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CAPTOPRIL REMOVAL BY RABBIT LUNG *IN VIVO**

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Abstract—Removal of [^{14}C]captopril by the lungs of anesthetized rabbits was measured by the multiple indicator dilution technique. After coinjection of indocyanine green (ICG) and [^{14}C]captopril into the jugular vein of anesthetized rabbits, serial blood samples were collected from the carotid artery and each was analyzed for its content of both substances. Percent removal (R) of captopril after its initial injection of 10 nmoles captopril/kg (calculated at the peak of the ICG outflow curve) was 40.2 ± 2.5 (S.E.M.) and was significantly greater than R after a second injection of 10 nmoles captopril/kg (20.1 ± 2.4) 1 hr later. Removal of 70 nmoles captopril/kg (5.8 ± 3.0 after first injection, 6.4 ± 2.2 after second injection) was significantly lower than R of 10 nmoles captopril/kg. During a single pulmonary passage of either dose of captopril, R was inversely related to the calculated fractional concentration of intravascular captopril. Pulmonary metabolism of the angiotensin converting enzyme (ACE) substrate [^{3}H]benzoyl-Phe-Ala-Pro ([^{3}H]BPAP) was 70.1 ± 1.7% in the absence of captopril, and was reduced significantly to 27.4 ± 2.4% by 10 nmoles captopril/kg and 7.6 ± 0.2% by 6 μmoles BPAP/kg. BPAP (6.4 ± 0.6 μmoles/kg) significantly reduced R of the first and second injections of 10 nmoles captopril/kg but this effect was selective, since BPAP did not reduce pulmonary removal of [^{14}C]serotonin. These data indicate that pulmonary removal of captopril *in vivo* is saturable and may primarily reflect binding of the drug to pulmonary endothelial ACE.

Hydrolysis of blood-borne angiotensin I to the potent vasconstrictor angiotensin II [1] reflects activity of angiotensin converting enzyme (ACE), located on the luminal surface of pulmonary capillary endothelial cells [2]. Captopril, a selective and potent inhibitor of this enzyme [3–5], inhibits both the formation of angiotensin II and degradation of bradykinin in the lung. These processes, at least in part, may account for the antihypertensive actions of the drug in various animal models and in man [6–10]. It was therefore of interest to assess the removal of captopril by the lung *in vivo*, since this process might reflect binding to endothelial converting enzyme and thus provide information on the mechanism of the antihypertensive effect of the drug. To determine whether uptake of captopril was associated with inhibition of ACE, we also studied hydrolysis of a specific substrate for the enzyme, [^{3}H]benzoyl-Phe-Ala-Pro ([^{3}H]BPAP) [11], which has been successfully used previously in multiple tracer studies to measure ACE kinetic activity *in vivo* [12–14].

METHODS

Animal preparation. The preparation of animals and the application of multiple indicator dilution methods have been described previously in detail [12, 14]. Briefly, rabbits (4 kg) were anesthetized by i.v. administration of 6 ml of a urethane (50 mg/ml) and allobarbital (200 mg/ml) solution. They were heparinized (1000 units) and the neck of each animal was infiltrated with 1% xylocaine, after which a tracheostomy was performed and the animals were ventilated with oxygen-enriched room air. The right jugular vein and left carotid artery were cannulated with Tygon tubing to the level of the right atrium and aortic arch respectively. Animals then received an additional 1000 units of heparin and were allowed to stabilize before beginning experiments. Arterial blood pressure was monitored continuously.

