AN ENDOTHELIUM-BOUND ANGIOTENSIN CONVERTING ENZYME-BASED ASSAY AND NOVEL COMPUTERIZED NONINVASIVE METHODS TO STUDY THE EFFECTS OF ANTIHYPERTENSIVE DRUGS

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PREFACE AND ACKNOWLEDGMENT

Since 1984 I have been involved in phase I-IV human clinical pharmacological studies at the First Department of Medicine, Medical University of Pecs, Hungary. My interest in clinical pharmacology was greatly stimulated by my excellent clinical supervisors Prof. Javor Tibor , Dr. Nagy Lajos and Dr. Radnai Bela. At that time we investigated several antihypertensive compounds, mostly angiotensin converting enzyme (ACE) inhibitors according to regulations of GCP (good clinical practice). We have been forced to develop novel noninvasive methods and introduce them into the clinical pharmacological practice. At the same time Prof. Mozsik Gyula inspired me to take a deeper insight of the cardiovascular regulation in physiological and pathological conditions. He has also encouraged and supported me to apply for a fellowship that gave me a great opportunity to investigate this issue.

With the great support of Dr. Marczin Nandor, I had the privilege to accept the invitation of Prof. John D. Catravas in the Vascular Biology Center at the Medical College of Georgia , Augusta Georgia U.S.A. I was keen to learn some more about the role of locally generated angiotensin II in cardiovascular regulation.

The present thesis comprises the major results of my 8 years of work, in the field of human clinical pharmacology, and 2.5 years of research work in the laboratory of Prof. John D. Catravas. First and foremost, I would like to express my deepest gratitude to my supervisor Prof. John D.Catravas. This work could not be completed without the establishment of a truly stimulating scientific atmosphere in his laboratory, without his caring supervision and without his recruiting of established collaborators during the development of various projects.

I greatly appreciate my colleque Dr. Horvath Ivan for his continuous help and indispensable cooperation. The prerequisite for successful completion

of the studies was the establishment of an interacting research group inside the department. I am grateful to my colleques Dr. Hunyady Bela and Dr. Rinfel Jozsef to provide me with this atmosphere, and to help me to carry out our ideas. I would like to thank Dr. Nemes Janos, Dr. Andreas Papapetropoulos and Dr. Gene Fisher for their continuing interest and for exciting discussions.

I am further indebted to the research assistants involved in this work. I greatly appreciate Mr. James Parkerson for his excellent technical assistance. I am pleased to acknowledge the skillful technical assistance of Mr. Sami U. Khan, Ms. Bereczk Orsolya and Ms. Horvath Maria. I would like to thank Dr. Kerekes Endrene, Nyikos Zsigmondne, Szalay Edit and Ms. Annie Cruz for typing my manuscripts.

INTRODUCTION

Hypertension, in Hungary, is one of the leading indications both for office visits to physicians and for the use of prescription drugs. This leading position reflects the increase in the number of people with hypertension who have been identified and brought under active treatment. Approximately 15-20 % of the young adult population in Hungary are or can be considered to be hypertensive patients (including borderline and slight hypertension), but over the age of 55 years this ratio increases up to 50-60 % (72,74).

The risks of elevated blood pressure have been determined largely from large scale epidemiological surveys. Data from the Framingham study - relating blood pressure to cardiovascular morbidity and mortality - document a number of important points (53). High blood pressure puts an immediate, direct burden on the heart and the resistance arterioles, so that all forms of cardiovascular disease are more frequent, especially the incidence of myocardial infarction and heart failure.The risk of stroke is particularly ominous. Unfortunately, with the possible exception of the very old, morbidity and mortality - mostly related to cardiovascular disease - increase progressively with each increment in blood pressure (72,90,131,).Therefore, it is remarkably important to initiate an effective antihypertensive treatment in order to prevent late organ damages. The vast majority of hypertension - approximately 95 % of all hypertension - is called essential hypertension. (However, any hypertension without evident cause can correctly be called primary). Essential hypertension is often regarded as a multifactorial disease, resulting from a number of diverse genetic and environmental factors (72).

Patients with primary or essential hypertension need to take a lifelong antihypertensive medication with all the adverse effects of these drugs.The objective of the antihypertensive therapy is at least threefold: a) to achieve normal blood pressure; b) to prevent late organ damage; and c) to provide an acceptable quality of life.

OVERAL OBJECTIVES

Angiotensin Converting Enzyme

Angiotensin converting enzyme (ACE, canines II) acts as a dipeptidyl carboxypeptidase and is involved in the metabolism of two major vasoactive peptides, angiotensin II and bradykinin. ACE generates the potent vasopressor hormone angiotensin II by cleaving the carboxyl-terminal dipeptide from angiotensin I and inactivates the vasodepressor hormone bradykinin, by sequential removal of two carboxyl-terminal dipeptides (73,96). The favoured ACE substrate is bradykinin, for which the Michaelis-Menten constant (K_m) is approximately 80 times lower than for angiotensin I (0.2 versus 16 µmol / L respectively; (12,13,14). In most mammalian organs ACE is primarily located along the luminal surface of endothelial cells.The vast majority of the enzyme and higher substrate availability is believed to exist in the pulmonary microvasculature relative to other tissues. ACE activity, although has been found in the vascular endothelium of the lungs, also occurs in other vascular beds and in many other tissues including the myocardium and coronary arteries (4).

Measurement of ACE activity utilizing either angiotensin I or bradykinin is complicated by the fact that both compounds are also metabolized by endogenous aminopeptidases or endopeptidases (114,115,116). A synthetic substrate, benzoyl-Phe-Ala-Pro (BPAP) has been demonstrated to be a highly specific substrate for blood, lung, and urine ACE (9,10,31). In the presence of ACE, BPAP is converted to benzoyl-Phenylalanine and Alanyl-Proline; *in vitro* the K_m for BPAP is approximately $2X10^{-6}$ M, slightly higher than the reported value for bradykinin but lower than the K_m for angiotensin I.

Angiotensin Converting Enzyme Inhibitors

The discovery of ACE inhibitors is one of the major therapeutic advances of the last decade. There is no doubt that ACE inhibitors have multiple sites of action. The chief and best understood mechanism is inhibition of ACE, not only of the circulating enzyme, but also of that found in the various tissues, particularly the vascular beds (96,46,74,123). There is proof in humans that the tissue renin-angiotensin system plays a decisive role as a site of action of ACE inhibitors. Direct estimations of drug and membrane-bound enzyme interactions, *in vivo*, would provide important information about the mode of action of different ACE inhibitors under various clinical conditions (9,118).

Therefore, our first aim was to compare and contrast the inhibitory effects of different ACE inhibitors on pulmonary capillary endothelium-bound and serum ACE activity, in vivo, in rabbits, and selected tissue ACE activities ex vivo. On the basis of these experiments, we developed indicator dilution techniques to estimate the enalaprilat-induced inhibition on pulmonary capillary endothelium-bound vs. serum ACE activities in human subjects.

In addition, we also aimed at investigating the influence of altered LAD coronary artery blood flow on coronary endothelium-bound ACE activity in dogs. The endothelium-bound ACE activities were determined from the single pass transpulmonary hydrolysis of the specific ACE substrate ^{3}H-BPAP.

After we had obtained enough consistent data from the animal experiments, a human study was performed. We compared coronary and pulmonary endothelium-bound ACE activities in patients undergoing

coronary arterial bypass graft surgery, before and after graft connection.

Clinical pharmacology is the branch of the medical sciences which is most concerned with the rational development, the effective and safe use, and the proper evaluation of drugs and other chemical entities in humans for the diagnosis, prevention, alleviation, and cure of disease and disease syndromes (73). There are four well specified phases of human clinical pharmacological studies, which may overlap with each other but are designed to progressively reveal the drug's beneficial and adverse properties. The aim of human phase I clinical pharmacological studies is to establish a minimum effective dose to achieve activity without significant adverse reactions. Pharmacokinetic measurements of absorption, half-life, and metabolism are often done in phase I studies. Noninvasive monitoring of cardiovascular parameters is a cornerstone in the phase I clinical pharmacological studies. The most valuable tools and methods are 2-dimensional Doppler echocardiography, impedance cardiography, and ambulatory blood pressure monitoring system which provide exact and reproducible data on the effects of different compounds. These methods also provide us an excellent opportunity to measure blood pressure lowering effects, as well as to recognize adverse effects of different antihypertensive drugs in the course of phase IV human clinical pharmacological studies.

Impedance cardiography is a feasible method for noinvasive calculation of stroke volume from beat to beat. In serial measurements to determine the changes in the stroke volume, cardiac output, peripheral vascular resistance, systolic time intervals provide us with a follow-up determination of these important hemodynamic parameters (60).

Ambulatory blood pressure monitoring (ABPM) is a widespread method for the diagnosis and differential diagnosis of high blood pressure and to estimate the effect of antihypertensive treatment accurately.The most of these devices -

using oscillometry principles - can measure and calculate numerous useful blood pressure parameters automatically (84,85,86).

Thus, our second major goal was to adopt to human clinical pharmacological studies (from phase I to phase IV) newly developed noninvasive techniques. We introduced and applied routinely in human clinical pharmacological studies the method of programmable impedance cardiography.

We also investigated the importance of ten different blood pressure parameters, provided by 24-hour ambulatory blood pressure monitoring, and considered to be characteristic of the patients' diurnal blood pressure behavior. In this study the hypertensive patients were classified by PRIMA (Pattern Recognition by Independent Multicategory Analysis) method.

CHAPTER I

APPLICATION OF INVASIVE METHODS IN EXPERIMENTAL MODELS AND IN HUMAN SUBJECTS TO INVESTIGATE THE ENDOTHELIUM-BOUND ACE AND THE EFFECT OF ACE INHIBITORS ON ENDOTHELIUM-BOUND ACE ACTIVITY

1. INHIBITION OF PULMONARY ENDOTHELIUM-BOUND AND SERUM ACE ACTIVITY IN VIVO AND TISSUE ACE ACTIVITIES EX VIVO

INTRODUCTION

Angiotensin converting enzyme (canines II) is a dipeptidyl-carboxypeptidase present in most mammalian tissues. In the lung, ACE is primarily located along the luminal surface of endothelial cells and is responsible for the processing of angiotensin I and bradykinin. It has recently been shown that the components of the renin-angiotensin system (RAS) are generated locally in several organs involved in cardiovascular regulation (61). Thus, angiotensin II acts not only as circulating hormone, but also as a locally generated modulator of organ function at the tissue level. Preclinical and clinical trials of the potencies of a new ACE inhibitors mostly rely on comparing systemic blood pressure to intravenously administered angiotensin I before vs. after administration of drugs. (2,8,15,16, 54,)

The aim of this study was to compare the ACE inhibitory profile of acutely and chronically administered trandolaprilat (Knoll Pharmaceuticals) and enalaprilat (Merck Sharp & Dohme) as reflected by the inhibition of a) pulmonary capillary-bound ACE activity, *in vivo*, b) pressure responses to iv.

angiotensin I and bradykinin, and c) tissue ACE activities, *ex vivo*. Furthermore, since combined treatment of trandolaprilat with a calcium channel blocker (verapamil) has been suggested as a promising alternative to the management of hypertension (89), the effects of the combination of trandolaprilat and verapamil on the aforementioned parameters were also studied.

METHODS

Experimental Design.

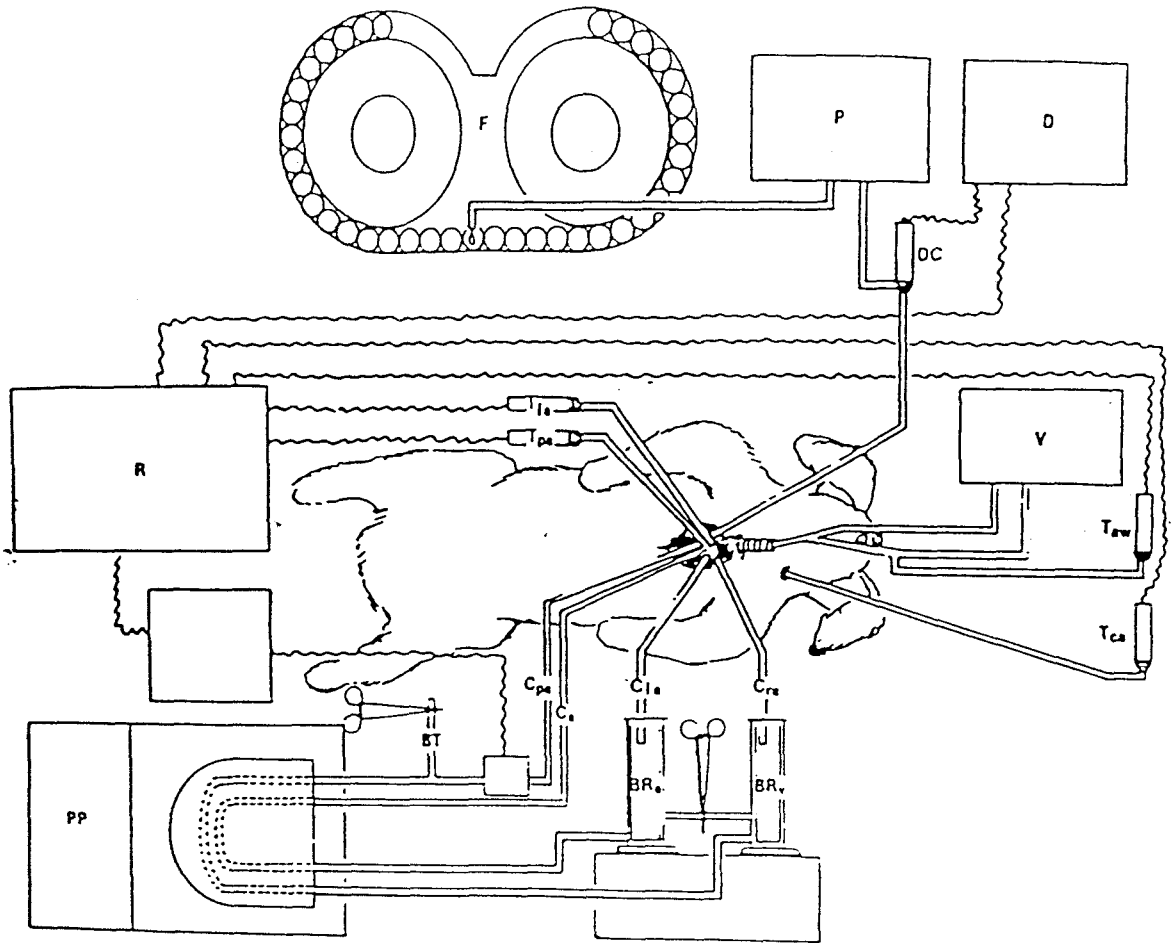
Acute Study. The purpose of this study was to compare and contrast the inhibitory effects of hemodynamically equiactive doses of trandolaprilat (alone and combined with verapamil) and enalaprilat on pulmonary capillary endothelial-bound ACE activity, *in vivo*, and selected tissue ACE activities *ex vivo*. First, the doses of trandolaprilat and enalaprilat that caused a 50% inhibition of the pressor response to angiotensin I (1μg/kg i.v.) were determined and were used subsequently in this study. Our preliminary experiments showed that 8 μg/kg trandolaprilat and 10 μg/kg enalaprilat were equiactive. Anesthetized rabbits were placed on total heart-bypass (26,27) to allow precise control of systemic and pulmonary blood pressure and cardiac output. All drugs were given as boluses via the catheter placed into the pulmonary artery. Systemic mean arterial pressure responses to angiotensin I (1 μg/kg iv.) and bradykinin (0.25 μg/kg i.v.) and changes in pulmonary endothelium-bound ACE activity were determined immediately before the administration of different ACE inhibitors (baseline) and twenty minutes after inhibitor administration. The following groups were studied: trandolaprilat, 8 μg/kg (n=7); trandolaprilat, 8μg/kg + verapamil, 100 μg/kg (n=6); enalaprilat, 10 μg/kg (n=6). Two hours after the administration of ACE inhibitor the following tissues were removed for the determination of ACE activity: plasma, lung, cardiac ventricles and atria, kidney, aorta, and pulmonary artery.

Chronic Study. The purpose of this study was to compare and contrast the inhibitory effects of chronic administration of trandolaprilat (alone as well as with verapamil), and enalaprilat on pulmonary capillary endothelium-bound ACE, *in vivo*, and selected tissue ACE, *ex vivo*. The following treatments were employed: trandolaprilat 8 μg/kg/day (n=7); trandolaprilat 8 μg/kg/day + verapamil 100μg/kg/day (n=6); enalaprilat 8μg/kg/day (n=5); enalaprilat 10μg/kg/day (n=5); and vehicle, saline (n=5). Drugs were administered daily into the marginal ear vein (0.5 ml/injection) for eight days. On day nine, twenty-four hours after the last dose of inhibitor, rabbits were placed on total heart-bypass, and the same parameters were studied as in the acute study.

Animal Preparation.

Animal handling and euthanasia were in accordance with guidelines approved by the Institutional Committee on Animal Use for Research and Education. New Zealand White rabbits weighing 3.2 - 4.2 kg were used in this study. Three animals were used for each individual experiment: two as blood donors to prime the cardiovascular perfusion system and one for the actual experiment. Figure 1. shows the sketch of the rabbit heart bypass model. All animals were anesthetized with a mixture of urethane (200 mg/ml) and 5,5-diallylbarbituric acid (50 mg/ml), i.v. The trachea was intubated and connected to a small animal respirator, the left carotid artery was cannulated, pancuronium bromide (1 mg) was given, and the chest was opened. Indomethacin, 5.5 μmol/kg (2 mg/kg), was then administered. This dose is sufficient to inhibit rabbit lung cyclooxygenase completely and prevent the synthesis and release of thromboxane and subsequent pulmonary hypertension, frequently seen in rabbits undergoing extensive surgical manipulations. By means of left atrial, aortic, pulmonary arterial, and right atrial cannulas, the animals were connected to an extracorporeal peristaltic pump that fully supported both pulmonary and systemic circulations. Systemic and pulmonary arterial, airway and left atrial pressures were continuously recorded throughout the experiment.Between enzyme function determinations, blood flow was kept

RABBIT HEART BYPASS MODEL



BT	BUBBLE TRAP	F	FRACTION COLLECTOR
BR_a	ARTERIAL BLOOD RESERVOIR	P	SAMPLE WITHDRAWAL PUMP
BR_v	VENOUS BLOOD RESERVOIR	PP	PERFUSION PUMP
C_a	CANNULA TO AORTA	R	MULTICHANNEL RECORDER
C_{la}	CANNULA FROM LEFT ATRIUM	T_{aw}	PRESSURE TRANSDUCER, AIRWAY
C_{pa}	CANNULA TO PULMONARY ARTERY	T_{ca}	PRESSURE TRANSDUCER, CAROTID ARTERY
C_{ra}	CANNULA FROM RIGHT ATRIUM	T_{la}	PRESSURE TRANSDUCER, LEFT ATRIUM
D	DENSITOMETER	T_{pa}	PRESSURE TRANSDUCER, PULMONARY ARTERY
DC	DENSITOMETER CUVETTE	V	VENTILATOR

FIG 1. Rabbit heart bypass model

constant at 400 ml/min. Arterial blood gas values (pH, PO_2, PCO_2), and hematocrit were recorded in every experiment before drug administration and immediately after the estimation of pulmonary capillary endothelium-bound ACE activity.

Measurement of ^{3}H-BPAP hydrolysis by pulmonary capillary endothelium-bound ACE, in vivo.

Single-pass transpulmonary metabolism of the synthetic tritiated tripeptide substrate, [^{3}H]benzoyl-Phe-Ala-Pro (^{3}H-BPAP), by endothelial plasmalemmal ACE was measured *in vivo*, according to the indicator-dilution principles under first-order reaction conditions (28,29). During the 20 sec of each indicator-dilution experiment, the ventilator was turned off at end expiration, so that airway pressure was 0 mmHg and lungs were at Zone 3 condition (i.e., pulmonary arterial pressure > left atrial pressure > airway pressure). A 0.9-ml saline aliquot containing 0.36 mg indocyanine green (Cardiogreen, CG), and trace amounts (2 μCi) of ^{3}H-BPAP (25 Ci/ mmol;Hycor Laboratories) was injected as a fast bolus into the pulmonary arterial line. Simultaneously, blood was withdrawn at a rate of 36 ml/min by means of a peristaltic pump from a site in the left atrial outflow cannula 3 cm distal to the left atrium into a fraction collector equipped with 13 x 100-mm borosilicate tubes advancing at the rate of 1 tube per 0.6 sec. The appearance of the bolus injection on the arterial side was monitored by a CG densitometer cuvette positioned in series with the withdrawal pump. Each collection tube contained 0.85 mg/ml EDTA and 0.765 mg/ml 8-(OH) quinoline in 2 ml of normal saline to stop the metabolism of unmetabolized ^{3}H-BPAP by plasma ACE. The samples were centrifuged at 3,000 rpm for 10 min to separate cells from plasma. Aliquots (0.5 ml) from the supernatant of each tube were transferred to two sets of 7-ml polyethylene scintillation vials. Total ^{3}H activity (plasma ^{3}H) was measured in one set of vials in the presence of 5 ml Ecoscint A scintillation cocktail (National Diagnostics, Atlanta, GA) by a liquid scintillation spectrometer (model LS 7500, Beckman Instruments). The other 0.5-ml aliquot in the second set of vials was mixed with 2.5 ml of 0.12 N

hydrochloric acid and radioactivity was estimated in the presence of 3 ml of 4 g/L Omnifluor (Dupont, Boston, MA) in Toluene (Baxter, Muskegon, MI) after 48 h of undisturbed equilibration in the dark. In this way, 62% of the metabolite ([^{3}H]benzoyl-Phe) and <5% of the unmetabolized substrate ([^{3}H]BPAP) were extracted into the toluene (counting) phase, the precise fraction determined by concurrently assayed pure [^{3}H]-BPAP and [^{3}H]-BPhe. The amount of metabolite in each sample was calculated according to:

$$[^3H]BPhe = (\text{toluene } ^3H - f_s \cdot \text{plasma } ^3H)/(f_p - f_s) \quad (1)$$

where f_p is the fractional extraction of product [^{3}H]BPhe into the counting phase (toluene) of the second set of vials and f_s is the fractional extraction of the substrate [^{3}H]BPAP into the counting phase. Ten microliters of injectate were added to five sample tubes blood containers collected before the appearance of any radioactivity into the arterial side to determine f_s; f_p was similarly determined by adding 10 µl previously synthesized [^{3}H] BPhe into five different blood sample tubes and processing them as all other samples. Calculations of enzyme kinetics from indicator-dilution experiments have been discussed previously.

Metabolite disintegrations per minute (dpm) per milliliter of plasma were calculated for each sample collected and percent metabolism of the injected substrate (%M) was calculated as the integral of [^{3}H]BPhe/([^{3}H]BPAP + [^{3}H]BPhe), each in units of disintegrations per minute per milliliter of plasma over a single transpulmonary passage.

Determination of tissue ACE activity.

Animals were sacrificed with an overdose of anesthetic, tissues were removed, quickly blotted on filter paper to remove excess fluid, weighed, and transferred into glass tubes containing 100 µl buffer (0.1 M HEPES and 0.15 M NaCl, pH 7.4) per milligram wet weight. An equal volume of the buffer, containing Triton X-100 (0.1%), was then added; the tissue was homogenized; and the tubes were capped and allowed to stand overnight at 4°C.

Subsequently, they were centrifuged at 3,000 rpm for 30 minutes (4°C), and the supernatant was transferred into another glass tube. Preparations thus obtained were kept at 4°C and were assayed within a few days. The utilization of the specific ACE substrate ^{3}H-BPAP (25 Ci/mmol [final activity in the reaction volume, 0.1 μCi/ml]) in different tissues was determined under first-order kinetics. Enzyme activity was calculated using the integrated first-order equation (118).

$$V_{max}/K_m = [\ln([S_o]/[S])] / t \qquad (2)$$

where V_{max}/K_m is the first-order rate constant. $[S_o]$is the initial concentration of the substrate,[S] is the remaining concentration of the substrate at time t, the time of incubation. They were then expressed as units, where 1 unit is the V_{max}/K_m value for 1% substrate metabolism in 1 min (= 0.01 min^{-1}). After protein determination of tissue homogenates the tissue ACE activities were expressed as units / mg protein.

Determination of serum ACE activity.

The serum was diluted to 1:40 with 0.05 M. HEPES buffered saline (pH 8.3). Radioactive working solution was prepared: 2 μl [^{3}H]-BPAP (2 μCi/μl) in 398 μl HEPES buffered saline. 500 μl from the serum sample and 20 μl of radioactive working solution were mixed and incubated for 15 min at 37 °C. The reaction was stopped by 2.9 ml 0.12N HCL. The samples were centrifugated. Totals and metabolites were prepared and counted as described previously. Enzyme activity was calculated according to equation (2) and data were expressed as units /ml serum.

Protein measurements.

Protein content in the supernatant of the centrifuged tissue homogenates was measured by the Bradford method (7). Sample aliquots were combined with the protein binding dye (Bio-Rad Laboratories, Richmond, CA), and optical density was determined at 630 nm. Bovine albumin (fraction V, Sigma)

dissolved in homogenization buffer was used as standard.

Statistics.

Data are presented as means ± SEM. Statistical calculations were performed using one or two way analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test unless indicated otherwise. Differences were considered significant at $p<0.05$.

