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TRICHLORETHYLENE AND HALOTHANE INHIBIT UPTAKE OF 5-HYDROXYTRYPTAMINE IN THE ISOLATED PERFUSED RAT LUNG

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Abstract—Lung uptake of 5-hydroxytryptamine (5-HT) was determined in isolated perfused and ventilated rat lung, and was found to decrease with time according to a two-compartmental model. When the lungs were exposed to either trichlorethylene (TRI) or halothane, the uptake of 5-HT was drastically reduced. Both TRI and halothane gave log dose inhibition curves, which were superimposed, i.e. they were equally potent to inhibit lung uptake of 5-HT. At a concentration TRI of 18,000 ppm, the extraction of 5-HT was inhibited by 80 ± 2 ($\bar{x}$ ± S.E.M.) per cent, at 8500 ppm the inhibition was 65 ± 6 per cent and 25 ± 1 per cent at 3000 ppm. When the lungs were exposed to halothane, the inhibition was 85 ± 6 per cent at 40,000 ppm, 48 ± 1 per cent at 6000 ppm, and 15 ± 0.3 per cent at 2000 ppm. When exposure to the solvent was discontinued, extraction of 5-HT was rapidly normalized. There was no detectable displacement of [^{3}H]-5-HT from lungs saturated with the amine when they subsequently were exposed to solvent-containing atmosphere. This inhibition of lung uptake of 5-HT from the circulation is therefore postulated as to be an effect dependent on concentration solvent in the tissue, and is probably due to a reversible membrane stabilization of the endothelium.

Trichlorethylene (TRI) is a widely used solvent in industry, and the world production in 1973 was 1 million tons [1]. It is a potentially hazardous chemical with reported toxicity to the liver [2], skin [3], the central nervous system [4], and the heart [5]. It has caused deaths by ventricular fibrillations, which in part could be due to the observed increased effect of endogenous catecholamines on the myocardium [6]. This is a well documented cause of death among solvent abusers [7]. TRI also has uses as a gaseous anesthetic, and as the chemical structure is very similar to that of halothane, it is probable that the two solvents have properties in common.

The lungs have been shown to play an important role in the inactivation of endogenous substances (for review see [8]), like noradrenaline (NA) [9], 5-hydroxytryptamine (5-HT) [10], and prostaglandins [11], as well as drugs like propranolol [12], lidocaine [13] and tricyclic antidepressant drugs [14]. Most endogenous substances are metabolised by the lung after uptake, whereas most drugs are released back into the circulation without detectable metabolic degradation. The major mechanism for uptake of drugs is simple diffusion (for review see [15]), the lung therefore can be viewed as a pharmacokinetic pool for these substances. In 1953, Gaddum found that 5-HT was inactivated in the pulmonary circulation is isolated perfused cat lung [16]. With radioautographic techniques, it was later shown that the substance after uptake is located in the endothelial cells of the capillaries [17]. The uptake is an active process, dependent on sodium and energy [18], and after uptake the substance is deaminated intracellularly by monoamine oxidase (MAO) to 5-hydroxyindolacetic acid (5-HIAA) [19], which enters the blood and is excreted by the kidney. Uptake and metabolism of 5-HT are separate processes, i.e. if MAO is blocked with e.g. iproniazid, the uptake process only is working [18]. Wiersma and Roth [20] have recently demonstrated that hepatic degradation of 5-HT *in vivo* is only one third that of the lung, despite the fact that the amount of hepatic metabolizing enzymes is ten times that of the lung. The reason for this is that the clearance by an organ is determined not only by the enzymic or intrinsic activity, but also by the rate of perfusion [21]. The relatively low content of MAO in the lung is therefore compensated by the high perfusion rate, i.e. the total cardiac output.

Several drugs have been reported to interact with pulmonary uptake of 5-HT and NA. Tricyclic antidepressants inhibit the monoamine uptake, probably by a blockade of the carrier [8], whereas the inhalation anesthetic halothane, on the other hand, probably inhibits the uptake [23, 24] by producing a general membrane stabilization [22]. The reduced pulmonary clearance of the vasoactive hormones can hypothetically cause cardiovascular side effects such as arrythmias [25], headache [26] and thrombosis [27]. Environmental influence on these functions of the lung is still, however, a poorly investigated area.