Protocol. Each animal received three bolus injections (0.9 ml) at hourly intervals. The composition of the bolus varied according to the requirements of each experiment. Injections were given rapidly into the jugular vein and were followed by 1 ml of heparinized saline. Injections contained [^{14}C]captopril, (sp. act. 12.8 μCi/mmole; 10 or 70 nmoles/kg) and indocyanine green (ICG, 94 μg/kg; Cardiogreen, Hynson, Westcott and Dunning, Baltimore, MD) in saline or, in separate experiments, [^{14}C]captopril, ICG and either [^{3}H]BPAP (sp. act. 20 Ci/nmoles; 18 pmoles/kg; Ventrex, Portland, ME) or unlabeled BPAP (6 μmoles/kg; Vega Biochemicals, Tuscon, AZ). When unlabeled BPAP was used, the bolus was prepared with ethanol instead of saline. Therefore, injections of [^{14}C]captopril and ICG in ethanol were used in appropriate control experiments. For an

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additional group of animals, the bolus injection contained [^{14}C]serotonin (sp. act. 58 μCi/mmole; 13 nmoles/kg; Amersham, Chicago, IL), ICG and 6 μmoles BPAP/kg in ethanol; BPAP was omitted from control injections.

Withdrawal of blood (20 ml/min) from the carotid artery was begun immediately after each injection and continued for 20 sec at a rate of one sample/sec. Samples were collected in tubes placed in a fraction collector (Gilson Escargot, Middletown, WI). Collection tubes containing blood, 3 ml of 10 μM (unlabeled) captopril and 10 units of heparin were inverted and centrifuged to obtain diluted plasma. Ten microliters of each injectate was added to four additional tubes with blood collected prior to the appearance of radioactivity and processed in a similar manner. These samples allowed calculation of the total radioactivity and ICG injected and also the apparent, spontaneous hydrolysis of parent [^{3}H]BPAP.

After centrifugation, the total ^{14}carbon of a 0.5-ml aliquot of the supernatant fraction mixed with 4 ml scintillation mixture was measured by liquid scintillation spectrometry. The concentration of ICG in a second set of aliquots was measured spectrophotometrically at 805 nm. A third set of aliquots was used to separate metabolite [^{3}H]BPhe and parent [^{3}H]BPAP by toluene extraction, after which the radioactivity of each was measured.

Calculations. ^{14}Carbon and tritium counts per minute were corrected for background radioactivity, spillover and counting efficiency, and the resulting dpm values were expressed per ml of blood by use of the appropriate dilution factors. The fractional concentration of radiolabel in each sample was determined by dividing the dpm per ml in each sample by the calculated total dpm injected. Optical density measurements were also converted to fractional concentrations of ICG per ml of blood in each sample by division by the total amount injected.

Pulmonary removal of [^{14}C]captopril (or [^{14}C] serotonin) was calculated from the following equation:

$$\% \text{ Removal} = ([FC_{ICG} - FC_{cap}]/FC_{ICG}) \times 100,$$

where FC_{ICG} and FC_{cap} (or $FC_{5\text{-}HT}$) are fractional concentrations of ICG, captopril or 5-HT, respectively, in each blood sample. Removal was calculated from samples at the peak of each ICG outflow curve (peak removal), and over the integral up to the peak of each ICG outflow curve (integral removal). The ICG data were used to calculated pulmonary mean transit time, blood flow and volume of distribution of the reference [15]. The metabolite of [^{3}H]BPAP ([^{3}H]benzoyl-phenylalanine) was determined as described previously [14] and percent metabolism was then calculated as

$$[[^{3}H]BPhe/([^{3}H]BPhe + [^{3}H]BPAP)] \times 100.$$

All data are presented as means ± S.E. Data were analyzed for statistical differences using Student's unpaired *t*-test at the 95% level of confidence. [^{14}C]-Captopril and unlabeled captopril were supplied by Mr. S. J. Lucania, Squibb Institute, Princeton, NJ.

RESULTS

As shown in Table 1, there were no significant differences in blood flow, mean pulmonary transit time or volume of distribution of ICG, or in pH, hematocrit or aortic blood pressure, between measurements made after injections of the high and the low dose of [^{14}C]captopril or between measurements made after the initial and the subsequent injections of either dose of [^{14}C]captopril.