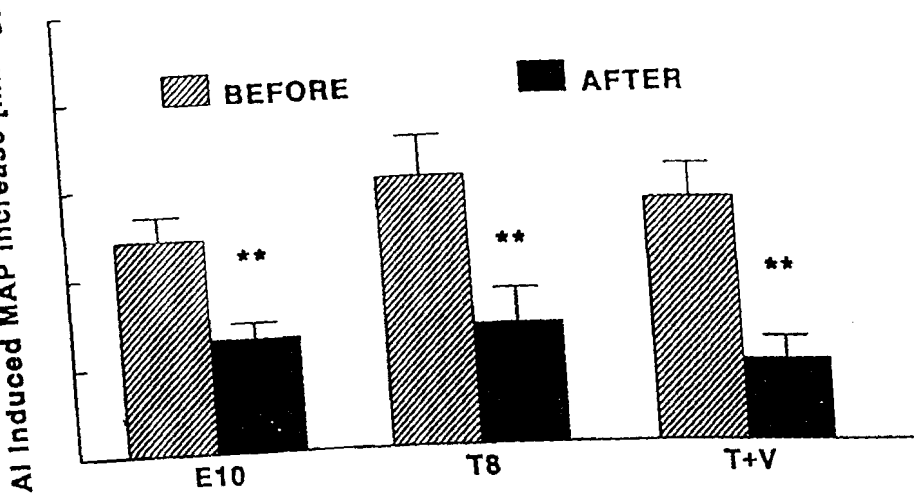
RESULTS

Arterial blood gas and hematocrit values are summarized in Table 1. The values remained stable throughout the experiments.

Pressure responses to Angiotensin I and Bradykinin.

Acute Study. In the acute study, each animal served as its own control. Trandolaprilat alone, and in combination with verapamil, as well as enalaprilat monotherapy, significantly reduced the mean arterial pressure response to angiotensin I (Figure 2a). Twenty min after iv. administration of 10 µg/kg enalaprilat, the MAP increase in response to angiotensin I was 12.8±1.8 mmHg vs. 24±2.7 mmHg in the absence of ACE inhibitor. In the presence of trandolaprilat the MAP increase in response to AI was 13.5±3.9 mmHg vs. 30.3±4.6 mmHg (control). Trandolaprilat together with verapamil reduced the MAP increase in response to AI to 9.1±2.5 mmHg vs. 27.1±3.9 mmHg at baseline ($p< 0.01$). Further a small, but significant increase in the inhibition of AI-pressor response by the trandolaprilat-verapamil combination was observed ($p<0.05$; Figure 2b). As compared to twenty minutes after the i.v. administration of ACE inhibitors, a significant potentiation of the bradykinin (0.25 µg/kg)-induced MAP decrease was observed. In the presence of enalaprilat, the bradykinin-induced MAP decrease was 25.8±1.6 mmHg vs. 17.6±1.4 mmHg in the absence of this ACE-inhibitor ($p<0.05$). Trandolaprilat caused a 31.0±2.3 mmHg MAP decrease vs. 20.5±2.9 mmHg at baseline, while the

a



b

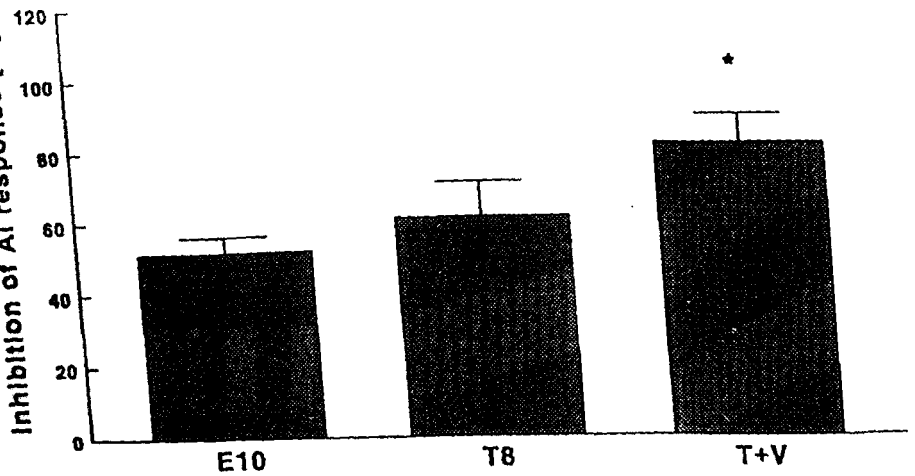
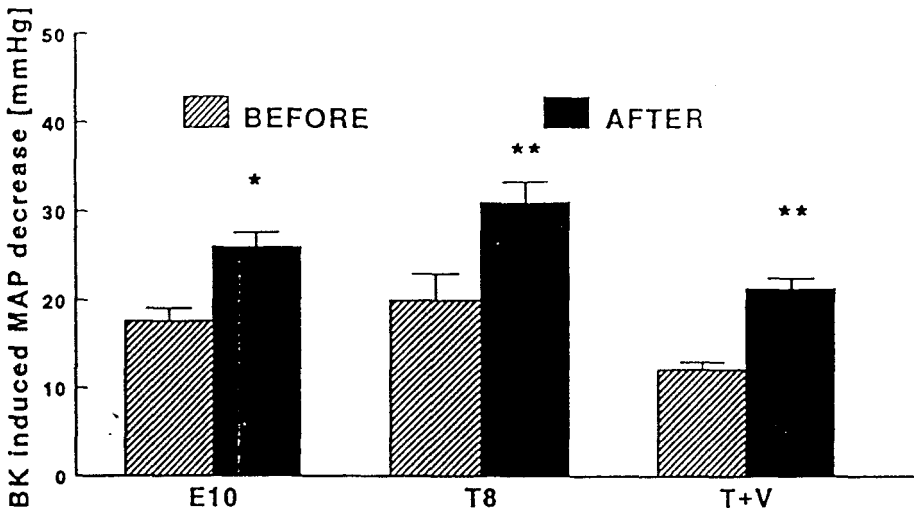


FIG. 2. Effects of acutely administered ACE inhibitors on 1 µg/kg angiotensin I- (AI) induced systemic mean arterial pressure (MAP) increase *in vivo*,(a) and percent inhibition of (AI) responses (b). Treatments: 10 µg/kg enalaprilat (E10; n=6), 8 µg/kg trandolaprilat (T8; n=7), 8 µg/kg trandolaprilat + 100 µg/kg verapamil (T+V; n=6) administered into the pulmonary artery. BEFORE: AI-induced change in MAP just before administration of ACE inhibitors. AFTER: AI-induced MAP changes 20 min. after drug administration. **:p< 0.01 from corresponding BEFORE values for panel a. *:p< 0.05 from E10 and T8 values for panel b. Data shown are means ± SEM.

a



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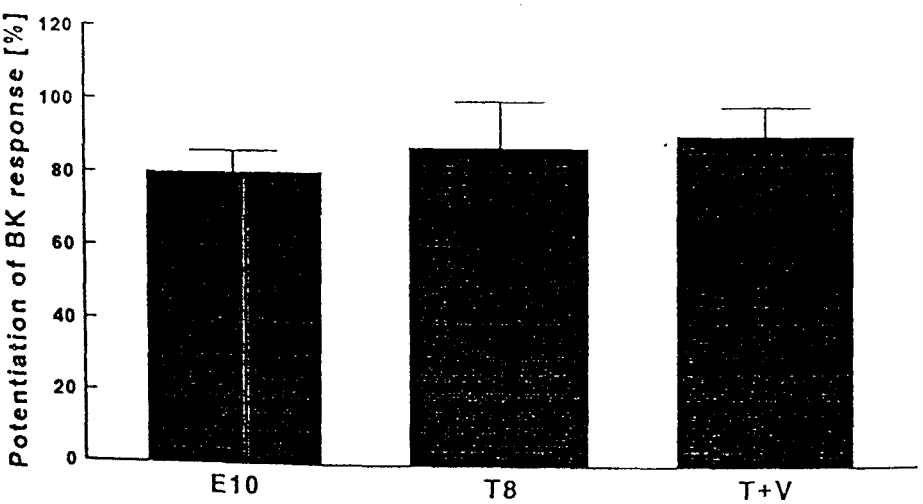


FIG. 3. Effects of acutely administered ACE inhibitors on 0.25 μg/kg bradykinin (BK)-induced systemic mean arterial pressure (MAP) decrease *in vivo*, (a), and percent potentiation of (BK) responses (b). Same treatments as in Figure 1. **:P<0.01 and *:p< 0.05 from corresponding BEFORE values

Table 1. Arterial blood gas and hematocrit values.

		ACUTE STUDY			CHRONIC STUDY				
		T8 n=7	T+V n=6	E10 n=6	T8 n=7	T+V n=6	E8 n=5	E10 n=5	Vehicle n=5
A	pH	7.42±0.02	7.41±0.04	7.42±0.02	7.36±0.03	7.40±0.02	7.39±0.05	7.39±0.2	7.38±0.03
	pO_2 (TORR)	301±43	320±44	382±35	391±33	369±51	383±45	372±47	376±26
	pCO_2 (TORR)	38.3±6	39.5±4	44.8±6	37.4±7	45.8±6	42.9±3	38.5±6	40.2±5
	Hct(%)	28.5±3.1	23.2±2.1	28.9±3	28.2±4.1	24.1±2.9	27.9±2.1	25.1±4.8	26.4±2
B	pH	7.38±0.06	7.40±0.02	7.39±0.01	7.41±0.05	7.37±0.06	7.36±0.05	7.39±0.03	7.42±0.05
	pO_2 (TORR)	392±38	356±42	381±39	402±49	390±28	307±32	359±41	318±29
	pCO_2 (TORR)	38.9±2	37.9±8	44.5±6	43.1±9	45.4±8	39.3±8	37.9±4	46.2±7
	Hct(%)	28.2±5	23.4±4	25.4±3	24.5±4	24.2±3.1	23.7±1.2	26.3±5	24.8±4.1

Values are means ± SE. T8 = 8μg/kg trandolaprilat iv; T + V = 8μg/kg trandolaprilat + 100 μg/kg verapamil iv; E8 = 8μg/kg enalaprilat iv; E10 = 10 μg/kg enalaprilat iv. A = Baseline; before administrationn of ACE inhibitor.
B = After administration of ACE inhibitor, and estimation of pulmonary endothelial-bound ACE-activity.

trandolaprilat- verapamil combination caused a 21.3±1.1 mmHg MAP decrease vs. a 12.1±0.8 mmHg at baseline ($p<0.01$; Figure 3a). There were no significant differences in the percent potentiation of bradykinin induced MAP responses among the three groups (Figure 3b).

Chronic Study. In the vehicle-treated group, angiotensin I (1 μg/kg) caused a 29.9±3.7 mmHg increase in MAP. This change was similar to what was observed at baseline in the acute study. All three drug-treatments significantly reduced the AI-induced MAP increase. Enalaprilat attenuated the MAP increase to 20.3±2.3 mmHg, and 19.1 ± 2.7 mmHg at 8 (E8) and 10 (E10) μg/kg, respectively. Trandolaprilat alone (8 μg/kg) attenuated the MAP increase to 9.7±1.4 mmHg and, in combination with verapamil, to 7.9±1.5 mmHg. ($p< 0.01$from either E8 or E10 values; Figure 4a). Comparable differences were observed in the potentiation of the BK-induced decrease in MAP (Figure 4b). Moreover, the trandolaprilat and verapamil combination appeared to be more potent than the other treatments.

^{3}H-BPAP metabolism by pulmonary capillary endothelium-bound ACE.

Acute Study. Transpulmonary BPAP metabolism was reduced from 77.0±2.2% to 36.5±3.6% after 10 μg/kg enalaprilat, and from 75.0±4.5% to 30.0 ± 5.0%, and 77.3±2.8% to 24.8±4.6% in the T8 and T+V groups, respectively ($P<0.01$; Figure 5a). In Figure 5b, data are expressed as percent decrease from baseline BPAP metabolism, and show that enalaprilat was less effective in inhibiting BPAP metabolism than trandolaprilat either alone or in combination with verapamil.($P<0.05$).

Chronic Study. BPAP metabolism was 82.5±2.8% in the vehicle-treated group. Drug treatments reduced BPAP metabolism to 62.1±2.1% (E8), 57.3±2.3% (E10), 47.0±26.1 (T8), and 49.4±3.5% (T+V)($p<0.01$ from the vehicle group). BPAP metabolism was significantly lower in the T8 and T+V groups compared to the E8 group; ($P<0.01$ and $P<0.05$, respectively;

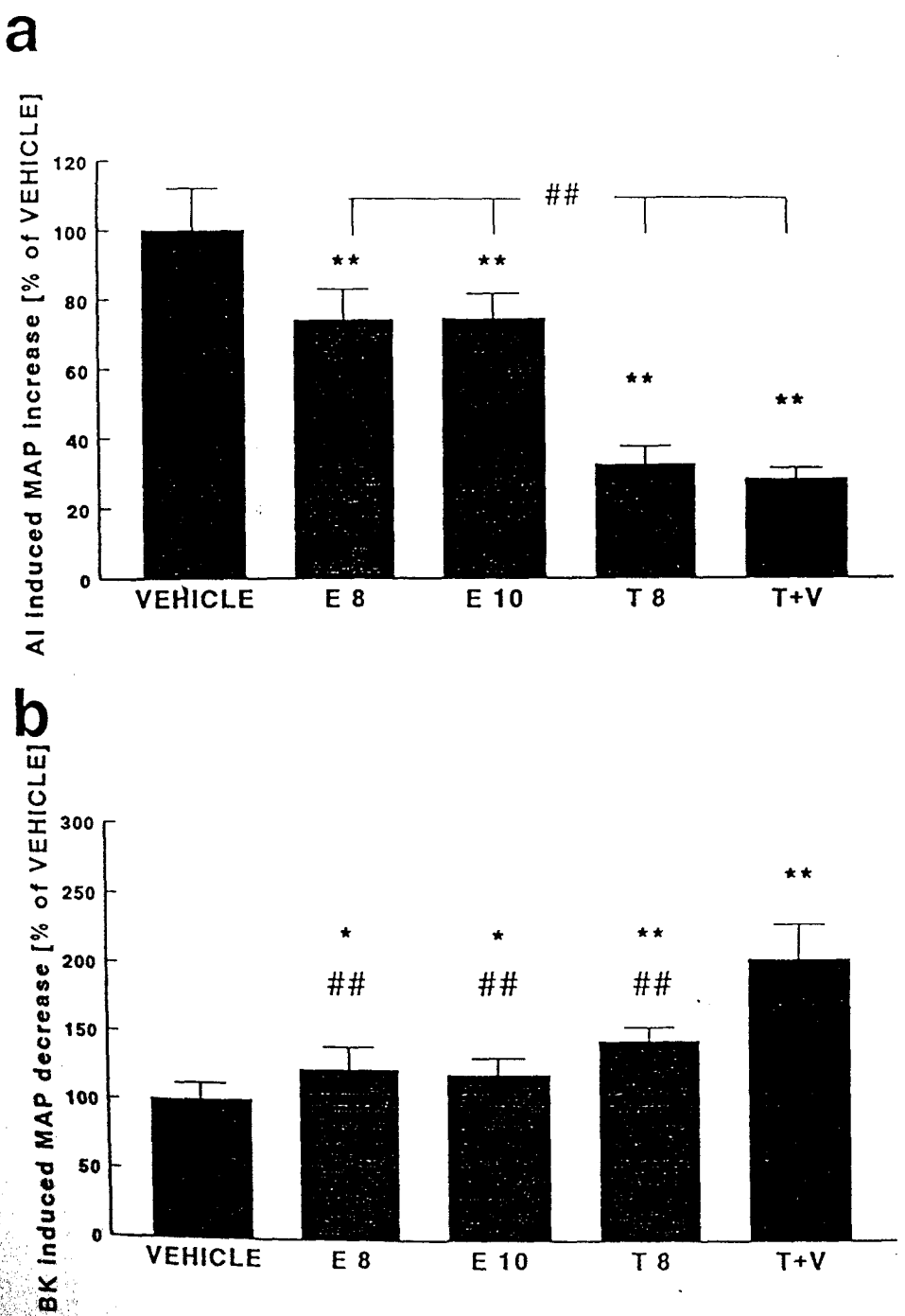
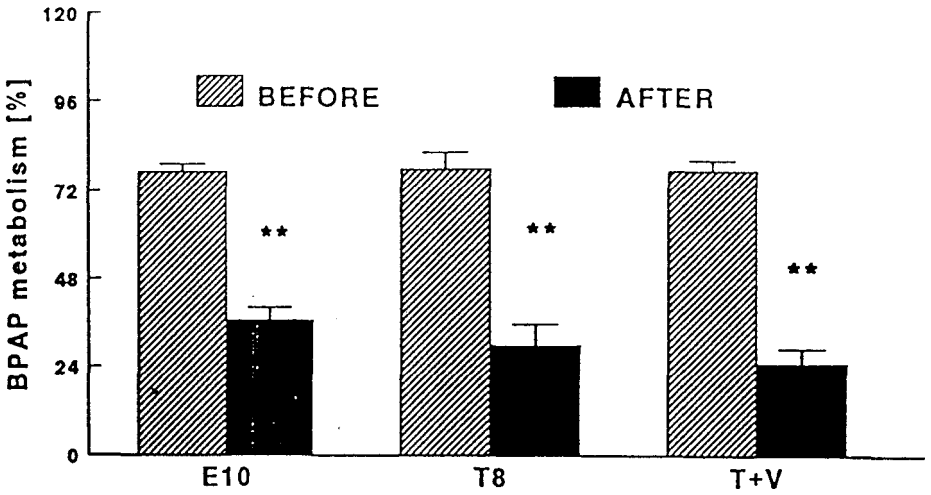


FIG. 4. Effect of chronic administration of ACE inhibitors on 1 µg/kg angiotensin I (AI; panel "a") or 0.25 µg/kg bradykinin (BK; panel "b")-induced changes in systemic mean arterial pressure (MAP). Animals were treated once daily for eight days with drugs administered i.v. and MAP responses were recorded 24 hours after the last drug dose. Treatments: VEHICLE = Saline (N=5), E8= 8µg/kg/day enalaprilat (N=5), E10= 10 µg/kg/day enalaprilat (N=5), T8= 8 µg/kg/day trandolaprilat (N=7), T+V= 8µg/kg/day trandolaprilat + 100 µg/kg/day verapamil (N=6). **:p<0.01 from vehicle group,##:p<0.01 between enalaprilat and trandolaprilat groups, for panel a. **:p<0.01 and *:p<0.05 from vehicle group, ##:p<0.01 from T+V group for panel b. Data shown are means ± SEM

a



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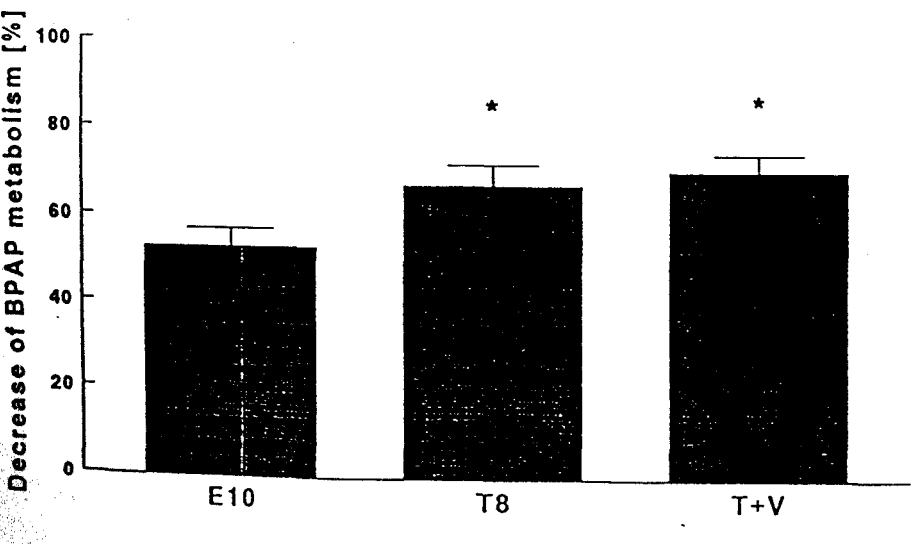

FIG. 5. Effects of acutely administered ACE inhibitors on BPAP metabolism *in vivo*, (a), and percent inhibition of BPAP metabolism by ACE inhibitors (b). Same treatments as in figure 1. **:$p < 0.01$ from appropriate BEFORE values for panel A. *:$p < 0.05$ from E10 values for panel b. Data shown are means ± SEM.

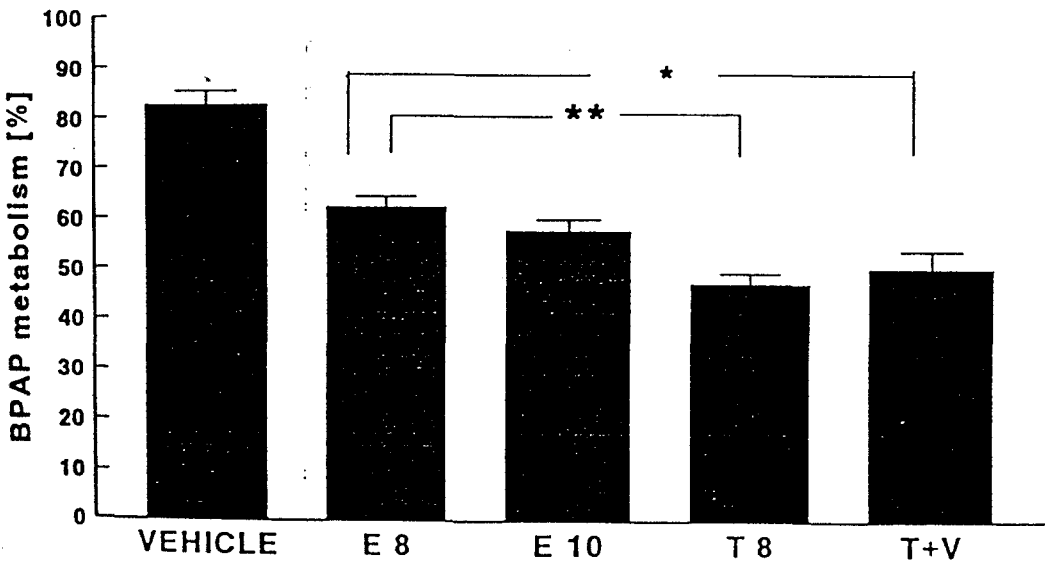

FIG. 6. Effect of chronic administration of ACE inhibitors on percent BPAP metabolism. See legend of figure 3 for details. **:$p<0.01$ from T8 group. *:$p<0.05$ from T+V group. Data shown are means ± SEM.

Figure 6).

Tissue and serum ACE activities (Table 2).

In vehicle-treated rabbits, the substrate hydrolysis by serum ACE was 30.5±3.2 unit/ml serum. In the acute study, 8 μg/kg trandolaprilat was 3-fold more effective in inhibiting serum ACE than 10 μg/kg enalaprilat (1.6±0.3 U/ml serum vs. 5.1±0.7 U/ml serum). In the chronic study 24 hours after the last drug dose, this difference was even more pronounced. No significant differences were found between the E8 and E10 groups.

In the vehicle treated group, lung ACE-activity was 998±47 units/mg protein. In the acute study, 8 μg/kg trandolaprilat caused a significantly higher reduction in the lung ACE activity as compared to 10 μg/kg enalaprilat (164±22 U/mg protein vs. 413±61 U/mg protein, $p< 0.01$). Similarly, in the chronic study, 8 μg/kg trandolaprilat was more effective in reducing lung tissue ACE activity as compared to either 8 μg/kg or 10 μg/kg enalaprilat (259±21 U/mg protein vs. 559±82 U/mg protein and 545±35 U/mg protein, respectively).

As in other species, higher ACE activities were observed in the left ventricle than in the right ventricle (134). In both ventricles, trandolaprilat caused significantly higher reduction of ACE activity than enalaprilat, in both the acute and chronic studies. Notably, the reduction of ACE activity in the left ventricle caused by trandolaprilat 2 hours after i.v. drug administration (acute study) almost remained unchanged 24 hours later (chronic study: 145±16 U/mg protein vs. 158±25 U/mg protein). Similar results were observed with atrial ACE. Trandolaprilat caused the highest reduction of ACE activity in the aorta. In the vehicle-treated group, ACE activity in the aorta was 1521±186 U/mg protein. In the acute study, aortic ACE activity was reduced to 891±40 U/mg protein in E10 group vs. 160±24 U/mg protein in T8 group ($p<0.01$). In the chronic study larger differences were obtained: 1279±98 U/mg protein vs. 1206±104 U/mg protein vs. 177±26 U/mg protein for E8, E10 and T8 groups respectively; ($p<0.01$). ACE activities were also measured in the renal cortex and the medulla. The lowest changes of ACE activity were

Tissue ACE activity values.

	ACUTE STUDY		CHRONIC STUDY			
	E10 n=6	T8 n=7	VEHICLE n=5	E8 n=5	E10 n=5	T8 n=7
PLASMA	5.1±0.7**	1.6±0.3	30.5±3.2	14.3±2.1##	13.2±0.6##	2.4±0.5
LUNG	413±61**	164±22	998±47	559±82##	545±35##	259±21
LEFT VENTRICLE	511±64**	145±16	899±42	610±48##	587±62##	158±25
RIGHT VENTRICLE	307±42**	114±18	520±57	395±74##	371±31##	154±19
LEFT ATRIUM	394±62**	118±23	561±42	450±50##	456±46##	139±24
RIGHT ATRIUM	331±15**	240±22	550±67	449±55##	428±35##	259±21
AORTA	891±40**	160±24	1521±186	1279±98##	1206±104##	177±26
PULMONARY ARTERY	808±78**	337±49	1928±157	1043±84##	1039±132##	460±78
RENAL CORTEX	419±48**	282±28	541±99	431±46##	449±81##	268±28
RENAL MEDULLA	463±49**	266±42	624±51	486±76##	473±65##	280±28

Values are means ± SE. Plasma ACE activity is expressed as units/ml plasma. All the other tissue ACE activities are expressed as units/mg protein. E8 = 8 μg/kg enalaprilat iv; E10 = 10 μg/kg enalaprilat iv; T8 = 8 μg/kg trandolaprilat iv. ** = $p<0.01$ vs. T8 group in the acute study. ## = $p<0.01$ vs. T8 group in the chronic study.

obtained in this tissue and drug treatment caused changes similar to those observed in other tissues.

