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# CATECHOLAMINE TRANSPORT IN ISOLATED LUNG PARENCHYMA OF PIG

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**1** Lung parenchyma strips of the pig incubated at 37°C with [$^3$H]-(−)-noradrenaline ([$^3$H]-NA) or [$^3$H]-(±)-isoprenaline ([$^3$H]-Iso), accumulated radioactivity via saturable, high affinity uptake processes. Apparent saturation constants ($K_m$) for [$^3$H]-NA and [$^3$H]-Iso were $1.34 \times 10^{-6}$ M and $1.63 \times 10^{-6}$ M respectively, while apparent transport maxima ($V_{max}$) were 4.86 and $1.63 \times 10^{-9}$ mol min$^{-1}$ g$^{-1}$ respectively.

**2** Cellular accumulation of radioactivity from radiolabelled catecholamines was greatly reduced by lowering the temperature to 7°C, pretreatment with ouabain (100 μM), phentolamine (15 μM) or phenoxybenzamine (80 μM). However, accumulation of radioactivity derived from [$^3$H]-NA was inhibited selectively by cocaine (10 μM) and desipramine (1 μM), while normetanephrine (80 μM) and 3-O-methylisoprenaline (50 μM) caused much greater reductions in cellular radioactivity from [$^3$H]-Iso than from [$^3$H]-NA. Taken together with information from kinetic studies, the results indicate that these amines are transported by separate uptake processes.

**3** Cocaine (50 μM) which selectively reduced [$^3$H]-NA transport, had no significant effect on the sensitivity ($EC_{50}$) of isolated parenchyma lung strips of the pig to the contractile effects of cumulative concentrations of NA. The catechol-*O*-methyl transferase (COMT) inhibitor, U-0521 (60 μM), also failed to alter the potency of NA, while normetanephrine (80 μM) caused a 2 fold decrease in potency.

**4** Phentolamine (15 μM), which reduced the cellular accumulation of radioactivity derived from [$^3$H]-Iso by 64%, caused a small potentiation of Iso-induced relaxations of porcine lung strips. Normetanephrine (80 μM) and 3-O-methylisoprenaline (50 μM), which also depressed the accumulation of cellular radioactivity from [$^3$H]-Iso by $> 50\%$, caused rightward shifts in Iso concentration-effect curves as a result of β-adrenoceptor blockade. In sharp contrast, cortisol (80 μM) and U-0521 (60 μM), which caused smaller reductions in the cellular accumulation of radioactivity derived from [$^3$H]-Iso, both caused an approximately 9 fold potentiation of responses to Iso in isolated lung strips.

**5** The results indicate that the major sites of uptake and metabolism of NA in porcine parenchyma strip are remote from α-adrenoceptors mediating NA-induced contraction. Similarly, some major sites of uptake of Iso are remote from β-adrenoceptors mediating Iso-induced relaxation. However, β-adrenoceptors are apparently in close proximity to a compartment containing COMT activity.

## Introduction

Catecholamines are taken up by human lung *in vivo* (Boileau, Campeau & Biron, 1972; Shenfield, Evans & Paterson, 1976; Sole, Drobac, Schwartz, Hussain & Vaughan-Neil, 1979) and by lung from several species both *in vivo* and *in vitro* (Axelrod, Weil-Malherbe & Tomchick, 1959; Whitby, Axelrod & Weil-Malherbe, 1961; Mathé, Vachon & Bookbinder, 1975; Gillis, 1976; Junod & Ody, 1977). In asthmatics, isoprenaline (Iso), administered by inhalation, causes effective bronchodilatation (Paterson, Woolcock & Shenfield, 1979). Subsequent inactivation of Iso involves uptake into cells with catechol-*O*-methyltransferase (COMT) activity (Blackwell, Briant, Connolly, Davies & Dollery, 1974). Adrenaline and noradrenaline (NA) are also rapidly *O*-methylated in cat lung (Axelrod *et al.*, 1959; Whitby *et al.*, 1961), presumably at extraneuronal sites (Gillis, 1976).

