

Epinephrine Fails to Hasten Hemodynamic Recovery in Fully Developed Canine Anaphylactic Shock

Edgar Bautista^{a,b} F. Estelle R. Simons^{c,d} Keith J. Simons^e Allan B. Becker^{c,d}
Krika Duke^{a,b} Michelle Tillett^{a,b} Wayne Kepron^{a,b,d} Steven N. Mink^{a,b,d}

^aSection of Respiriology, ^bDepartment of Internal Medicine, ^cDepartment of Pediatrics and Child Health, ^dFaculty of Medicine, ^eFaculty of Pharmacy, University of Manitoba, Winnipeg, Man., Canada

Key Words

Distributive shock · Allergy · Pharmacokinetics · Vasoactive agents · Histamine

Abstract

Background: Epinephrine (Epi) is the treatment of choice for reversing cardiovascular collapse in anaphylactic shock (AS). However, there are few data supporting its use in this condition, and most treatment guidelines have been anecdotally derived. In the present study, the time course of hemodynamic recovery from maximal hypotension was investigated in a canine model of AS in which Epi was administered by the intravenous (IV), subcutaneous (SQ) and intramuscular (IM) routes on different occasions. The findings obtained with Epi treatment were compared to those in a nontreatment study. **Methods:** Ragweed-sensitized dogs were examined in respective studies approximately 5 weeks apart in which Epi was administered by one of the above routes in a randomized design. Either Epi (0.01 mg/kg) or placebo was administered at maximal hypotension, and hemodynamics were followed for 3 h after shock. The animals were studied while ventilated and anesthetized. Mean arterial pressure (MAP), cardiac output, stroke volume (SV), pulmonary wedge pressure (Pwp) and plasma Epi concentrations were obtained at each measurement in-

terval. **Results:** In the IV study, Epi produced a transient immediate increase in MAP, SV and Pwp as compared to the nontreatment study (144 vs. 52 mm Hg; 32 vs. 12 ml; 9 vs. 5 mm Hg; $p < 0.01$), but no differences were observed 15 min after shock. Hemodynamics were not different between Epi and no treatment at any intervals when Epi was given by the SQ and IM routes. AS compared with the placebo study, plasma Epi concentrations were higher in the IV and IM studies, but not in the SQ study. **Conclusions:** Although higher Epi concentrations were observed in the IM and IV studies, a sustained benefit in hemodynamic recovery was not observed in this anesthetized, ventilated canine model. In AS, when administered during maximum shock after mediators have already been released, a single IM, IV or SQ dose of Epi may have limited utility in the treatment of cardiovascular collapse. Earlier administration of Epi, before maximal hypotension occurs, may produce a more beneficial effect.

Copyright © 2002 S. Karger AG, Basel

Introduction

Anaphylactic shock (AS) is a type I or IgE-mediated hypersensitivity reaction that develops immediately after allergen exposure in an individual sensitized to a specific

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2002 S. Karger AG, Basel
1018–2438/02/1282–0151\$18.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Steven N. Mink
Health Sciences Centre
GF-221, 700 William Ave
Winnipeg, Manitoba R3E 0Z3 (Canada)
Tel. +71 204 787 2914, Fax +1 204 787 1932, E-Mail minksn@cc.umanitoba.ca

allergen [1, 2]. Shock develops because of the rapid release of potent vasoactive mediators, such as histamine and leukotrienes, from tissue mast cells or peripheral blood basophils. This release leads to vascular permeability, vasodilation and hypovolemia because of leakage of plasma from the intravascular space. Mediators released in AS may also directly impair cardiac function, which may further compound the effect of circulatory abnormalities contributing to the hypotension that characterizes the late stages of this condition [3, 4]. Additionally, these mediators may produce bronchoconstriction and laryngeal edema, and the ensuing hypoxemia and hypercapnia may potentiate the cardiovascular dysfunction observed [5]. The overall population at risk for anaphylaxis is enormous and is estimated to lie between 1.2 and 16% in the United States [6]. Food allergy, insect stings and bites, latex rubber allergy and medication allergy are the major provoking factors [2, 7].

Epinephrine (Epi) is the treatment of choice in AS [8–10]. Currently, 0.8% of the general population in some areas carries Epi as first treatment for anaphylaxis [11]. Early studies performed in *in vitro* systems showed that when administered prior to allergen challenge, β -adrenergic stimulation of mast cells by Epi inhibited subsequent mediator release [12]. This inhibition was due to an enhanced production of adenylyl cyclase, which converts adenosine triphosphate to cyclic 3',5'-adenosine monophosphate (AMP). In turn, cyclic AMP stabilizes mast cell membranes, which prevents mediator release. Based on these *in vitro* studies and the fact that the α_1 -adrenergic effect of Epi would increase left ventricular preload by reducing venous capacitance, while its β -adrenergic effect would increase cardiac inotropy and promote bronchodilation, Epi has assumed an important role in the treatment of AS in clinical medicine [5, 8].

Nevertheless, it is important to recognize that the possible beneficial effects of Epi in attenuating AS have primarily been investigated in *in vitro* studies where Epi was administered before allergen challenge [12]. The beneficial effects of Epi administered during circulatory collapse *in vivo* when mediators have already been released have not been adequately investigated. The few studies performed so far have not demonstrated a beneficial effect on hemodynamic recovery [13–15]. In one study, Smith et al. [13] investigated the hemodynamic consequences when Epi was administered to patients undergoing desensitization to Hymenoptera venom allergen who developed shock during allergen rechallenge. There was no apparent benefit of Epi on systemic hemodynamics, and the time course of shock appeared unaltered by bolus Epi treat-

ment in these patients. In another study, Mink et al. [15] showed that when intravenous (IV) bolus Epi was given during maximal hypotension in an anesthetized, ventilated canine model of AS, there was little beneficial effect of Epi on improving the time course of recovery of cardiovascular collapse as compared with no treatment. In fact, IV Epi appeared to augment the release of thromboxane and leukotrienes during allergen challenge, suggesting that in this model, it may have had a deleterious effect in the treatment of AS once mediator release had evolved.

Furthermore, the route of Epi administration in AS is controversial. IV, intramuscular (IM) and subcutaneous (SQ) routes have been advocated in this condition [5, 8]. There is some evidence that more rapid systemic absorption and higher peak plasma levels occur after IM than after SQ administration, so that the former may be preferred over the latter route [16, 17]. However, a beneficial effect of one route over the other in terms of hemodynamic consequences has not been delineated. Because there is limited evidence to show the efficacy of Epi in improving hemodynamic recovery in AS, we compared the effect of Epi given by IV, IM and SQ routes on cardiovascular function in ragweed-sensitized dogs [16, 17]. In this model, IV ragweed challenge produces cardiovascular collapse without producing airway obstruction and respiratory dysfunction [16, 17]. We compared the time course of hemodynamic recovery from maximal hypotension when Epi was administered by the different routes during IV allergen challenge and assessed the relationship between plasma Epi concentrations and the hemodynamic recovery observed.

Materials and Methods

Anaphylaxis Model

This study was approved by the Central Animal Committee at the University of Manitoba. This investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

The ragweed model used in this study has been described previously [18, 19]. Briefly, newborn mongrel dogs received 0.5 mg of ragweed mixed with 30 mg of $\text{Al}(\text{OH})_3$ intraperitoneally within 24 h after birth. Injections were repeated weekly for 8 weeks, at biweekly intervals for approximately 30 weeks and then at monthly intervals. Sensitized animals develop bronchoconstriction in response to inhalation of the allergen and cardiovascular collapse in response to IV administration [18]. The animals were examined at approximately 1 year of age. Furthermore, it should be noted that this model is used without being entirely sure that the observed responses are not affected by anesthesia and without assurance that the results can be extrapolated to humans.

Ragweed Shock Protocol

Ragweed-sensitized dogs were examined on six different occasions approximately 3–5 weeks apart. The 3- to 5-week interval was chosen because challenges are reproducible when performed over this interval [15]. For each sensitized dog, the IV dose of ragweed needed to produce shock was known and the same dose was used in all studies. All studies were performed in a randomized manner in which either Epi (treatment study) or a similar volume of diluent (normal saline solution; placebo study) was administered by IV, IM or SQ routes. The dose of Epi administered was 0.01 mg/kg. This is the recommended dose described in the algorithm for the treatment of acute anaphylaxis in children and adults (to a maximum of 0.3 mg) [8]. By the IV route, Epi was administered through a central vein over 5–6 s as described by Smith et al. [13]; by the IM and SQ routes, Epi was administered into the left buttock [10]. For a specific route of Epi administration (i.e. IV, IM and SQ), the same dogs were examined when placebo and treatments were compared. However, the number of experiments varied between the different routes; not all of the dogs could participate in the six studies (see below), either because of the death of an animal or because of enrollment of the animal in other protocols.