DISCUSSION

It has been recently shown that ACE inhibitors exert beneficial cardiovascular actions not only by reducing high blood pressure, but also by inhibiting ACE in various target organs of cardiovascular control (15,32,41,54 134).

In the present study we measured pulmonary capillary endothelium-bound ACE activity *in vivo,* from the single pass transpulmonary hydrolysis of the specific ACE substrate ^{3}H-BPAP. The total heart bypass rabbit model allows precise control of systemic and pulmonary blood pressures and cardiac output and in addition the ability to correlate pulmonary capillary endothelial-bound ACE activity with hemodynamics (i.e. pressure responses to angiotensin I and bradykinin) as well as with tissue ACE activity obtained from different target organs(13,125,126). Twenty minutes after drug administration, 8 μg/kg trandolaprilat and 10 μg/kg enalaprilat caused a similar degree of inhibition of the pressure responses to AI or BR, although trandolaprilat at the same time caused a slightly higher inhibition of pulmonary capillary endothelial-bound ACE activity (42). In the chronic study, 24 hours after the last treatment 8 μg/kg trandolaprilat was significantly more effective than 10 μg/kg enalaprilat in reducing both the MAP responses to angiotensin I or bradykinin and in reducing pulmonary capillary endothelial-bound ACE activity.

These findings correlate with the pattern of inhibition of ACE activity measured ex vivo in different tissues. In rabbit plasma, the inhibitory effect of trandolaprilat was 3-fold more effective than that of enalaprilat. Moreover, the inhibition of plasma ACE activity achieved with i.v. trandolaprilat 2 hours after drug administration remained almost unchanged 24 hours later. In all tissues studied 8μg/kg trandolaprilat appeared to be more effective than 8 or 10 μg/kg enalaprilat. The mechanism of this action of trandolaprilat could be related to

a) a longer half-life of the drug, which could thereby inhibit the locally generated and newly synthesized enzyme, and/or b) its longer association to the enzyme. The largest differences in tissue ACE activities were observed in the aorta, the left ventricle, the left atrium and the lung; in these tissues, 8 μg/kg trandolaprilat was 5.5-, 3.5- 3.3- and 2.5-fold more effective than enalaprilat. In the kidney, trandolaprilat was 1.4-fold more effective in the medulla and 1.7-fold more effective in the cortex.

The present findings agree with previous studies on the inhibitory effect of trandolapril and enalapril on serum ACE activities in normotensive rats (16). Trandolapril was 6-10 times more potent than enalapril and had a more prolonged effect on serum, aorta, heart ventricle, lung and kidney ACE activity (34). In spontaneously hypertensive rats, chronically administered trandolapril was approximately threefold more potent than enalapril in inhibiting angiotensin I-induced pressor response. In addition, trandolapril was effective in reducing left ventricular hypertrophy (34). These data together with our results strongly suggest that trandolaprilat has a much greater affinity for tissue ACE than enalaprilat (15,32).

Trandolaprilat in combination with verapamil did not significantly add to the inhibition of pulmonary capillary endothelial-bound ACE activity. Our finding suggest that the effects of trandolaprilat on pulmonary capillary endothelial-bound ACE - activity correspond to its hemodynamic effect and to its action on tissue ACE - activity. Accordingly trandolaprilat is more potent than enalaprilat. The small improvement in inhibiting the pressor response to angiotensin I by trandolaprilat in combination with verapamil appears to be independent of its effect on the pulmonary capillary endothelial-bound ACE-activity.

In summary, trandolapril reduces blood pressure over a 24 hour period and has an apparently high affinity for ACE in several organs that are involved in cardiovascular regulation (87). Our results indicate that trandolapril treatment may be useful in preventing the occurrence of complications and further damage of target organs in hypertensive patients.

2.QUANTIFICATION OF PULMONARY ENDOTHELUIM-BOUND AND SERUM ACE INHIBITION BY ENALAPRILAT IN PATIENTS.

INTRODUCTION

ACE inhibitors have been extensively studied and available for clinical use for fifteen years (61,96). There is no doubt that ACE inhibitors act at multiple sites of action in the cardiovascular system. The best understood mechanism of their action is the inhibition of the renin-angiotensin system, not only of the circulating components but most likely also those found in the various tissues, particularly the vascular bed. Compared to other antihypertensive drugs, ACE inhibitors possess a very favorable hemodynamic profile: they lower blood pressure by reducing total peripheral vascular resistance, without influencing cardiovascular reflexes. Therefore, they appear acceptable first-line antihypertensive agents and can be used in a variety of co-existing disease states (6,34,35,). The therapeutic value of ACE inhibitors is well known in different heart diseases (38). They can reduce left ventricular hypertrophy in hypertensive patients and have very favorable effects in congestive heart failure when compared to vasodilators (17). ACE inhibitors can also diminish the occurrence of reperfusion and post-infarct arrhythmias and improve the remodeling of the myocardium (50,51).

We reported a technique for measuring apparent Michaelis-Menten kinetics of pulmonary capillary endothelium-bound ACE for a synthetic substrate 3H-benzoyl-Phenylalanyl-Alanyl-Proline (BPAP) in anaesthetized rabbits (10,11,13, 98). In addition we have investigated the effects of different ACE inhibitors on the pulmonary capillary endothelium-bound ACE activity, serum ACE activity and selected tissue ACE activities in experimental model (22,23,). Currently, serum ACE is the only routinely used source of ACE for measurements to evaluate ACE inhibitors in humans. We have developed indicator dilution techniques to compare the activity of ACE inhibitors in their

ability to reduce pulmonary capillary endothelium-bound vs. serum ACE in humans (29,30).

Therefore the first aim of this study was to compare the ACE inhibitory effect of chronically administered enalaprilat as reflected in the inhibition of a) pulmonary capillary-bound ACE activity, b) tissue ACE activity in hypertensive patients. We also designed to investigate the inhibitory effect of intravenously administered enalaprilat on the pulmonary capillary endothelium-bound and serum ACE activities in normotensive subjects.

METHODS

Experimental Design.

Chronic study. The purpose of this study was to compare and contrast the inhibitory effects of chronic administration of enalaprilat on pulmonary capillary endothelium-bound and serum ACE. Six patients undergoing diagnostic video assisted thoracic surgery with mild essential hypertension (diastolic blood pressure 90-104 mmHg) were enrolled in this study. Previously, all patients were orally treated with enalapril maleate (tabl.Vasotec; Merck Sharp & Dohme) at a dose of 10 mg/day for three weeks. Seven other patients, without any ACE inhibitor medication or manifest lung disease undergoing coronary arterial bypass graft surgery (CABG) served as control group.

Acute study. The inhibitory effect of 1.5 μg / kg intravenously administered enalaprilat (inj.Vasotec ; Merck Sharp & Dohme) on the pulmonary capillary endothelium-bound and that on serum ACE activities in eleven normotensive patients undergoing thoracic surgery were determined. The ACE inhibitor was injected in the left subclavian vein, then the hydrolysis of ^{3}H-BPAP by pulmonary endothelium-bound and serum ACE were measured before (0 h), 15 min and 2 hours after intravenous administration of

the ACE inhibitor.

All patients enrolled in these studies signed an informed consent form approved by the institution's Human Assurance Committee. In addition, this study was conducted according to the principles expressed in the Declaration of Helsinki, which has been endorsed by The American Society for Clinical Investigation.

Determination of arterial blood gas, hematocrit and blood pressure values.

Both in the chronic and acute studies arterial pO2 and pCO2, % O2 saturation, pH and hemoglobin were assayed immediately after each transpulmonary measurement (1304 pH / Blood Gas Analyzer; Instrumention Laboratory). Blood pressure was continuously monitored via a catheter placed in the radial artery, at the same time heart rate and ECG curve were continuously recorded.

Measurement of transpulmonary hydrolysis of ^{3}H-BPAP.

A specific ACE substrate (^{3}H-BPAP ; 40 μCi or 2 nM), was injected as a bolus into a central vein via a catheter (7 fr.x 20 cm multi lumen catheter, Arrow International Inc., Reccling, PA) inserted in the left subclavian vein and immediately blood was withdrawn from a radial artery catheter (20 ga. Angiocath, Critikon MI) via a peristaltic pump (24 ml/min) into a fraction collector equipped with tubes advancing at the rate of one every 2.4 sec. for 60 sec. Each sample tube contained 2 ml of 3mM 8-hydroxyquinoline -5- sulfonic acid and 1 mM EDTA solution in normal saline to prevent any further metabolism by serum ACE.

Determination of ^{3}H-BPAP hydrolysis (v) by the pulmonary capillary endothelium-bound ACE.

See pages 19-20 for detailed description of the method. Percent metabolism of BPAP (%M) was calculated as the integral of [^{3}H]BPhe/([^{3}H]B-PAP + [^{3}H]BPhe), each in units of disintegrations per minute per milliliter of plasma, over a single transpulmonary passage.

The-single pass transpulmonary substrate hydrolysis of ^{3}H-BPAP (v) was calculated by applying the integrated Henri-Michaelis-Menten equation, under first order reaction conditions (38), as proposed by Segel, Ryan and Catravas (39,118):

$$v = \ln([S_0]/[S]) = [E] \cdot t_c \cdot k_{cat} / K_m$$

where [E], t_c, and k_{cat} being the microvascular enzyme concentration, reaction time (microvascular mean transit time), and catalytic rate constant, respectively, while K_m is the Henri-Michaelis-Menten constant. $[S_0]$ is the initial substrate concentration ([BPAP] + [BP]) and [S] is the surviving substrate concentration [BPAP] in the effluent arterial plasma estimated in dpm/ml.

Estimation of ^{3}H-BPAP hydrolysis by the serum ACE.

At the same time blood was taken to determine the hydrolysis of ^{3}H-BPAP by the serum ACE. See page 21 for details.

Statistics

Data are presented as means ± SEM. Statistical calculations were performed using paired t test and one or two way analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test unless indicated otherwise. Differences were considered significant at $p<0.05$.

RESULTS

Arterial blood gas (pO2, pCO2, % Sat O_2) and hematocrit values are summarized in table 3.They all remained stable throughout the experiments.

Table 3. Changes in the arterial blood gas and hematocrit values in patients after intravenous administration of enalaprilat.

	T_1	T_2	T_3
pO_2 (torr)	**359.3±19**	**355.1±21**	**361.4±17**
pCO_2 (torr)	**41.6±1.5**	**40.1±1.2**	**38.7±0.9**
pH	**7.39±0.006**	**7.38±0.005**	**7.39±0.002**
% Sat O_2	**96.8±0.5**	**96.4±0.15**	**94.4±0.67**
Hct (%)	**42.1±0.3**	**40.7±0.4**	**39.5±0.7**

Data are means ± SE. T_1 = before iv. administration of enalaprilat (1.5 μg/kg); T_2 = 15 min. after iv. administration of enalaprilat (1.5 μg/kg); T_3 = 2 h. after iv. administration of enalaprilat (1.5 μg/kg).

^{3}H-BPAP hydrolysis (v) by the pulmonary endothelium-bound and serum ACE in the chronic study.

In hypertensive patients, three weeks after enalaprilat treatment the hydrolysis of 3H-BPAP (v) by the pulmonary capillary endothelium-bound ACE were significantly reduced compared to the control group (0.54±0.1 vs 1.35±0.17 ; $p < 0.01$); (Figure 7 a). Similarly, significant difference was observed in the serum. (1.3±0.1 U/ml in enalaprilat treated group vs. 2.75±0.2 U/ml in control group ; Figure 7b). In figure 7c. data of pulmonary capillary endothelium-bound vs. serum ACE inhibition are expressed as percent inhibition from control group values. The percent inhibition of pulmonary capillary endothelium-bound ACE by chronic administration of enalaprilat was significantly larger than that of serum ACE (66.9±4.2 % vs 51±5.1% ; p <

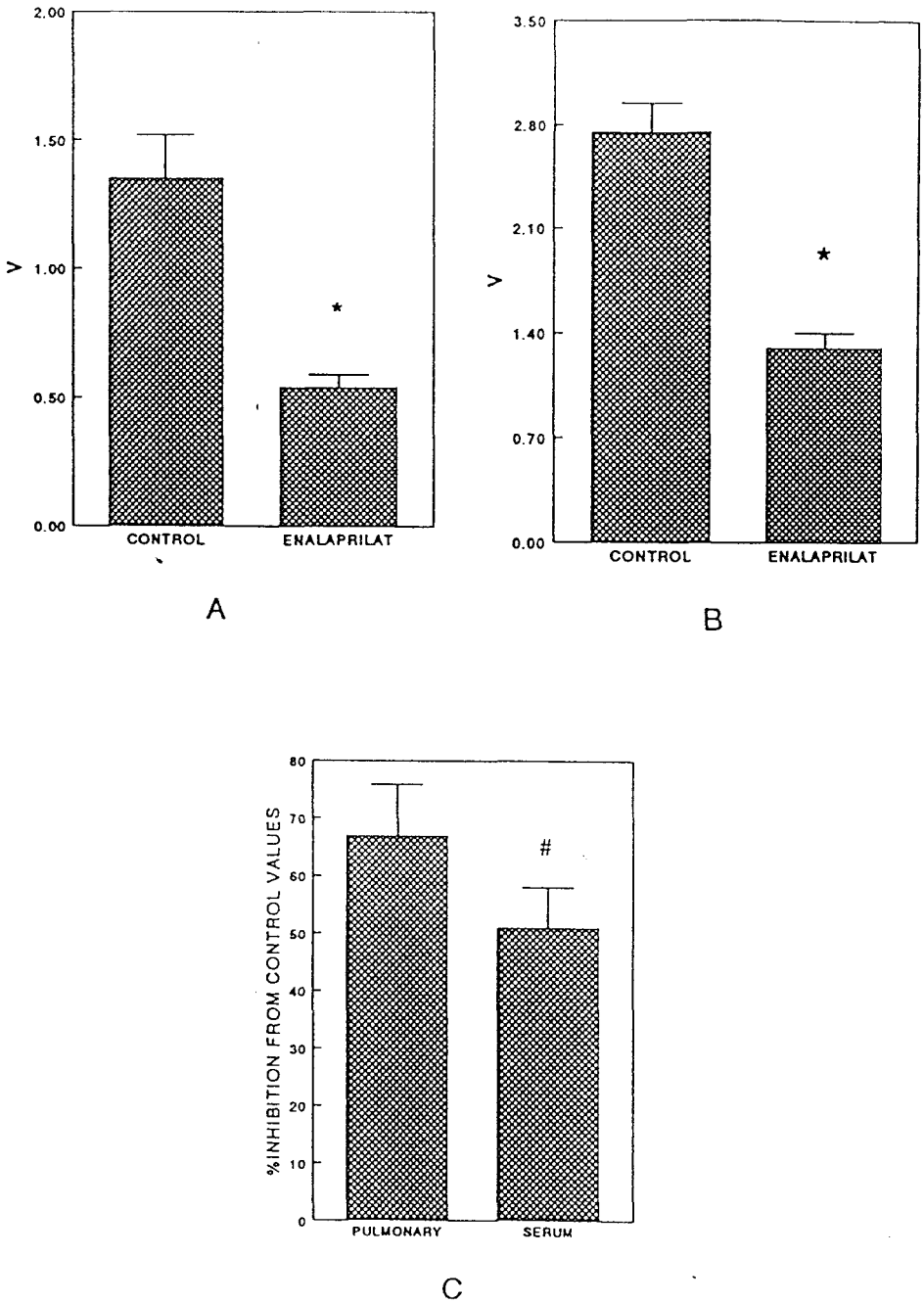

FIG. 7A and B. Decrease in ^{3}H-BPAP hydrolysis (v) by pulmonary capillary endothelium-bound and serum ACE in patients under chronic ACE inhibitor treatment. Panel C. Pulmonary vs. serum ACE inhibition in subjects chronically treated with enalaprilat. CONTROL group = untreated patients without manifest lung disease (n = 7). ENALAPRILAT group = 10 mg / day enalaprilat administered orally for three weeks (n = 6). Data are means ± SEM. * = $p < 0.01$; # = $p < 0.05$.

0.05).

^{3}H-BPAP hydrolysis (v) by the pulmonary endothelium-bound and serum ACE in the acute study.

In this study 1.5 μg/kg of enalaprilat was administered intravenously via a catheter placed in the subclavian vein over a five minute infusion (Fig. 8). Arterial blood pressure values were continuously recorded via a catheter placed in the radial artery. Systemic mean arterial pressure values were stable throughout the surgical procedure. This dose of enalaprilat did not alter significantly the systemic mean arterial pressure (91±3 vs. 86±4 vs. 88±3 mmHg for 0 h, 15 min, and 2 h, respectively);(Fig.9).

However, 1.5 μg/kg dose of enalaprilat significantly decreased the transpulmonary hydrolysis of ^{3}H-BPAP (v) in normotensive patients. When normalized to pre-drug (0h) levels, enalaprilat was found to inhibit the transpulmonary ^{3}H-BPAP hydrolysis by 76.9 ± 5.8 % vs. 60.9 ± 5.1 % at 15 min. as compared to the values obtained 2 h after administration of the ACE inhibitor. Figure 10. illustrates the inhibition of ^{3}H-BPAP hydrolysis by the serum ACE. Similarly, enalaprilat significantly decreased the serum ACE activity. However, 15 min. after administration of enalaprilat, ^{3}H-BPAP hydrolysis decreased by 68.8 ± 4.7 %, after 2 hours the inhibition in serum ACE activity was lessened to 38.1 ± 3.8 %.

DISCUSSION

The physiologic and potential pathologic roles of the local renin-angiotensin system are under intense investigation. Several functions have been proposed, including (1) regulation of regional vascular tone and blood flow; (2) development of vascular hypertrophy; (3) contribution to the vascular response to inflammation and injury; and (4) response to pharmacologic

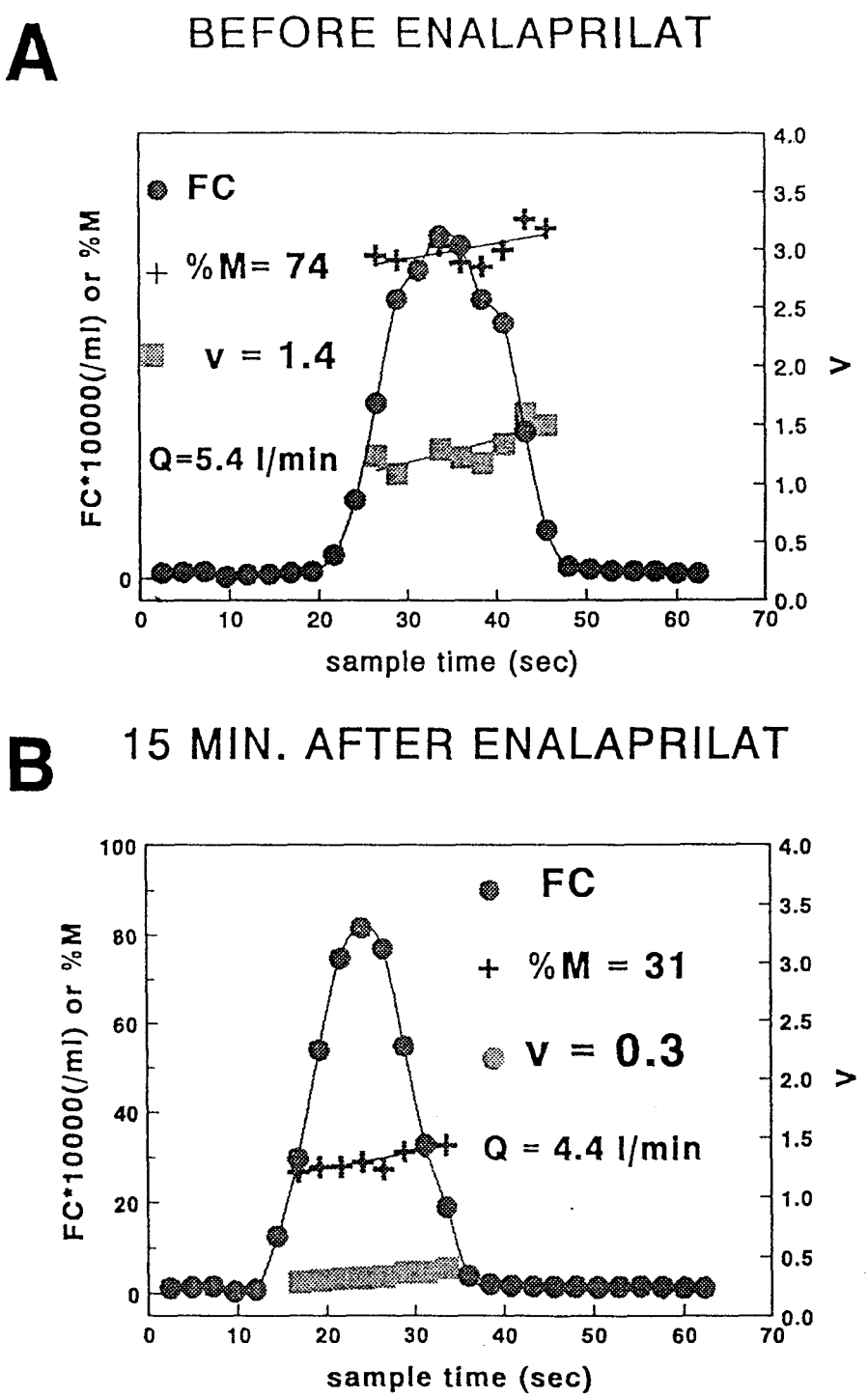

FIG. 8. Determination of ^{3}H-BPAP hydrolysis (v) by pulmonary capillary endothelium-bound ACE in a normotensive subject before (panel A) and 15 min after administration of enalaprilat (panel B). ^{3}H-BPAP was injected as a bolus into the subclavian vein and immediately blood was withdrawn from a radial artery catheter via a peristaltic pump (24 ml/min) into a fraction collector. Fractional concentration of total tritium in arterial plasma (FC), percent metabolism of ^{3}H-BPAP (% M) and substrate hydrolysis (v) were calculated at each sample and integrated over the entire arterial outflow curve.

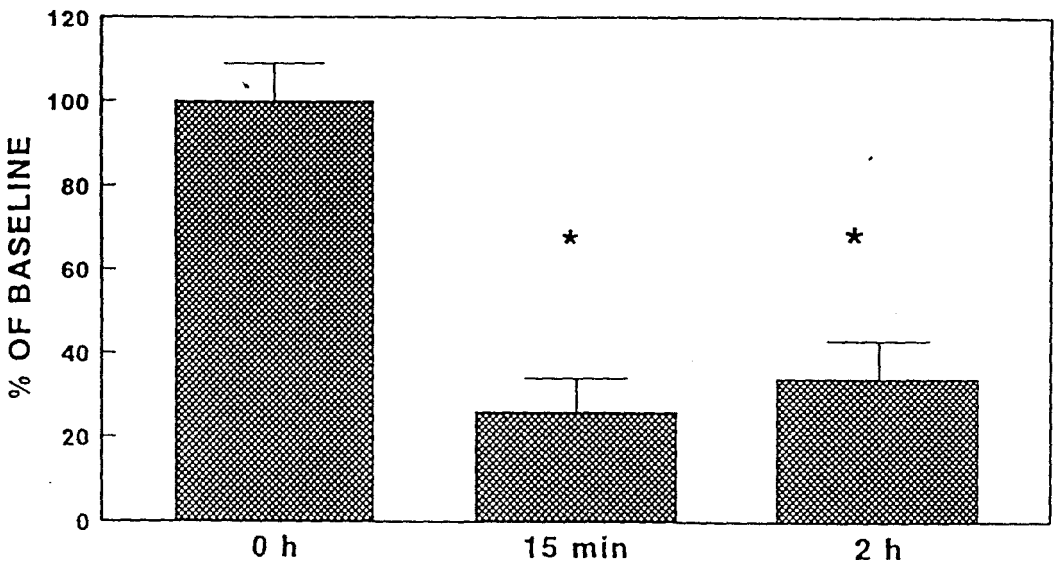

FIG. 9. Inhibition of pulmonary capillary endothelium-bound ACE by enalaprilat (1.5 µg / kg) administered intravenously. 0h = baseline, 15 min = 15 min after iv. administration of enalaprilat, 2h = 2 hours after administration of enalaprilat (n = 11). Means ± SEM. * = $p < 0.01$.