It was of interest to test the suitability of an incubation system as a model for lung accumulation of catecholamines, since most previous studies have involved isolated perfused lung. In perfusion systems, catecholamines are sequestered almost exclusively in vascular endothelial cells, since airways smooth muscle is not exposed to the infused amine. NA is removed preferentially in perfused rabbit lung, adrenaline (Adr) and Iso being removed at much lower rates (Gillis, 1976). Indeed, *in vivo*, in man and in animals, neither Adr nor Iso is removed from the pulmonary blood (Bakhle & Vane, 1974; Gillis & Roth, 1976; Sole *et al.*, 1979). However, when catecholamines are administered by inhalation, air-

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ways rather than vascular smooth muscle is initially exposed to amine. Using an incubation system, all cellular components and thus all potential uptake sites, are exposed to administered catecholamines. It was envisaged that the use of [$^3$H]-NA and [$^3$H]-Iso would help to define the properties of catecholamine uptake processes in lung and provide insight into the potential role of lung parenchyma in the disposition of sympathomimetic bronchodilators.

Inhibition of either the extraneuronal uptake or the metabolism of catecholamines, in both isolated airways and vascular smooth muscle, can result in a marked potentiation of amine-induced responses (Foster, 1968; 1969; Kalsner, 1969; Hapke & Green, 1970; Pun, McCulloch & Rand, 1973). Isolated strips of lung parenchyma respond to agonists as if they contained significant amounts of both airway and vascular smooth muscle (Mirbahar & Eyre, 1981). It was of interest to determine whether inhibition of uptake or metabolism of catecholamines in lung parenchyma strips resulted in changes in the potency of amines thought to stimulate primarily adrenoceptors in either airway or vascular smooth muscle.

## Methods

### *Radio-tracer studies*

Wedges of tissue, approximately 6 × 3 cm, were cut from the major lobes of lung from freshly slaughtered pigs. Fine strips of parenchyma tissue approximately 40 × 1 × 1 mm were dissected out distal to the major bronchioles and blood vessels. Lung strips were pooled to provide samples weighing 40–60 mg. These were tied to small, silastic-coated weights and individual preparations incubated in 3 ml of Krebs Henseleit solution aerated with 5% $CO_2$ in $O_2$ at 37°C. Preparations were allowed to equilibrate for 1 h. At least 4 such samples of lung from different pigs were used for each experiment.

In some experiments, lung strips were incubated with [1-$^3$H]-(+)-sorbitol (6 μg/ml; $3.3 \times 10^{-5}$ M; 100 nCi/ml) for 1–80 min to determine the apparent volume and rate of filling of the extracellular space. In other experiments, preparations were incubated with [7-$^3$H]-(−)-noradrenaline or [7-$^3$H]-(±)-isoprenaline (5–3200 ng/ml; $24 \times 10^{-9}$–$19.4 \times 10^{-6}$ M; 100 nCi/ml) for 1–10 min. Some preparations were exposed to uptake inhibitors for 30 min and then incubated in the presence of [$^3$H]-catecholamines (400 ng/ml) for 5 min. Following incubation with tracer, parenchyma strips were blotted dry, weighed, placed in glass vials and digested in 1 ml of NaOH (1 M) for 2 h at 75°C. After cooling, 100 μl of hydrogen peroxide 27% w/w (8 M) was added to each vial as a decolourizing agent. After approximately 1 h, vials were re-incubated at 75°C to remove excess hydrogen peroxide. Fifteen ml of 2:1 toluene-Triton X-100 mixture containing 0.4% PPO was added to each vial, followed by 100 μl of HCl (12 M). After this mixture had clarified, radioactivity was measured in a Nuclear Chicago Isocap 200 liquid scintillation spectrometer. A 1 ml sample of each bath solution was also taken for estimation of radioactivity. Quench correction was employed using the external standards ratio (ESR) facility.