In the present study, shock was defined as a reduction in mean arterial pressure (MAP) to approximately 50% of that measured at baseline (prior to allergen challenge). Since this represents severe shock, this restricts the analogy with humans to only the most severe cases of anaphylaxis. In this model, after shock was produced and when a plateau in MAP had occurred, Epi or placebo was administered by one of the three routes. Previous studies have indicated that this plateau lasts for approximately 10–15 min after challenge [15, 20]. The nadir of MAP was examined because it would be analogous to the emergency room presentation of a patient in the late stages of AS. Hemodynamics were measured before allergen challenge (i.e. at baseline), at the point of the maximal hypotensive response, immediately after treatment (approximately 1–2 min after shock) and 15, 30, 45, 60, 90, 120, 150 and 180 min after treatment. In about one half of the experiments in each study, mixed venous blood samples (see below) were collected prior to each of the hemodynamic determinations from the distal port of the Swan-Ganz catheter to assess Epi plasma levels, which were later measured by high-performance liquid chromatography [16, 21] (see below). Because of equipment problems, this measurement was obtained in the latter half of the experiments that were performed in the different studies. Furthermore, in a subset of the experiments in which the IM and SQ routes were examined, all measurements were additionally obtained 5, 10 and 20 min after shock in order to assess whether treatment produced more immediate changes in hemodynamics and Epi concentrations than those observed 15 min after shock.

Animal Preparation and Procedures

The animals were studied while anesthetized with sodium pentobarbital (30 mg/kg, IV). Based on previous studies [22], the half-life of pentobarbital anesthesia in dogs is approximately 8 h. Preshock plasma pentobarbital concentrations average <25 mg/l at baseline [23], and although concentrations fall slightly, they remained fairly constant over the course of the study (17–20 mg/l). If additional anesthesia was needed in the 120- to 180-min period, 60 mg of pentobarbital was administered over 1–2 min at least 10 min prior to obtaining the next measurement when concentrations would have plateaued.

The animals were studied while placed in the supine position. The trachea was intubated with an endotracheal tube and the lungs were mechanically ventilated at a volume of 20 ml/kg (Harvard Apparatus). The ventilator rate was increased as needed to maintain the blood pH between 7.3 and 7.4, since slight metabolic acidosis develops during challenge [15, 20]. Oxygen at a concentration of 40–50% was given to maintain arterial oxygen tension above 150 mm Hg, as was substantiated in previous studies [15, 20]. Hemodynamic measurements (see below) were obtained at end expiration with the ventilator turned off for 3 s.

All procedures were performed under sterile conditions. A thermolubrication balloon-tipped catheter was used to measure mean pulmonary arterial pressure, mean pulmonary capillary wedge pressure (Pwp) and mean right atrial pressure as well as to determine thermolubrication cardiac output (CO) (Columbus Instruments, Ohio, USA). The average of three CO determinations was reported. A polyethylene catheter was placed into the femoral artery and advanced to the mid-descending aorta to measure MAP and to withdraw samples of blood for arterial blood gases. All catheters were connected to transducers (Cobe Laboratories) and were referenced relative to the left atrium. All transducers were connected to a chart recorder (Astro Med, West Warwick, R.I., USA). Stroke volume (SV) was calculated from the following formula: $SV = CO/\text{heart rate}$. Systemic vascular resistance was calculated from the following formula: $\text{systemic vascular resistance} = [(MAP - \text{mean right atrial pressure})/CO] \times 80 \text{ dyn s/cm}^5$. Pulmonary vascular resistance was calculated from the following formula: $\text{pulmonary vascular resistance} = [(\text{mean pulmonary arterial pressure} - Pwp)/CO] \times 80 \text{ dyn s/cm}^5$. Baseline measurements were made after 30 min of stabilization. Baseline Pwp was approximately 5 mm Hg, and no fluids were administered throughout the study. Following completion of the experiment, vascular accesses were removed and hemostasis was obtained. The animals were then given prophylactic antibiotics (cloxacillin, 10 mg/kg, IV, and gentamicin, 2 mg/kg, IV) and returned to their cages.

Sham Shock Protocol

In another group of studies, Epi was administered by either the IV, IM or SQ route to dogs in a nonshocked condition and the protocol was repeated. In the sham shock protocol, the procedures and randomization were identical to those used in the ragweed shock protocol, but normal saline solution rather than allergen was administered. Hemodynamics were monitored for 3 h after shock. The objective of the sham shock protocol was to compare hemodynamics and plasma Epi concentrations when Epi was administered by the different routes in the shocked versus the nonshocked condition.

Epi Plasma Concentrations

When hemodynamic measurements were obtained, 3 ml of mixed venous blood were withdrawn from the distal port of the pulmonary artery catheter for determinations of plasma Epi concentrations. Samples were placed into a tube with EDTA and centrifuged at 4°C. Then, plasma was transferred into a labeled polypropylene tube with a screw cap, frozen promptly in an upright position and stored at –20°C until analysis. After thawing the plasma, solid/liquid phase extraction was performed, with an efficiency of 75–80%. Epi plasma levels were measured with a high-performance liquid chromatography [16–21] reverse-phase system (Waters Corp., Milford, Mass., USA) with electrochemical detection. With modification of this assay, it was possible to detect as little as 5 pg/ml (0.025 nmol/ml) of Epi. Calibration curves were linear over the range 25–1,000 pg/ml,