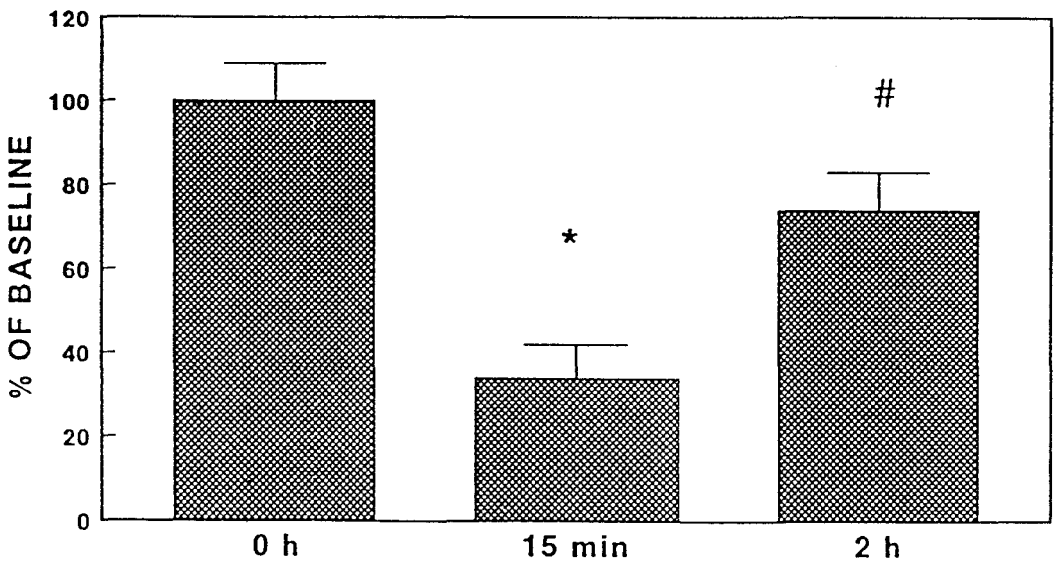

FIG. 10. Inhibition of serum ACE activity by enalaprilat (1.5 µg / kg) administered intravenously. 0h = baseline, 15 min = 15 min after iv. administration of enalaprilat, 2h = 2 hours after administration of enalaprilat (n = 11). Means ± SEM. * = $p < 0.01$; # = $p < 0.05$.

inhibitors of renin-angiotensin system (61).

Several experiments compared and contrasted the inhibitory effect of enalapril on serum ACE *in vivo* and on selected tissue ACE *ex vivo* (15,16,68,134). In normotensive rats 10, 30, 100, and 300 μg/kg of enalapril were administered orally. The maximal inhibitory effect was obtained 2 hours after administration of drug. The ID_{50} values obtained from the serum and different tissues indicates that enalapril appeared to be 3 - 5 times more potent on tissue ACE inhibition, especially in the kidney, lung and in the heart. In another clinical pharmacological study enalapril was given in single oral doses of 2.5 and 5 mg to healthy human volunteers. The peak serum concentration of enalaprilat and the maximum inhibition of serum ACE was reached after two to four hours (48,58).

Enalapril is the first clinically available prodrug, nonsulfhydryl ACE inhibitor. Several studies have confirmed the antihypertensive effect of enalapril in patients with uncomplicated mild to moderate essential hypertension (48,58,124). In addition, recent large-scale trials have demonstrated the beneficial effects of ACE inhibitors in congestive heart failure (43). In the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) I, a large, randomized and placebo- controlled trial, two-hundred fifty-three patients with congestive heart failure (NYHA functional class IV) were treated with enalapril at a dose of 5 - 20 mg. There was a 50 % reduction in deaths from progressive congestive heart failure in the enalapril-treated patients compared to the placebo group (17). In Studies of Left Ventricular Dysfunction (SOLVD) after a follow-up period of 48 months, there were 461 cardiovascular deaths in the placebo group compared with 399 in the enalapril group, with a risk reduction of 18 % (122).

In clinical practice, serum is currently the only source to assay human ACE activity in response to ACE inhibitors. The aforementioned studies have clearly indicated, however, that in humans, as well as in other mammals, the tissue-bound ACE - mostly pulmonary - is responsible for the conversion of angiotensin I to angiotensin II. Consequently, tissue ACE is a remarkably important locus of action of ACE inhibitors in the treatment of hypertension. We

developed indicator-dilution techniques to estimate the pulmonary capillary endothelium-bound ACE activity in patients. In this study we compared the inhibitory effects of acutely and chronically administered enalaprilat on pulmonary capillary endothelium-bound vs. serum ACE activity in normotensive and hypertensive patients. In chronically treated patients the hydrolysis of ^{3}H-BPAP by the pulmonary capillary endothelium-bound and serum ACE were significantly reduced by enalaprilat compared to a group of untreated patients. However, the inhibition of pulmonary capillary endothelium-bound ACE was significantly greater than that of serum ACE. In the acute study, 15 min after iv. administration of enalaprilat the ^{3}H-BPAP hydrolysis by the pulmonary capillary endothelium-bound and serum ACE was significantly decreased. However, two hours after administration of enalaprilat the inhibition of serum ACE was significantly lower than that of pulmonary capillary endothelium-bound ACE, suggesting tissue specificity for the inhibitory actions of enalaprilat.

In summary, we demonstrated the usefulness of an indicator dilution technique-based method to determine the changes in pulmonary capillary endothelium-bound ACE activities by enalaprilat in patients. This procedure can be used to a) distinguish between serum and tissue-bound effects of ACE inhibitors; b) aid in the development of tissue-specific ACE inhibitors; and c) quantify the efficacy and duration of action of different ACE inhibitors.

3. THE EFFECT OF LEFT ANTERIOR DESCENDING CORONARY ARTERY OCCLUSION ON CORONARY ENDOTHELIUM - BOUND ACE ACTIVITY IN DOGS.

INTRODUCTION

The genetic information, localization and density of the ACE are defined in different organs, as well as in the heart. Using an autoradiographic localization of ACE in the heart was demonstrated that the high density of the ligand is associated with the coronary arteries of the left ventricle. The physiologic and potential pathologic roles of the local renin-angiotensin system in cardiovascular regulation are under intense investigation and several functions have been proposed thus far. It has been suggested that the local RAS may be implicated in the following processes: a) development of cardiac hypertrophy; b) potentiation of coronary vasoconstriction; c) increased contractility; and d) a propensity toward ventricular arrhythmias during myocardial reperfusion (97). Locally produced angiotensin may influence vascular tone through paracrine or autocrine effects (61);(Table 4).

Table 4. Effect of angiotensin on vascular tone mediated by autocrine or paracrine mechanisms

SITE	AUTOCRINE	PARACRINE
Endothelium	Production of PGE_2, PGI_2, or endothelium-derived relaxing factor	Vascular smooth-muscle conctraction
Smooth muscle	Vascular smooth-muscle conctraction	Increased norepinephrine release

PGE_2, prostaglandin E_2; PGI_2, prostaglandin I_2.

(From Greenwald L. and Becker C.R. : Expanding the paradigm of the renin-

angiotensin system and angiotensin-converting enzyme inhibitors (1994). ***Am. Heart J.*** 128: 997-1009.

Vascular angiotensin II can produce vasoconstriction by directly affecting smooth-muscle cells and by amplifying the vasoconstriction induced by the symphatetic nervous system. In humans, angiotensin II has a direct vasoconstrictor effect on the coronary arteries that is independent of sympathetic innervation. Perondi et al. found that in patients with coronary artery disease, ACE inhibitors attenuated vasoconstriction after symphatetic stimulation (112). They concluded that the removal of angiotensin II's enhancing effect on symphatetic vasomotor tone was the responsible mechanism. Conducting experiments with coronary flow in the isolated rat heart, Vanhaecke et al. found that captopril may have more than one effect on the coronary vasculature, including an indirect effect shared by all ACE inhibitors and caused by the decreased breakdown and subsequent accumulation of bradykinin (128). (Bradykinin-induced vasodilation appears to be mediated by prostaglandins.) In concordance with this theory, it was published that prostaglandin I_2 synthesis increased with ACE inhibitor administration (51).

By infusing angiotensin II into rat coronary arteries significant increase was found in vascular permeability caused by contraction of endothelial cells and separation of intercellular junctions (59).

Other studies confirm the beneficial effect of intracoronarily administered enalaprilat in patients with dilated cardiomyopathy. In this study, 0.05 mg / min. of enalaprilat was administered as bilateral coronary infusion (50,51). The results demonstrate that this ACE inhibitor has significant coronary vasodilator properties, which can be elicited without stimulating the peripheral renin - angiotensin system (51).

We decided to investigate further the changes of the locally generated coronary endothelium-bound ACE under altered coronary flow conditions. Therefore, we developed a method to measure directly the coronary endothelium-bound ACE activity.

The aim of this study was to investigate whether the measurements of

coronary endothelium-bound ACE can be used to determine alterations in perfused coronary capillary surface area. In order to quantify coronary endothelium-bound ACE activity, indicator dilution technique was developed. The coronary capillary endothelium-bound ACE activity was determined from the single pass transpulmonary hydrolysis of the specific ACE substrate ^{3}H-BPAP.

The specific aims of this study were a) to determine coronary endothelium-bound ACE activity in a selected area supplied by LAD; b) to compare coronary vs. pulmonary endothelium-bound ACE activities; c) to compare coronary endothelium-bound ACE activities during altered flow conditions; d) to investigate the influence of altered LAD coronary artery blood flow on the parameter A_{max}/K_m (proportional to dynamically perfused coronary capillary surface area). e) to estimate changes in A_{max}/K_m after artificially decreased coronary capillary surface area by mechanical occlusion of one branch of LAD.

METHODS

Animal preparation.

11 mongrel dogs were enrolled in this study. All animals were anesthetized utilizing intravenously administered sodium pentobarbital with a dose of 30 mg /kg. The trachea was intubated and connected to a laboratory animal respirator. Each experimental animal was ventilated with Harward respirator (Harvard Apparatus, Mills MA) using room air with O_2 to maintain physiologic blood gas parameters. The airway pressure was continuously measured and recorded via a pressure transducer (Statham Intsruments, Hato Riley, PR) connected to the ventilator tubing. Polyethylene cannulas were inserted into the femoral vein for maintaining deep surgical anesthesia (stage 3) that was regularly evaluated by the absence of palpebral, corneal and pedal reflexes. Concurrently, the femoral artery was cannulated for continuous monitoring and recording of systemic blood pressure (Gould 2400, Gould

Instruments, Columbus, OH). After a transverse chest incision the pericardium was opened. In situ, on a beating heart fixed by pericardial cradle, the following surgical procedures were carried out: Polyethylene cannulas were inserted into the left and right atria then distal and proximal segments of the left anterior descendent artery (LAD) were dissected from the coronary artery bed. The occluder and electromagnetic flow probe were placed around the proximal segment of LAD, and the flow probe was connected to a flowmeter (Cliniflow II, CarolinasMedical Electronics, King, NC), then the distal segment of the LAD was cannulated with a small polyethylene cannula.

Experimental protocol

After the surgical procedure had been completed eight measurements were performed. Pulmonary measurements have been carried out before (P1) and after (P2) coronary measurements. Second (C2), fourth (C4), and sixth (C6) coronary measurements were performed at 50 % of LAD occlusion , 75 % of LAD occlusion, and total occlusion respectively, of an anterior ventricular branch. First (C1) third (C3) and fifth (C5) coronary measurements were carried out before each LAD occlusion and served as controls for C2, C4, and C6.

Pulmonary measurements.

For each pulmonary measurement, 2 μCi of the synthetic ACE substrate BPAP was injected into the right atrium and blood was immediately withdrawn from the catheter placed in the right atrium at a rate of 0.52 ml. / tube by means of roller pump. A fraction collector was equipped with 13 X 100- mm borosilicate tubes advancing at the rate of 1 tube per 0.7 sec.

Coronary measurements.

Before coronary measurements were performed, right atrial polyethylene cannula had been replaced into the coronary sinus. For each coronary measurement, 2 μCi of the synthetic ACE substrate BPAP was injected into the segment of the LAD controlled by the flowmeter. Immediately after injection,

blood was withdrawn from the cannula placed in the coronary sinus.

Determination of ^{3}H-BPAP hydrolysis by coronary and pulmonary capillary endothelium-bound ACE.

See pages 19-20. and 29-30. for details.

Calculation of the perfused microvascular surface area.

Angiotensin converting enzyme is distributed homogeneously over the endothelial surfaces. Therefore, the metabolism of an ACE substrate reflects the actively perfused microvascular surface area. Under first order enzyme reaction conditions A_{max} / K_m (proportional to dynamically perfused microvascular surface area) was calculated using the integrated form of Henri-Michaelis-Menten equation:

$$A_{max} / K_m = E \cdot k_{cat} / Km = Q \cdot \ln ([S_0] / [S])$$

where Q is plasma flow (calculated according to the indicator dilution curve), $[S_0]$ and [S] are the initial and surviving substrate concentrations, respectively, A_{max} is the product of enzyme mass, k_{cat} is the catalytic rate constant and K_m is the Michaelis constant. During the course of the experimental protocol, (and under first-order reaction conditions) changes in perfused microvascular surface area, as reflected by changes in enzyme mass, are thus indicated by changes in the A_{max} / K_m ratio (98,125,126).

Statistical Analysis

Data are presented as means ± SEM. Statistical calculations were performed using one way analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test unless indicated otherwise. Differences were considered significant at $p<0.05$.

RESULTS

Typical findings from one experiment are shown in fig. 11. Fractional concentration of tritium in the effluent blood, percent metabolism of ^{3}H-BPAP (% M) and transpulmonary ^{3}H-BPAP hydrolysis (v) in the pulmonary vascular bed (panel a) and in the coronary vascular bed (panel b) are plotted. In the pulmonary vascular bed we did not find significant changes in the pulmonary blood flow (1889±120 ml/min vs. 1671±141 ml/min), enzyme activity (1.51±0.07 vs. 1.54±0.05) and A_{max} / K_m (1542±140 vs.1460±120) between the first and the last measurement, indicating the stability of the preparation. Fig.12. shows the results of the first transcoronary measurement under moderately reduced LAD flow by having tightened the ligature placed around the LAD. As shown, the flow was reduced significantly from 17.9 ± 1 ml/min to 7 ± 0.9 ml/min and the A_{max} / K_m decreased from 6.3 ± 0.9 to 2.75 ± 0.4 ($p < 0.01$). However, the enzyme activity remained unchanged. Similar results were obtained after more severe reduction in LAD flow, approximately by 75 % (fig. 13). The LAD flow was reduced from 17.9 ± 2 ml/min to 3.1 ± 0.8 ml/min and A_{max} / K_m decreased from 6 ± 0.8 to 0.98 ± 0.02 ; $p < 0.01$. We did not find significant changes in the enzyme activity.

In a different approach to reduce LAD flow, one side branch of LAD was machanically occluded. Fig. 14. shows the results after this maneuvers. We achieved a significant flow reduction in LAD (from 22 ± 2 ml/min to 7.2 ± 1 ml/min ; $p < 0.01$) and a significant decrease in A_{max} / K_m (from 6.6 ± 0.8 to 2.1 ± 0.4; $p < 0.01$). The enzyme activity again remained unchanged.

DISCUSSION

To investigate the potential role of the local renin-angiotensin system on

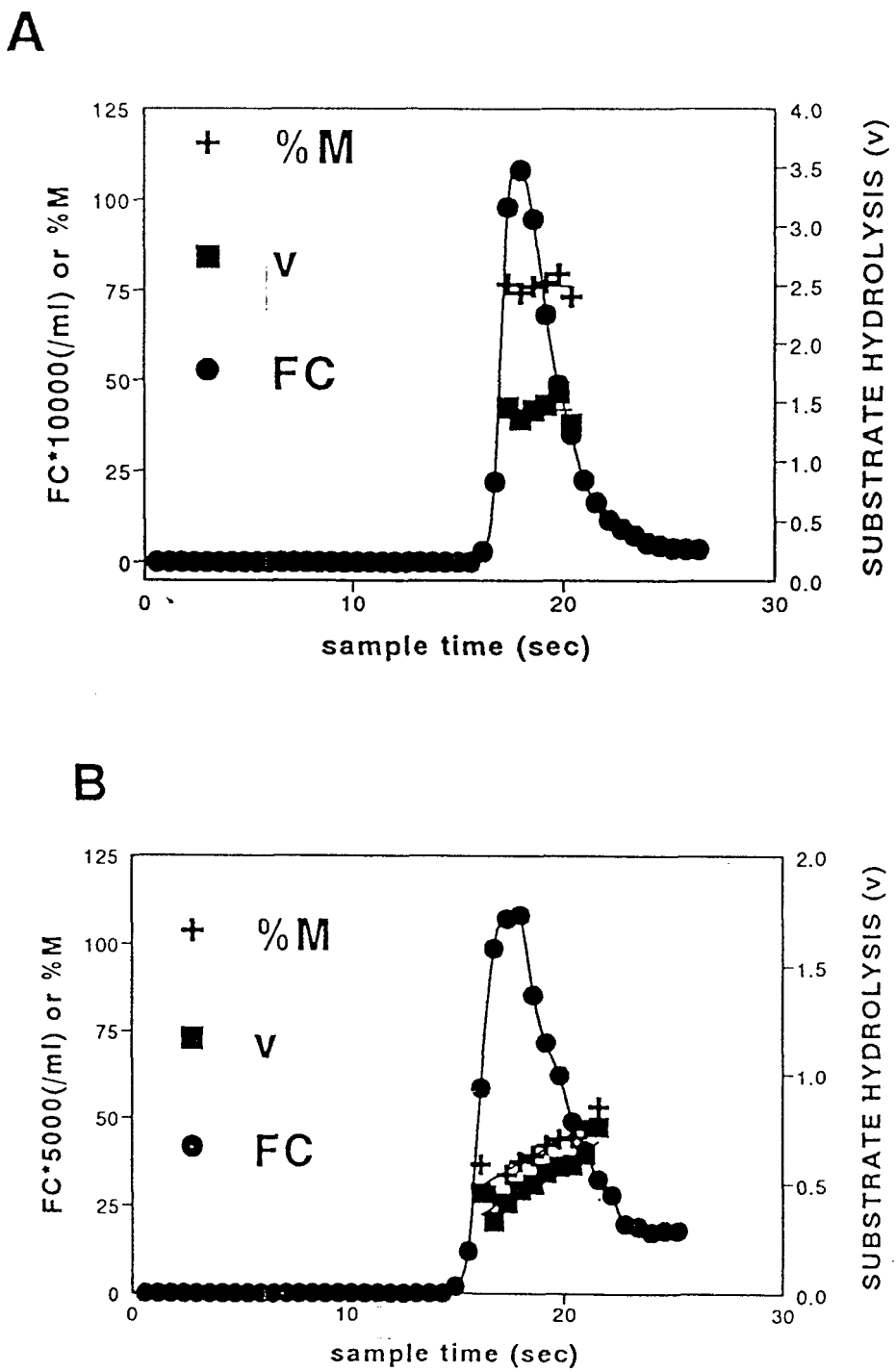

FIG. 11. Indicator dilution curve of ^{3}H-benzoyl-Phe-Ala-Pro (BPAP) in the pulmonary (panel A) and in the coronary (panel B) vascular beds. Fractional concentration of total tritium in arterial plasma (FC), percent metabolism of ^{3}H-BPAP (% M) and substrate hydrolysis (v) were calculated at each sample and integrated over the entire arterial outflow curve.

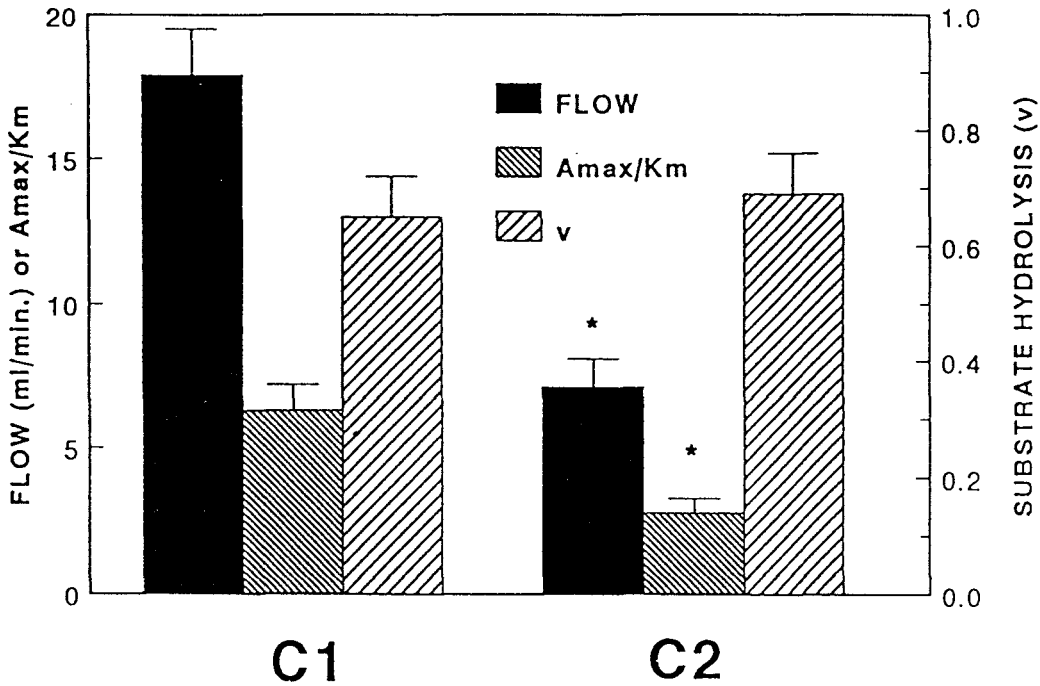

FIG. 12. Changes in coronary blood flow, substrate hydrolysis (v) and A_{max} / K_m (proportional to perfused coronary capillary surface area) after moderately reduced LAD flow of approximately by 50 %. Means ± SEM. * = $p < 0.01$

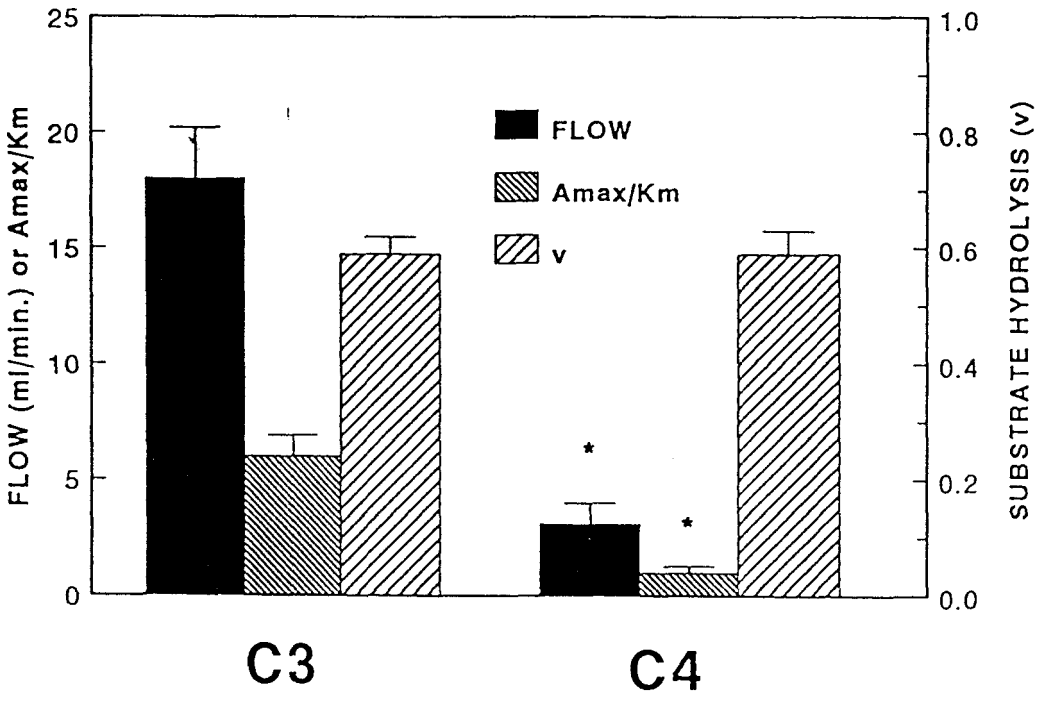

FIG. 13. Changes in coronary blood flow, substrate hydrolysis (v) and A_{max} / K_m (proportional to perfused coronary capillary surface area) after more severe reduction in LAD flow of approximately by 75 %. Means ± SEM. * = $p < 0.01$

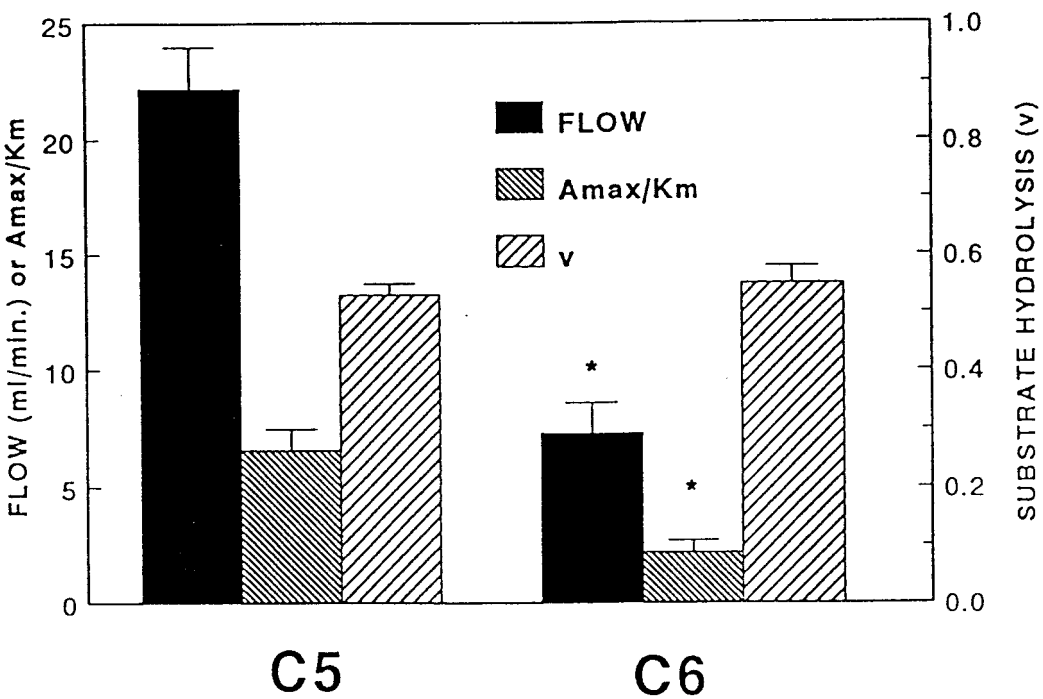

FIG. 14. Changes in coronary blood flow, substrate hydrolysis (v) and A_{max} / K_m (proportional to perfused coronary capillary surface area) after mechanical occlusion of one side branch of LAD. Means ± SEM. * = $p < 0.01$

the coronary microvessels is difficult since coronary blood flow depends greatly on the loading conditions of the left ventricle and the myocardial oxygen needs. In this study, we present a useful, indicator dilution technique to measure coronary endothelium-bound ACE activity in dogs (63). At the same time, similar measurements were performed in the pulmonary vascular bed which served as control. The pulmonary blood flow, pulmonary endothelium-bound enzyme activity, and the dynamically perfused pulmonary capillary surface area remained unaltered.