### *Pharmacological studies*

Strips of lung parenchyma approximately 25 × 5 × 2 mm were dissected from the perimeters of central lobes of pig lung. Preparations were suspended initially and maintained under 500 mg tension in Krebs Henseleit solution aerated with 5% $CO_2$ in $O_2$ at 37°C. Tissues were allowed to equilibrate for 60–90 min. Changes in resting tension were measured using a Grass force-displacement transducer (FT03C) coupled to a Rikadenki pen recorder (model B28L). Two cumulative concentration-effect curves to either Iso or NA were produced at 45 min intervals and the second curve used to determine control tissue sensitivity to the amine ($EC_{50}$ control). Responses were measured as a % of the maximal response ($E_{max}$ = 100%). Preparations were then equilibrated for 1 h in the presence of a single concentration of test drug and concentration-effect curves to catecholamines re-established. The ratio $EC_{50}$ control: $EC_{50}$ test drug was taken as a measure of altered tissue sensitivity to catecholamine.

The drugs used were cocaine hydrochloride (M & B); cortisol sodium hemisuccinate (Glaxo); desipramine hydrochloride (Ciba); (±)-isoprenaline hydrochloride (Sigma); [7-$^3$H]-(±)-isoprenaline (New England Nuclear); 3-O-methylisoprenaline (synthesized at Victorian College of Pharmacy, Melbourne); nialamide (Pfizer); (−)-noradrenaline bitartrate, normetanephrine hydrochloride (Sigma); [7-$^3$H]-(−)-noradrenaline (New England Nuclear); ouabain (Arnaud); phenoxybenzamine hydrochloride (S.K.F.); phentolamine mesylate (Ciba); [1-$^3$H]-(+)-sorbitol (New England Nuclear); U-0521 (Upjohn). All radioactive amine concentrations are expressed in terms of the base.

## Results

### *Radio-tracer experiments*

*[$^3$H]-sorbitol* At 37°C, [$^3$H]-sorbitol diffused in lung parenchyma strips such that equilibrium with the extracellular fluid (ECF) was complete at 80 min (Figure 1). The estimated ECF volume was 63 ± 1.5% ($n$ = 4) of the tissue mass giving a derived

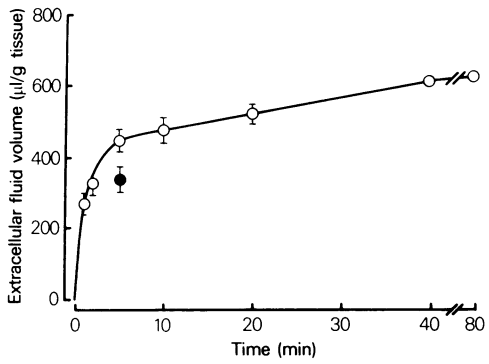


**Figure 1** Relationship between the apparent extracellular fluid volume of pig lung parenchyma strips and incubation time, determined at 37°C (○) and at 7°C (●) using [$^3$H]-(+)-sorbitol. Points represent means of at least 4 experiments; vertical bars show s.e.means.

cellular content of 0.37 g/g wet wt. lung tissue. At shorter time intervals, [$^3$H]-sorbitol values were used to estimate the volume of ECF into which $^3$H-catecholamines might be expected to diffuse and thus enable their mean concentration in the ECF at any time to be calculated. Estimates of tissue radioactivity levels were corrected for extracellular amine. The catecholamines did not themselves alter [$^3$H]-sorbitol distribution. The apparent ECF volume was smaller in lung parenchyma strips incubated for 5 min at 7°C than in strips incubated for 5 min at 37°C. This may have reflected slower diffusion of [$^3$H]-sorbitol at 7°C than at 37°C (Figure 1).

*Kinetics of $^3$H-catecholamine transport* Lung parenchyma accumulated radiactivity derived from [$^3$H]-NA against concentration gradients at all concentrations tested (Figure 2a). At low amine concentrations, cellular: extracellular radioactivity ratios of nearly 6:1 were obtained. Ratios decreased with increasing concentration of [$^3$H]-NA at all incubation times used, suggesting saturation of the accumulation of radioactivity. Similar results were obtained with [$^3$H]-Iso (Figure 2b) although much lower cellular: extracellular radioactivity ratios were achieved. The initial velocity ($V_i$) of accumulation of both amines was determined from velocity versus time (t) curves at $t = 0$. $V_i$ was not altered by inhibition of monamine oxidase (MAO) with nialamide (100 μM), inhibition of COMT with U-0521 (60 μM) or by simultaneous inhibition of both enzymes. As previously described (Cornish, Goldie & Miller, 1978), using 1 min mean extracellular catecholamine concentrations ($S$), a plot of $S/V_i$ versus $S$ (Figure 3) indicated that initial uptake of both catecholamines obeyed Michaelis-Menten kinetics. Values for apparent $K_m \times 10^{-6}$ M and apparent $V_{max} \times 10^{-9}$