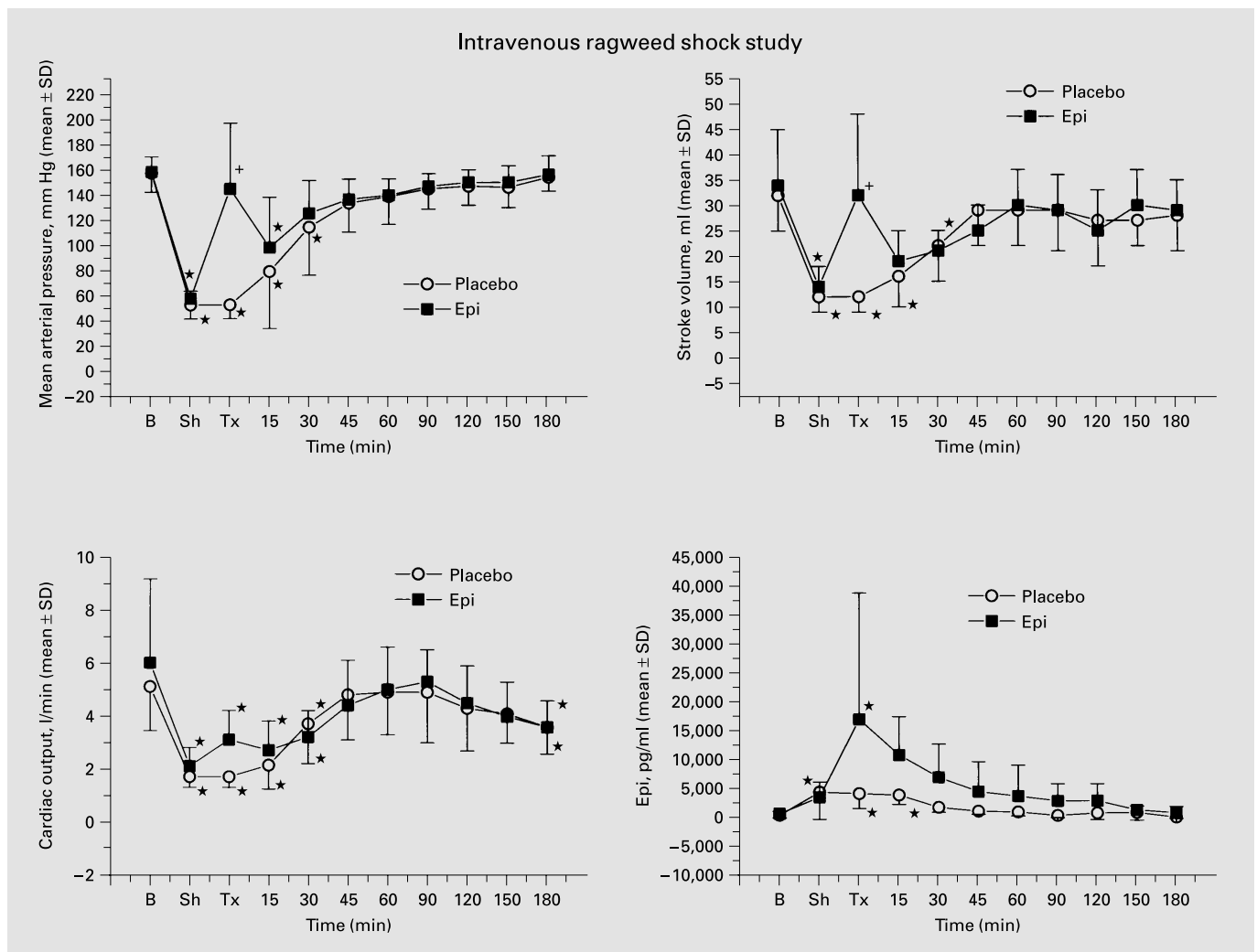


Fig. 1. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the IV Epi ragweed shock study. Measurements were obtained at baseline (B), shock (Sh), treatment (Tx) and until 3 h after shock. $n = 6$ for all parameters at all intervals. Statistics by two-way ANOVA and SNK: $^+ p < 0.05$ between studies; $*$ $p < 0.05$ versus baseline.

with a coefficient of variation of 3% at 1,000 pg and 10% at 25 pg. Epi concentrations were obtained in approximately one half of the experiments in each study.

Statistical Analyses

Each treatment study was compared to a placebo study by means of a two-way analysis of variance (ANOVA) for two repeated measures [i.e. factor A (treatments) and factor B (time periods)] in which the interaction between the two factors was assessed (Northwest Analytical, Portland, Oreg., USA). If the $A \times B$ interaction term was significant ($p < 0.05$), then this indicated that the studies proceeded differently over the course of the experiment [24]. In that case, Student Newman-Keuls' multiple-range test (SNK) was used to determine at which specific time interval a difference between studies occurred. An SAS program (Proc Univariate, SAS Institute, Cary,

N.C., USA) was used to test whether there was a normal distribution of the data; for all of the variables analyzed, the data did not violate this assumption [25]. A one-way ANOVA for repeated measures and SNK were used to ascertain differences between time conditions for a given study (i.e. between baseline and maximal hypotensive response intervals). Because we were mainly interested in differences between treatment and control studies, we did not report all the statistics for a given study over the 3-hour course of the study. The area under the curve (AUC) for Epi concentrations was calculated over the interval of the study [16]. A one-way ANOVA was used to compare the results obtained in the Epi and placebo studies. During shock, our sample size was of 90% power to detect a MAP difference between the treatment and placebo studies of 15 mm Hg, a CO difference of 1 liter/min and an SV difference of 7 ml. Results are reported as mean $\pm$ 1 SD.

Table 1. Pwp (mm Hg, mean $\pm$ SD) in the different studies

	Baseline	Ragweed	Tx	5	10	15	20	30	45	60	90	120	150	180
a Shock with placebo														
IV (n = 6)	9±1	5±1	5±1	–	–	5±1	–	6±1	6±1	7±2	7±1	7±1	7±1	8±2
IM (n = 9)	10±3	6±2	6±1	6±1	6±1	6±2	7±2	8±2	8±2	8±3	8±2	9±2	8±2	8±3
SQ (n = 11)	10±2	6±2*	7±2*	7±2*	7±2	6±2	7±1	7±2	8±1	8±2	8±2	8±2	8±2	9±2
b Shock with Epi														
IV (n = 6)	10±3	6±3	9±5 ⁺	–	–	7±2	–	7±2	7±2	10±2	8±3	8±2	7±2	9±2
IM (n = 9)	10±2	7±1	6±1	5±6	6±1	6±2	6±1	7±1	8±2	8±2	8±1	8±1	8±2	9±2
SQ (n = 11)	9±2	6±3*	7±2*	7±1	7±1	6±1	7±1	7±2	7±2	8±1	8±2	8±2	8±2	8±2
	Baseline	Sham	Tx	15	30	45	60	90	120	150	180			
c Sham shock with placebo														
IV (n = 6)	11±1	11±2	11±2	10±1	9±1	9±2	9±3	9±2	8±2	9±3	10±3			
IM (n = 9)	10±1	10±2	10±2	9±2	9±1	9±2	9±2	9±2	9±2	9±3	9±3			
SQ (n = 11)	10±1	10±2	10±2	9±2	9±1	9±2	9±2	9±2	8±2	9±3	9±3			
d Sham shock with Epi														
IV (n = 6)	9±4	9±3	21±6 ^{*, +}	8±2	8±3	8±2	8±3	8±3	8±5	8±4	8±2			
IM (n = 9)	7±2	8±1	8±1	8±2	8±2	8±2	8±2	8±3	8±2	8±1	8±1			
SQ (n = 11)	9±1.5	10±2	10±2	10±2	9±1	9±1	10±2	10±3	8±2	8±2	9±2			

The numbers in the header of each part of the table represent posttreatment time (in minutes). Tx = Treatment. ⁺ p < 0.05 for Epi versus placebo study; * p < 0.05 versus baseline (ANOVA and SNK).

Results

For each dog, the same dose of ragweed (range 1–8 mg, mean $\pm$ SD, 3.7 $\pm$ 0.6 mg) was used in each study. The results observed in the IV ragweed shock protocol are shown in figure 1. The declines in MAP, CO, SV (fig. 1) and Pwp (table 1) between baseline and shock were similar in the placebo and treatment studies. In the placebo study, after the initial fall in hemodynamic parameters after shock, there was a gradual return from maximal hypotension to preshock values over the 3-hour course of the experiment. MAP, CO, SV and Pwp had returned to baseline values by 45 min after shock.

During shock, plasma Epi increased from approximately 500 pg/ml at baseline to approximately 4,000 pg/ml after challenge in both the placebo and Epi studies. In the treatment study, peak Epi increased to approximately 17,000 pg/ml after treatment, after which there was a gradual decline in concentrations over the remainder of the study. As assessed by the AUC, Epi concentrations after treatment were significantly higher than those found after placebo (table 2). In terms of hemodynamics, when Epi was administered, there were significant transient elevations in MAP, SV and Pwp as compared with the placebo

Table 2. AUC for plasma Epi concentrations (ng/ml, mean $\pm$ SD)

	Placebo	Epi
Ragweed shock		
IV	267.90 $\pm$ 113.4	854.90 $\pm$ 508.90 ⁺
IM	230.65 $\pm$ 775.90	397.35 $\pm$ 268.30
SQ	267.90 $\pm$ 113.38	319.52 $\pm$ 229.50
Sham shock		
IV	78.69 $\pm$ 25.62	144.02 $\pm$ 53.15
IM	92.96 $\pm$ 29.09	159.12 $\pm$ 42.51 ⁺
SQ	92.53 $\pm$ 29.02	127.49 $\pm$ 70.31

⁺ p < 0.05 for Epi versus placebo study by repeated-measures ANOVA.

bo study. However, these changes were transient. By 15 min after shock, all parameters in the treatment study were similar to those observed in the placebo study and there were no differences between the two studies over the remainder of the experiment.