In this study, transcoronary hydrolysis of the synthetic ACE substrate ^{3}H-BPAP remained unchanged over the studied range of LAD blood flow. Reduction in LAD blood flow produced proportional decreases in dynamically perfused coronary capillary surface area. We conclude, that the measurement of the coronary endothelium-bound ACE activity could be used to determine short-term alterations in dynamically perfused coronary capillary surface area in the heart.

4. DETERMINATION OF CHANGES IN CORONARY AND PULMONARY ENDOTHELIUM - BOUND ACE ACTIVITIES IN PATIENTS UNDERGOING CORONARY ARTERIAL BYPASS GRAFTING.

INTRODUCTION

Angiotensin converting enzyme is distributed homogeneously over the endothelial surface of the coronary vessels. Angiotensin II can be generated locally from activation of angiotensin I by the vascular endothelium, and there is even a possibility that renin might be produced by heart muscle itself. In humans, angiotensin II exerts a direct coronary vasoconstrictor effect independent of sympathetic innervation. However, it is capable of modulating and amplifying sympathetic coronary vascular control is unknown (39-42)

Most studies have described the results of ACE inhibition in different types of patients with or without cardiac decompensation who are sometimes on various concomitant medications (50,104). Not surprisingly, the results have been varied, although many of them commented on the clear relationship between the change in coronary flow and the reduction in hemodynamic load consequent to ACE inhibition. The coronary vasodilation induced by intracoronary ACE inhibitor have been studied by Foult and coworkers. Their results with enalaprilat demonstrated that this particular ACE inhibitor has significant coronary vasodilator properties,without stimulating the peripheral renin - angiotensin system (50).

Patients with ischemic hart disease scheduled for coronary artery bypass graft (CABG) surgery have significant occlusion in the coronary vascular bed which is corrected after surgery. Therefore, there is a need to delineate more precisely the influence of altered coronary blood flow on coronary endothelium - bound ACE activity. Because of its pathophysiological and clinical importance, we investigated this in patients undergoing coronary artery bypass grafting.

Thus, the aims of this study were: a) to determine percent metabolism of ^{3}H-BPAP and coronary endothelium-bound ACE activity from the single pass transcoronary hydrolysis of the specific ACE substrate in twelve anesthetized patients undergoing coronary arterial bypass graft surgery before and after graft connection; b) to compare the changes in the transcoronary and transpulmonary hydrolysis of the specific ACE substrate ^{3}H-BPAP before and after graft connection; c) to investigate the influence of altered coronary blood flow on the parameter A_{max}/K_m in the coronary vascular bed.

METHODS

Patients

12 anesthetized patients (age: 47-72 yrs) undergoing CABG surgery have been enrolled in this study.Table 5. shows the clinical characteristics of patients scheduled for CABG surgery. Measurements were performed before and after graft connection in the coronary vascular bed, and at the same time, similar measurements were performed in the pulmonary vascular bed.

Measurement of transcoronary hydrolysis of ^{3}H-BPAP.

The specific ACE substrate (^{3}H-BPAP ; 4 μCi or 0.2 nmol) was injected as a bolus into the root of aorta via an aortic root catheter (14 ga. cardioplegia cannula ; DLP. Grand Radios, MI), which was inserted two centimeters above the right coronary orifice. Blood was withdrawn immediately from the retrograde coronary sinus cardioplegia cannula (12 ga. D.L.P. Grand Rapids, MI) placed in the coronary sinus through the right atrial wall. The coronary sinus cannula was connected to a fraction collector, equipped with tubes advancing at the rate of every 2.4 sec. for 60 sec. Blood collection in 1 ml aliquots from coronary sinus started (total about 30 ml) and BPAP injected into aortic root as ascending aorta above the injection site was occluded for 5 systoles for the maximum delivery of BPAP to the coronaries.

Table 5. Characterization of patients undergoing CABG surgery.

N^0 of patient	Gender	Age (years)	N^0 of saphenus vein grafts	Coexisting disease
1	M	59	4	Hypertension
2	M	53	2	Diabetes mellitus
3	F	60	3	-
4	F	51	3	Heart failure
5	F	72	3	Hypertension
6	M	62	5	-
7	M	60	2	Increased serum cholesterin level
8	F	61	3	Diabetes mellitus Atrial fibrillation
9	M	47	2	Hypertension Ulcus ventriculi
10	M	57	5	Increased serum cholesterin level
11	F	54	2	-
12	M	58	2	Increased serum cholesterin level

Measurement of transpulmonary hydrolysis of ^{3}H-BPAP.

The specific ACE substrate (^{3}H-BPAP ; 40 μCi or 2 nM) was injected as a bolus into a central venous catheter (7 fr.x 20 cm Multi lumen catheter, Arrow International Inc., Reccling, PA) inserted in the left subclavian vein. Blood was immediately withdrawn from a radial artery catheter (20 ga. Angiocath, Critikon MI) using a peristaltic pump (24 ml/min) into a fraction collector equipped with tubes advancing at the rate of one every 2.4 sec. for 60 sec. Each sample tube contained 2 ml of 3mM 8-hydroxyquinoline -5- sulfonic acid and 1 mM EDTA solution in normal saline to prevent any further metabolism by serum ACE.

Determination of ^{3}H-BPAP hydrolysis by coronary and pulmonary capillary endothelium-bound ACE.

Sere pages 29-30. for details .

Calculation of the perfused microvascular surface area.

See page 39. for details.

Statistical Analysis

Data are presented as means ± SEM. Statistical calculations were performed using the two way analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test unless indicated otherwise. Differences were considered significant at $p<0.05$.

RESULTS

Arterial blood gas, hemoglobin and hemodynamic parameters were determined immediately after each coronary and pulmonary measurement and are summarized in table 6.

Fig. 15. demonstrates a typical indicator dilution curve of ^{3}H-BPAP obtained from the pulmonary vascular bed (upper panels; P1 and P2) in a patient

Table 6. Arterial blood gas, hemoglobin, blood pressure and hemodynamic parameters in patients undergoing CABG surgery.

	P_1	C_1	P_2	C_2
O_2 (TORR)	428 ± 18	412 ± 20	394 ± 17	388 ± 25 *
CO_2 (TORR)	37 ± 4	38 ± 5	39 ± 5	39 ± 6
pH	7.40 ± 0.009	7.41 ± 0.005	7.41 ± 0.007	7.4 ± 0.008
O_2 SAT (%)	96.9 ± 0.7	95.6 ± 0.9	94.6 ± 1.7	94.1 ± 0.8
Hgb (g/dl)	12.1 ± 0.5	11.2 ± 0.4	9.3 ± 0.4 **	9.2 ± 0.6 **
MAP (mmHg)	82 ± 4	81 ± 3	76 ± 4 *	71 ± 5 **
Heart rate 1/min	78 ± 3	80 ± 4	84 ± 5 *	86 ± 5 **
CVP (cm water)	6.9 ± 0.8	7.4 ± 0.5	7.8 ± 0.7 **	7.6 ± 0.8 **

MAP, systemic mean arterial pressure; CVP, central venous pressure;
P_1 and C_1, pulmonary and coronary measurements before graft connection;
P2 and C2, pulmonary and coronary measure- ments after graft connection.
Data are means ± SEM.
* = $p < 0.05$ from the corresponding P_1 value; ** = $p < 0.01$ from the corresponding P_1 value.

who underwent coronary arterial bypass graft surgery. As shown in this figure we did not find significant differences in the percent ^{3}H-BPAP metabolism (%M) , in the substrate hydrolysis (v) and in the pulmonary blood flow (Qp) before (P1) and after (P2) graft connection.

With this patient, the following procedure was performed: Aorto-coronary bypass grafting with reverse saphenous vein grafts to the left anterior descending artery, first diagonal artery and sequential grafting of reverse saphenous vein grafts to the posterior descending artery from the left anterior descending artery graft. In fig. 15. the bottom panels (C1 and C2) illustrate a typical indicator dilution curve of ^{3}H-BPAP obtained from the coronary vascular bed before (C1) and after (C2) connection of the saphenous vein grafts. As shown in this figures after connection of the saphenous vein grafts we found significant increases in percent ^{3}H-BPAP metabolism (%M), substrate hydrolysis (v) and in the coronary blood flow (Qc), respectively.

Fig.16. illustrates the changes in blood flow in the coronary vs. pulmonary vascular beds before and after graft connection. After surgery coronary blood flow was increased significantly, by 40.6 % (354 ± 32 ml/min to 498 ± 42 ml/min ; $p < 0.01$), whereas pulmonary blood flow remained unchanged (4.9 ± 0.2 L/min to 5.2 ±0.3 L/min). Fig. 17. summarizes the changes in BPAP metabolism in the coronary vs. pulmonary vascular bed. Overall, the transpulmonary BPAP metabolism remained unaltered before and after graft connection (72.4 ± 3 % vs. 76.5 ± 4 %), whereas the transcoronary BPAP metabolism increased significantly (49.9 ± 3 % to 77.2 ± 2 % ; $p < 0.01$). Similar changes in the transpulmonary substrate hydrolysis (v) were observed in the pulmonary vs. coronary vascular bed (Fig. 18). There were no significant changes in the pulmonary ACE activity before vs. after graft connection (1.39 ± 0.2 vs 1.40 ± 0.3). However, the transcoronary ACE activity increased significantly (0.67 ± 0.2 to 1.43 ± 0.1). Fig. 19. illustrates the changes in A_{max} / K_m. In the pulmonary vascular bed pregraft vs. postgraft A_{max} / K_m values did not change significantly (3912 ± 120 vs. 4253 ±150). However, in the coronary vascular bed a significant increase was found (151 ± 20 vs. 442 ± 60 for pregraft vs. postgraft values).

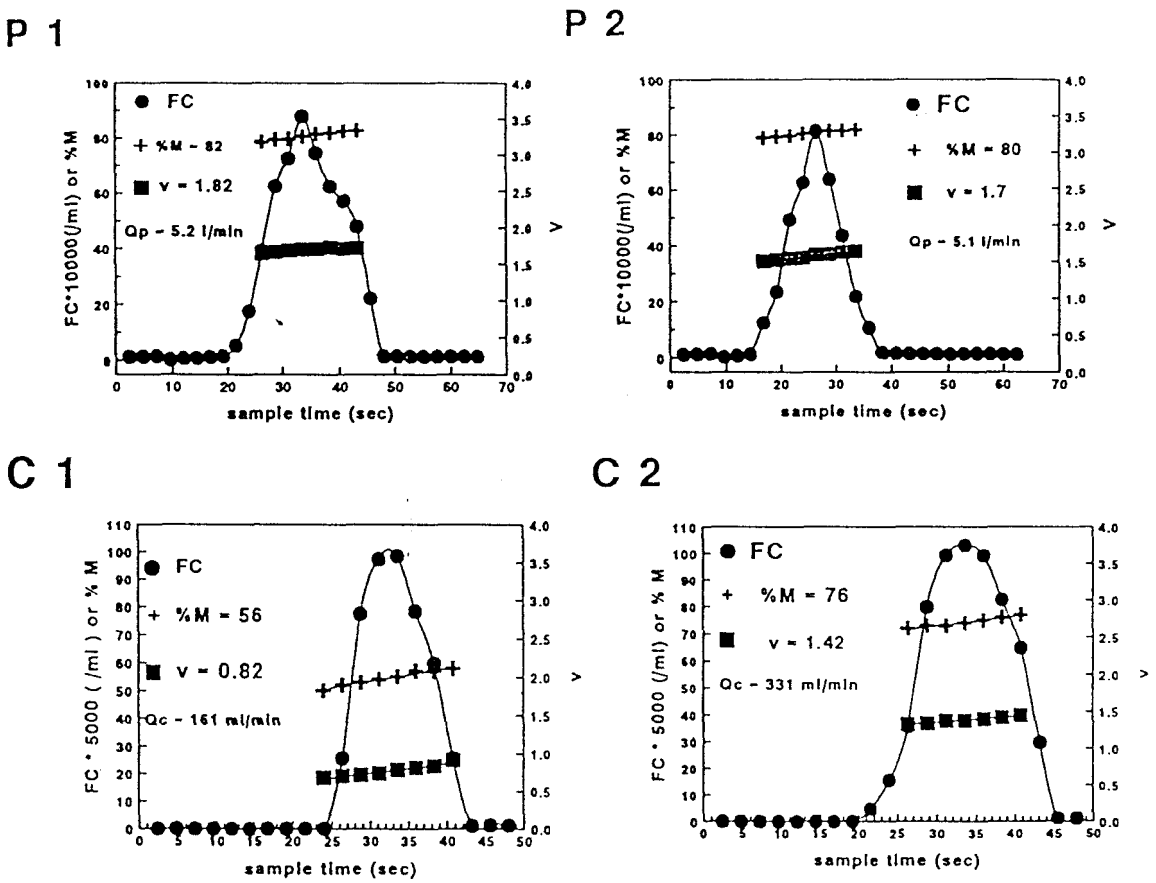

FIG. 15. Transpulmonary (upper panels ; P1 and P2) and transcoronary (bottom panels ; C1 and C2) hydrolysis of tritiated BPAP. Indicator dilution curves of ^{3}H-benzoyl-Phe-Ala-Pro (BPAP) were obtained before (P1 ; C1) and after (P2 ; C2) connection of grafts. The percent matabolism of BPAP (%M), substrate utilization (v), were calculated for each sample and integrated over the entire arterial outflow concentration curve. Qp = pulmonary blood flow. Qc = coronary blood flow. FC = Fractional concentration of total tritium in arterial plasma.

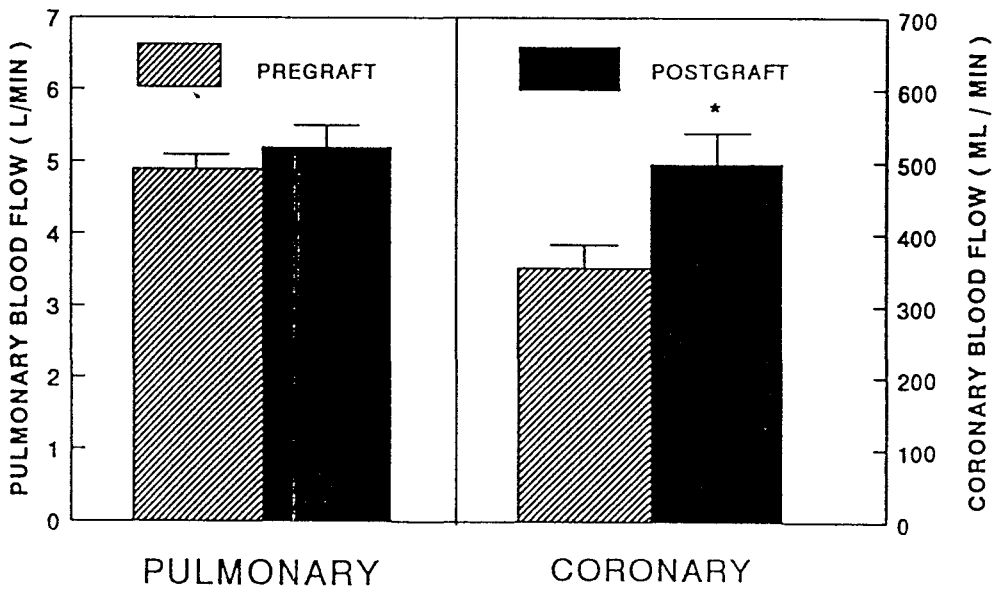

FIG. 16. Changes in pulmonary vs. coronary blood flow before (PREGRAFT) and after (POSTGRAFT) connection of the saphenous vein grafts in patients undergoing CABG. Means ± SEM. * = $p < 0.01$

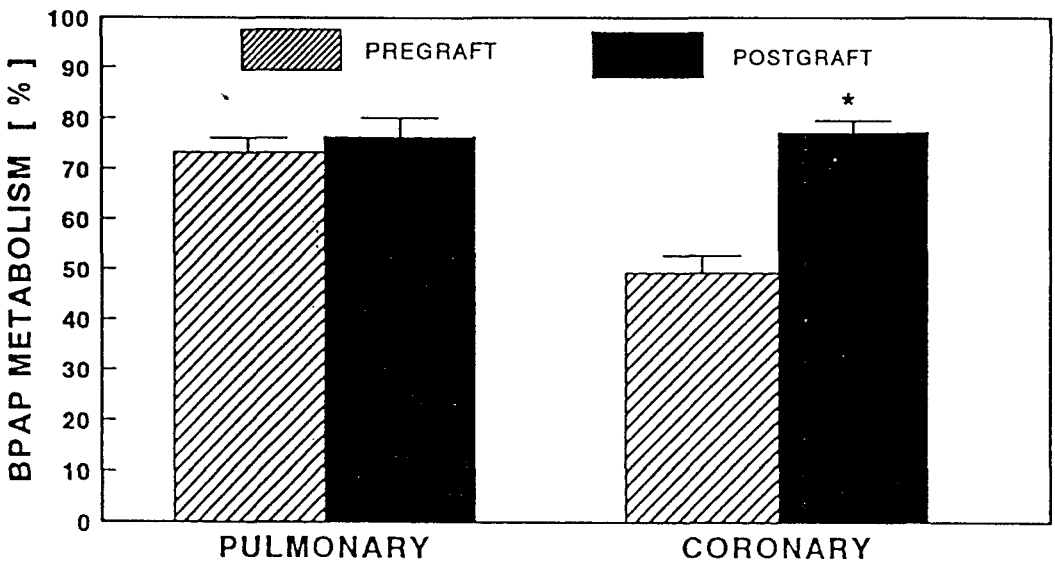

FIG. 17. Changes in percent ^{3}H-BPAP metabolism by capillary endothelium-bound ACE in the pulmonary vs. coronary vascular beds before (PREGRAFT) and after (POSTGRAFT) connection of the saphenous vein grafts in patients undergoing CABG. Means ± SEM. * = $p < 0.01$

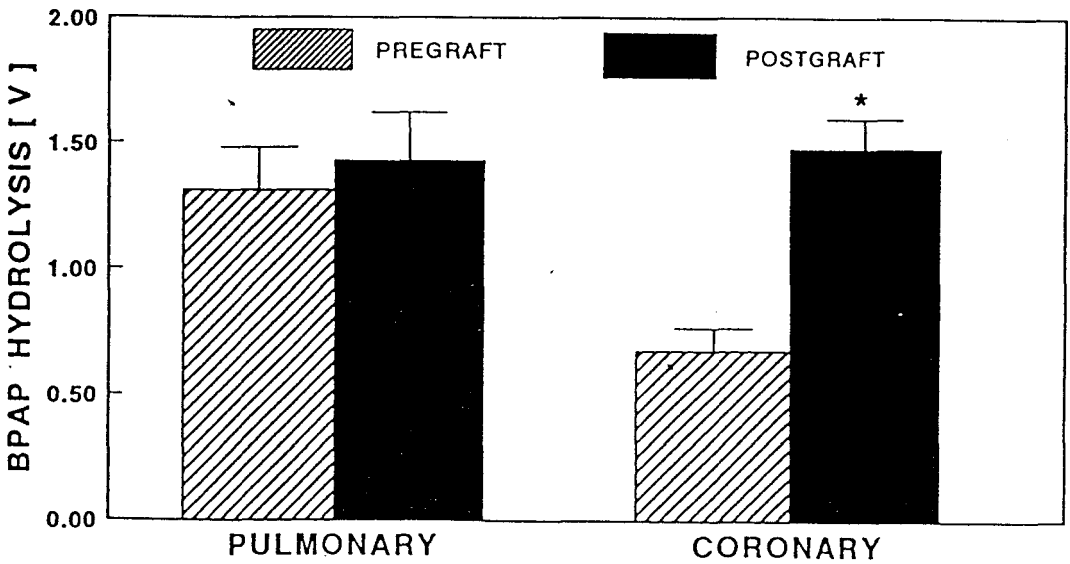

FIG. 18. Changes in ^{3}H-BPAP hydrolysis (v) by pulmonary and coronary endothelium-bound ACE before (PREGRAFT) and after (POSTGRAFT) connection of the saphenous vein grafts in patients undergoing CABG. Means ± SEM. * = $p < 0.01$

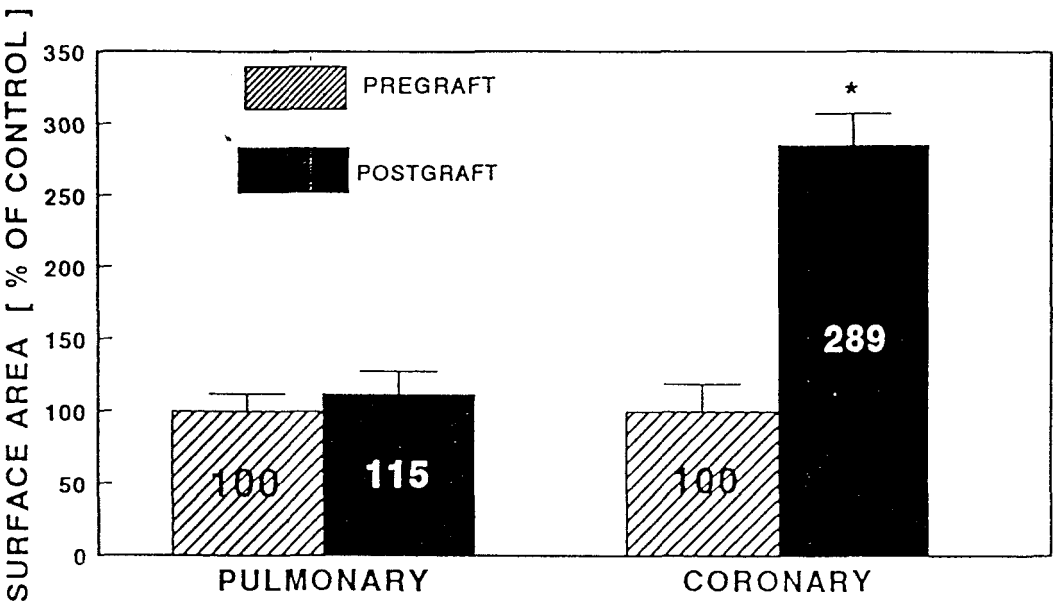

FIG. 19. Changes in A_{max} / K_m in the pulmonary vs. coronary vascular beds before (PREGRAFT) and after (POSTGRAFT) connection of the saphenous vein grafts in patients undergoing CABG. Means ± SEM. * = $p < 0.01$

DISCUSSION

Ertl and co-workers have investigated the effect of ischaemia-reperfusion on coronary microvessels and the extend of myocardial infarction in mongrel dogs. They found that coronary conduit vessels are relatively tolerant to myocardial ischaemia with or without reperfusion (4,45).

The control of the coronary arterial tone and coronary flow may be influenced by circulating and locally released vasoactive compounds. The response to many of these vasoactive compounds is modulated by the endothelium. Ischaemia and reperfusion occur in several clinical situations. These include variant angina, unstable angina, myocardial infarction with either spontaneous or therapeutic recanalization, and after coronary arterial bypass graft surgery. The effect of ischaemia and reperfusion on the coronary microcirculation is less known. Although microvessels play a central role in the regulation of myocardial perfusion, their function after ischaemia and reperfusion may be particularly important (57).

Thus ACE, a typical endothelial ectoenzyme, is distributed on the endothelial surface of the coronary vessels. The site of the enzyme reaction is the surface of the coronary microvasculature rather than that on the large conduit vessels. After we had obtained enough encouraging data from the animal experiments in this study we determined the coronary endothelium-bound ACE activities in patients with ischemic heart disease undergoing coronary arterial bypass grafting (28). Coronary endothelium-bound ACE activity was found to increase significantly while pulmonary endothelium-bound ACE activities remained unaltered. In the coronary vascular bed we also found a significant increase in A_{max} / K_{m}, (which is proportional to the dynamically perfused microvascular surface area), after graft connection. However, during reperfusion, the microvasculature is exposed to higher-than-normal perfusion pressure, thus altered endothelial function may also be due to sudden changes in perfusion pressure.

We conclude, that the indicator dilution technique utilized in this study is routinely usable and can provide a quantitative measurement of the coronary endothelium bound ACE activity with altered coronary blood flow in patients with ischemic heart disease undergoing therapeutic recanalization.