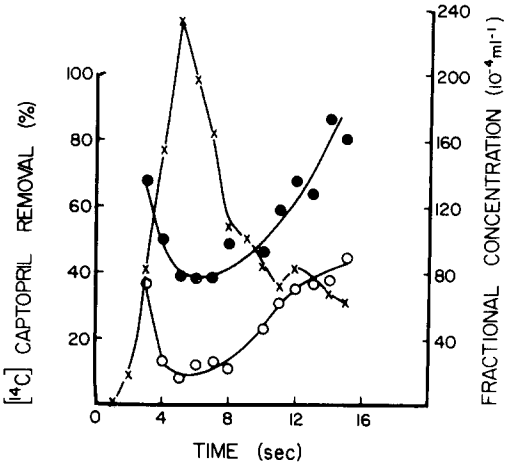

Fig. 1. Instantaneous percent removal of [^{14}C]captopril [10 nmoles/kg (●) or 70 nmoles/kg (○)], and corresponding fractional concentrations of indocyanine green (×), during a single pulmonary transit. Only one indocyanine green outflow curve is illustrated, although both are congruent.

Table 1. Physiological parameters during repeated injections of [^{14}C]captopril*

Dose (nmoles/kg)	Order of administration	N	$\dot{Q}$ (ml/min)	$\bar{t}$ (sec)	V_D (ml)	P_A (mm Hg)	Hct (%)	pH (units)
10	1st	3	408.8 ± 41.2	7.9 ± 0.3	53.7 ± 4.2	114.4 ± 6.1	38.3 ± 2.9	7.57 ± 0.04
70	1st	3	424.1 ± 52.1	7.0 ± 0.3	49.3 ± 6.3	119.9 ± 10.9	37.0 ± 1.0	7.57 ± 0.06
10	2nd	3	325.9 ± 35.9	8.5 ± 0.8	45.6 ± 3.0	100.5 ± 6.4	37.0 ± 2.5	7.51 ± 0.03
70	2nd	2	368.3 ± 27.1	7.2 ± 0.2	44.1 ± 2.0	105.8 ± 9.5	37.0 ± 1.0	7.45 ± 0.03
10	3rd	3	331.5 ± 12.8	8.3 ± 0.8	46.0 ± 6.2	82.3 ± 10.4	33.0 ± 1.7	7.37 ± 0.05
70	3rd	2	292.6 ± 6.6	9.6 ± 0.2	33.4 ± 0.0	95.0 ± 2.3	34.0 ± 0.0	7.40 ± 0.01

* $\dot{Q}$ = pulmonary blood flow; $\bar{t}$ = mean pulmonary transit time; V_D = volume of distributionof indocyanine green; P_A =mean carotid arterial blood pressure; Hct = hematocrit; and pH = carotid arterial blood pH. There were no significant differences. Data correspond to Fig. 2.

Table 2. Effects of dose and repeated administration of [^{14}C]captopril on its pulmonary removal

Dose injected (per kg)	Order of administration	N	Percent removal	
			Peak	Integral
10	1	3	40.2 ± 2.5	30.1 ± 1.3
10	2	3	20.1 ± 2.4*	25.1 ± 1*
10	3	3	24.2 ± 2.1*	16.9 ± 5.3*
70	1	3	5.8 ± 3.0†	11.8 ± 3.4†
70	2	2	6.4 ± 2.2†	9.0 ± 0.5†
70	3	2	0	3.3 ± 0.2*†

* Significantly ($P < 0.05$) lower than the corresponding value for the *first* injection of the same dose.

† Significantly ($P < 0.05$) lower than the corresponding value for 10 nmoles [^{14}C]-captopril/kg.

Analysis of [^{14}C]*captopril removal during a single transpulmonary passage.* Typical outflow data after injection of ICG and [^{14}C]captopril into the jugular vein are illustrated in Fig. 1. After injection of either 10 or 70 nmoles [^{14}C]captopril/kg, the instantaneous percent removal of captopril during a single pulmonary passage was inversely related to the calculated intravascular concentration of the drug (i.e. $FC_{ICG} \times$ amount of captopril injected). However, at each time point percent removal of 70 nmoles [^{14}C]-captopril/kg was less than the corresponding value for 10 nmoles [^{14}C]captopril/kg.