The results obtained in the IM study in the ragweed shock protocol are shown in figure 2. The declines in

Intramuscular ragweed shock study

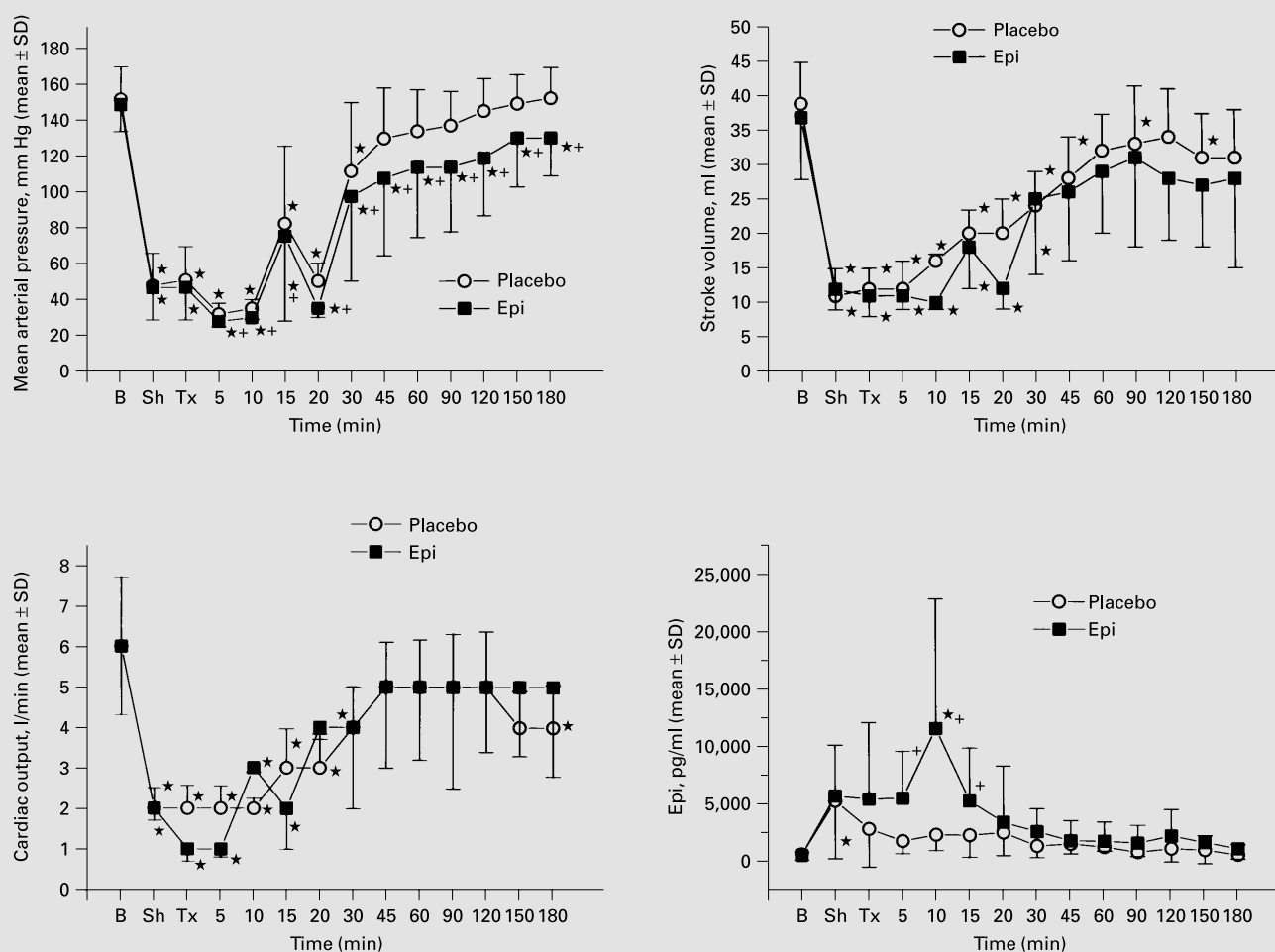


Fig. 2. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the IM Epi ragweed shock study. Measurements were obtained at baseline (B), shock (Sh), treatment (Tx) and until 3 h after shock. For the hemodynamic measurements, $n = 9$ for all parameters at baseline, maximal hypotensive response, treatment, 15, 30, 45, 60, 90, 120, 150 and 180 min, and $n = 3$ at 5, 10 and 20 min. For the Epi measurements, $n = 6$ at baseline, maximal hypotensive response, 15, 30, 45, 60, 90, 120, 150 and 180 min, and $n = 3$ at 5, 10 and 20 min. Statistics by two-way ANOVA and SNK: + $p < 0.05$ between studies; * $p < 0.05$ versus baseline.

MAP, CO, SV (fig. 2) and Pwp (table 1) between baseline and shock were similar in the placebo and treatment studies. Over the course of the experiment, CO, SV and Pwp were not different in the two studies. In the treatment condition, MAP was often lower than that found in the placebo study over the course of the study, even though MAP measured at maximal shock was nearly identical between studies.

In both the treatment and placebo IM studies, serum Epi increased from approximately 500 pg/ml at baseline to approximately 4,000 pg/ml during shock. As compared with the placebo study, higher plasma Epi was observed in the treatment group 5, 10 and 15 min after shock, after which there were no differences between the two studies.

The results obtained in the SQ study in the ragweed shock protocol are shown in figure 3. The declines in

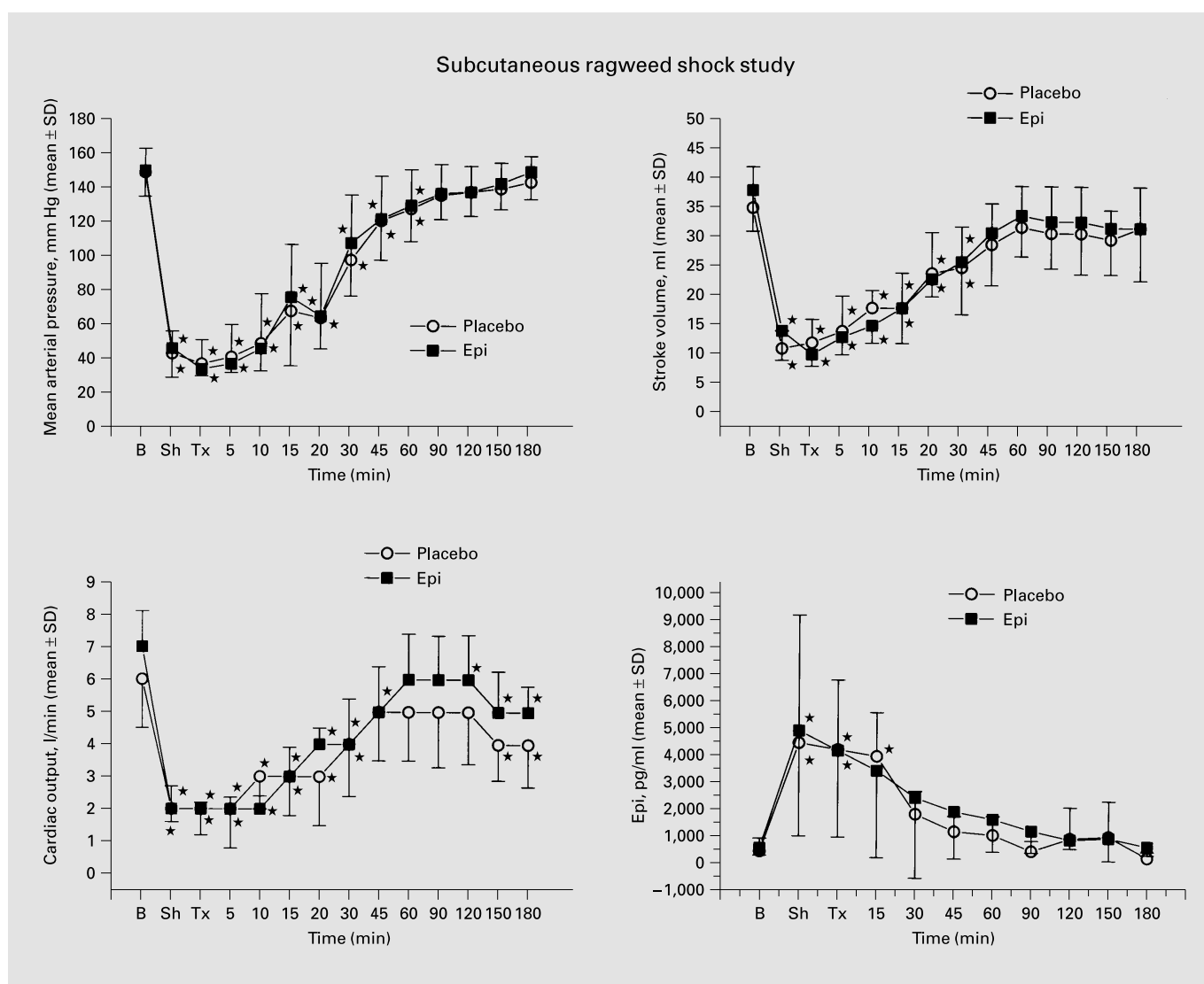


Fig. 3. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the SQ Epi ragweed shock study. Measurements were obtained at baseline (B), shock (Sh), treatment (Tx) and until 3 h after shock. For the hemodynamic measurements, $n = 11$ for all parameters at baseline, maximal hypotensive response, 15, 30, 45, 60, 90, 120, 150 and 180 min, and $n = 5$ at 5, 10 and 20 min. For the Epi measurements, $n = 9$ at baseline, maximal hypotensive response, 15, 30, 45, 60, 90, 120, 150 and 180 min, and $n = 3$ 5, 10 and 20 min after shock. Statistics by two-way ANOVA and SNK: * $p < 0.05$ versus baseline.