5.SUMMARY OF THE RESULTS DESCRIBED IN CHAPTER I.

1. We utilized the rabbit heart bypass model to compare the inhibitory effect of two different ACE inhibitors (trandolaprilat and enalaprilat) in acute and chronic study as reflected a) changes in pressure responses to i.v. angiotensin I and bradykinin, b) changes in the inhibition of pulmonary capillary endothelium-bound ACE activity, *in vivo,* c) changes in the serum ACE activities and d) changes in tissue ACE activities. Our data demonstrated that trandolaprilat has greater affinity for pulmonary capillary endothelium-bound and tissue ACE than enalaprilat.

2. We developed and utilized indicator dilution techniques using ^{3}H-BPAP, a specific synthetic ACE substrate to determine pulmonary capillary endothelium-bound ACE activity in normotensive and in hypertensive patients.

3. We compared the effects of chronically administered enalapril on the pulmonary capillary endothelium-bound and serum ACE activities in patients with essential hypertension.

4. We also compared the inhibitory effects of acute, intravenous administration of 1.5 μg / kg enalaprilat on the pulmonary capillary endothelium-bound and serum ACE activities in normotensive patients.
We demonstrated significant differences in the pulmonary capillary endothelium-bound vs. serum ACE inhibition.

5. We developed a method to estimate coronary endothelium-bound ACE activity in a selected area supplied by LAD in anaesthetized mongrel dogs. We demonstrated, that the measurement of the coronary endothelium-bound ACE activity could be used to determine short-term alterations in the dynamically perfused coronary capillary surface area.

6. We developed indicator dilution technique to study the metabolism of ^{3}H-BPAP and the coronary endothelium-bound ACE activity in patients with ischemic heart disease, before and after coronary arterial bypass graft surgery.

7. We demonstrated a significant increase in coronary capillary endothelium-bound ACE activities after connection of the saphenous grafts.

8. We also demonstrated a significant increase in A_{max} / K_m, (which is proportional to the dynamically perfused microvascular surface area), after graft connection.

CHAPTER II.

APPLICATION OF NONIVASIVE METHODS IN HUMAN CLINICAL PHARMACOLOGICAL STUDIES (FROM PHASE I TO IV)

Safety, accuracy and reproducibility are the most important requirements for methods of measurement in clinical pharmacological studies (73). To achieve these requirements, we connected an impedance cardiograph (ICG-M401 ASK Ltd., Budapest, Hungary) with an automatic blood pressure monitoring device (MEDITECH ABPM, Meditech Ltd., Budapest, Hungary). and developed a simple noninvasive method of measurement that we have named programmable impedance cardiography (PIC).

The principle of impedance cardiographic (ICG) measurements is well known (3,60,77,79,106,107). The impedance cardiograph measures the change in the impedance of the tissues against a high-frequency and low-intensity, i.e. biologically inert current(60). On the basic impedance curve (Z_0) an amplitude modulation appears, parallel with the pumping function of the heart, and proportional to the amount of blood pulsed out. For calculation of the stroke volume (SV) the original formula of Kubicek has been rearranged to :

$$SV = k * L^2 * LVET * dZ/dt_{max} / Z_0^2$$

where SV is the stroke volume (cm^3), k is a constant (Ωcm), L is distance between the electrodes, LVET is left ventricle ejection time (sec), dZ/dt_{max} is the maximum of the first derivative of the impedance cardiogram (Ω/sec) and Z_0 is the basic impedance (5,39,77,78).

In serial beat-to-beat determination of the systolic intervals, stroke volume, cardiac output and systemic vascular resistance provide

reproducible measurements of these important hemodynamic parameters(39). The reproducibility, and the accuracy make ICG measurements a valuable tool in clinical pharmacological practice to evaluate the effect of antihypertensive drug treatment (102).

It has been proved that clinical sphygmomanometric readings provide only limited information on treatment-induced changes in the 24-hour blood pressure profile. Moreover, clinical blood pressure measurements are often affected by the doctor's presence ("white coat effect"), and this reaction causes a rise in blood pressure which may be both large and unpredictable.

The description of methods of non-invasive ambulatory blood pressure monitoring (ABPM) has spurred interest in blood pressure variability during the past 20 years. These methods have permitted observation of blood pressure for 24-hour periods and measurement of day and night variations.

ABPM offers a number of advanteges over clinical readings. For example, automated blood pressure measurements delivered by non-invasive monitors do not elicit an alerting reaction and a rise in blood pressure. Furthermore, ABPM allows the effectiveness of a given antihypertensive drug to be tested not just in the artificial environment of the physician's office, but under exposure to a variety of physical and psychological stimuli in daily life (83-87). A further advantage of ABPM in evaluating antihypertensive treatment is the absence of placebo effect (or, in some cases, only a minor effect) to modify the 24-hour average blood pressure. Finally, by using ABPM, precise and detailed information can be obtained on the time-course of the blood pressure fall induced by antihypertensive drugs (100).

To take further advantages of ICG and ABPM, we developed and applied to clinical pharmacological studies a novel noninvasive method of programmable impedance cardiographic measurement (PIC). ICG and ABPM were connected by appropriate software that allowed measurement of the changes in blood pressure and hemodynamics concurrently in hypertensive patients. With PIC we were able to obtain more precise information about the efficacy of the investigated antihypertensive compound.

INVESTIGATION OF THE ANTIHYPERTENSIVE EFFECT OF A NEW POSTSYNAPTIC VASCULAR ALPHA - ADRENORECEPTOR ANTAGONIST USING THE PROGRAMMABLE IMPEDANCE CARDIOGRAPHY.

INTRODUCTION

A newly developed alpha-adrenoreceptor antagonist called GYKI-12743 exerted marked antihypertensive effect in several experimental hypertension models without causing tachycardia. In vitro receptor binding studies revealed the $alpha_1$- and $alpha_2$ - adrenergic receptor affinity of the compound. In isolated organs, GYKI-12743 was a competitive antagonist of both subclasses of postsynaptic alpha - adrenoreceptors. In isolated canine saphenous vein preparation its competitive antagonist potency was about 10 times greater than that of idazoxan at the postsynaptic $alpha_2$ - adrenoreceptors (109).

Generally, the aim of human phase I/A clinical pharmacological studies is to establish a minimum effective dose to achieve activity without significant adverse reactions. Pharmacokinetic measurements of absorption, half-life, and metabolism are often done in phase I studies. In the course of this human phase I/A clinical pharmacological study, our first aim was to investigate the blood pressure lowering and hemodynamic effects of GYKI-12743 using programmable impedance cardiographic measurements (PIC). In addition, we compared the pharmacodynamic effect of GYKI-12743 to the serum concentration of the compound obtained from pharmacokinetic analysis.

METHOD

Subjects

Eight male healthy volunteers (age: 20-25 years) were involved in this randomized, placebo controlled, double blind study. All patients enrolled in this study signed an informed consent form approved by the institution's Human Assurance Committee. The conduct of this study complies with the principles expressed in the Helsinki Declaration, which has been endorsed by The Hungarian Society for Clinical Investigation.

Study Protocol

A 10 mg dose of the compound under investigation (GYKI-12743) was administered orally and PIC measurements were taken at baseline then at every ten minutes for two hours after administration of GYKI-12743. Blood pressure readings were taken by automatic blood pressure monitor, using the oscillometric principle (MEDITECH ABPM, Meditech LTD Budapest, Hungary) and hemodynamic parameters were estimated noninvasively by impedance cardiography (ICG-M401 ASK Ltd. Budapest Hungary). Cardiac output (CO), rate pressure product (RPP) and total peripheral resistance (TPR) were calculated according to following equations:

CO (l/min) = SV x HR

where SV is the stroke volume (ml/min) and HR is the heart rate (beats/min).

RPP = Systolic blood pressure x HR

TPR = (MAP x 80) / cardiac output, where MAP is the systemic mean arterial pressure. (Normal range : 800 - 1200 dyn x sec x cm^{-5}).

Blood was withdrawn at baseline, 15, 30, 45, 60, 90, min, and 2, 3, 4, 6, 12 and 24 hours after administration of GYKI-12743 to estimate the concentration of the compound in the serum. Analysis of the samples was done by the HPLC method (LKB, Model 2105, Bromma, Sweden) using MEDUSA software package (version 1.3).

RESULTS

Table 7. shows the changes in blood pressure, hemodynamic parameters and the serum concentrations of the GYKI-12743 after oral administration of a 10 mg dose of the compound in healthy volunteer number 6. As shown in table 7 the concentration of GYKI-12743 in the serum was detectable only 20 minutes after drug intake and peak concentration (110.19 ng/ml) was measured at 50 minutes. The serum concentrations of the alpha-adrenoreceptor antagonist GYKI-12743 correlated with the blood pressure lowering and hemodynamic effects of this compound. As listed in table 7. peak serum concentration of GYKI-12743 coincided with the nadir of systolic and diastolic blood pressure, TPR and RPP which recorded at 50 min. after administration of the drug. However, the CO and HR did not change significantly.

Table 8. summarizes the changes in the pharmacokinetic, blood pressure and hemodynamic parameters in the study group. According to the pharmacokinetic parameters (C_{max} and T_{max}) the eight healthy volunteers can be classified clearly into two different groups. In volunteers number 1, 4 and 8 the maximum serum concentrations after the 10 mg orally-administered dose of GYKI-12743 (C_{max}) occurred between 1.5 and 2 hours (T_{max}). In volunteers number 2,3,5,6 and 7 the C_{max} values were developed within 40 - 50 minutes. As shown in table 8 maximum reduction of the MAP and TPR values coincided with C_{max}.

Table 7. Changes in the serum concentration of GYKI-12743, systolic and diastolic blood pressure, TPR and RPP in volunteer number 6.

Time (min.)	SBP (mmHg)	DBP (mmHg)	HR (1/min.)	RPP	CO (l/min.)	TPR (dynexs x cm^{-5})	GYKI-12743 (ng/ml)
0	130	71	64	8320	4.7	1452	-
10	111	69	69	7659	4.6	1446	-
20	109	51	68	7412	5.0	934	84.5
50	95	48	70	6650	5.0	873	110.19
120	113	57	62	7006	5.1	1178	33.0
240	124	66	66	8184	4.7	1407	5.8

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; RPP, rate-pressure product; CO, cardiac output; TPR, total peripherial resistence;

Table 8. Changes in the pharmacokinetic parameters, MAP and TPR after single oral dose of 10 mg of GYKI-12743.

N° of volunteer	T_{max} (min.)	C_{max} (ng/ml)	AUC (hourng/ml)	t_{max} (min.)	$MAP_{max}\downarrow$ (% of b)	$TPR_{max}\downarrow$ (% of b)
1	120	17.43	41.10	120	15	12
2	50	100.12	193.29	60	29	20
3	50	98.32	11.48	50	27	16
4	90	24.95	42.12	100	19	19
5	50	56.11	108.45	50	23	18
6	50	110.19	143.62	50	30	21
7	45	63.09	62.60	50	14	9
8	90	57.20	107.05	100	17	11

T_{max}, time of maximum concentration of GYKI 12743 in the serum; C_{max}, maximum concentration of GYKI 12743 in the serum; AUC, area under the curve; t_{max}, time of the maximum decrease in the MAP and TPR; $MAP_{max}\downarrow$, maximum decrease in systemic mean arterial pressure; $TPR_{max}\downarrow$, maximum decrease in total peripherial resistence; % of b, percent of baseline;

EVALUATION THE EFFECT OF CALCIUM ANTAGONIST NIFEDIPINE ON BLOOD PRESSURE AND HEMODYNAMICS MEASURED BY PROGRAMMABLE IMPEDANCE CARDIOGRAPHY.

INTRODUCTION

Calcium antagonists became widely used as antihypertensive agents in the late 80's. They work by inhibiting the entry of calcium into cardiac and smooth muscle cells through calcium-permeable channels in the cell plasma membranè. The movement of calcium through these channels is much slower than that of sodium during depolarization, so they are referred to as "slow channels". Calcium antagonists act primarily to reduce peripheral vascular resistance, aided by an initial diuretic effect that persists, at least in the case of isradipine. No negative inotropic effect can be detected in patients with initially normal myocardial function.

As early as1986 three calcium antagonists were available in clinical practice: nifedipine, verapamil and diltiazem. All three prototypical calcium antagonists, especially nifedipine, cause modest increases in plasma catecholamines and small elevations of plasma renin activity as a counter-regulatory effect. More calcium antagonists are likely to become available soon, some with a more prolonged duration of action, for example nitrendipine, izradipine and others with more specific sites of action, like nimodipine.

The currently available calcium antagonists differ both in their sites and modes of action upon the slow channel, as well as their effects upon various other cardiovascular functions. Calcium antagonists may be selected as initial monotherapy, especially if there are other indications for these agents, such as angina pectoris, Raynaud's phenomenon, or supraventricular tachycardia. While they are all effective antihypertensive agents, nifedipine is

the most potent peripheral vasodilator and, it has little effect on atrioventricular conduction. In addition, nifedipine proved to be a useful antihypertensive drug in case of emergency (95).

The aim of this study was to investigate the acute effect of sublingual administration of 10 mg nifedipine on the blood pressure and hemodynamics in hypertensive patients.

MATERIALS AND METHODS

Patients

Ten essential hypertensive patients (6 men and 4 women ; age : 47.7 ± 5.1 years), were involved in this study. The hypertensive patients were chosen from patients who were examined at the outpatient clinic of the First Department of Medicine, Medical University of Pecs, Hungary and met the following criteria: systolic blood pressure exceeded 170 mmHg and/or diastolic blood pressure exceeded 110 mmHg and this elevated blood pressure still existed after 30 min. of recumbent seat. All patients enrolled in this study signed a statement of informed consent which was approved by the institution's Human Assurance Committee. In addition, the conduct of this study complies with the principles expressed in the Declaration of Helsinki, which has been endorsed by the Hungarian Society for Clinical Investigation.

Protocol

A 10 mg. dose of nifedipine (cordaflex) was administered sublingually to every patient. Programmable impedance cardiographic (PIC) measurements were taken before administration of nifedipine and every minute after drug intake for ten minutes. After ten minutes the PIC measurements were taken at five minutes intervals for 2 hours.

Methods

Blood pressure and hemodynamic parameters were taken automatically by PIC measurement at every preprogrammed point of time, and at the same time stroke volume (SV), cardiac output (CO) and total peripheral vascular resistance (TPR) were estimated as previously described.

Two- dimensional Doppler echocardiography was performed with the patient in partial left decubitus position, using a Picker SE 151 B 2-D Doppler echocardiograph with 2.25 MHZ and continuous wave transducer in order to determine cardiac output parallel to impedance cardiographic measurements.

$$CO = A \times TAI \times HR$$

where CO is the cardiac output, A is the cross sectional area of the left ventricle outflow tract, TAI is the time velocity integral and HR is the heart rate (47,80).

Statistical analysis

Data were analyzed by Student's paired test and expressed as means ± SEM. The differences were considered significant at $p<0.05$.

RESULTS

Fig. 20 shows the maximum percent decrease in systolic (21.5 ± 2 %) and diastolic blood pressure (16.6 ± 1.5 %) and TPR (15.8 ± 3 %) compared to the baseline. In this study the maximum blood pressure lowering effect of sublingually administered nifedipine was found between 45-60 minutes.

We compared the changes in TPR, SV, CO and HR values measured at time of the maximum blood pressure decrease to the corresponding baseline values. As shown in fig. 21 nifedipine significantly reduced the TPR from 1881 ± 108 dyn x sec/cm^5 to 1563 ± 93 dyn x sec/cm ; $p < 0.01$) and at the same time the SV increased significantly from 71 ± 3 to 80 ± 2 ; $p <$

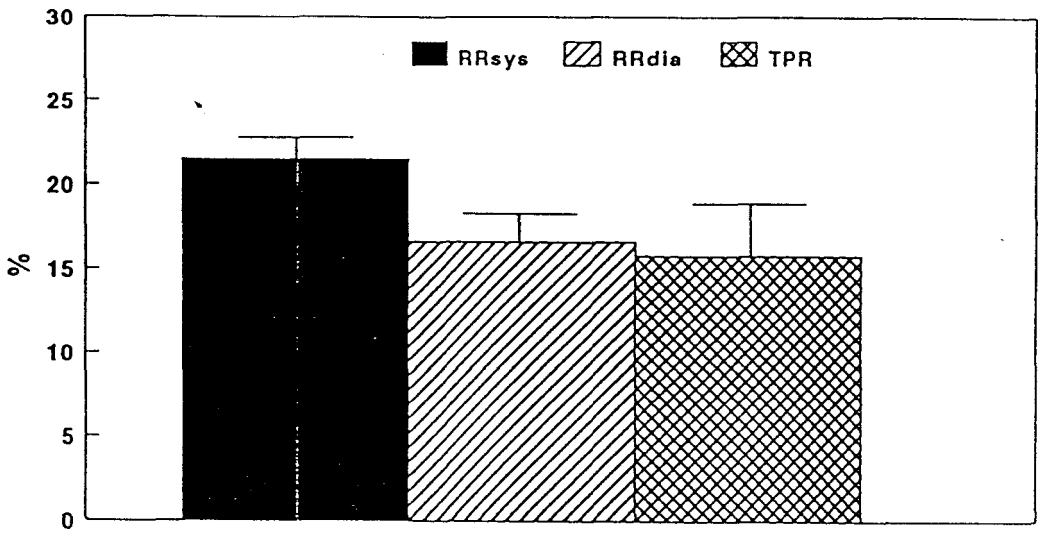

FIG. 20. Maximum decrease in systolic (RRsys), diastolic (RRdia) blood pressure and total peripheral vascular resistance (TPR) in hypertensive patients. Data are expressed as percent decrease from the baseline. Data are means ± SEM.

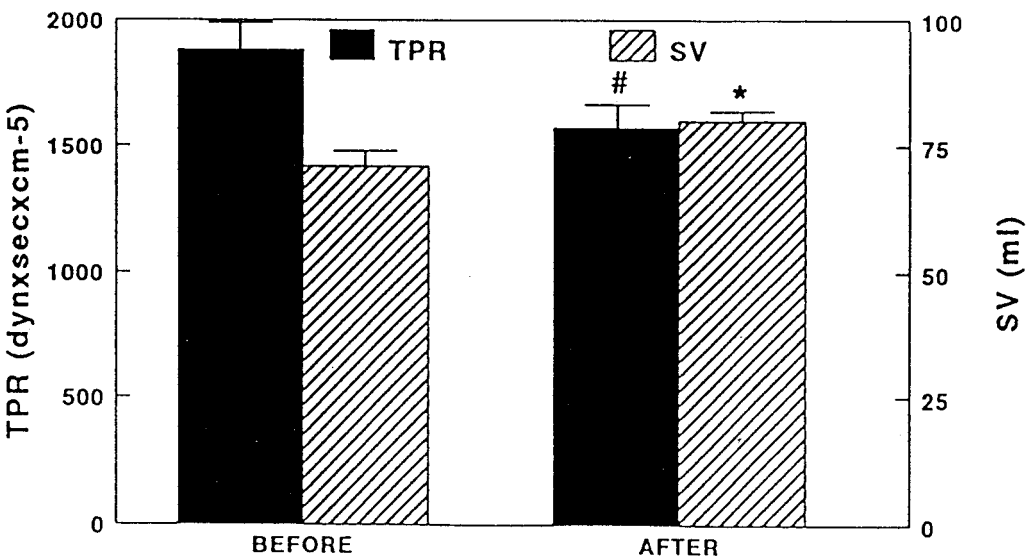

FIG. 21. Changes in total peripheral resistance (TPR) and stroke volume (SV) before and ater sublingual administration of nifedipine. The "after" values represent the peak hemodynamic rersponses to 10 mg of nifedipine. Data are means ± SEM. # = $p < 0.01$; * = $p < 0.02$.

0.02. CO was measured by both ICG and 2-D Doppler echocardiography. According to ICG measurements CO was increased significantly from 5.09 ± 0.2 l/min to 5.38 ± 0.3 l/min ($p< 0.01$). Similarly, significant increase was found in CO, measured by 2-D Doppler echocardiography (5.1 ± 0.2 l/min vs. 5.41 ± 0.4 l/min ; $p<0.02$). However, heart rate values did not change significantly (74 ± 5 beats/min vs. 80 ± 6 beats/min).

SUMMARY

We developed and introduced to the human clinical pharmacological studies the programmable impedance cardiographic measurements a feasible, entirely automatic, noninvasive method. Serial measurements, of beat-to-beat stroke volume and estimation of CO, TPR and RPP provide several important pieces of information on cardiac and peripheral hemodynamic function.

In the course of human phase I/A study we demonstrate the blood pressure lowering effect of once daily treatment with 10 mg of GYKI-12743 a newly developed alpha adrenoreceptor antagonist. The peak blood pressure reducing effect of GYKI-12743 was developed in different time in accordance with the pharmacokinetic parameters.

In the course of human phase IV study we demonstrated the blood pressure lowering effect of 10 mg nifedipine administered sublingually. In addition we also demonstrated the beneficial hemodynamic effects of nifedipine as reflected in TPR, SV, CO and HR values.

EVALUATION THE EFFECT OF CILAZAPRIL TREATMENT ON BLOOD PRESSURE AND HEMODYNAMICS MEASURED BY PROGRAMMABLE IMPEDANCE CARDIOGRAPHY AND 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors provide an excellent approach to the treatment of patients with hypertension. Compared with other antihypertensive drugs, ACE inhibitors possess a very favourable hemodynamic profile : they lower blood pressure by reducing total peripheral resistance (TPR), without influencing cardiovascular reflexes (1,2). Consequently, ACE inhibitors are acceptable first-line antihypertensive agents and can be used in the presence of a variety of co-existing cardiovascular diseases.

Left ventricular systolic and diastolic dysfunction are often the consequences of increased afterload and left ventricular hypertrophy in patients with systemic hypertension. Left ventricular hypertrophy detected as an increase in echocardiographic left ventricular mass, is a primary risk factor associated with cardiovascular mortality and morbidity. ACE inhibitors can reduce left ventricular hypertrophy in hypertensive patients and have very favourable effects in congestive heart failure beyond those of other vasodilators (3). Also, they can improve impaired diastolic performance of left ventricle observed in hypertensive patients (4,5,6).

Cilazapril is a relatively recent addition to a class of the non-sulfhydryl ACE inhibitors. As a prodrug, it is converted mainly in the liver and blood to its active form cilazaprilat which has a long terminal half-life with a longer duration of action. The calculated terminal half-life in hypertensive patients with normal renal function is 3 hours for cilazapril and 8 hours for cilazaprilat (8). However other data suggest a terminal half life of 37 to 86 hours (7).

Essential hypertension is often regarded as a multifactorial disease, resulting from a number of diverse genetic and environmental factors. Physiologically, the mean arterial pressure (MAP) is given by : MAP = CO X TPR, where CO = cardiac output and TPR = total peripheral resistance. Estimations of the blood pressure lowering effect of cilazapril rely mostly on 24-hour systolic and diastolic blood pressure averages obtained from 24-hour ambulatory blood pressure (ABP) recording.

Therefore, in the present study we investigated the effect of orally administered Cilazapril on blood pressure, hemodynamics, and the systolic and diastolic performance of the left ventricle in essential hypertensive patients. To estimate concurrently the acute (first 24-hour) effect of orally administered Cilazapril on TPR, and the blood pressure, we developed and applied to this clinical pharmacological study a novel noninvasive method of programmable impedance cardiographic (PIC) measurement by connection of the programmable blood pressure monitor with the impedance cardiograph. In the chronic study, twenty-four-hour noninvasive ambulatory blood pressure monitoring (ABPM) was performed to estimate long-term blood pressure lowering effect of orally administered Cilazapril. In addition to estimate the changes in 24-hour mean systolic and diastolic blood pressure values other clinically relevant parameters, such as systolic and diastolic hypertensive index and impact were studied.

METHODS

Patient Population

Twenty-four patients (11 men and 13 women ; age : 45.7 ± 4.9 years), were included in the study. They were chosen from patients examined at the outpatient clinic of the First Department of Medicine, Medical University of Pecs, Hungary and met all the following criteria : 1) 24-hour mean diastolic blood pressure > 90 mmHg < 115 mmHg at the end of 2-week placebo period. 2) no antihypertensive drugs for at least 4 weeks, 3) good quality of echocardiographic tracings, 4) absence of clinical, ECG, or

echocardiographic evidence of ischemic coronary artery disease, valvular disease (2-D echocardiography), or renal disease. Nineteen patients of total twenty four were classified as moderate hypertensive subjects (105 mmHg ≤ 24-hour mean diastolic blood pressure < 115 mmHg) and five patients belonged to mild hypertensive group (90 mmHg < 24-hour mean diastolic pressure < 105 mmHg). All patients signed a statement of informed consent which was approved by the institution's Human Assurance Committee. Furthermore, this study was conducted according to the principles expressed in the Declaration of Helsinki which has been endorsed by the Hungarian Society for Clinical Investigation.

Study protocol; Examined parameters.

Blood pressure measurements. In this study 24-hour blood pressure monitoring was performed by an automatic blood pressure monitor (Meditech ABPM , Meditech LTD Budapest, Hungary) using the oscillometric principle. The unit was set to take readings automatically every 15 minutes throughout the 24 hours. 24-hour blood pressure readings were taken at the end of two-week placebo period (I) and during the first 24-hours after once daily treatment with 5 mg of Cilazapril (II ; first dose effect). The 24-hour ABPM was repeated after 8 (III) and 24 weeks (IV) of once daily treatment with 5 mg of Cilazapril.

