ml sample from the effluent, and dpm/total is dpm/l ml sample from the sample influent. One ml samples were taken from the perfusate at different

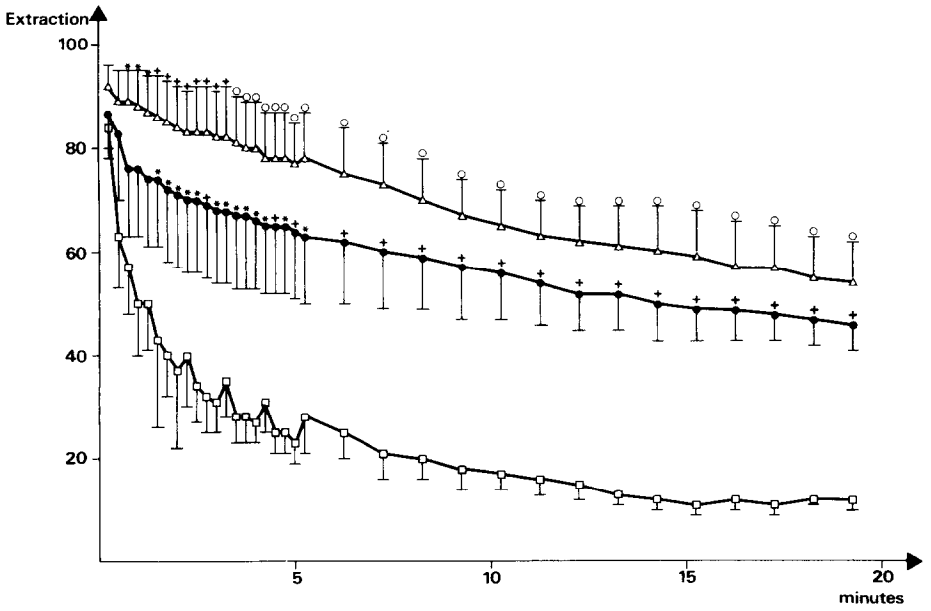

Fig. 1. Extraction of zimelidine at 0.5×10^{-5} M (△——△), 10^{-6}M (●——●) and 10^{-5} M (□——□) concentrations. $P < 0.05$ (★), $P < 0.01$ (+), $P < 0.001$ (○) compared to 10^{-5} M. Bars indicate S.E.M.

Table 1. Constants from washout experiments after 20 min perfusion with 10^{-6} or 10^{-5} M zimelidine*

	$Concn_{effluent} = A \times e^{-\alpha \times t} + B \times e^{-\beta \times t}$					
	A(M)	$\alpha(min^{-1})$	$t_{1/2\alpha}$ (min)	B(M)	$\beta(min^{-1})$	$t_{1/2\beta}$ (min)
10^{-6} M	$0.35 \cdot 10^{-7} \pm 0.21 \cdot 10^{-7}$†	-0.679 ± 0.128 (n.s.)	1.16 ± 0.24	$0.21 \cdot 10^{-7} \pm 0.05 \cdot 10^{-7}$ (n.s.)	-0.045 ± 0.011†	23 ± 10
10^{-5} M	$0.76 \cdot 10^{-6} \pm 0.05 \cdot 10^{-6}$†	-1.14 ± 0.18 (n.s.)	0.66 ± 0.11	$0.30 \cdot 10^{-6} \pm 0.07 \cdot 10^{-6}$ (n.s.)	-0.106 ± 0.013†	6.9 ± 0.8

* Lungs were, after 20 min perfusion with 10^{-6} M or 10^{-5} M zimelidine, perfused with buffer without zimelidine during the subsequent 45 min. The effluent curves were with the best fit adapted the equation. Values given are mean ± S.E.M.; $n = 6$.
† $P < 0.02$.

time intervals, mixed with 10 ml Instagel (Packard Instrument, IL) and counted in a liquid scintillation spectrometer with internal standardization for correction of quenching.