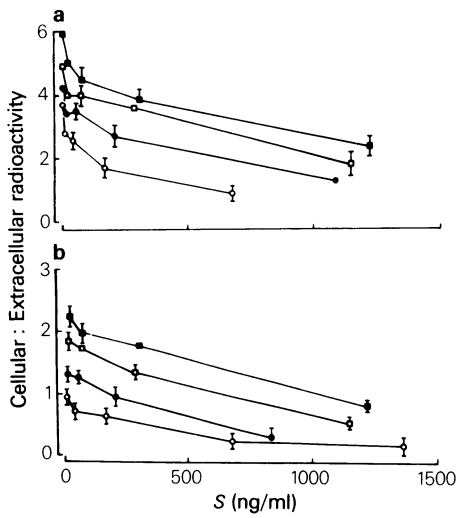


**Figure 2** Relationship between the mean extracellular $^3$H-catecholamine concentration (S) and the cellular: extracellular radioactivity concentration ratio in pig peripheral lung strips incubated with [$^3$H]-noradrenaline (a) or [$^3$H]-isoprenaline (b) for 1 min (○), 2 min (●), 5 min (□) or 10 min (■). Points represent means and vertical bars the s.e.means of at least 4 experiments.

mol min$^{-1}$ g$^{-1}$ for [$^3$H]-NA were 1.34 and 4.86 respectively and for [$^3$H]-Iso were 1.63 and 1.63 respectively.

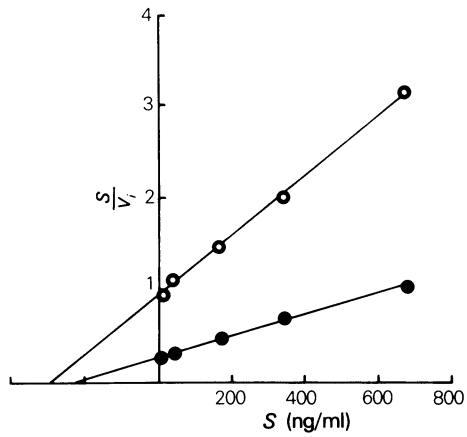


**Figure 3** Linear transformation of the Michaelis-Menten equation as applied to an analysis of the accumulation of radioactivity derived from [$^3$H]-noradrenaline or [$^3$H]-isoprenaline (○) in pig peripheral lung strips. $S$ = mean extracellular $^3$H-catecholamine concentration after 1 min incubation. $V_i$ = initial accumulation velocity as determined from velocity versus time curves.

*Effect of uptake inhibitors on $^3$H-catecholamine transport* Lung strips were exposed to $^3$H-catecholamines (400 ng/ml) for 5 min, so that mean molar extracellular amine concentrations which approximately half-saturated uptake, were obtained. In the absence of uptake inhibitors, parenchyma strips accumulated radioactivity equivalent to [$^3$H]-Iso or [$^3$H]-NA, 361 ± 18 and 1031 ± 30 ng/g cells respectively. At 7°C, accumulation of radioactivity derived from [$^3$H]-NA was reduced by 76% and was abolished virtually in the case of [$^3$H]-Iso (Table 1), while ouabain (100 μM) reduced accumulation by 88% and 54% respectively. Phentolamine (15 μM) or phenoxybenzamine (80 μM) which inhibit both neuronal and extraneuronal uptake (Foster, 1968; 1969), reduced [$^3$H]-NA accumulation by 81% and 75% respectively and had similar effects on [$^3$H]-Iso transport. Although the relatively selective extraneuronal uptake inhibitors normetanephrine (80 μM) and 3-O-methyl isoprenaline (50 μM) (Iversen, 1965; Mireyless & Foster, 1973) markedly reduced the accumulation of radioactivity derived from [$^3$H]-Iso, the effect of 3-O-methyl isoprenaline on cellular radioactivity derived from [$^3$H]-NA transport was not statistically significant ($0.2 > P > 0.1$; non-paired $t$ test). Cortisol (80 μM) which selectively inhibits extraneuronal uptake in cat heart (Goldie, 1976; Graefe, 1976; Cornish *et al.*, 1978), increased the accumulation of cellular radioactivity derived from [$^3$H]-NA by about 23% ($P < 0.001$) and reduced cellular radioactivity from [$^3$H]-Iso by only 16%. The neuronal uptake inhibitors, cocaine (10 μM) and desipramine (1 μM), caused highly significant reductions in cellular radioactivity derived from [$^3$H]-NA ($P < 0.001$), while the accumulation of tritium from [$^3$H]-Iso was not significantly altered ($P > 0.05$). The COMT inhibitor U-0521 (60 μM) produced small but significant reductions in cellular radioactivity derived from both [$^3$H]-NA and [$^3$H]-Iso ($0.05 > P > 0.02$).