Effects of repeated administrations and dose upon pulmonary removal of [^{14}C]*captopril.* The results obtained from repeated injections of 10 or 70 nmoles [^{14}C]captopril/kg at 1-hr intervals are summarized in Table 2. Repeated injection of the same dose of [^{14}C]captopril was associated with reduced percent removal. In addition, percent removal of the higher dose was significantly ($P < 0.05$) less than that of the lower dose after both initial and subsequent injections.

Effect of [^{14}C]*captopril on pulmonary angiotensin converting enzyme activity.* As can be seen in Table 3, control [^{3}H]BPAP metabolism was 70.1 ± 1.7% at the peak of the ICG outflow curve and 67.3 ± 2.0% over the integral of the *entire* curve. After co-injection of 10 nmoles [^{14}C]captopril/kg with [^{3}H]BPAP, metabolism of the latter was reduced significantly ($P < 0.05$) to 27.4 ± 2.4% (peak) and 26.7 ± 1.8% (integral). In one animal, co-injection of 70 nmoles [^{14}C]captopril/kg with [^{3}H]BPAP completely eliminated [^{3}H]BPAP metabolism. In addition, [^{3}H]BPAP metabolism was reduced significantly ($P < 0.05$) to 7.6 ± 0.2% (peak) and 14.3 ± 2.4% (integral) after co-injection of 6 μmoles unlabeled BPAP/kg with trace [^{3}H]-BPAP.

Effect of BPAP on pulmonary removal of [^{14}C]-*captopril.* Data from experiments in which unlabeled BPAP was injected with [^{14}C]captopril are illustrated in Fig. 2. Removal after the initial injection of

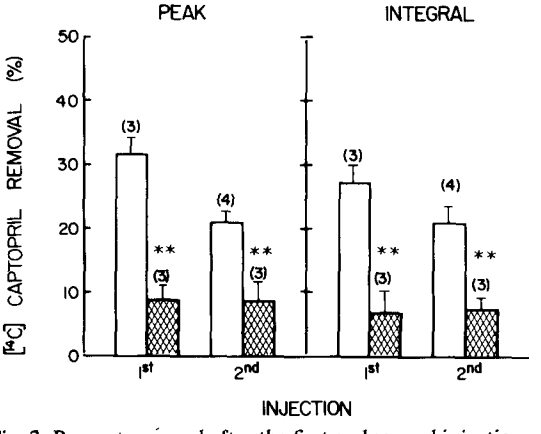

Fig. 2. Percent removal after the first and second injections of [^{14}C]captopril (10 nmoles/kg) with (▩) or without (□) BPAP (6.4 ± 0.6 μmoles/kg) measured at the peak and over the integral of each indocyanine green outflow curve. N values are indicated in parentheses. A double asterisk (**) indicates a significant ($P < 0.05$) difference between injections with and without BPAP.

Table 3. Effects of [^{14}C]captopril and unlabeled BPAP on percent metabolism of [^{3}H]BPAP*

Injection	N	Percent metabolism of [^{3}H] BPAP	
		Peak	Integral
[^{3}H]BPAP (control; 18 pmoles/kg)	9	70.1 ± 1.7	67.3 ± 2.0
[^{14}C]Captopril (10 nmoles/kg)	3	27.4 ± 2.4†	26.7 ± 1.8†
BPAP (6.0 μmoles/kg)	3	7.6 ± 0.2†	14.3 ± 2.4†

* [^{3}H]BPAP metabolism was measured at the peak and over the integral of each indocyanine green venous outflow curve.

† Significant difference from control ($P < 0.05$).