The perfusion buffer and extracts of lungs homogenized after 60 min recirculation of 100 ml buffer containing 0.5×10^{-6} M zimelidine were analysed for metabolites of zimelidine by the HPLC method described by Westerlund *et al.* [11]. The lower detection limit for zimelidine, norzimelidine and the primary amine, with this method, is 30 pmole injected into the HPLC system.

Student's *t*-test was used for calculating statistical differences.

Zimelidine was taken up in a concentration-dependent manner (Fig. 1), and the initial uptake of the drug was higher than 90% in the first sample, representing the first 15 sec of exposure, at a concentration of 0.5×10^{-6}M. The uptake then declined gradually during the subsequent 20 mins, to 54 ± 8% (mean ± S.E.M.) at the end of the perfusion period. In the experiments with the higher concentrations of zimelidine, the initial extraction was almost as high as that at 0.5×10^{-6} M, but it declined more rapidly. The uptake was 46 ± 5% at 20 min with 10^{-6} M zimelidine and 10 ± 3% with 10^{-5} M zimelidine.

From these results, it was concluded that the uptake, at least in part, occurred at some saturable binding sites. Such sites have been postulated to exist for other basic amines, resulting in displacement phenomena both *in vitro* [5, 12, 13] and *in vivo* [14]. The present study demonstrates the same type of displacement as the effluent concentration of zimelidine after 20 min perfusion with 0.5×10^{-6} or 10^{-6} M, increasing instantaneously after a bolus injection of 3.7×10^{-6} mole lidocaine (Fig. 3). After the displacement peaks, there was an immediate return of radioactivity back to a level slightly below that before the bolus injection. This is in agreement with displacement of nortriptyline by lidocaine [13], and is probably the result of a higher affinity of zimelidine for the binding sites than is the case for lidocaine.

After 60 min perfusion with buffer containing 0.5×10^{-6} M zimelidine, none of the metabolites reported by Westerlund *et al.* [11] were detectable.

When the lungs, after a 20 min uptake period without displacement by lidocaine, were shifted over to a buffer with no zimelidine added, radioactivity decreased in the effluent according to a 2-compartment model represented by the formula:

$$Concn_{effluent} = A \times e^{-\alpha \times t} + B \times e^{-\beta \times t}$$

The values of the constants and the corresponding half-lives of the two components of elimination are given in Table 1. The results indicate that the drug is released from more than one compartment, and taken together with the displacement results that one of the compartments could be a binding site with some selectivity and a limited capacity. Local anaesthetic agents [15] and tricyclic antidepressant agents [16] have been shown to bind to phospholipids, which is probably the common saturable binding site for these types of drugs. The lipophilic nature and the existence of charged molecules at physiological pH [17] in the phospholipid phase of the cellular membranes are properties probably favouring binding of basic amines. The free base, which is highly lipid soluble, could equilibrate with the lipophilic part of the phospholipids, and the cationic form combine with the negatively charged sites. Lung uptake of xenobiotic amines could therefore not only change with the type of amine, but also if the nature of the binding site for these substances is altered. This is poorly investigated, however, but we have previously found that smokers, compared to non-smokers, have a statistically significant increased lung uptake of lidocaine [18], which might be an effect of the smoking on the nature of these binding sites.