The aim of this study was to examine the influence of organic solvents on the pulmonary uptake of 5-HT. The halogenated hydrocarbons trichlorethylene (TRI) and halothane were chosen as model substances for this purpose.

MATERIALS AND METHODS

Organ preparation. Sprague–Dawley rats of either sex weighing 250–350 g were anesthetized with pentobarbital (Mebumal vet®, ACO läkemedel, Sweden) (40 mg/kg body weight given i.p.). A dose of 500 I.U. heparin (KABI AB Sweden) was administered intravenously to prevent thrombosis. At full anesthesia, the animals were tracheotomized, the chest rapidly opened, and the pulmonary artery and vein canulated. The pulmonary circulation was immediately perfused with 10 ml of a modified Krebs buffer with the following composition: Polymerized peptides from gelatine, 3.5 g/100 ml, (Haemaccel (Behringwerk AG, FRG)), and in mM concentrations: NaCl 195, KCl 4.02, $CaCl_2$ 6.3, $MgCl_2$ 1.2, $NaHCO_3$ 15.4, KH_2PO_4 1.2 and glucose 5.5. The buffer was equilibrated with 95% O_2 + 5% CO_2 to pH 7.4. It also contained iproniazid (Sigma Chem. Co., St. Louis, MO, U.S.A.) (5×10^{-4} M) to block the activity of MAO. The lungs were dissected free and placed in a perfusion chamber with humidified air at 37°, and they were perfused single pass with the buffer by using a peristaltic pump (Multiperpex 2115, LKB, Bromma, Sweden) and ventilated with air by an animal respirator (Model 680, Harvard Apparatus, MA, U.S.A.). The respiratory rate was 70 cycles/min, the respiratory volume 2 ml and the perfusion rate 10 ml/min. After equilibration for 15 min, the lungs were perfused with buffer containing 10^{-11} M [^{3}H]-5-hydroxytryptamine creatinine sulphate (generally labeled. sp. act. 12 Ci/mmole, The Radiochemical Centre, Amersham, U.K.).

Analysis of 5-HT uptake. Samples were taken from the perfusate during 30 sec intervals. One ml of the samples was mixed with 10 ml Instagel (Packard Instrument, Ill. USA) and counted in a Packard Tri Carb 3375 liquid scintillation spectrometer. Lung uptake of 5-HT was calculated using the formula:

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

where dpm/sample is dpm/1 ml sample from effluent and dpm/total is dpm/1 ml sample influent. The quenching of the samples was determined by internal standardization, and generally varied in the range 30–35 per cent.

Generating and measuring the inhaled air–gas mixtures. During the 5 min exposure phase, the air from the ventilator was passed over a solution of TRI (Merck, Darmstadt, FRG) or halothane (Hoechst AG, Frankfurt am Main, FRG) in a three necked glass flask. To achieve the desired solvent concentrations, the flask was kept at different temperatures (see Table 1). During the exposure phase, samples of the inhaled gas mixture (less than 10%) were continuously pumped through a carbonfilter (Charcoal tubes, SKC Inc.) by a portable Sipin pump (Model SP-15, Anatok J. Sipin Co., NY, U.S.A.). The tubes were kept in a freezer at −20° until analyzed. The carbon filter was then dissolved in CS_2, which was injected into a GLC-system (Varian model 3700, FID-detector, Varian Associates Inc. CA, U.S.A.).

RESULTS

In control experiments with no exposure to any of the solvents, the uptake of 5-HT was measured during ventilation with air for 15 min. The metabolism of 5-HT was, as in all experiments, blocked with the MAO-inhibitor iproniazid (5×10^{-4} M). The extraction of 5-HT from the perfusate was 60 per cent at 0.5 min, decreasing during the subsequent perfusion to a level of 12 per cent after 15 min (Fig. 1a). From the semilogarithmic plot of the extraction (Fig. 1b), it was estimated that the decline of uptake with time followed a 2-compartment pharmacokinetic model. No attempt was made to further characterize the kinetics of the uptake of 5-HT, as this was not the goal of the present investigation. The results do, however, confirm that our results are comparable to those of other investigations on lung uptake of 5-HT [19, 28].