MAP, CO, SV (fig. 3) and Pwp (table 1) between baseline and shock were similar in the placebo and treatment studies. Plasma Epi increased from approximately 500 pg/ml at baseline to approximately 4,000 pg/ml during shock in both studies. There were no differences in hemodynamics or in plasma Epi concentrations between the two studies over the course of the experiment.

The results obtained in the IV sham shock study are shown in figure 4 and tables 1 and 2. In the placebo study, there were no changes in hemodynamics or Epi concentrations over the course of the study. When Epi was administered in the treatment study, there were transient increases in MAP, CO, Pwp and SV as compared with the values found in the placebo study. Immediately after treatment, plasma Epi concentrations in the treatment

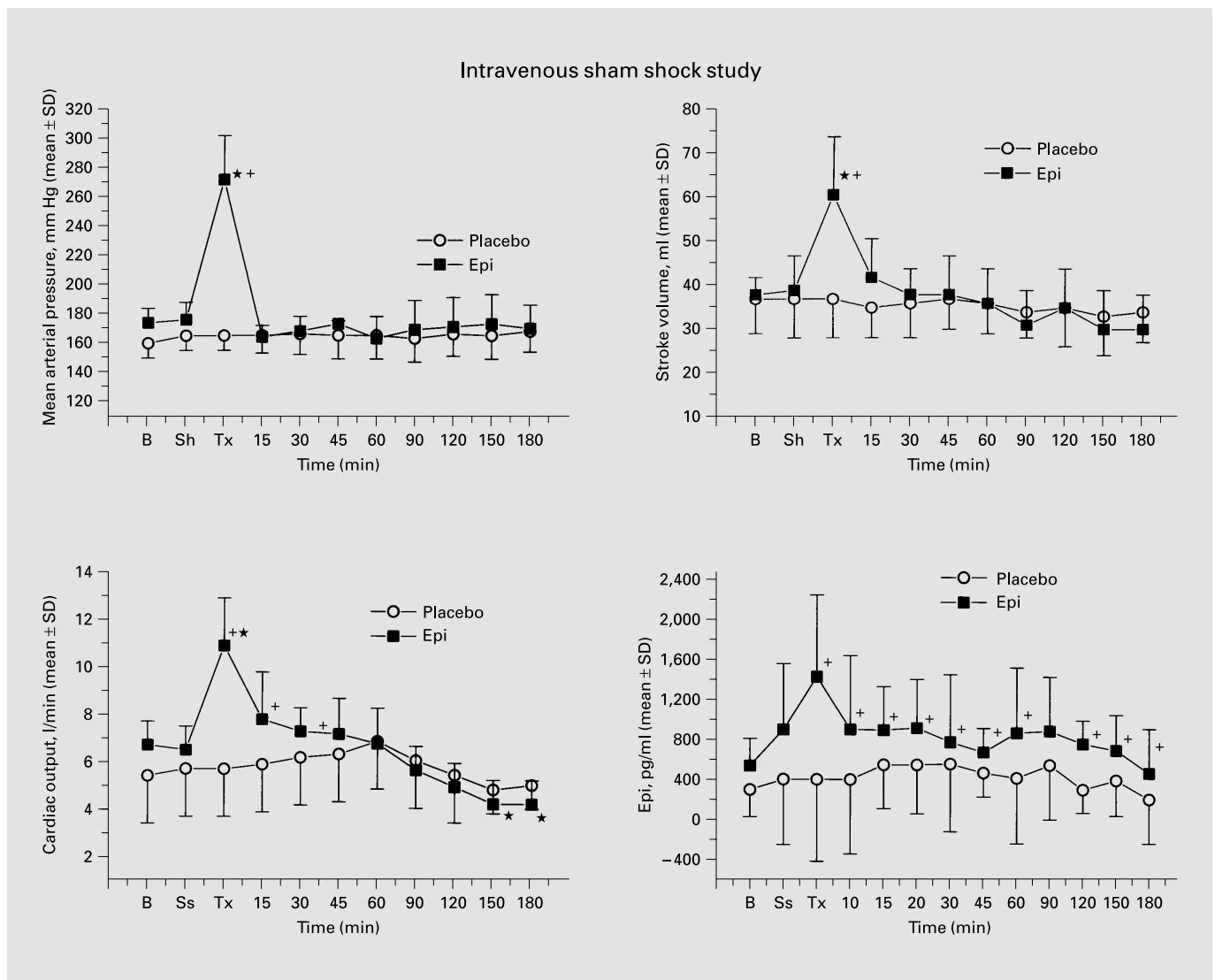


Fig. 4. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the IV Epi sham shock study. Measurements were obtained at baseline (B), sham shock (SS), treatment (Tx) and until 3 h after shock. For the hemodynamic measurements and Epi measurements, $n = 6$ for all parameters. Statistics by two-way ANOVA and SNK: $+$ $p < 0.05$ between studies; $*$ $p < 0.05$ versus baseline.

study peaked as compared with the placebo study and then slowly declined over the course of the experiment.

The results obtained in the IM sham shock study are shown in figure 5 and tables 1 and 2. MAP was not different over the course of the study between the placebo and Epi studies. CO in the treatment study was slightly higher than that found in the placebo study 120, 150 and 180 min after treatment. Epi concentrations in the treatment study were generally higher than those found in the placebo study, but no clear peak in Epi concentrations

was observed in the treatment study. As assessed by the AUC, Epi concentrations in the treatment study were significantly higher than those found in the placebo study (table 2).

The results obtained in the SQ sham shock study are shown in figure 6 and tables 1 and 2. There were no differences in hemodynamics or plasma Epi concentrations between the two studies over the course of the experiment.