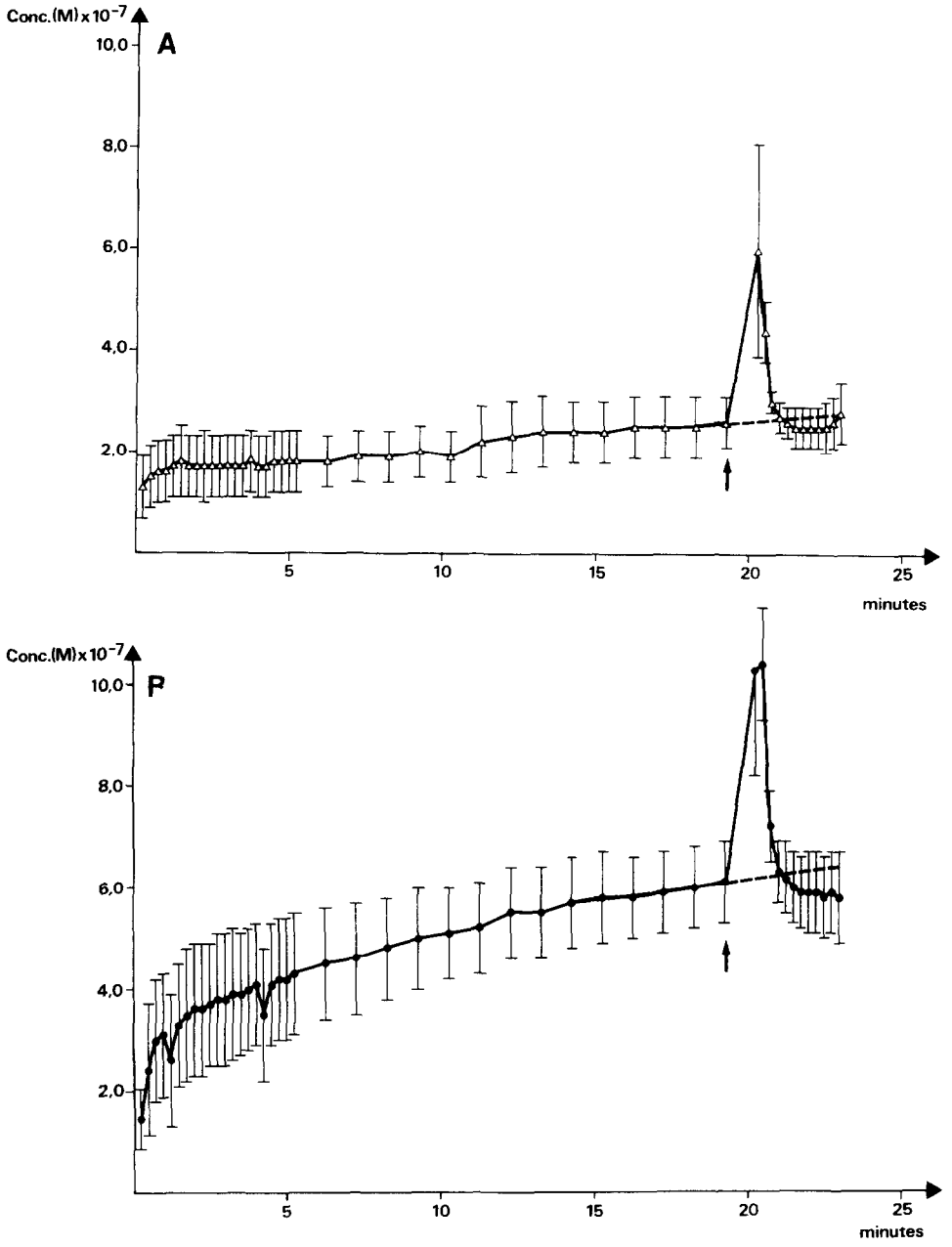

Fig. 2. (A) Effluent concentration of zimelidine, where 3.7×10^{-6} mole lidocaine was injected rapidly into the influent buffer, as indicated by arrow. Influent concentration was 0.5×10^{-6} M. Dashed line represents extrapolation of effluent concentration after lidocaine. Bars indicate S.E.M. (B) The same type of experiment as in Fig. 2A; influent concentration was 10^{-6} M; 3.7×10^{-6} mole lidocaine was injected as bolus. Bars indicate S.E.M.

Uptake of 5-HT at a concentration of 10^{-7} M declined with respect to time when no zimelidine was added; the value was 51 ± 6% in the first sample (Fig. 2), and 18 ± 2% at 20 min. When zimelidine was added to the buffer, the uptake of 5-HT declined more rapidly than in the control experiments with 5-HT only. Both 0.5×10^{-6} M and 10^{-6} M concentrations of zimelidine inhibited lung extraction of 5-HT. At 20 min, the value was 7 ± 1 and 5 ± 1%, respectively (Fig. 2). The concentrations used in the present investigation are within the range reported to be clinically effective for the treatment of endogenous depression [8]. The possible consequence of this inhibition is difficult to assess, as 5-HT is an amine with a large variety of pharmacological actions. This effect of zimelidine on normal physiological functions of the lung should, however, be taken into consideration.