To study the effect of TRI and halothane on the pulmonary uptake of 5-HT, the uptake was measured before, during and after exposure to either solvent in order to make it possible to use each lung as its own control. Experiments were also performed in the order solvent, air, and solvent, to compensate for the possibility of displacement of bound [^{3}H]-5-HT by the solvent. The pulmonary uptake of 5-HT decreased dramatically when the lungs were shifted over from ventilation with air only to air/TRI-mixtures, and in the reversed type of experiments, where 18,000 ppm TRI was used, similar results were obtained (Fig. 4). Experiments were also performed where the lungs were perfused with buffer containing [^{3}H]-5-HT for 5 min and then shifted over to non-radioactive buffer and exposed to 18,000 ppm TRI. These experiments showed no detectable displacement of radioactivity from the pulmonary circulation. The degree of inhibition was calculated as the relative inhibition at 5 min, i.e. by extrapolating back from the minimum extraction during the solvent exposure for 5 min, and then comparing the two levels of extraction (see Fig. 2a). When ventilated with 18,000 ppm TRI, the extraction was inhibited by 80 ± 2 ($\bar{x}$ ± S.E.M.) per cent (Fig. 2a), at 8500 ppm by 65 ± 6 per cent (Fig. 2b) and at 3000 ppm by 25 ± 1 per cent (Fig. 2c). After exposure to the air–solvent mixtures, the lungs were again ventilated with air and uptake of 5-HT was then normalized. The effect of halothane was very similar to that of TRI. Thus 40,000 ppm halothane inhibited pulmonary uptake of 5-HT by 85 ± 6 per cent (Fig.

Table 1. Concentration solvent at different temperatures

Solvent temperature (°C)	Solvent concentration (ppm) in inhaled air/gas mixtures
TRI	
+20	18,000
+4	8500
−15	3000
Halothane	
+20	40,000
−10	6000
−48	2000

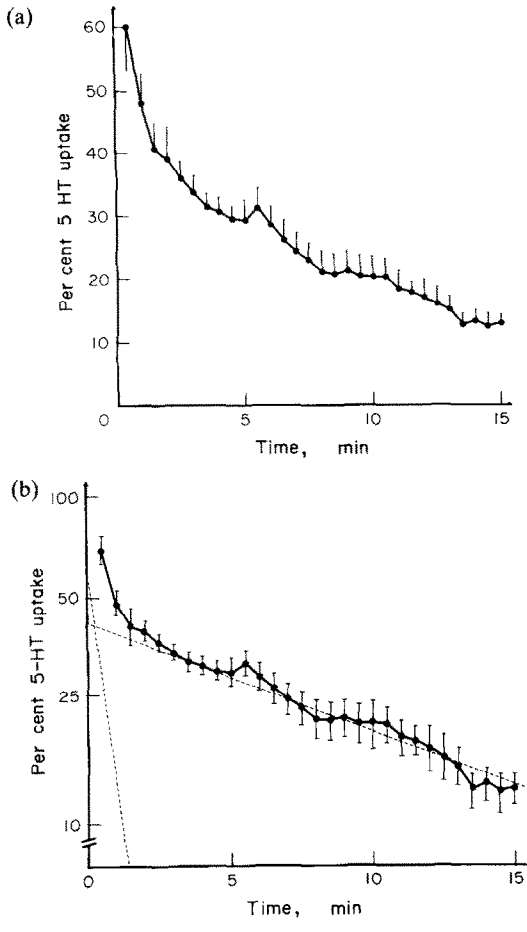

Fig. 1. (a) Uptake curve of 5-HT (10^{-11} M) in isolated perfused rat lung when ventilated with air ($n = 3$). Bars indicate S.E.M. (b) The same as in (a) when transformed to a semilogarithmic plot. Dashed lines indicate two compartments of uptake.

3a). At the lower doses 6000 ppm (Fig. 3b) and 2000 ppm (Fig. 3c), the inhibition was 48 ± 1 and 15 ± 0.3 per cent respectively. The reason for the sharp peaks of extraction seen in some of the figures, is that the perfusion was stopped prior to changes in ventilation between air and air–solvent mixtures. This procedure was also simulated in the control experiments, and the increases of extraction was also observed there.

When the relative inhibition of lung uptake of 5-HT was plotted as a function of log concentration of TRI or halothane, both solvents gave equal log dose–response curves (Fig. 5), indicating similar properties in this respect.