The following parameters were computed: (1) mean 24-hour systolic BP (SYSM), (2) mean 24-hour diastolic BP (DIAM), (3) systolic hypertensive time index (SYSIND), (4) systolic hypertensive impact (SYSIMP), (5) diastolic hypertensive time index (DIAIND) and (6) diastolic hypertensive impact (DIAIMP). For systolic blood pressure the hypertensive index is the ratio of the time of systolic blood pressure exceeding 140 mmHg to the whole measurement time, expressed as a percentage. For diastolic blood pressure the hypertensive time index is the ratio of the time of diastolic blood pressure exceeding 90 mmHg to the whole measurement time, expressed as percentage.The systolic hypertensive impact is the integral of the parts of the systolic curve exceeding 140 mmHg, standardized to one day.

The diastolic hypertensive impact is the integral of parts of the diastolic curve exceeding 90 mmHg, standardized to one day. The dimension of hypertension impact is mmHg*hour / day.

Evaluation of total peripheral vascular resistance (TPR) rate pressure product (RPP) and cardiac index (CI).

Hemodynamic parameters were estimated noninvasively by impedance cardiograph (ICG-M401 ASK Ltd. Budapest Hungary). PIC measurements (TPR and RPP) were performed before administration of Cilazapril (0 h) and 4h, 12h, and 24h after oral administration of 5 mg Cilazapril. TPR and RPP were estimated as previously described.

Echocardiographic measurements.

Two-dimensionalional Doppler echocardiography was performed at the end of the placebo period and after 24 weeks of once daily treatment with 5 mg of Cilazapril. The measurements were performed with the patient in partial left decubitus position, using a Picker SE 151 B echocardiograph with 2.25 or 2.5 MHZ transducers. Mitral flow velocities were recorded from an apical four-chamber view. The peak early diastolic (E) and atrial contraction (A) velocities were measured by averaging five cardiac cycles to avoid a respiratory influence on LV filling dynamics; isovolumic relaxation time (IVRT), early diastolic velocity time integral (EDVTI) and late diastolic velocity time integral (LDVTI) were calculated. In addition the left ventricle percent fractional shortening (FS) and the end systolic stress (ESS) of the left ventricle were calculated according to the following equations:

$$FS\ \% = (LVIDd - LVIDs) \ / \ LVIDd \ X \ 100$$

where LVIDd is the internal diameter of the end-diastolic dimension of the left ventricle and LVIDs is the internal diameter of the end-systolic dimension of the left ventricle.

$$ESS = 0.334 \times SBP \times LVIDs / PW_{ths} \times (1 + PW_{ths} / LVIDs)$$

where SBP is the systolic blood pressure and Pw_{ths}, the posterior wall thickness of the left ventricle.

Statistical analysis.

Data are presented as means ± SEM. Data were analyzed by Student's paired test. Also, one way ANOVA followed by Newman-Keuls test was utilized as required and the differences were considered significant at $p<0.05$.

RESULTS

Acute study

Fig. 22. and 23. summarize the changes in blood pressure and hemodynamic parameters recorded by PIC measurements at 0, 4, 12 and 24 hours after oral administration of 5 mg Cilazapril. As shown in fig.22. the TPR was decreased significantly at 4 hours after administration of Cilazapril (1996 ± 167 dyn x sec/cm^{-5} vs. 2867±180 dyn x sec/cm^{-5} at 4h and 0 h, respectively, $p < 0.01$). In addition a further reduction in the TPR values was found at 12 h and 24 h compared with the 4h value (1198±156 dyn x sec/cm^{-5} and 1256±178 dyn x sec/cm^{-5} vs. 2867±180 dyn x sec/cm^{-5}, $p<0.01$). The lowest value in TPR was recorded at 12 h after administration of cilazapril and TPR remained significantly reduced after 24 hours compared to baseline. Similarly, the MAP values decreased significantly after cilazapril treatment (121±7 mmHg, 110±5 mmHg, 98±4 mmHg and 111±4 mmHg at 0, 4h, 12h and 24h respectively ; $p < 0.01$). However, cilazapril treatment did not alter the CI values significantly throughout the 24- hour observation period (3.12±0.2 l/min/m^2, 3.0±0.1 l/min/m^2, 3.2±0.2 l/min/m^2 and 3.18±0.2 l/min/m^2 at 0h,4h ,12h and 24 h, respectively).

As shown in fig. 23. the RPP values were significantly lower at 4h, 12h and 24h after administration of cilazapril (11200 ±798, 10560±765 and

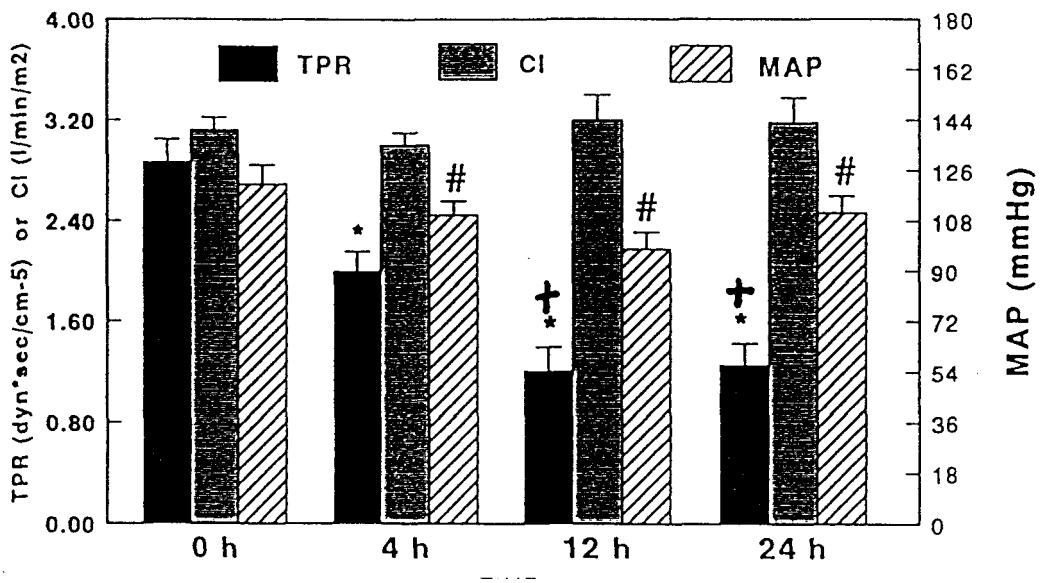

FIG. 22. Changes in total peripheral resistance (TPR), cardiac index (CI) and systemic mean arterial pressure (MAP) 4, 12 and 24 hours after administration of cilazapril. The pretreatment (0 h) values were recorded immediately before administration of cilazapril. Data are means ± SEM. *, # = $p < 0.01$ from the corresponding "0 h"values. + = $p < 0.01$ from the corresponding "4 h" values.

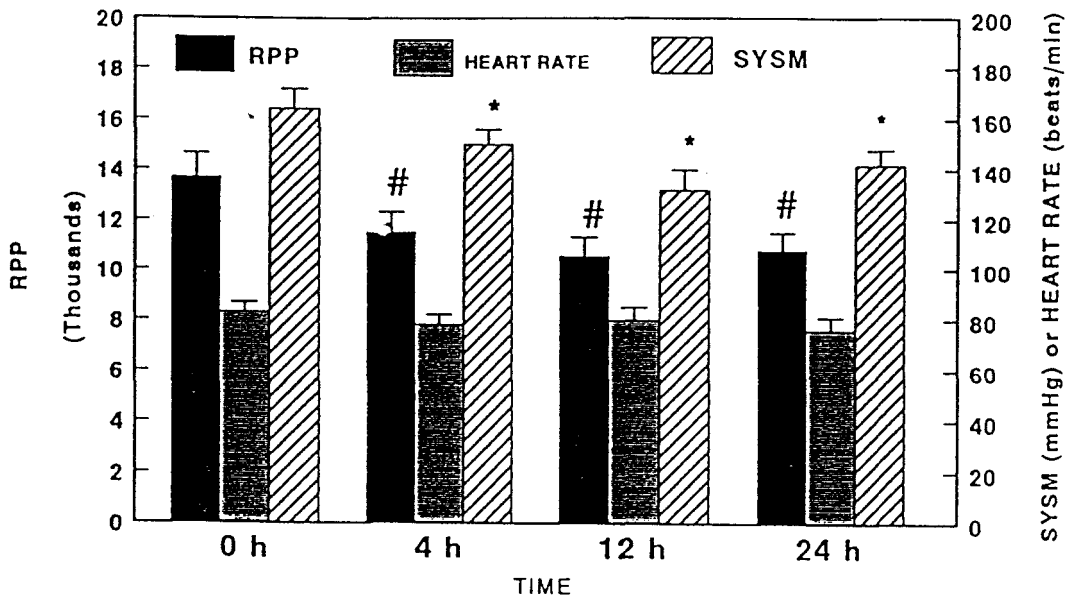

FIG. 23. Changes in rate pressure product (RPP), heart rate and the 24-hour mean systolic blood pressure (SYSM) 4, 12 and 24 hours after administration of cilazapril. The pretreatment (0h) values were recorded immediately before administration of cilazapril. Data are means ± SEM. *, # = $p < 0.01$ from the corresponding "0 h" values.

10792±698) compared with 0h value (13678±989 ; $p<0.01$). Similar changes were observed in the SYSM values (164±8 mmHg, 144±6 mmHg, 132±8 mmHg and 142±6 mmHg at 0h, 4h, 12h and 24h respectively). A further significant decrease was found at 12h compared with the 4h value (132±8 mmHg vs. 144±6 mmHg). The heart rate values did not change significantly throughout the 24-hour period (83±4 beats/min, 78±5 beats/min, 80±3 beats/min, 76±5 beats/min at 0h, 4h, 12h and 24h respectively).

Chronic study

Fig. 24. summarizes the changes in blood pressure values during cilazapril treatment at week 8 and 24. Both systolic and diastolic blood pressure values decreased significantly compared to the blood pressure values recorded at the end of the placebo period. Systolic blood pressure values decreased from 164±8mmHg as recorded at the end of the placebo period to 148±9mmHg at week 8 and 139±8mmHg at week 24 ; $p<0.01$). Similarly, diastolic blood pressure values decreased significantly throughout the observation period (88±5 mmHg, 86±4mmHg vs. 108±7mm at week 8 and 24 vs. placebo; $p<0.01$).

As shown in fig. 25. the SYSIND value was 66±9 % at the end of the placebo period and decreased significantly to 24±4% and to 25±3% at week 8 and 24 ; $p<0.01$. The DIAIND values also decreased significantly from 58±7 to 19±3 after 8 weeks and to 17±2 after 24 weeks ; $p<0.01$.

As shown in fig. 26. the SYSIMP values showed a significant decrease from 365±20 mmHg*hour/day to 114±12 mmHg*hour/day after 8 weeks and to 109±11 mmHg*hour/day after 24 weeks ;$p<0.01$. Also, the DIAIMP values decreased significantly from 256±24 mmHg*hour/day to 87± mmHg*hour/day after 8 weeks and to 81 mmHg*hour/day after 24 weeks of therapy with cilazapril.

Long- term effect of cilazapril treatment on left ventricular systolic and diastolic functions.

Table 9. summarizes the long term effect of cilazapril treatment on left

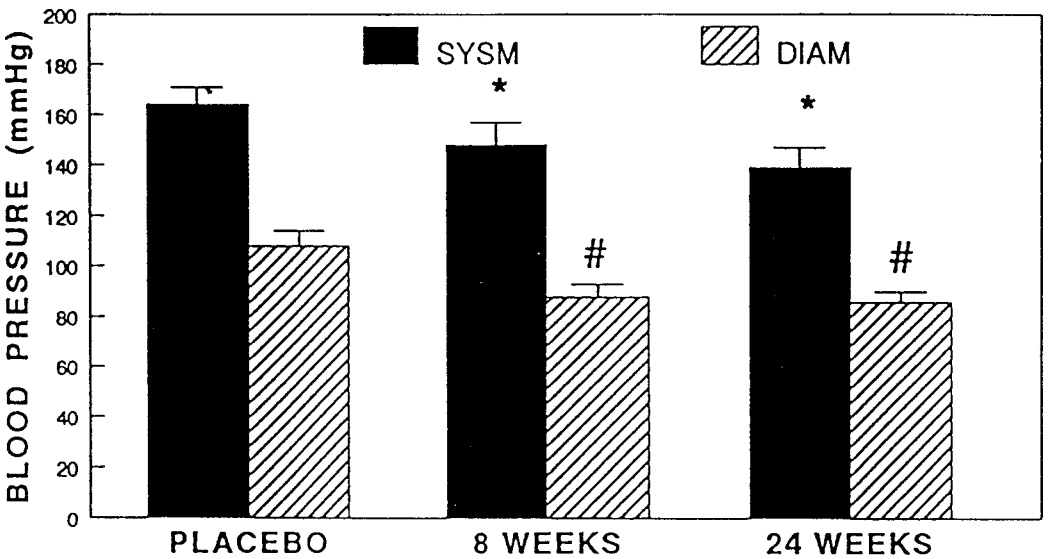

FIG. 24. Changes in the 24-hour mean systolic (SYSM) and diastolic (DIAM) blood pressure following 8 and 24 weeks of continuous oral administration of cilazapril. Treatments are compared to the blood pressure values measured in the the placebo period. Data are means ± SEM. *, # = $p < 0.01$ from the corresponding placebo values.

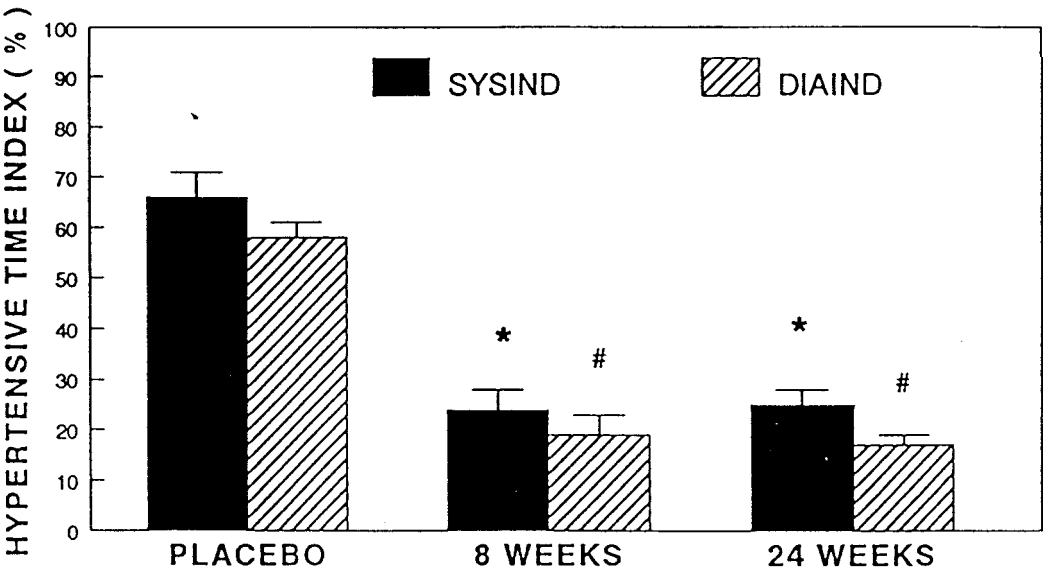

FIG. 25. Changes in the systolic (SYSIND) and diastolic (DIAIND) hypertensive time index values following 8 and 24 weeks of continuous oral administration of cilazapril. Treatments are compared to the hypertensive time index values measured in the placebo period. Data are means ± SEM. *, # = $p < 0.01$ from the corresponding placebo values.

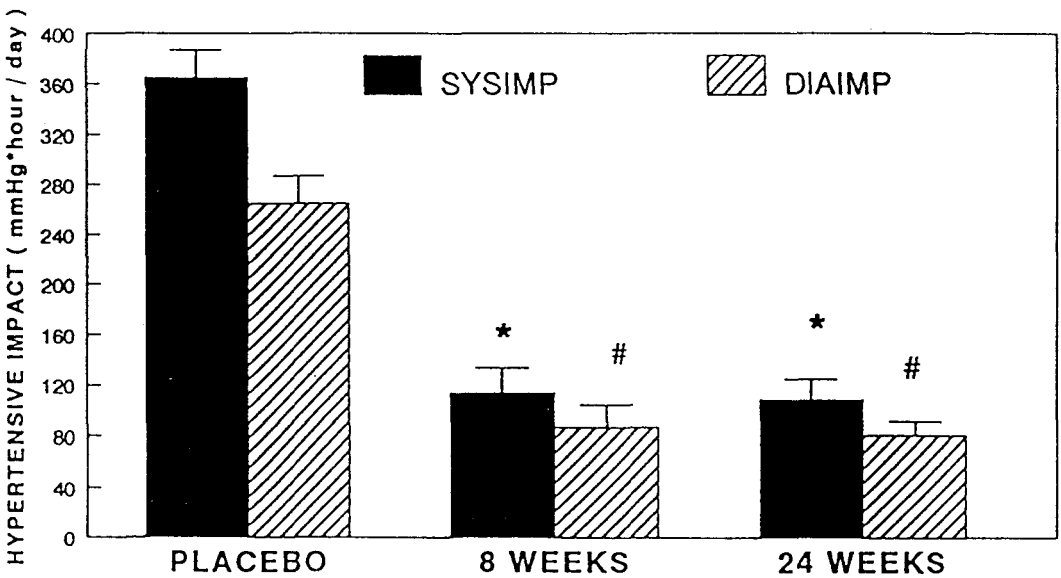

FIG. 26. Changes in the systolic (SYSIMP) and diastolic (DIAIMP) hypertensive impact values following 8 and 24 weeks of continuous oral administration of cilazapril. Treatments are compared to the hypertensive impact values measured in the placebo period. Data are means ± SEM. *, # = $p < 0.01$ from the corresponding placebo values.

Table 9. Long-term effect of cilazapril treatment on systolic and diastolic functions of the left ventricle in hypertensive patients.

	PLACEBO	24 WEEKS
IVRT (ms)	109 ± 3	86 ± 2 *
EDTVI (ms)	7.2 ± 0.2	8.5 ± 0.2 *
LDTVI (ms)	6.9 ± 0.3	4.9 ± 0.2 *
EF (%)	47.3 ± 3	48.5 ± 4
ESS (10^3 X dyn/cm^2)	54.6 ± 3	43.2 ± 2 *

PLACEBO, at the end of the placebo period; 24 WEEKS, 24 weeks after cilazapril treatment; IVRT,isovolumic relaxation time; EDTI, early diastolic velocity time integral, LDTVI, late diastolic velocity time integral, EF, ejection fraction; ESS, end-systolic left ventricular wall stress

ventricular systolic and diastolic functions. As shown in this table IVRT and ESS decreased significantly from 109±3 ms to 86±2 ms and from 54.6±3 10^3 x dyn x cm^2 to 43.2±2 10^3 x dyn x cm^2 after 24 weeks. Similarly, significant changes were found in the EDTVI (from 7.2±0.2 ms to 8.5±0.2 ms) and in the LDTVI (from 6.9±0.3 ms to 4.9±0.2 ms) after 24 weeks of therapy with cilazapril.The EF values did not change significantly after 24 weeks compared to the value measured at the end of the placebo period.

DISCUSSION

In this study we combined the programmable blood pressure monitoring device with the impedance cardiograph and performed the programmable impedance cardiographic measurements to evaluate the antihypertensive effect of the ACE inhibitor cilazapril in hypertensive patients. This method provides us with simultaneous accurate and highly reproducible data about the blood pressure lowering and hemodynamic effects of this drug. In essential hypertensive patients 5 mg of cilazapril significantly reduced the MAP , TPR and the RPP values. However, the CI and the heart rate remained unchanged.

In clinical practice the 24-hour ABPM has been used in the evaluation of blood pressure response to antihypertensive treatment. In addition to the routinely recorded ABPM parameters, the hypertensive index and impact values were measured in this study to evaluate of the effect of the treatment with 5 mg cilazapril in hypertensive patients. It is known that some behaviors (such as eating, drinking, or mental work when performed in the presence of stress and other daytime behaviors) may raise blood pressure, but nighttime sleep, daytime sleep, and postprandial digestion cause hypotension. Emotion can cause a slight blood pressure rise, when mild, and a pronounced and prolonged pressure rise, when more marked and long - lasting. Furthermore, 24-hour blood pressure variability can be divided into an irregular component, originating from the cardiovascular response to environmental stimuli, and several blood pressure oscillations that are intrinsic to the

cardiovascular system. Presumably, measurement of systolic or diastolic hypertensive impact, which includes both blood pressure and time seems to provide important information about the duration of the irregular component originating from the cardiovascular response to environmental blood pressure raising stress stimuli. In this study we found a marked, significant decrease in the hypertensive impact and the hypertensive index values after 8 and 24 weeks of therapy with cilazapril.

The diastolic properties of the left ventricle are the first to be modified during the course of arterial hypertension (1). Whether or not these modifications are dependent on coronary heart disease is debatable, but there is no doubt that they occur before systolic dysfunction. Recently, it has been suggested that in chronic pressure overload , myocardial stiffness and its biological counterpart, left ventricular collagen concentration, depend on hormonal control, and in particular on angiotensin II and the aldosterone plasma level, together and independently (15,16). Although, several studies have shown an improvement in diastolic function with antihypertensive therapy, there is also inconsistency in response to antihypertensive drugs. Studies on the effect of beta-blocking agents 4,5), calcium antagonists (6,7), are controversial. Probably, the best improvement of the left ventricular diastolic function can be obtained by long term administration of an ACE inhibitor. In this study we found a significant improvement in the diastolic function of the left ventricle after 24 weeks of treatment with cilazapril.

In summary, this study demonstrates that once daily treatment with 5 mg of cilazapril decreased significantly the blood pressure, and maintained favourable hemodynamics in patients with mild and moderate hypertension. Serial, automatic determination of TPR, RPP, CI, MAP, heart rate by means of PIC measurements is a valuable tool in the evaluation of antihypertensive treatment.

IMPORTANCE OF THE BLOOD PRESSURE PARAMETERS OBTAINED BY 24-HOUR AMBULATORY BLOOD PRESSURE MONITORING IN THE CLASSIFICATION OF HYPERTENSIVE PATIENTS

INTRODUCTION

Patients with hypertension, even those with mild elevation of blood pressure, are at increased risk of other cardiovascular disease, whether or not symptoms are present. High blood pressure is one of the major risk factors for premature death and is associated with a higher incidence of myocardial infarction and heart failure.

Diagnosis of hypertension and planning therapy are facilitated by the correct grading of patients with high blood pressure as well as the classification of hypertension. In the rush to identify and treat everyone with high blood pressure, there is a need for caution not to falsely and inappropriately label a large number of people. Casual blood pressure measurements do not give the best estimate of blood pressure, since they provide readings for a single time point only. They are subject to a "white-coat" effect, may show a significant placebo effect and are not reproducible(69,70,85). Ambulatory blood pressure monitoring (ABPM) overcomes these problems and offers the possibility of obtaining reliable, reproducible and detailed information on blood pressure over a 24- hour period. The clinical usefulness of ABPM rests on the original report of Sokolow about patients with moderately severe hypertension (121).

The main clinical interest in this approach is the potential for providing a more precise diagnosis of the blood pressure elevation occurring in a given patient, and thus, a sharper definition of blood pressure- related risk (83,86). Several previously published studies have demonstrated the relationship between blood pressure measured by ambulatory monitoring and left

ventricular hypertrophy in essential hypertension (110). Most of these studies are based on nighttime and daytime systolic and diastolic blood pressure averages, as well as 24-hour arterial blood pressure averages (129,130). Organ damage bears a direct relation to blood pressure elevation occurring at work, the number of daytime blood pressure peaks, the nighttime blood pressure values, and the 24-hour blood pressure variability (100).

Beyond these aforementioned parameters, there are others considered to be important in the characterization of patients' daily blood pressure values. Therefore, the objective of this study was to estimate the importance of ten different blood pressure parameters that are obtained by 24-hour ambulatory blood pressure monitoring and considered characteristic of the patients' diurnal blood pressure behavior. In addition, the ten blood pressure parameters were used to classify hypertensive patients and the discrimination power of each parameter was measured to characterize its clinical importance.

METHODS

Patient Population

One-hundred seventy-four patients (91 men and 83 women , age 45.7 ± 4.9 years) were studied. Forty-six were normotensive.The hypertensive patients were consecutively chosen from patients who were examined at the outpatient clinic of the First Department of Medicine, Medical University of Pecs, Hungary, and met all of the following criteria : 1) casual blood pressure recording greater than 140 / 90 mmHg, 2) no antihypertensive drugs within the last 4 weeks, 3) good quality of echocardiographic tracings, 4) absence of clinical, ECG or echocardiographic evidence of ischemic coronary artery disease, valvular disease (2-D echocardiography) or renal disease. The casual blood pressure values were determined by a physician at the office and used for classification of hypertensive patients. All patients have signed a statement of informed consent which was approved by the institution's

Human Assurance Committee. The conduct of this study complied with the principles expressed in the Helsinki Declaration which has been endorsed by the Hungarian Society for Clinical Investigation.