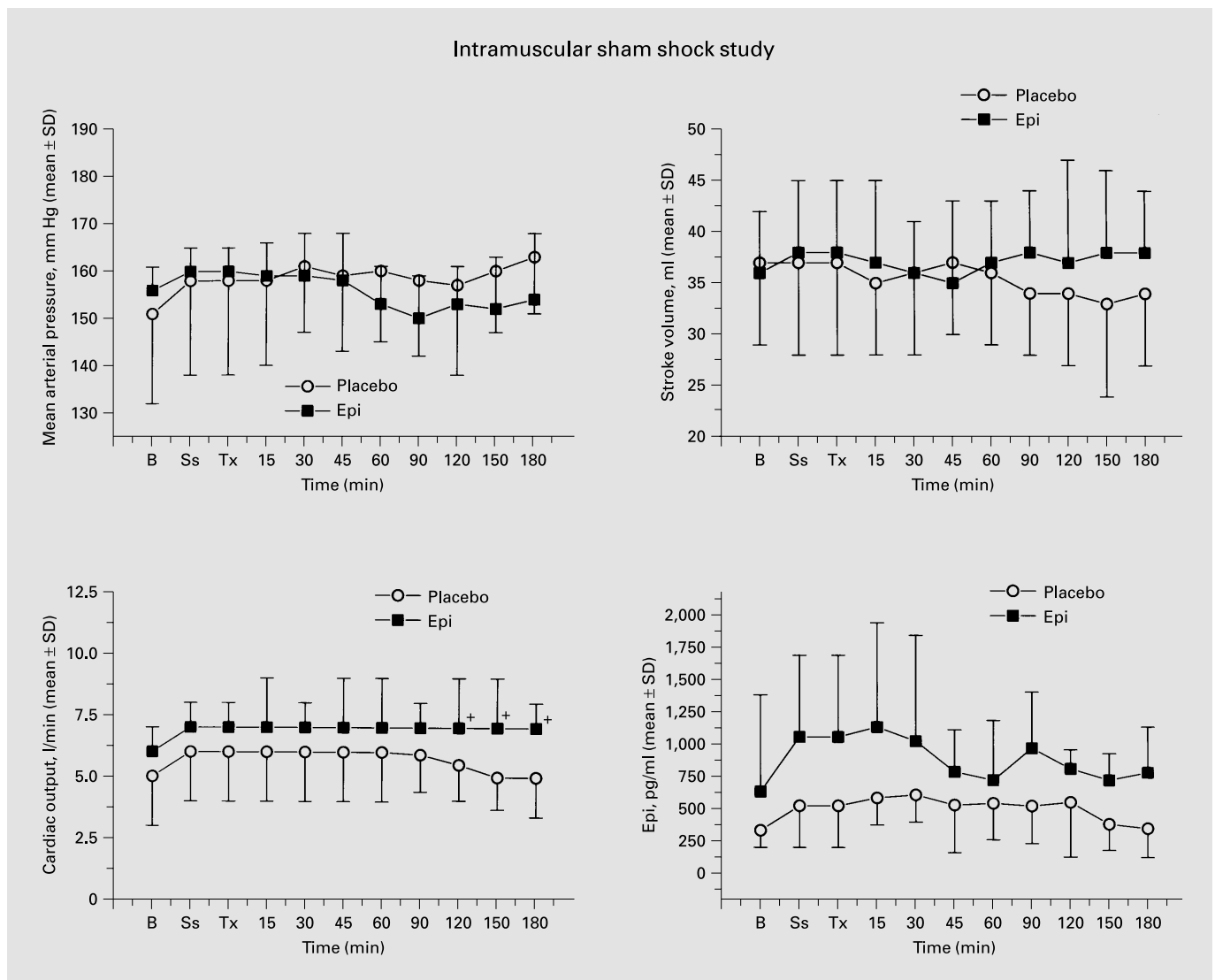


Fig. 5. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the IM Epi sham shock study. Measurements were obtained at baseline (B), sham shock (SS), treatment (Tx) and until 3 h after shock. For the hemodynamic and Epi measurements, $n = 6$ for all parameters. Statistics by two-way ANOVA and SNK: $^+ p < 0.05$ between studies.

Discussion

The primary objective of this study was to determine the effect of Epi on hemodynamic recovery in a canine model of AS when Epi was given by the IV, IM and SQ routes. Epi was administered during maximum cardiovascular collapse, when mediators had already been released, so that the protocol would simulate the emergency treatment of the late stage of AS in humans [2]. The Epi dose chosen was based on current recommendations for

the initial treatment of anaphylaxis [8], which is less than the 1-mg dose used for cardiopulmonary resuscitation [26]. Even in humans, the dose of Epi has been empirically derived, and the pediatric dose of 0.01 mg/kg chosen was previously shown to produce hemodynamic changes when given by the IV route in a canine study [15]. Based on the latter results, we used the same dose in the different treatment conditions in the present study.

In both the IV and IM ragweed shock studies, plasma Epi concentrations in the treatment study were higher

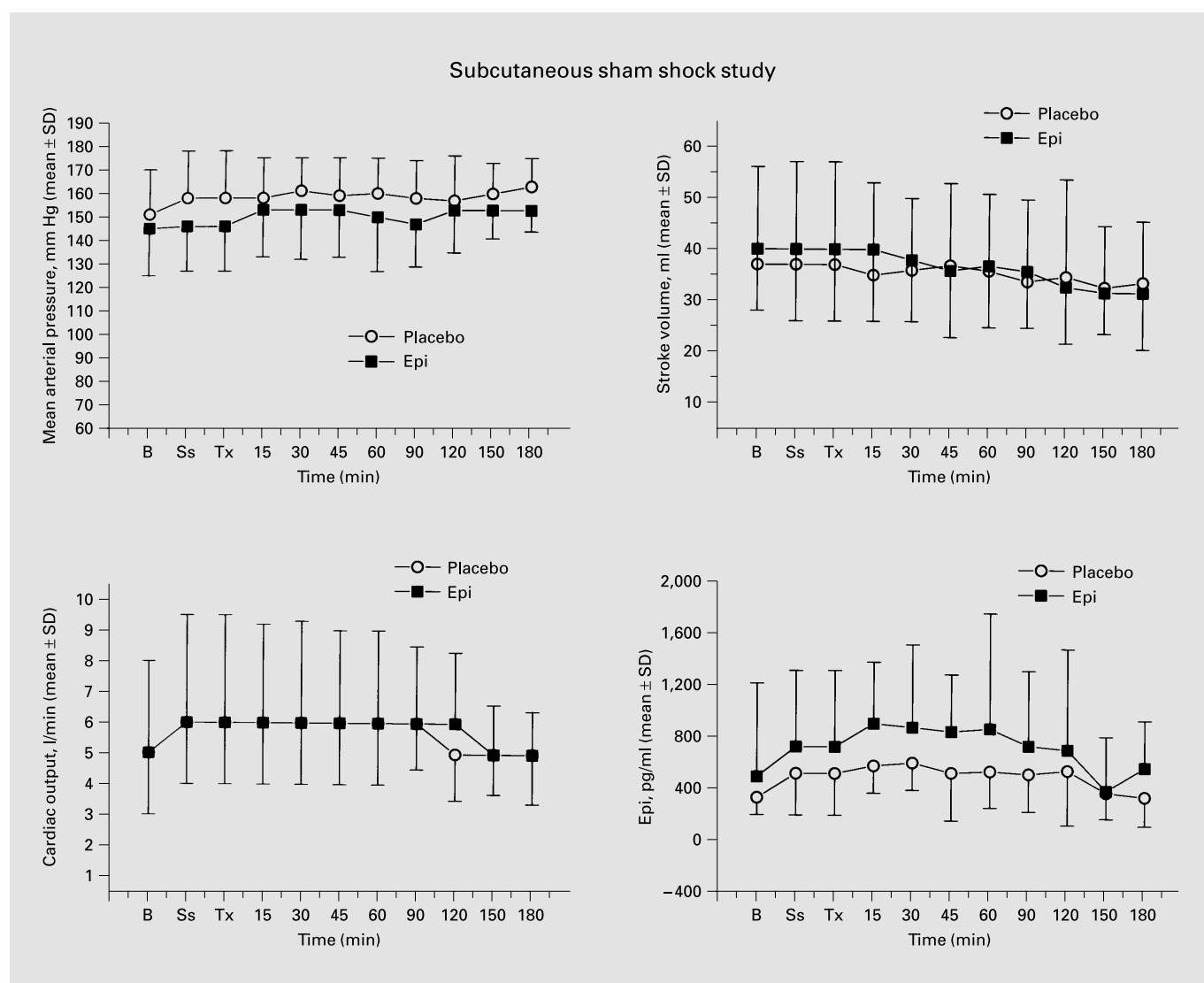


Fig. 6. Hemodynamic parameters and plasma Epi concentrations for experiments performed in the SQ Epi sham shock study. Measurements were obtained at baseline (B), sham shock (SS), treatment (Tx) and until 3 h after shock. For the hemodynamic and Epi measurements, $n = 6$ for all parameters.

than those found in the placebo study. In the IV treatment study, Epi produced an immediate improvement in MAP, SV and Pwp as compared to the placebo study. In terms of the protocol, without any treatment, the reduction in MAP found after ragweed challenge would otherwise last for 10–15 min. Once this plateau was recognized, either Epi or the placebo was given approximately in the middle of the plateau. In the treatment study, an immediate increase in hemodynamics was observed; however, by 15 min after shock, there was no difference between the

treatment and nontreatment studies. As much as possible, the interval between shock and 15 min after shock would reflect the identical period of ongoing anaphylaxis in the two studies.

In the IM treatment study, Epi produced no improvement in cardiovascular parameters, even though concentrations 5, 10 and 15 min after shock were higher than those found in the placebo study. In the SQ treatment study, no changes in hemodynamic recovery or Epi concentrations were observed between the placebo and treat-

ment studies. Thus, although higher Epi concentrations were observed in the IM and IV studies, a sustained benefit in hemodynamic recovery was not observed in this anesthetized, ventilated canine model.

The rationale for the use of Epi in the treatment of AS is based on the understanding that β -adrenergic agents such as epinephrine increase cellular levels of cyclic AMP [12]. The cyclic AMP system has been shown to modulate the immunological release of histamine and other mediators in isolated tissue preparations. Studies with human lung and nasal polyp fragments demonstrated a bidirectional effect of tissue levels of cyclic AMP, with elevations suppressing mediator release and diminutions enhancing release [27, 28]. β -adrenergic agents increase cellular levels of cyclic AMP and inhibit the antigen-induced release of histamine and other anaphylactic mediators. In contrast, α_1 -adrenergic stimulation causes a decrease in tissue levels of cyclic AMP, enhancing the release of anaphylactic mediators during allergen challenge [12]. In addition to its favorable effect on mediator release, Epi has a beneficial hemodynamic profile that would be useful for the treatment of AS. Epi causes an increase in cardiac inotropy and venous and systemic vasoconstriction and a decrease in laryngeal edema and promotes bronchodilation [5]. All of these attributes might be life-saving in the treatment of AS.