DISCUSSION

Uptake of 5-hydroxytryptamine (5-HT) in isolated perfused rat lung was 60 per cent at 0.5 min. The extraction thereafter declined according to a 2-compartment model (Fig. 1b), indicating saturation of the tissue or the uptake mechanism. This confirms previous investigations [19, 28] where also a 2-compartment model was observed for lung uptake of

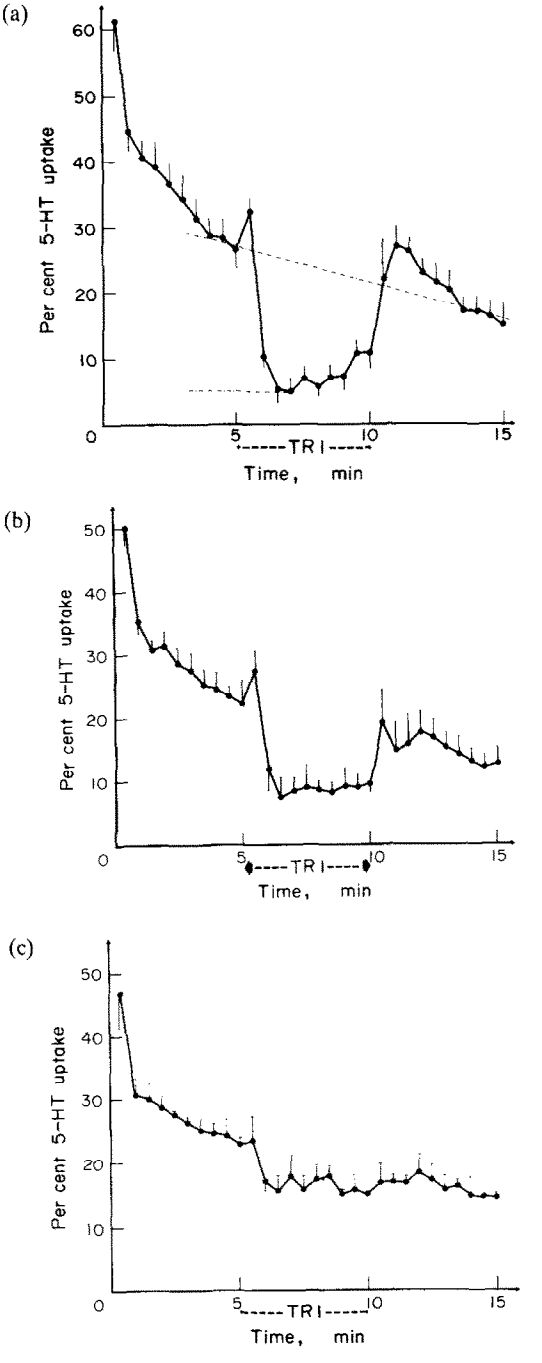

Fig. 2. Uptake of 5-HT (10^{-11}M) in isolated perfused rat lung. (---TRI---) indicates exposure to trichloroethylene. Bars indicate S.E.M. (a) 18,000 ppm TRI; (-------) indicates estimated differences of uptake of 5-HT when exposed to air only (upper line) and TRI (lower line) ($n = 4$). (b) 8500 ppm TRI ($n = 4$). (c) 3000 ppm TRI ($n = 4$).

5-HT. In other experiments, after 5 min ventilation with air only, the lungs were changed over to air with different concentrations of the industrial solvent trichlorethylene (TRI) or the gaseous anesthetic halothane. Pulmonary uptake of 5-HT from the per-