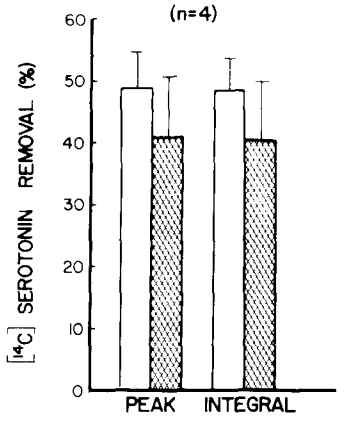

Fig. 3. Percent removal of [^{14}C]serotonin (13 nmoles) after injection with (▩) or without (□) BPAP (5.9 ± 0.1 μmoles/kg), measured at the peak and over the integral of each indocyanine green outflow curve. There were no significant differences.

10 nmoles [^{14}C]captopril/kg in the absence of BPAP (i.e. ethanol vehicle control) was 31.8 ± 2.4% (peak) and 27.1 ± 2.9% (integral). In another group of animals, removal after initial co-injection of 10 nmoles [^{14}C]captopril/kg with BPAP (6.2 ± 1.1 μmoles/kg) was reduced significantly ($P < 0.05$) to 8.7 ± 2.3% (peak) and 6.8 ± 3.44% (integral). Removal after a second injection of 10 nmoles [^{14}C]captopril/kg in the absence of BPAP (ethanol vehicle control) was 20.9 ± 1.7% (peak) and 20.8 ± 2.7% (integral). Removal after the second co-injection of 10 nmoles [^{14}C]captopril/kg with BPAP (6.6 ± 0.8 μmoles/kg) was reduced significantly ($P < 0.05$) to 8.7 ± 2.8% (peak) and 7.3 ± 1.7% (integral). It was found that [^{14}C]captopril removal was significantly lower with an ethanol vehicle than with a saline vehicle (Table 2 and Fig. 2). In addition, as reported previously [14], BPAP did not alter the flow, mean transit timer or volume of distribution of the injectate, pH, hematocrit or arterial blood pressure.

Effect of BPAP on pulmonary removal of [^{14}C]-*serotonin.* As illustrated in Fig. 3, [^{14}C]serotonin removal was not affected by unlabeled BPAP (6.0 μmoles/kg).

Examination of other possible sources of [^{14}C]-*captopril removal.* Injection and withdrawal catheters were connected to a mixing chamber containing 50 ml saline. ICG and [^{14}C]captopril were coinjected into the chamber and serial samples were collected. There was no calculated removal of [^{14}C]captopril in this system, indicating that all [^{14}C]captopril removal described above occurred within the pulmonary circulation and was unrelated to non-specific uptake within tubing or glassware.

DISCUSSION

We have demonstrated that captopril is removed from blood by the rabbit lung *in vivo*. The doses of [^{14}C]captopril were sufficient to significantly inhibit pulmonary endothelial converting enzyme activity ([^{3}H]BPAP metabolism), indicating that removal of the drug was accompanied by pharmacological activity. There was significantly less percent removal of the higher dose of captopril (70 nmoles/kg) than of the lower dose (10 nmoles/kg), suggesting that pulmonary removal of the drug is a saturable process. This suggestion receives support from the fact that during the first transpulmonary passage of each injection of captopril, the instantaneous percent removal at each sample time was inversely proportional to the intravascular concentration of captopril, resulting in percent removal versus time curves with a concave-upward shape. Similar concave-upward extraction versus time curves have been explained previously as reflecting non-linear (saturable) pulmonary removal of [^{14}C]serotonin and prostaglandin E_1 [16–19].

Pulmonary removal of a circulating compound also may be influenced by several factors, including the rate of pulmonary blood flow, the residence time within the lung, and perfused capillary surface area [16]. Accordingly, during each experiment we monitored the pulmonary blood flow, mean transit time and volume of distribution of indocyanine green, as well as arterial blood pressure, pH and hematocrit. Although there were no statistically significant changes in any of these potential contributing factors (Table 1), perhaps because of the small number of observations and wide variation in values, we cannot rule out the possibility that changes in one or more of these parameters occurred during the course of the experiments.

