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Angiotensin II inhibits the uptake and removal of [^{3}H]metaraminol by rat lung *in vitro*

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Recently we demonstrated that the rat lung tissue is able to take up and concentrate metaraminol by an active transport mechanism (Davila & Davila, 1975a). We also showed that this process may be blocked by various drugs (Davila & Davila, 1975b). It is now well recognized that angiotensin II is able to inhibit uptake of noradrenaline (Palaic & Khairallah, 1967; Peach, Cline & others, 1970) or metaraminol (Davila & Khairallah, 1970; Peach & others, 1970) in several organs. The present studies show *in vitro* evidence for such an inhibitory effect of angiotensin II on the storage and removal of [^{3}H]metaraminol by rat lung.

For this purpose pieces of lung tissue were incubated in Krebs solution according to Davila & Davila (1975a). The uptake of [^{3}H]metaraminol (±)-metaraminol-[7-^{3}H] (N), New England Nuclear Corp., Boston, Mass., USA; specific activity 6·72 Ci mM^{-1}) was measured when it was present in the incubation medium alone or in the presence of angiotensin II (asparagine-1, valine-5 angiotensin II amide, Hypertensin, Ciba-Geigy, Basle, Switzerland). In some experiments the removal of [^{3}H]metaraminol from lung tissue was measured.

Changes in [^{3}H]metaraminol in lung tissue incubated with angiotensin II are presented in Table 1. The octapeptide from 3 to 300 ng ml^{-1} caused a good dose-dependent inhibition of uptake of radioactive metaraminol; the maximal inhibition is about 70%. In a few experiments the rate of removal of [^{3}H]metaraminol from the lung tissue was followed. These experiments were performed with the dose of tested polypeptide which showed about 50% of inhibition of [^{3}H]metaraminol uptake (see Table 1). As shown in Table 2 there is an initial rapid loss of the radioactivity during the first 3 min of the washout period. This is followed by a further slow disappearance of radioactivity. In the presence of angiotensin II during washout the

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Table 1. *The effect of angiotensin II on the uptake of [^{3}H]metaraminol by rat lung.* Equilibration time: 30 min. [^{3}H]Metaraminol: 10 ng ml^{-1}. 5–6 pieces of lung tissue for incubation at 37°. Oxygenation: 95% O_2 and 5% CO_2. Incubation time: 15 min. *P* values: Dunnett's *t*-test in comparison with control.

Dose (ng ml^{-1})	[^{3}H]metaraminol d min^{-1} g^{-1} × 10^3	n*	Inhib. %	*P*
Control				
—	33·23 ± 1·79**	15	0	—
Angiotensin II				
3	32·50 ± 1·29	8	2	>0·05
10	24·20 ± 0·89	10	27	<0·01
30	14·95 ± 1·13	6	55	<0·01
100	8·98 ± 1·71	8	73	<0·01
300	8·96 ± 2·01	5	73	<0·01

* Number of determinations.
** Mean ± s.e.m.

Table 2. *Removal of [^{3}H]metaraminol from rat lung.* Lung tissue was incubated at 37° in the presence of tritiated metaraminol (10 ng ml^{-1}) for 15 min. After incubation lung tissue was washed with fresh metaraminol-free Krebs solution (control) or containing angiotensin II (30 ng ml^{-1}) for 1–20 min. The radioactivity removed from lung tissue was expressed as percentage of the initial radioactivity at zero time (100%). Numbers represent the mean of 6–10 determinations.

% of removed radioactivity						
0	1	3	5 (min)	10	15	20
Control						
0	40	92	99	98	99	99
Angiotensin II						
0	20	45	55	55	56	55

radioactivity remaining is much higher than in the controls.

The inhibitory effect of angiotensin II on noradrenaline or metaraminol uptake was also manifested in the present experiments in lung tissue. Inhibition was greater than that obtained in perfused rabbit hearts (Peach, Bumpus & Khairallah, 1969) in which maximal inhibition of metaraminol uptake was about 50%. In the present experiments angiotensin II inhibited the uptake of [^{3}H]metaraminol in lung to about 70% in the doses used. From the washout experiments the rate of removal of radioactivity was followed. The loss of radioactivity from lung is rapid at the beginning of the washout, thereafter there is slow disappearance and in controls declined to about zero during the first 5 min of washout. In this case the radioactivity remaining after 20 min of washout was about 1%. When angiotensin II was present in the medium during the washout period the amount of radioactivity remaining was much higher, i.e. about 45%. The initial slope of the disappearance curves can be used for the determination of the half-life of [^{3}H]metaraminol, i.e. the time in which the amount of radioactivity drops to 50% was 1–2 min for control, and about 10 min for angiotensin II.

The present study has demonstrated that angiotensin II inhibits the uptake of [^{3}H]metaraminol in rat lung. The findings obtained in washout experiments indicate the possibility of the existence of two different sites at which metaraminol is bound in the lung tissue.

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The fate of prostaglandin A_1-17,18-^{3}H in the dog

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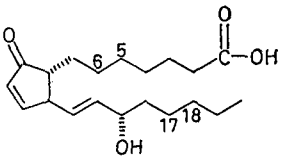
An earlier communication described the metabolic fate of the commercially available PGA_1-5,6-^{3}H in the rat (Wickrema Sinha & Shaw, 1977). This material which bears the tritium-label on C-5 and C-6 of the prostaglandin was deemed unsuitable for metabolism studies in man, due to the significant loss of its tritium label during metabolism (Wickrema Sinha & Shaw, 1977). The loss of tritium was attributed to β-oxidative cleavage of the carboxy side chain of the prostaglandin. Consequently, radioactive PGA_1 bearing the tritium label on C-17 and C-18 was synthesized (Hsi, 1977) and the fate of the PGA_1-17,18-^{3}H was investigated in the dog.



Each of four fasted Beagle dogs (two males and two females; average weight 9 kg) received a bolus injection of 2 ml of a solution of PGA_1-17,18-^{3}H (specific activity 0·153 Ci mg^{-1}) in 0·15 M sodium chloride with 0·1% sodium bicarbonate, into the left jugular vein. The bolus contained 335 μg of PGA_1 ($113·4 \times 10^6$ d min^{-1}) (38 μg kg^{-1}). Plasma, urine and faeces samples were collected and analysed (Wickrema Sinha & Shaw, 1977). The titriated water content of the urine and plasma were determined (Wickrema Sinha & Shaw, 1977).

Peak plasma concentrations of radioactivity equivalent to 516 ng of PGA_1 ml^{-1} were observed 5 to 10 s after administration of the prostaglandin, with a second peak at 10 to 15 min after drug administration (see Table 1). The second peak may correspond to the appearance of metabolites of PGA_1 in the plasma, or the release of PGA_1-^{3}H related material from a tissue depot. The initial plasma PGA_1-^{3}H radioactivity disappearance half-life in dog was estimated to be 1 min, suggesting rapid clearance of intact PGA_1. The half-life of 70 min associated with a terminal portion of the disappearance curve probably corresponds to the clearance of the metabolites of PGA_1.

The tritiated water found in the time interval plasma samples of the PGA_1-17,18-^{3}H treated dog is presented in Table 1. The appearance of tritiated water was indicative of the loss of the tritium label from C-18 due to ω-oxidation of PGA_1 (or one of its metabolites) followed by β-oxidative cleavage of the two terminal

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