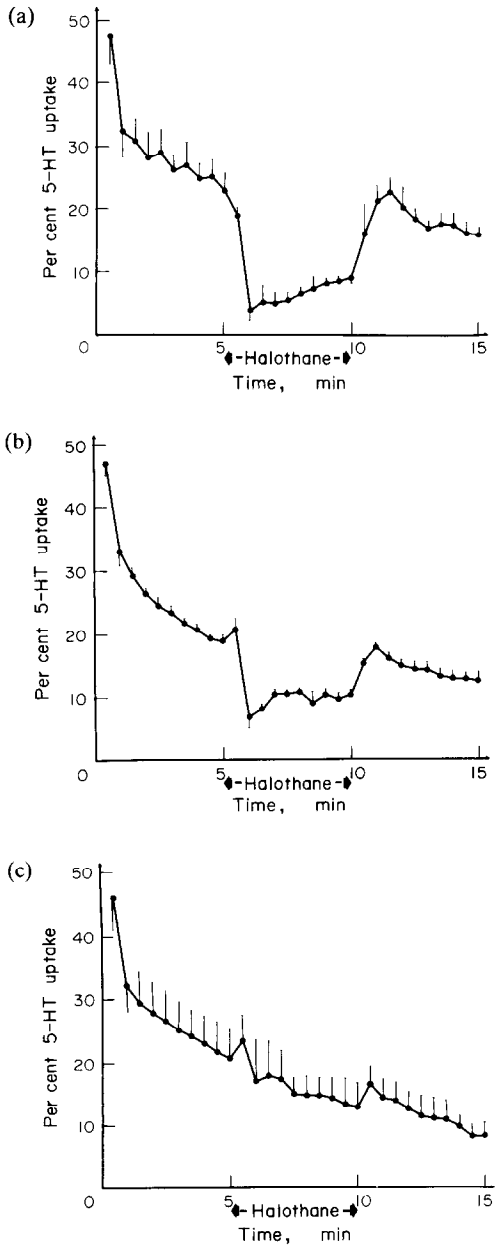

Fig. 3. Uptake of 5-HT (10^{-11} M) in isolated perfused rat lung. (---Halothane---) indicates exposure to halothane. Bars indicate S.E.M. (a) 40,000 ppm halothane ($n = 4$). (b) 6000 ppm halothane ($n = 4$). (c) 2000 ppm halothane ($n = 3$).

fusate then decreased instantaneously (Figs. 2a–c and 3a–c). From Fig. 1(b) it seems probable that the second compartment exhibits the major uptake at 5 min, and it should therefore be uptake into this compartment that is inhibited in Figs. 2 and 3. The observed inhibition of lung extraction of 5-HT by both solvents could, however, hypothetically be explained as a displacement phenomenon of [^{3}H]-5-HT taken up during the first 5 min when the lungs are ventilated with air, especially as MAO is inhibited by iproniazed and 5-HT is therefore not

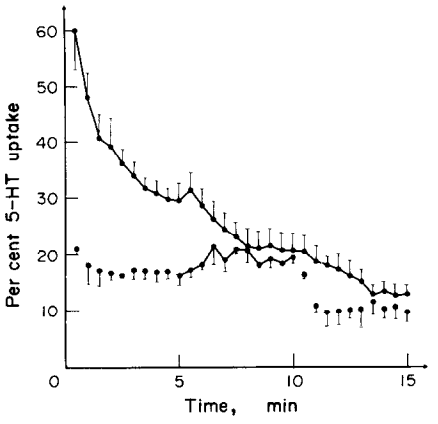

Fig. 4. Uptake of 5-HT (10^{-11} M) in isolated perfused rat lung. The upper curve shows the uptake of 5-HT when ventilated with air (see Fig. 1a), and the lower curve shows the uptake of 5-HT when ventilated with TRI (18,000 ppm), air and TRI (18,000 ppm). Dashed curve indicates exposure to TRI ($n = 3$). Bars indicate S.E.M.

metabolized. In a system with MAO working at normal pace, on the other hand, this would be highly unlikely. Figure 4 shows that the extraction of 5-HT during the first 5 min of solvent exposure, where no [^{3}H]-5-HT previously taken up is available, is inhibited by the solvent exposure. The two curves are converging towards the end of the 5 min exposure, as the extraction in the control experiments goes down. When ventilation is shifted over to air, extraction is increased up to the level of the control

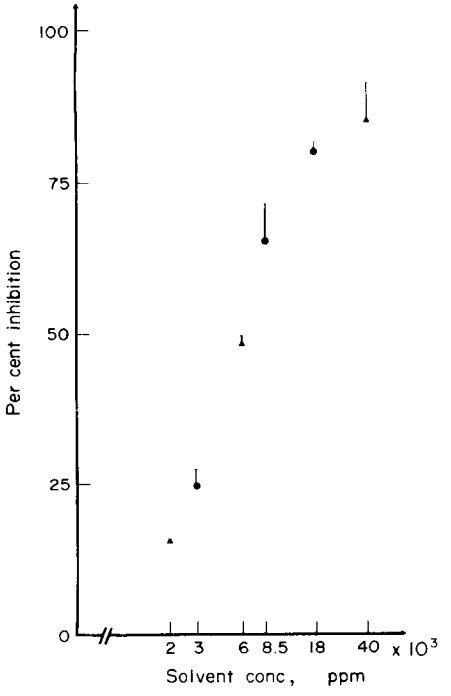

Fig. 5. Relative inhibition of lung uptake of 5-HT when exposed to TRI (●) and halothane (▲). Bars indicate S.E.M.