*Effect of uptake inhibitors on responses of parenchyma strip to catecholamines*

Iso invariably caused propranolol-sensitive, concentration-dependent decreases in spontaneously developed tone in porcine lung parenchyma strips (Table 2), while NA always caused contractions that were inhibited by phentolamine.

*Isoprenaline relaxations* Phentolamine (15 μM) which markedly inhibited the accumulation of radioactivity derived from [$^3$H]-Iso in porcine lung parenchyma (Table 1), caused a significant but small increase in the sensitivity of lung strips to Iso (Table 2). Cocaine (10 μM) and desipramine (1 μM) which had no significant effect on the cellular accumulation of radioactivity derived from [$^3$H]-Iso, had no significant effect on Iso concentration-effect curves. Furthermore, normetanephrine (20–80 μM) and 3-O-methyl isoprenaline (5–200 μM) which selectively inhibited the accumulation of cellular radioactivity derived from [$^3$H]-Iso uptake in lung parenchyma, caused a marked, concentration-related, inhibition of Iso relaxations ($K_B$ normetanephrine = 1.8 ± 0.3 × $10^{-5}$ M; n = 7; $K_B$ 3-O-methyl isoprenaline = 4.2 ± 0.6 × $10^{-6}$ M; $n = 11$). In contrast, cortisol (80 μM) and U-0521 (60 μM) which caused small reductions in cellular radioactivity from [$^3$H]-Iso, induced a 9.5 fold and 8.7 fold potentiation of Iso responses of lung parenchyma respectively (Table 2).

**Table 1** Effect of reducing temperature or of inhibitors on the accumulation of radioactivity derived from [$^3$H]-noradrenaline ([$^3$H]-NA) or [$^3$H]-isoprenaline ([$^3$H]-Iso)

| | *[$^3$H]-NA* | *[$^3$H]-Iso* |
|---|---|---|
| Control accumulation (ng/g cells) at 37°C | 1031 ±30 | 361 ±18 |
| *Inhibitor* | | *% inhibition* |
| 7°C | 76.4± 1.3 | 98.1± 1.3 |
| Ouabain (100 μM) | 87.6± 1.4 | 53.8± 2.4 |
| Phentolamine (15 μM) | 81.1± 2.1 | 63.8± 4.8 |
| Phenoxybenzamine (80 μM) | 74.8± 2.8 | 68.6± 3.7 |
| Normetanephrine (80 μM) | 25.1± 6.6 | 58.7± 4.1 |
| 3-O-Methylisoprenaline (50 μM) | 12.0± 7.6† | 51.9± 5.3 |
| Cortisol (80 μM) | −22.9± 4.7‡ | 16.0± 3.0 |
| Cocaine (10 μM) | 91.4± 2.3 | 19.8± 8.3† |
| Desipramine (1 μM) | 65.6± 2.1 | 13.4±10.8† |
| U-0521 (60 μM) | 13.4± 4.3 | 18.3± 3.9 |

Except where indicated, experiments were conducted at 37°C.
Data recorded as mean ± s.e. of mean of observations from 4–8 lung samples from different animals. All % inhibitions were significant ($P < 0.05$; non-paired $t$ test) unless otherwise indicated:
† % inhibition not significant ($P > 0.05$); ‡ significant increase in uptake ($P < 0.001$).