Nevertheless, although Epi has assumed an important role in the treatment of AS in clinical medicine, there are few data to support its use once shock has developed, especially at the dose (i.e. 0.01 mg/kg) usually recommended for AS [10]. Smith et al. [13] investigated the hemodynamic consequences of Epi in patients undergoing desensitization to Hymenoptera allergen who developed shock during allergen rechallenge. Of the 22 patients studied, 14 patients developed systemic anaphylaxis. In 11 of the 14 patients, the reactions were entirely cutaneous, whereas in the remaining 3 patients, hypotension developed. Thus, 3 of the 22 patients or approximately 14% of the population who were challenged developed significant symptoms that required treatment with Epi. Smith et al. [13] found that standard therapy with IV bolus Epi and fluids did not immediately reverse the hemodynamic abnormalities observed. There was a gradual recovery in blood pressure after shock that did not seem directly related to any specific therapeutic intervention administered. Muelleman et al. [14] also examined the effects of IV bolus Epi on hemodynamics in a rabbit model of anaphylactic shock. Epi resulted in an increased MAP over normal saline 2 min after treatment, but there were no differences in MAP detected beyond 4 min after

treatment between the two groups. Survival also was not different between the saline- and Epi-treated groups.

In a related study, Mink et al. [15] assessed the effect of Epi on hemodynamic recovery and mediator release in ragweed-sensitized dogs. Serum mediators of anaphylaxis, including histamine, leukotrienes, metabolites of prostaglandins and thromboxane, were measured after shock over the time course of the study. After IV bolus Epi treatment, the hemodynamic results were similar to those found in the present study, in that there was only a transient effect in improving hemodynamics, and no sustained effect on recovery was found. In that study, since IV Epi caused an increase in the concentrations of leukotrienes and thromboxane after shock as compared with no treatment, the authors concluded that Epi may have a deleterious effect on mediator release in AS.

Accordingly, although in vitro data suggest that Epi may be of value in the treatment of AS, the extent to which Epi may hasten recovery in vivo when mediators have already been released and when hemodynamic collapse is maximal is not clear. The results of the present study would argue that Epi administered in the context of full-blown shock does not hasten hemodynamic recovery in this model. In the IV treatment study, the hemodynamic consequences were short-lived and were related to the very high Epi concentrations achieved. In the IM and SQ studies, no beneficial effect of Epi on hemodynamics was observed as compared with the placebo studies, although modestly higher Epi concentrations were observed in the IM treatment study as compared with the placebo study. The results of the present study would argue that when administered after mediators have been released, Epi does not have a sustainable impact on improving hemodynamic recovery in this model of AS. It should also be noted that in a related study [29], we examined the effects of histamine H_1 and H_2 blockers, a leukotriene inhibitor and a cyclooxygenase inhibitor on cardiovascular collapse in this model. When the animals were pretreated with the H_1 blocker, a higher dose of ragweed was required to produce shock as compared to animals in the nontreated condition. However, once shock was produced, the degree of shock was not different between animals in the treated and untreated conditions. Thus, antihistamines produced little effect once full-blown shock was produced.

In the literature, the choice of route and dose of Epi administration is for the most part based on anecdotal experience. Recently, Simons et al. [16] prospectively compared the SQ and IM routes of Epi administration in allergic children at risk for anaphylaxis to food, Hyme-

noptera venom or other substances. They injected a dose similar to the one used in the present study either subcutaneously or intramuscularly in respective groups of children and monitored plasma Epi concentrations, heart rate, blood pressure and adverse effects over the course of the study. In subjects who received SQ Epi, the mean maximum plasma concentrations were delayed (mean time of peak concentrations was 34 min) as compared with the IM route (8 min). Mean peak concentrations were found to increase from approximately 400 pg/ml at baseline to 1,802 pg/ml in the SQ study and 2,136 pg/ml in the IM study. In a more recent study, similar results were reported in adults [17].

In the present study, plasma Epi profiles observed in the IM and SQ sham shock studies in this canine model were similar to those found by Simons et al. [16] in humans. In the IM sham shock study, the Epi concentrations observed 5, 10 and 15 min after shock in the treatment study were approximately 1,000–1,500 pg/ml higher than those in the placebo study, although there was wide variation between dogs. In the SQ sham shock study, although significant differences in Epi concentrations between the treatment and the placebo study could not be observed, Epi concentrations between 30 and 180 min generally paralleled those reported by Simons et al. [16].

Simons et al. [16] also monitored heart rate and blood pressure intermittently as part of their protocol. They observed that these parameters increased in only a few children at the time of peak Epi concentrations in their study. In another study in children weighing 15–30 kg and at risk for anaphylaxis, the EpiPen Auto-Injector (0.3 mg of Epi; Allerex Laboratory Ltd., Kanata, Ont. Canada) was compared with the EpiPen Jr (0.15 mg of Epi; Allerex). Side effects, including an increase in systolic blood pressure and tremor, anxiety and palpitations, were greater at the higher dose and peaked 30 min after the dose was given [30]. The authors concluded that for the out-of-hospital treatment of anaphylaxis, additional premeasured, fixed doses of Epi would facilitate more precise dosing in young children. In the present study, the recommended IM and SQ Epi dose of 0.01 mg/kg was given in all experiments, and this dose was insufficient to produce hemodynamic changes in the nonshocked condition.

Accordingly, the relevant questions to address in the treatment of AS are how much Epi is needed to promote hemodynamic recovery when mediators have been released and what is the physiological rationale for the use of Epi in this condition? As shown in figures 1–3, when shock occurs, the endogenous Epi response is an elevation in plasma Epi concentrations from approximately 500 pg/

ml at baseline to nearly 5,000 pg/ml during challenge. Yet, even though the endogenous response was quite large, it was insufficient to immediately reverse the cardiovascular collapse associated with allergen challenge. In the IM ragweed shock study, exogenous Epi caused an additional increase of 1,000–2,000 pg/ml at 5–15 min as compared to the values found in the placebo study, while in the SQ study, the increase in Epi was trivial. Thus, compared to the endogenous Epi release, the additional amount of Epi provided by IM and SQ routes is minuscule. However, in the IV ragweed shock study, Epi treatment caused a very large increase in concentration that was sufficient to augment hemodynamics. Yet, this augmentation was short-lived, and when Epi plasma concentrations declined, hemodynamic parameters also decreased.

These results would therefore suggest that in the treatment of anaphylaxis, once mediators have been released, Epi acts predominantly as a vasopressor in augmenting hemodynamic recovery and has no special pharmacological properties to hasten immunological recovery. Once full-blown anaphylaxis occurs, Epi appears to enhance hemodynamic recovery, acting mainly through its α_1 -adrenergic properties. Smith et al. [13] reached similar conclusions in the treatment of AS due to Hymenoptera allergen rechallenge, where an agent such as norepinephrine with predominantly α_1 -adrenergic effects produced similar increases in MAP as those found with IV Epi bolus. Thus, the rationale of using Epi based on in vitro studies may not be relevant to the situation when shock has already occurred.

In the present study, it is important to note that we were only concerned with the hemodynamic consequences of anaphylaxis, since respiratory changes are minor when IV allergen is given in this model. We maintained arterial oxygen tension at >150 mm Hg and pH within the normal range, to prevent hemodynamic deterioration due to respiratory consequences. In clinical AS, it is acknowledged that bronchospasm and laryngospasm may benefit from Epi treatment [5]. It is also recognized that hemodynamics were examined while the animals were anesthetized, since it would be impossible to study these animals in the conscious condition. Of the anesthetic agents available, all would affect some aspect of the anaphylactic response in our model (i.e. histamine release and/or bradycardia by narcotic agents [31] and vasodilation by inhalational agents [32]). Since pentobarbital anesthesia is often used for investigating hemodynamics in allergic models [33], we administered this anesthesia in the present study. The dosing of pentobarbital anesthesia was identical in all studies, and the dose used was shown

to not produce myocardial dysfunction in a previous study [23]. Since it is not possible to exclude an effect of anesthesia on our results, we recognize that our findings must be interpreted in that context.