experiments, and is inhibited again at the beginning of the last exposure to TRI. This therefore demonstrates that the effect of 5-HT on lung extraction is on the uptake mechanisms rather than displacement of bound [^{3}H]-5-HT from the pulmonary tissue. Both solvents showed a concentration-dependent inhibition of lung uptake of 5-HT, and the log dose–response curves were superimposed (see Fig. 4). As the curves asymptotically go towards both 100 and 0 per cent inhibition of 5-HT uptake, it is difficult to assess whether exposure at levels around maximal allowable concentrations would have this effect or not. It is equally difficult to say which concentration is needed for complete inhibition. It would be of interest to compare a number of solvents in this respect, as well as their influence on lung uptake of other substances, both endogenous and exogenous, to find a correlation between the inhibiting capacity and their physico-chemical properties. This would provide information on what type of chemicals might interfere with the non-respiratory functions of the lung, an effect with possible toxicological implications.

The physiological effect of 5-HT is very complex [29], and the consequence of inhibition of pulmonary uptake of the amine is therefore not clear. Such functions as platelet aggregation [30], vasoconstriction of cerebral blood vessels [26] and vasodilation in, e.g., skeletal vessels [31] are influenced by 5-HT. The substance is clearly connected to the development of vascular headache of the migraine type [32], and the headache induced by solvents could therefore be an effect of impaired lung uptake of 5-HT. The potential cardiac toxicity of solvents [5], however, is probably not directly mediated by 5-HT, as the amine has very little effect on cardiac function [29]. A recent paper by Göthert *et al.* [33], on the other hand, reports that 5-HT can liberate NA locally in the heart, and NA has the potential of inducing cardiac dysrythmias and even ventricular fibrillation [34]. The solvent might also, of course, inhibit lung uptake of NA, which has indeed been shown to occur during exposure to halothane and nitrous oxide [23, 24], and we have, in preliminary studies, made similar observations regarding TRI, and why this mechanism would be possible.

Upon discontinuing exposure to citalopram, a selective blocker of the neuronal as well as lung uptake of 5-HT, there is a very slow return towards normalized lung uptake of 5-HT [35]. This is probably the result of persistence of the drug at receptors, where such inhibition occurs. Our results do, however, show that inhibition by solvents is almost immediately reversed, probably because they are rapidly cleared from the tissue due to their volatility. The mechanism of uptake inhibition by citalopram and other drugs, is probably inhibition of specific energy-dependent uptake processes for 5-HT (for review see [36]). Even though we made no attempt to elucidate the mechanism of the uptake inhibition by TRI and halothane, it is very tempting to make such speculations. Uptake of 5-HT is at least in part mediated by Na^+–K^+-dependent ATPase, but the activity of this enzyme has not been reported to be decreased by gaseous anesthetics [22]. Halothane can, on the other hand, increase levels of cyclic AMP in uterus [37], and this cyclic nucleotide has in smooth muscle been demonstrated to influence uptake of amino acids [38], which are transported by similar energy-dependent transport mechanisms to those of the endogenous vasoactive amines. It is therefore possible that the mechanism for inhibition of uptake of 5-HT by solvents might be mediated via such an effect on cyclic AMP, but the hypothesis remains to be tested. Another possible mechanism for inhibition of uptake of 5-HT, is the direct effect of the solvents on the membranes, whereby the structure and motility of, for instance, transport proteins could be changed. Trudell *et al.* [39] have demonstrated that the degree of order in artificial membranes decreases by halothane and methoxyflurane in a concentration-dependent manner, and this effect might be the mechanism for the decreased pulmonary clearance of 5-HT. It would be of interest to investigate whether not only substances with active transport mechanisms of uptake, but also xenobiotics of, for instance, the basic amine type are affected as well.

We are currently extending the studies to other solvents in order to establish a relationship between the physiochemical properties of the solvents and the inhibition of uptake of endogenous substances reported in the present investigation. We are also in the process of elucidating the connection between solvents and the cyclic nucleotides.

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