**Table 2** Effect of U-0521 or inhibitors of catecholamine uptake on the sensitivity of porcine parenchyma lung strip to isoprenaline (Iso)

| *Inhibitor* | *Iso $EC_{50} \times 10^{-7}$ M* | *Ratio* = $\frac{\text{Iso } EC_{50} \text{ control}}{\text{Iso } EC_{50} \text{ inhibitor}}$ |
|---|---|---|
| None | 1.80 ± 0.15 (40) | 1.00 |
| U-0521 (60 μM) | 0.21 ± 0.09 ( 6)** | 8.74 |
| Cortisol (80 μM) | 0.19 ± 0.08 ( 6)** | 9.47 |
| Phentolamine (15 μM) | 1.12 ± 0.26 ( 8)* | 1.61 |
| 3-O-methylisoprenaline (5 μM) | 5.00 ± 2.05 ( 4)† | 0.36 |
| (50 μM) | 31.8 ± 4.10 ( 4)** | 0.06 |
| ,, (200 μM) | 250 ± 104 ( 4)* | 0.007 |
| Normetanephrine (20 μM) | 2.52 ± 0.45 ( 5)† | 0.71 |
| ,, (80 μM) | 20.1 ± 3.6 ( 5)** | 0.09 |
| Cocaine (10 μM) | 2.53 ± 0.41 ( 4)† | 0.71 |
| Desipramine (1 μM) | 2.35 ± 0.26 ( 4)† | 0.77 |

Numbers in parentheses indicate number of experiments. Values are ± s.e.mean.
* $P < 0.05$; ** $P < 0.001$ (significantly different from non-pretreatment value; non-paired *t* test)
† $P > 0.05$ (not significantly different from non-pretreatment value, non-paired *t* test).

*Noradrenaline contractions* The sensitivity of lung parenchyma strips to noradrenaline was not altered in the presence of the COMT inhibitor, U-0521 (60 μM), cocaine (10 μM) or normetanephrine (80 μM) (Table 3).

## Discussion

Parenchyma lung strips of the pig accumulated radioactivity derived from [$^3$H]-NA or [$^3$H]-Iso via saturable, high affinity, temperature-sensitive uptake processes. Ouabain (100 μM) markedly reduced the transport of both amines suggesting that they were sequestered by energy-dependent processes. The COMT inhibitor, U-0521 (60 μM), caused only a small reduction in the accumulation of radioactivity derived from [$^3$H]-NA and [$^3$H]-Iso. This may have been caused by a direct inhibition of uptake rather than by a failure to store parent amine in lieu of O-methylated metabolites. U-0521 is known to be a weak inhibitor of extraneuronal uptake in rat isolated heart (Bönisch, Uhlig & Trendelenburg, 1974) and in kitten isolated coronary arteries (Cornish & Goldie, 1980).

Estimates of apparent $K_m$ for [$^3$H]-NA removal by perfused rabbit (Iwasawa, Gillis & Aghajanian, 1973) and rat lung (Nicholas, Strum, Angelo & Junod, 1974) are in close agreement with the present estimate. Although the values for apparent $K_m$ for both amines are very similar, $V_{max}$ estimates indicate that [$^3$H]-NA was transported via an uptake mechanism which was discrete from that transporting [$^3$H]-Iso. The relatively selective effects of some neuronal or extraneuronal uptake inhibitors support this speculation. Platelets and mast cells have previously been discounted as likely sites of [$^3$H]-NA accumulation (Junod & Ody, 1977). It is most unlikely that noradrenergic nerves were involved in the accumulation of any of these catecholamines, since sympathetic innervation of airways smooth muscle is sparse or absent below the level of the bronchi in rat (Zussman, 1966; El-Bermani, 1978), guinea-pig (O'Donnell, Saar & Wood, 1978), rabbit and pig lung (Mann,