Pulmonary removal of the initial high or low dose of captopril given to an animal was significantly greater than removal after subsequent, identical doses. Possibly, therefore, after the first transpulmonary passage of captopril, there was some retention of the drug at its site of removal, resulting in decreased removal of subsequent doses of captopril. A similar proposal was made [20] to explain the observation that after a single oral dose of captopril, plasma drug levels were two or three times higher in patients receiving chronic captopril therapy than in those who never received the drug. It was therefore suggested that bioavailability of captopril increased with chronic administration because of saturation of binding sites in the gastrointestinal tract and presystemic circulation [20]. Furthermore, the plasma levels of captopril measured in patients [20], 1.7 μM (0.36 μg/ml), were very similar to estimated rabbit plasma levels after the initial injection of 10 nmoles captopril/kg (1.9 μM, based upon the calculated volume of distribution). Thus, our results are consistent with the suggestion [20] that saturation of binding sites in the pulmonary circulation may be responsible for the higher plasma captopril levels in patients on chronic captopril therapy. Alternatively, decreased captopril removal during the course of our experiments may have been due to other temporal factors, such as duration of anesthesia or post-surgical events, rather than repeated administrations of the drug *per se*.

The likelihood that pulmonary removal of captopril may influence systemic plasma levels, and possibly, therefore, therapeutic effectivness of the drug, underscores the potentially critical role of the lung in regulating "downstream" concentrations of clinically-used drugs, including amphetamine, propran-

olol and imipramine [21–23]. Furthermore, altered pulmonary removal of captopril (which could possibly occur during various lung disorders [13, 22, 24]) may alter plasma levels and thus the therapeutic effectiveness of the drug.

Our results indicate that pulmonary removal of captopril is likely due primarily to binding of captopril to endothelial converting enzyme. Thus, BPAP significantly reduced pulmonary removal of captopril, but not [^{14}C]serotonin, suggesting specific competition of the converting enzyme substrate and inhibitor for access to the site of removal. A non-specific effect on endothelial membrane would also be expected to decrease serotonin uptake [22]. The dose of BPAP used did not completely inhibit pulmonary [^{3}H]BPAP metabolism or removal of captopril. It is therefore uncertain whether remaining captopril removal (in the presence of BPAP) reflects specific or non-specific uptake. However, recent ligand binding studies *in vitro* have demonstrated a high degree of specific binding of [^{3}H]captopril to lung tissue [25]. Furthermore, similar indicator dilution methods [26] allowed successful demonstration of specific binding of acetazolamide to pulmonary endothelial carbonic anhydrase. Nevertheless, an unresolved question is whether it is captopril bound to endothelial ACE, or the circulating captopril that is primarily responsible for the antihypertensive effect of the drug.

The magnitude of pulmonary captopril removal is influenced by the intravascular concentration of the free drug, which could be reduced by plasma binding and also, because of its reactive sulfhydryl group, by spontaneous oxidation of the drug to form a disulfide dimer, as occurs in rats, dogs and man [27, 28]. However, captopril is stable in rat plasma for up to 30 min [29]. Therefore, the magnitude of disulfide formation that occurs in the rabbit during a single transpulmonary passage of captopril (less than 20 sec) is likely to be small. We did not attempt to separate unchanged captopril from possible metabolites in blood, and any captopril bound to plasma proteins was included in the measurements of total ^{14}C activity. Probably, however, neither plasma-bound captopril nor the disulfide dimer would be available for binding to endothelial converting enzyme. Therefore, to the extent that these factors were of significance, we may have underestimated pulmonary removal of captopril. It should also be emphasized that our results were obtained from single pass measurements. Analysis of captopril removal under steady-state conditions may provide more accurate assessment of captopril disposition during chronic administration of the drug.

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