In summary, the present study does not demonstrate any sustained beneficial effect of a single dose of IM, IV or SQ Epi on hemodynamics when administered during maximal shock in a canine model of anaphylaxis. Nevertheless, it does not preclude the possibility that if Epi were given on a repetitive schedule or if a higher dose were used, a beneficial effect would be observed. Furthermore, it is important to note that this study does not reflect the full spectrum of anaphylaxis found in the clinical situation. Therefore, this study does not preclude the possibility that when given early by the IM route, Epi may be helpful in hypotension of mild to moderate severity. In addition, this study does not state that Epi should not be used in the treatment of anaphylaxis. Indeed, failure to inject Epi promptly has been identified as the most important factor contributing to death in patients with this disorder [34, 35]. Thus, it is possible that a beneficial effect of Epi could be observed if treatment were instituted immediately after allergen challenge at the onset of

AS, before the total release of mediators was completed. This research strengthens the pharmacological evidence for continuing to recommend that Epi should be given early rather than late in the treatment of AS in humans. In that case, the β -adrenergic effect of Epi may attenuate the cardiovascular collapse that accompanies AS. While the relationship of animal models to the human condition must be interpreted cautiously, the present results obtained in a canine model would support those reported by Smith et al. [13] in human subjects that a single dose of Epi does not hasten the time to hemodynamic recovery in severe AS. The role of Epi in the treatment of AS and the extent to which higher dosing and repetitive dosing are beneficial need to be further investigated in human and animal studies.

Acknowledgments

This study was supported by the Children's Hospital Research Foundation. The work was performed while E.B. was a Fellow of the Manitoba Lung Association.

References

- 1 Atkinson TP, Kaliner MA: Anaphylaxis. *Med Clin North Am* 1992;76:841–855.
- 2 Lieberman P: Anaphylaxis and anaphylactoid reactions; in Middleton E, Ellis EF, Yungster JW, Reed CE, Adkinson NF, Buse WW (eds): *Allergy: Principles and Practice*, ed 5. St. Louis, Mosby, 1998, pp 1079–1092.
- 3 Burke JA, Levi R, Guo ZG, Corey EJ: Leukotrienes C₄, D₄ and E₄: Effects on human and guinea-pig cardiac preparations in vitro. *J Pharmacol Exp Ther* 1982;221:235–241.
- 4 Felix S, Baumann G, Helmus S, Sattelberger U: The role of histamine in cardiac anaphylaxis: Characterization of histaminergic H₁- and H₂-receptor effects. *Basic Res Cardiol* 1988;83: 531–539.
- 5 Roizen ME, Levy J, Moss J: Anaphylactic and anaphylactoid reactions; in Hall JB, Schmidt GA, Wood LDH (eds): *Principles of Critical Care*, ed 2. New York, McGraw-Hill, 1998, pp 1595–1604.
- 6 Miller R, Ghatek A, Rothman P, Neugut A: Anaphylaxis in the United States: An investigation into its epidemiology. *J Allergy Clin Immunol* 2000;105:S349.
- 7 Slater JE, Mostello LA, Shaer C: Rubber-specific IgE in children with spina bifida. *J Urol* 1991;146:578–579.
- 8 Niclas RA, Bernstein IL, Li JT, Lee FE, Spector SL, Dykewics MS, Fineman S, Berger WE, Blessing-Moore J, Schuller DE: Diagnosis and management of anaphylaxis. *J Allergy Clin Immunol* 1998;101:S465–S498.
- 9 Barach EM, Nowak RM, Lee TG, Tomalano-vich MC: Epinephrine for treatment of anaphylactic shock. *JAMA* 1984;251:2118–2122.
- 10 Simons FER, Chad Z, Dean JM, Watson WTA: Position statement: Fatal anaphylactic reactions to food in children. *Can Med Assoc J* 1994;150:337–339.
- 11 Black CD, Peterson S, Simons FER: Epinephrine for outpatient treatment of anaphylaxis: A population based study. *J Allergy Clin Immunol* 2001;107:S58.
- 12 Orange RP, Kaliner MA, Laria PJ, Austen KF: Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. 2. Influence of cellular levels of cyclic AMP. *Fed Proc* 1971;30:1725–1728.
- 13 Smith PL, Kagey-Sobotka A, Bleecker ER, Traystman R, Kaplan AP, Gralnick H, Valentine MD, Permutt S, Lichtenstein LM: Physiologic manifestations of human anaphylaxis. *J Clin Invest* 1980;66:1072–1080.
- 14 Muelleman RI, Pribble JP, Salomone JA 3rd: Blood pressure effects of thyrotropin-releasing hormone and epinephrine in anaphylactic shock. *Ann Emerg Med* 1988;17:309–313.
- 15 Mink SN, Bands C, Becker A, Elkin J, Sharma S, Unruh H, Kepron W: Effect of bolus epinephrine on systemic hemodynamics in canine anaphylactic shock. *Cardiovasc Res* 1998;40: 546–556.
- 16 Simons FER, Roberts JR, Gu X, Simons KJ: Epinephrine absorption in children with a history of anaphylaxis. *J Allergy Clin Immunol* 1998;101:33–37.
- 17 Simons FER, Gu X, Simons KJ: Epinephrine absorption in adults. *J Allergy Clin Immunol* 2001;108:871–873.
- 18 Kepron W, Jackson C, Sehon A: A canine model for the study of hapten-specific suppression of IgE-mediated bronchoconstriction in anaphylaxis. *Int Arch Allergy Appl Immunol* 1987; 82:468–470.
- 19 Kepron W, James J, Kirk B, Sehon A, Tse K: A canine model for reaginic hypersensitivity and allergic bronchoconstriction. *J Allergy Clin Immunol* 1977;59:64–69.
- 20 Correa E, Mink S, Unruh H, Kepron W: Left ventricular contractility is depressed in IgE-mediated anaphylactic shock in dogs. *Am J Physiol* 1991;260:H744–H751.
- 21 Hjemdahl P: Plasma catecholamines – analytical challenges and physiological limitations. *Baillieres Clin Endocrinol Metab* 1993;7:307–353.

- 22 Frederiksen M, Henthorn T, Ruo T, Atkinson A: Pharmacokinetics of pentobarbital in dogs. *J Pharmacol Exp Ther* 1983;225:355–360.
- 23 Unruh H, Wang R, Bose D, Mink S: Does pentobarbital anesthesia depress left ventricular contractility in dogs? *Am J Physiol* 1991;261:H700–H706.
- 24 Snedecor GW, Cochran WG: *Statistical Methods*, ed 6. Ames, Iowa State University Press, 1967, pp 271–275.
- 25 Scholtzhauer SD, Littell RC: *SAS System for Elementary Statistical Analysis*. Cary, SAS Institute, 1987, pp 113–125.
- 26 Horowitz BZ, Panacek EA: Cardiopulmonary resuscitation; in Hall JB, Schmidt GA, Wood LDH (eds): *Principles of Critical Care*, ed 2. New York, McGraw-Hill, 1998, pp 71–78.
- 27 Austen KR: Systemic anaphylaxis in the human lung. *N Engl J Med* 1974;291:661–664.
- 28 Kaliner M, Orange RP, Austen KF: Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. 4. Enhancement by cholinergic and alpha adrenergic stimulation. *J Exp Med* 1972;136:556–567.
- 29 Mink S, Becker A, Sharma S, Unruh H, Duke K, Kepron W: Role of autacoids in cardiovascular collapse in anaphylactic shock in anaesthetized dogs. *Cardiovasc Res* 1999;43:173–182.
- 30 Simons FER, Gu X, Silver NA, Simons KJ: EpiPen Jr versus EpiPen in young children weighing 15 to 30 kg at risk for anaphylaxis. *J Allergy Clin Immunol* 2002;109:171–175.
- 31 Bailey PL, Stanley TH: Intravenous opioid anesthetics; in Miller RD (ed): *Anesthesia*. New York, Churchill Livingstone, 1994, pp 291–387.
- 32 Pisko E, Weniger F, DeGroat WC: The effect of halothane on preganglionic and postganglionic sympathetic activity in the cat. *Brain Res* 1970;20:330–334.
- 33 Wagner EM, Mitzner WA, Blecker ER: Peripheral circulatory alterations in canine anaphylactic shock. *Am J Physiol* 1986;251:H934–H940.
- 34 Sampson HA, Mendelson L, Rosen JP: Fatal and near-fatal anaphylactic reactions to food in children and adolescents. *N Engl J Med* 1992;327:380–384.
- 35 Pumphrey RSH: Lessons for management of anaphylaxis from a study of fatal reactions. *Clin Exp Allergy* 2000;30:1144–1150.