A way to restore lung uptake of 5-HT from the blood, would be to displace zimelidine from the lung by amines not inhibiting the uptake of 5-HT. Lidocaine has previously been shown not to affect uptake of noradrenaline *in vitro* [19], and the effect of displacing zimelidine by lidocaine on extraction of 5-HT was therefore followed. A slight, but

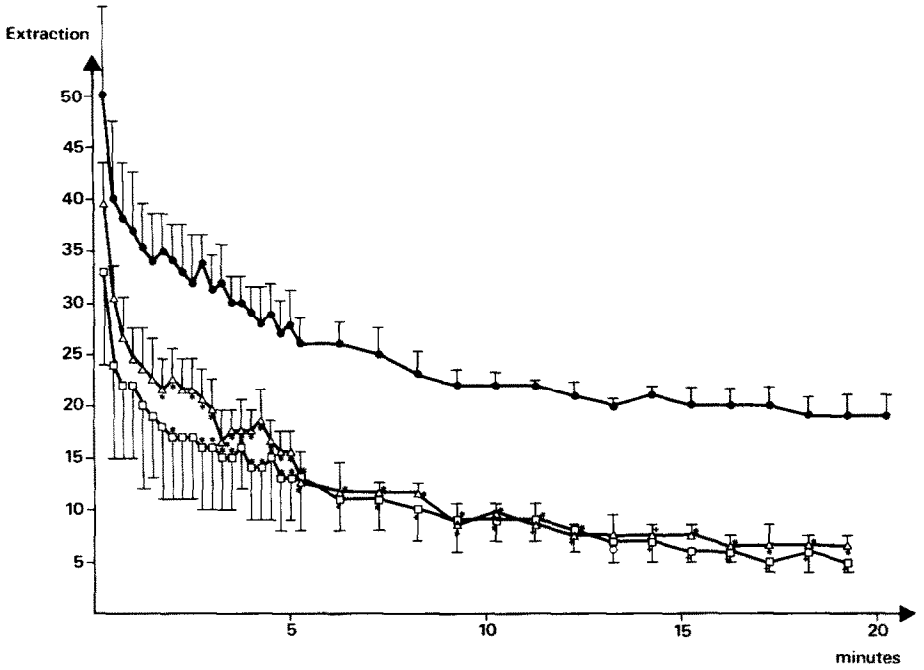

Fig. 3. Extraction of 5-HT at a concentration of 10^{-7} M without any zimelidine added (●——●), and at zimelidine concentration of 0.5×10^{-6} M (△——△) and 10^{-6} M (□——□). $P < 0.05$ (★), $P < 0.01$ (+) compared to the extraction where no zimelidine was added. Bars indicate S.E.M.

statistically insignificant, increase in lung extraction of 5-HT occurred immediately after the displacement of zimelidine. The nature of the displacement curves (Fig. 3), with a decline in concentration of zimelidine in the effluent compared to before the displacement, indicates that zimelidine is bound more strongly to the tissue than is lidocaine. Zimelidine is probably, therefore, not displaced in amounts sufficient to enhance the extraction of 5-HT, and rapid redistribution of zimelidine to these bindings sites within the tissue might also occur.

In summary, uptake of zimelidine from the perfusate in isolated perfused rat lung was concentration-dependent. Accumulated zimelidine was released from the lung according to a two-compartment model and lidocaine injected as a bolus did partially displace zimelidine. The properties of the displacement curves indicated, however, that the affinity to the lung tissue was greater for zimelidine than lidocaine. Uptake of 5-hydroxytryptamine added to the perfusion buffer was inhibited by zimelidine. The displacement of zimelidine by lidocaine did not, statistically, significantly alter the extraction of 5-hydroxytryptamine.

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