**Table 3** Effect of U-0521 or inhibitors of catecholamine uptake on the sensitivity of porcine parenchyma lung strip to noradrenaline (NA)

| *Inhibitor* | *NA $EC_{50} \times 10^{-6}$ M* | *Ratio* = $\frac{EC_{50} \text{ control}}{\text{NA } EC_{50} \text{ inhibitor}}$ |
|---|---|---|
| None | 2.53 ± 0.34 (15) | 1.00 |
| U-0521 (60 μM) | 2.85 ± 0.36 ( 4)† | 0.89 |
| Cocaine (10 μM) | 2.00 ± 0.84 ( 4)† | 1.27 |
| Normetanephrine (80 μM) | 5.73 ± 2.76 ( 4)† | 0.44 |

Numbers in parentheses indicate number of experiments. Values are given ± s.e.mean.
† Not significantly different from non-pretreatment value; non-paired *t* test ($P > 0.2$).

1971), leaving only the sparse innervation of the most peripheral pulmonary blood vessels. Thus it is likely that [$^3$H]-NA was transported primarily into capillary endothelium in incubated lung strips as it was in perfused lung (Hughes, Gillis & Bloom, 1969; Gillis, 1976) where cocaine and imipramine were potent uptake inhibitors (Iwasawa & Gillis, 1974; Nicholas *et al.*, 1974), while [$^3$H]-Iso was largely transported by a different process. Junod & Ody (1977) also speculated that [$^3$H]-NA was accumulated by endothelial cells in pig lung slices. Although we have not presented direct evidence for a morphological separation of the processes transporting these two amines, it is well established that Adr and Iso are not removed in significant amounts from the pulmonary circulation *in vivo* (Bakhle & Vane, 1974; Gillis & Roth, 1976; Sole *et al.*, 1979) or *in vitro* (Gillis, 1976). It is therefore unlikely that vascular endothelial cells played a major role in the uptake of [$^3$H]-Iso in this system.

Phentolamine (15 $\mu$M), normetanephrine (80 $\mu$M) and 3-O-methyl isoprenaline (50 $\mu$M) reduced the cellular accumulation of radioactivity derived from [$^3$H]-Iso by 50–60% (Table 1). However, phentolamine caused only a small potentiation of Iso-induced relaxations of porcine lung parenchyma strips, while the O-methylated amines had a weak $\beta$-adrenoceptor blocking activity consistent with similar effects in cardiac muscle, central airways smooth muscle and vascular smooth muscle (Bassett, 1971; Picken & Jarrott, 1975; Brine, Cornish & Miller, 1979). Cocaine (10 $\mu$M) and desipramine (1 $\mu$M), which did not significantly alter the cellular accumulation of radioactivity from [$^3$H]-Iso, also failed to alter significantly the Iso concentration-effect curves. In contrast, cortisol (80 $\mu$M) and the COMT inhibitor, U-0521 (60 $\mu$M) which caused only small reductions in cellular radioactivity derived from [$^3$H]-Iso (Table 1), caused 9.5 fold and 8.7 fold increases respectively, in tissue sensitivity to Iso. Since cortisol also inhibits COMT in kitten heart (Cornish & Goldie, 1980), potentiation of Iso responses in lung parenchyma caused by this steroid or by U-0521 may be attributed to an inhibition of amine metabolism rather than of uptake. The fact that inhibition of Iso uptake by phentolamine caused less than a 2 fold potentiation of amine responses indicates that phentolamine-sensitive uptake sites were remote from Iso-activated $\beta$-adrenoceptors. However, sites of uptake associated with U-0521-sensitive metabolism of Iso were apparently in close association with activated $\beta$-adrenoceptors.

Similarly, NA-stimulated $\alpha$-adrenoceptors were presumably remote from major sites of both COMT activity and cocaine-sensitive uptake, since neither U-0521 nor cocaine potentiated contractile responses to NA (Table 3). NA-induced responses of parenchyma strips may have been caused by contractions of arterioles and venules (Mirbahar & Eyre, 1981). Although autoradiographic or fluorescence histochemical studies are required to determine precisely the sites of $^3$H-catecholamine uptake, it seems likely that [$^3$H]-NA was transported primarily into capillary endothelial cells while [$^3$H]-Iso may have been mainly sequestered into airways tissues. If this is so, it may be that parenchyma lung tissues in man are important sites for the removal of Iso administered by inhalation.

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