Study protocol; Examined parameters

Blood pressure measurements. In this study 24-hour blood pressure readings were taken by MEDITECH ABPM (Meditech LTD Budapest, Hungary) automatic blood pressure monitor, using the oscillometric principle. The unit was set to take readings automatically every 15 minutes throughout the 24 hours. The following parameters were computerized : (1) average 24-hour systolic BP (SYSAVG mmHg), (2) average daytime (6:00 AM to 8:00 PM) systolic BP (SYSDTAVG mmHg), (3) average 24-hour diastolic BP (DIAAVG mmHg), (4) average daytime (6:00 AM to 8:00PM) diastolic BP (DIADTAVG mmHg), (5) systolic maximum BP (SYSMAX mmHg), (6) diastolic maximum BP (DIAMAX mmHg), (7) systolic hypertensive time index (SYSIND), (8) systolic hypertensive impact (SYSIMP), (9) diastolic hypertensive time index (DIAIND), (10) diastolic hypertensive impact (DIAIMP).

Electrocardiography

A standard 12-lead ECG was obtained from each patient in order to determine left ventricular hypertrophy. The recorded ECG variables included R-wave voltage in leads I,II,III, aVF,aVL and V_3 to V_6; S-wave voltage in V_1 to V_3; QRS frontal plane axis and duration, intrinsicoid deflection, left atrial abnormality, ST - T pattern of "strain", Sokolow-Lyon voltage criteria (113) ST, S-wave voltage in V1 plus R wave in lead V_5 or $V_6 \geq 35$ mm).

Echocardiography

Two-dimensional Doppler echocardiography was performed with the patient in partial left decubitus position, using a Picker SE 151 B echocardiograph with 2.25 and 2.5 MHZ transducers in order to calculate left ventricular mass (LVM) and determine diastolic performance of the left

ventricle. Left ventricular measurements were made at end-diastole and end-systole according to the recommendations of the American Society of Echocardiography (ASE). The left ventricular mass index was calculated according to the Devereux and Reichek formula (37) with a modified convention for determination of left ventricular dimensions (LVID), posterior wall thickness (PWT), and interventricular septal thickness (IVST), which excluded the thickness of endocardial echo lines from wall thicknesses and included the thickness of left septal and posterior wall endocardial echo line in LVID :

$$LVM = 1.04\ [(LVID + PWT + IVST)^3 - (LVID)^3] - 13.6\ gm$$

(Normal values : LVM $\leq$ 134 g/m^2 in males or LVM $\leq$ 110 g/m^2 in females.)

Measurement of left ventricular diastolic performance

Alterations in left ventricular diastolic function were indicated when the height of the mitral E wave was reduced and the height of the mitral A wave was increased, accompained by the prolongation of the isovolumic relaxation time, deceleration time, and the enlargement of left atrium (47,120).

Evaluation of systemic vascular resistance.

Hemodynamic parameters have been monitored noninvasively by impedance cardiography (ICG-M401 ASK Ltd., Budapest Hungary). Total peripheral resistance (TPR) was calculated according to the following equation described previously (36,64,77):

$$TPR = (MAP \times 80) / CO \quad (36,64,77).$$

Retinal abnormalities related to hypertension

The abnormalities of the fundus were also studied and graded according to the Keith-Wagener criteria (102,121).

Statistical analysis

All blood pressure values were analyzed by the PRIMA (Pattern Recognition by Independent Multicategory Analysis) method (71). The principle of pattern recognition is to achieve classification based on easily measurable quantitative features. Obviously, the measured properties must be characteristic for the classes. Briefly, this class modeling method derives in the learning phase for each class-independent decision rule that subsequently can be used for classification of samples of unknown origin (in our case those are blood pressure parameters of essential hypertensive patients). The decision rules are based on class distances. Classification was done by assigning the patients to that class, for which the class distance is minimal or smaller than a suitably selected limit value, the so-called class distance threshold. After the learning phase, discriminating power of different parameters (blood pressure values) have been calculated, which can be then used to characterize the importance of the given parameters and to select the relevant data from the point of view of the given classification. The efficiency of classification is characterized by the recognition ability which corresponds to the fraction of patients from the training set that are classified correctly.

RESULTS

Table 10A. shows the classification of patients according to their office diastolic blood pressure values. After this classification each patient was subjected to ABPM for 24 hours and ten ABP parameters (SYSAVG, SYSDTAVG, DIAAVG, DIADTAVG, SYSMAX, DIAMAX, SYSIND, SYSIMP, DIAIND, DIAIMP) were defined and used for subsequent PRIMA analysis. Table 10B. demonstrates the rearrangement of 174 patients by PRIMA analysis according to the ten ABP parameters used to distinguish between groups. As shown in this table, two patients were transferred from the normotensive group to the slightly hypertensive group and three patients

Table 10A. Classification of 174 patients according to the office blood pressure values.

	Diastolic BP	N^0 of Men	N^0 of Women	Total
Normotensive	< 90mmHg	20	26	46
Mild	90-104mmHg	11	17	28
Moderate	105-114mmHg	44	30	74
Severe	≥115mmHg	16	10	26
Total		91	83	174

Normotensive, group of the normotensive patients; Mild, group of the mild hypertensive patients; Moderate, group of the moderate hypertensive patients; Severe, group of the severe hypertensive patients. BP, blood pressure.

Table 10B. Reclassification of 174 patients by PRIMA analysis according to ten blood pressure parameters.

	N^0 of Rearranged Patients	N^0 of Men	N^0 of Women	Total
Normotensive	↓2	22	25	47
Mild	↑3 ; ↓1	12	19	31
Moderate	↑5 ; ↓1	42	30	72
Severe	↑3	15	9	24
Total	15	91	83	174

PRIMA = Pattern Recognition by Independent Multicategory Analysis. See legend of Table 1A for details.

from the slightly hypertensive group were reclassified and moved to the normotensive group. The largest changes in the classification of patients were observed in the mild hypertensive group. Four patients were transferred to different groups, and another seven were moved to this group from the normotensive (two patients) and moderate hypertensive (five patients) groups. Remarkably, eleven patients of a total of thirty-one in the mild hypertensive group, had to be reclassified by PRIMA analysis, when using ten ABP parameters for distinction. From the moderate hypertensive group, ten patients out of a total of seventy-two were reclassified. From the group with severe hypertension (n=24), three patients were transferred to the group of moderate hypertension and one patient was moved to the severe group from the group with moderate hypertension.

To diagnose an individual hypertensive complication, left ventricular hypertrophy was determined by ECG. Echocardiographic measurements were also performed to determine left ventricular mass and LV diastolic dysfunction. Concurrently, ocular fundoscopic abnormalities as well as increase in total peripheral resistance were investigated. Target organ damage and increased TPR were used to characterize hypertension in 127 patients (Table 11). LVH was determined by ECG. LVM and LV diastolic dysfunction were estimated by echocardiography.

Figures 27-29. show the distinction of different hypertensive groups by PRIMA method based on the measurement of ten ABP parameters. Figure 27. shows the separation of the normotensive group from the mild hypertensive group, as computed by PRIMA analysis. As shown in this figure, patients belonging to normotensive and mild hypertensive groups can be distinguished very clearly, according to the discriminative power of the ten ABP parameters. Figure 28. shows the separation of the mild hypertensive group from the moderate hypertensive group analyzed as in Figure 27. In a few cases, it was difficult to recognize and distinguish mild vs. moderate hypertensive patients because the class distances were smaller than those in Figure 27. and there were overlaps between the groups. Figure 29. demonstrates the classification and clear separation of moderate and severe

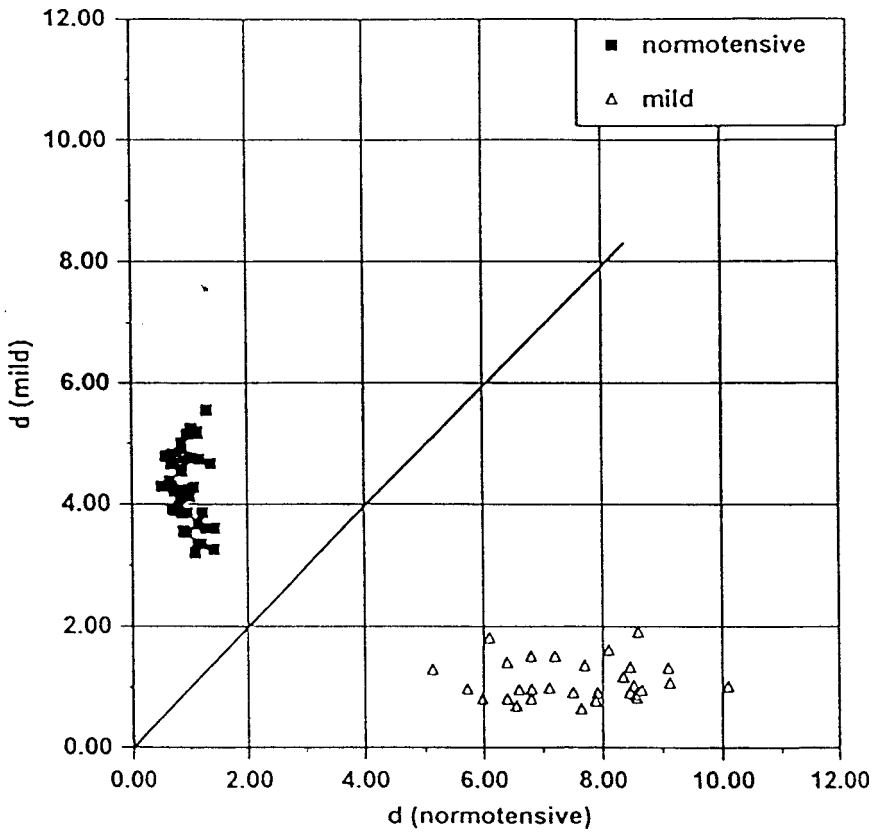

FIG. 27. Separation of normotensive group from mild hypertensive group by PRIMA (Pattern Recognition by Independent Multicategory Analysis) method according to ten blood pressure parameters, provided by 24-h ambulatory blood pressure recording. The "X" axis values represent the distance of a given patient from the normotensive group. The "Y" axis represents the distance of a given patient from the mild hypertensive group.

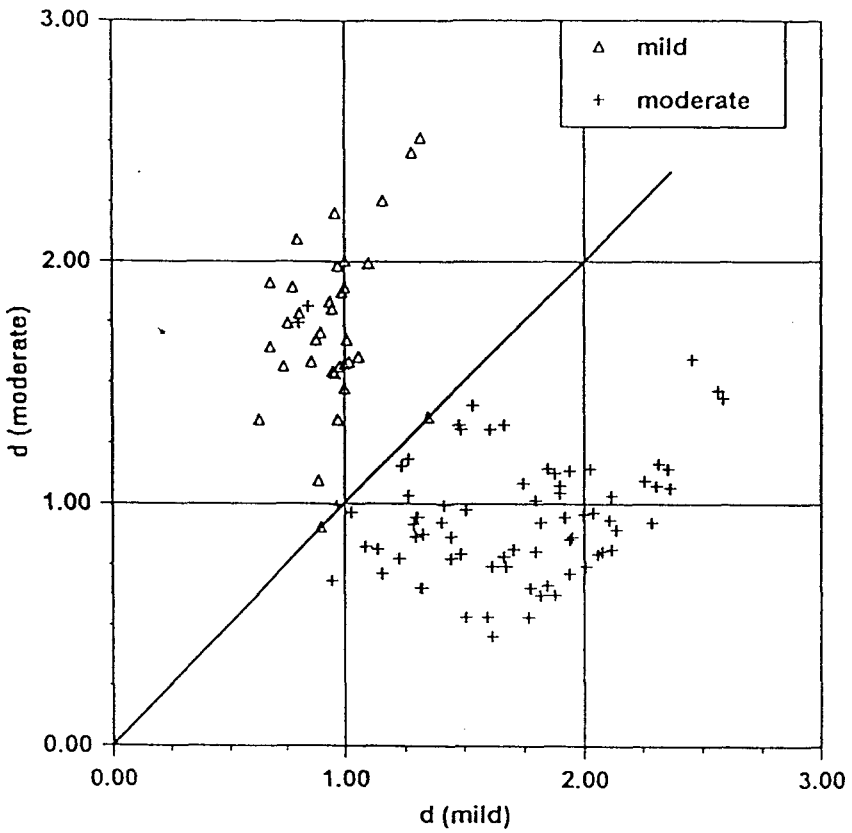

FIG. 28. Separation of mild hypertensive group from moderate hypertensive group by PRIMA (Pattern Recognition by Independent Multicategory Analysis) method according to ten blood pressure parameters, provided by 24-h ambulatory blood pressure recording. The "X" axis represents the distance of a given patient from the mild hypertensive group. The "Y" axis represents the distance of a given patient from the moderate hypertensive group.

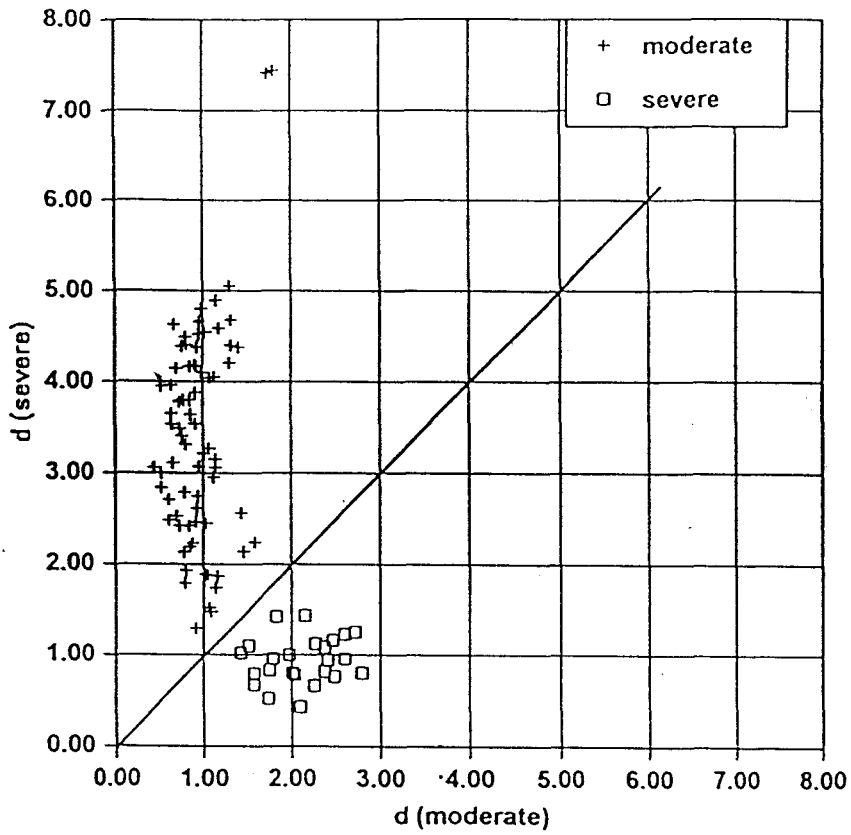

FIG. 29. Separation of moderate hypertensive group from severe hypertensive group by PRIMA (Pattern Recognition by Independent Multicategory Analysis) method according to ten blood pressure parameters, provided by 24-h ambulatory blood pressure recording. The "X" axis represents the distance of a given patient from the moderate hypertensive group. The "Y" axis represents the distance of a given patients from the severe hypertensive group.

Table 11. Characterization of 127 hypertensive patients according to target-organ damage and increased total peripheral resistance.

	LVH (ECG)	LVM↑ (ECHO)	Dysfunction in diastolic performance of LV (ECHO)	Ocular fundoscopic abnormalities	TPR↑ (ICG)
Mild hypertension (N=31)	5	-	-	12	1
Moderate hypertension (N=72)	30	28	23	52	37
Severe hypertension (N=24)	19	18	12	19	21
TOTAL (N = 127)	58	46	35	83	59

TPR, total peripheral resistance; LVH, left ventricular hypertrophy ; ECG, electrocardiography; LVM↑ = Inrease in the left ventricular mass; ECHO, 2-D echocardiography; TPR↑ = inrease in the total peripheral resistance; ICG = impedance cardiography.

Table 12. Mean values of ten blood pressure parameters obtained from 174 patients by 24-hour ambulatory blood pressure monitoring.

	NORMOTENSIVE (n = 47)	MILD (n = 31)	MODERATE (n = 72)	SEVERE (n = 24)
SYSAVG (mmHg)	115 ± 1.6	140 ± 1.7	146 ± 0.9	154 ± 1.3
SYSDTAVG (mmHg)	132 ± 1.5	143 ± 1.6	149 ± 0.9	160 ± 1.3
DIAAVG (mmHg)	77 ± 0.7	98 ± 0.8	109 ± 0.4	117 ± 0.2
DIADTAVG (mmHg)	83 ± 0.7	104 ± 0.9	114 ± 0.5	119 ± 0.3
SYSMAX (mmHg)	162 ± 1.8	184 ± 1.8	189 ± 1.3	198 ± 1.6
DIAMAX (mmHg)	92 ± 0.7	109 ± 0.9	116 ± 0.5	126 ± 0.6
SYSIND (%)	10 ± 0.9	49 ± 2.5	50 ± 1.0	79 ± 2.8
SYSIMP (mmHg*hour/day)	27 ± 2.8	215 ± 12.5	289 ± 4.7	373 ± 11.6
DIAIND (%)	4 ± 0.8	51 ± 2.6	63 ± 1.6	81 ± 2.7
DIAIMP (mmHg*hour/day)	11 ± 2.5	254 ± 12.4	294 ± 5.0	363 ± 14.4

Normotensive, group of the normotensive patients; Mild, group of the mild hypertension; Moderate, group of the moderate hypertension; Severe, group of the severe hypertension; SYSAVG, average 24-h systolic blood pressure; SYSDATVG, average daytime systolic blood pressure; DIAAVG, average 24-hour diastolic blood pressure; DIADTAVG, average daytime diastolic blood pressure; SYSMAX, systolic maximum blood pressure; DIAMAX, diastolic maximum blood pressure; SYSIND, systolic hyprtensive time index; SYSIMP, systolic hypertensive impact; DIAIND, diastolic hypertensive time index; DIAIMP, diastolic hypertensive impact. Data are means ± SEM.

Table 13. Discriminating power values of ten blood pressure parameters.

	NORMOTENSIVE (n = 47)	MILD (n = 31)	MODERATE (n = 72)	SEVERE (n = 24)
NORMOTENSIVE		1.DIAIMP (6.4) 2.DIAIND (6.2) 3.DIAAVG (4.7)	1.DIAIMP (8.2) 2.SYSIMP (7.6) 3.DIAAVG (7.4)	1.DIAAVG (10.7) 2.SYSIMP (8.8) 3.DIAIND (8.3)
MILD	1. DIAIMP (6.4) 2. DIAIND (6.2) 3. DIAAVG (4.7)		1.DIAAVG (3.2) 2.DIADTAVG(2.2) 3.DIAMAX (1.8)	1.DIAAVG (7.3) 2.DIADTAVG(4.9) 3.DIAMAX (4.1)
MODERATE	1.DIAIMP (8.2) 2.SYSIMP (7.6) 3.DIAAVG (7.4)	1.DIAAVG (3.2) 2.DIADTAVG(2.2) 3.DIAMAX (1.8)		1.DIAAVG (3.1) 2.SYSIND (2.7) 3.SYSIMP (2.2)
SEVERE	1.DIAAVG (10.7) 2.SYSIMP (8.8) 3.DIAIND (8.3)	1.DIAAVG (7.3) 2.DIADTAVG(4.9) 3.DIAMAX (4.1)	1.DIAAVG (3.1) 2.SYSIND (2.7) 3.SYSIMP (2.2)	

Discriminative power values of ten blood pressure parameters (in parentheses) were calculated by the PRIMA (Pattern Recognition by Independent Multicategory Analysis) method. See legend of table 12. for details.

hypertensive groups.

Concurrently, data obtained from ABP recording were computed in order to assess the discriminating power of the ten blood pressure parameters. Table 12. summarizes the mean values of ten ABP parameters in the hypertensive groups. Table 13. demonstrates the results obtained by ranking from one to three of the ABP parameters into six groups, on the basis of discriminating power (DP). Interestingly, fourteen diastolic, but only four systolic parameters were found among the eighteen most powerful discriminating properties, emphasizing the importance of diastolic blood pressure values in classification of hypertensive patients. As shown in Table 13, the values of DIAVG are found four times in first place and DIAIMP appears twice, indicating that these parameters have the highest DP values among the studied blood pressure parameters. It is also noteworthy that eight diastolic average blood pressure values (6 DIAAVG ; 2 DIADTAVG), five hypertensive impact values (3 SYSIMP ; 2 DIAIMP), five hypertensive index values (3 SYSIND ; 2 DIAIND), and two diastolic maximum BP values (DIAMAX) were also found among the most powerful discriminating parameters.

DIAIMP seemed to be the most powerful discriminating parameter (DP value = 6.4) to distinguish the normotensive group (11 ± 2.5 mmHg*hour / day) from the mild hypertensive group (254 ± 12.4 mmHg*hour / day) as well as from the moderate hypertensive group (294 ± 5 mmHg*hour / day ; DP value = 8.2); see Tables 12 and 13. DIAAVG had very powerful disriminating property to distinguish the severe hypertensive group from the normotensive (DP value = 10.7) and the mild hypertensive groups (DP value = 7.3); (see : table 12). DIAAVG values were 117 ± 0.2 mmHg , 77 ± 0.7 mmHg and 98 ± 0.8 mmHg for severe, normotensive, and mild hypertensive groups respectively (see : Table 11). DIAAVG values were found to be powerful in distinguishing the moderate hypertensive group from the mild (DP value = 3.2) and severe (DP value = 3.1) hypertensive group.

DISCUSSION

There is an increasing body of evidence indicating that single casual measurements of blood pressure may be inaccurate in providing a reliable index for the evaluation of hypertension. Measurement of blood pressure over a prolonged period of time is preferable. Casual blood pressure measurements may lead to misclassification of a hypertensive patient, or inappropriate antihypertensive treatment(84,86). Clinical ambulatory blood pressure monitoring has been used to improve the physician's diagnosis of hypertension, classification of hypertensive patients and evaluation of the effect of antihypertensive treatment. Currently, there is still uncertainty regarding the most appropriate analysis of ambulatory blood pressure data. The 24-hour average blood pressure, daytime and nighttime blood pressure values, daytime blood pressure peaks, and blood pressure variabilities are the most frequently cited parameters(75). Other blood pressure parameters, which presumably possess clinical relevance have not been investigated properly.

The first remarkable finding of this study using 24-hour ABPM + PRIMA is that 15 out of total 174 patients had to be reclassified when a more accurate statistical analysis (PRIMA method) was used for classification of hypertension based on ten different blood pressure parameters. This finding suggests that nine percent of the patients in this small group could have been misdiagnosed, with single casual blood pressure recordings.

Fourteen diastolic parameters out of eighteen were identified as powerful discriminating parameters. Our data confirm the priority and clinical importance of the 24-hour diastolic average to classify hypertensive patients accurately. It is known that some behaviors (such as eating, drinking, or mental work when performed in the presence of stress and other daytime behavior) may raise blood pressure, but nighttime sleep, daytime sleep, and postprandial digestion cause hypotension. Emotion can cause a slight blood

pressure rise when mild, and a pronounced and prolonged pressure rise when more marked and long-lasting. Furthermore, 24-hour blood pressure variability can be divided into an irregular component, originating from the cardiovascular response to environmental stimuli, and several blood pressure oscillations that are intrinsic to the cardiovascular system (83). Presumably, systolic and diastolic hypertensive impact, which includes both the blood pressure value and the time element seems to be an important blood pressure parameter. Measurements of the hypertensive impact values can provide information about the duration of the irregular component originating from the cardiovascular response to environmental blood pressure raising stress stimuli (86). In this study considerable importance of hypertensive impact values was found, especially in the separation of mild from moderate hypertensive groups.

In summary, this study on hypertensive patients presents a clinically relevant and feasible method to investigate the importance of different blood pressure parameters which help to avoid misclassification of hypertensive patients. Our data, in accordance with other authors' observations, confirm that further studies are necessary on a large number of hypertensive patients to determine the importance of all blood pressure parameters obtained from 24-hour ABPM recording. Concurrently, other prospective controlled trials are needed to investigate the predictive value of a various blood pressure parameter with respect to the target organ damage.

SIGNIFICANT RESULTS OF CHAPTER II.

1. We developed and introduced to human clinical pharmacological studies the programmable impedance cardiographic (PIC) measurements as a feasible, entirely automatic, noninvasive method.

2. By means of PIC measurements in a phase I/A study we demonstrate the blood pressure lowering effect of once daily treatment with 10 mg of GYKI-12743 a newly developed alpha adrenoreceptor antagonist. The peak blood pressure reducing effect of GYKI-12743 was developed in different time in accordance wit the pharmacokinetic parameters.

3. In phase IV human clinical pharmacological study we demonstrated the blood pressure lowering effect of 10 mg nifedipine administered sublingually. In addition we also demonstrated the beneficial hemodynamic effects of nifedipine as reflected in TPR, SV, CO and HR values in hypertensive patients..

4. By means of PIC measurements during a 24-hour period we demonstrated the blood lowering and beneficial hemodynamic effect of 5 mg orally administered cilazapril (first dose effect) in mild and moderate hypertensive patients. We also demonstrated a long-term effect of cilazapril treatment on left ventricular systolic and diastolic function. In addition to the commonly investigated ABPM parameters, the hypertensive index and hypertensive impact values (which include blood pressure and time elements) were investigated to estimate the efficacy of the antihypertensive treatment.

5. We investigated the clinical importance of ten different blood pressure parameters obtained from 24-hour ABPM and evaluated by PRIMA (Pattern Recognition by Independent Multicategory Analysis) method in classification

174 hypertensive patients. In this study we also investigated the discriminative power of ten blood pressure parameters and revealed the importance of the 24-hour diastolic average and hypertensive impact values in the precise classification of hypertensive patients.

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