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TIME SEQUENCE OF HISTAMINE RELEASE AND FORMATION IN RAT ENDOTOXIC SHOCK

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ABSTRACT—Increased histamine release and formation (induced histamine) are two hypotheses considered in the pathogenesis of endotoxic shock development. To prove both hypotheses a sequence of four randomized controlled studies in rats was performed. Histamine release was measured indirectly as a decrease in tissue-histamine contents (lung, liver, spleen, stomach); histamine formation was estimated directly as an increase in histidine decarboxylase (HDC) activity in the same organs. Changes in contents and enzymatic activities were determined 4 and 8 h after shock induction; in addition, at the time of death, the activity of HDC was measured in heart, kidney, and small intestine. 4 h after shock induction, there was a significant decrease in the tissue-histamine content as measured only in the liver, with the same trend in lung and spleen. 8 h after endotoxin application, however, histamine concentration increased in the liver (significantly $p < .05$) and lung compared to the NaCl control group. The manifestation of changes in HDC activity in various organs was selective (i.e., not all organs showed alterations), not uniform (decreased as well as increased activities were measured), and time-dependent (no increase in HDC activity in animals dying at >20 h). At 4 and 8 h, only the liver showed a strong increase in HDC activity which can explain the increase in histamine content. In lung, spleen, and stomach, a significant decrease occurred. The results on histamine release and formation let us conclude that histamine is involved in the pathogenesis of endotoxic shock development.

INTRODUCTION

Histamine is the mediator with the longest history of all active substances currently described in shock. In 1920 this amine was already considered as a "shock toxin," because of its ability to dilate small blood vessels, to increase capillary permeability and to produce a lethal shock syndrome when given in larger doses (1).

In 1960, Hinshaw's group introduced histamine specifically into the pathogenetic concept of septic shock (2). They recognized and emphasized the similarities between vascular actions of histamine and those of endotoxin; consequently, they interpreted their findings as support for the hypothesis that histamine release plays a major role in the progression of systemic hypotension following endotoxin application. Simultaneously, Schayer postulated a second hypothesis in which increased histamine formation by an inducible form of histidine decarboxylase (HDC) was considered important for the development of shock 3–5 h after endotoxin challenge (3). Together both hypotheses led histamine to be "the star of the show" during the World Congress of Physiology in Leyden 1962. Since then inconsistent experimental findings (4–7) and the identification of other, more potent, vasoactive mediators, such as arachidonic acid metabolites, cytokines, etc. made histamine lose in reputation. Today histamine is considered as one of numerous mediators in the multicausal pathogenetic concept of septic shock. The prevailing view still suggests histamine to be a "shock toxin." Also this hypothesis is discussed controversially (8–10).

To establish a cause and effect relationship of a mediator in a given disease, i.e., septic/endotoxic shock, it is generally accepted to use the four classical KOCH-DALE criteria (11). These include: 1) presence of a mediator in the disease; 2) absence of a mediator in health; 3) the possibility to elicit the disease by exogenous administration or endogenous release/formation and; 4) to block its effect(s) by inhibitors of synthesis/release or by specific antagonists and preventing or ameliorating the disease.

Knowing the effect of histamine on cardio-, pulmonary-, vascular-, and cellular systems it is biologically plausible to assign histamine a causal role in the pathogenesis of septic/endotoxic shock. However a comprehensive meta-analysis of the published literature investigating each KOCH-DALE criterium individually for histamine shows contradictory findings not only between, but also within a species (12). The aim of this paper was to investigate the time sequence of both histamine release and increased histamine formation in endotoxic shock development in a standardized rat model (KOCH and DALE criterium 1 and 2) with saline and methylprednisolone controls.

MATERIALS AND METHODS

Endotoxic shock model

All studies were performed with female Sprague Dawley rats (180–250 g). Shock was induced by application of either 5, 25, or 45 mg/kg b.w. *Escherichia*

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The experiments described in this paper were performed in adherence to National Institutes of Health guidelines for the use of experimental animals.

coli endotoxin i.p. (Difco 055:B5, Difco Lab. Detroit, MI) in ether narcosis. No analgesics were given during the whole experiment to avoid any interference with histamine release and/or histamine formation.

Study design

A total of four subsequent randomized controlled studies were performed to examine histamine release and formation with the standardized rat endotoxic shock model. Table 1 shows a summary of the study designs. In studies I–III histamine *release* was measured as decrease of tissue-histamine-concentration in different shock organs (lung, liver, spleen, and stomach) 4 and 8 h after shock induction, respectively. In addition histamine *formation* was measured as change of histidine decarboxylase (HDC) activity in the same organs and at the same time. The 4 h measurement was performed because the hypothesis (3, 28) stated a period of histamine predominance 3.5–5 h after endotoxin challenge. 8 h was chosen, because the mortality rate increases > 8 h thus leading to imbalances in study groups and to mixed effects.

Each study consisted of four groups: two test- and two observation groups with 20 animals/group. In study I an endotoxin group was compared with a NaCl treated control group after 4 h. Studies II and III both compare an endotoxin group (E) with methylprednisolone (MP) treated controls (Urbason®, 50 mg/kg b.w., intravenously) 4 and 8 h after endotoxin application, respectively. In testgroups A and C organ explantation with subsequent measurement of tissue-histamine-concentration and HDC activity was performed. Data on the effects of methylprednisolone will be reported elsewhere. The observation groups B and D were treated identically to the testgroups A and C. These groups were created for measurement of survival time and rate as well as parameters of behavior and condition. MP always served as a "positive control" as previous experiments showed a 95–100% survival rate in this model. Study IV was performed for additional information on HDC activity later than 8 h after endotoxin application. In study IV three different doses of endotoxin (5, 25, 45 mg/kg b.w.) were compared to a NaCl-control with five animals per group. HDC activity was measured in six different organs (lung, liver, stomach, heart, small bowel, kidneys) either at the point of death or at 96 h after shock induction (long-time survival).

Methods

Sample taking—Testgroup animals were sacrificed by transecting the carotid artery in ether narcosis. After laparotomy the organs were explanted, washed in ice cold 0.9% NaCl solution, and weighed.

Table 1. **Experimental design of studies on histamine release and increased formation in rat endotoxic shock development**

Studies	Testgroups*	Observation groups‡
I. NaCl versus E	A 0.9% NaCl	B 0.9% NaCl
(4 h)	C Endotoxin	D Endotoxin
II. E versus E+MP	A Endotoxin	B Endotoxin
(4 h)	C Endotoxin + MP	D Endotoxin + MP
III. E versus E+MP	A Endotoxin	B Endotoxin
(8 h)	C Endotoxin + MP	D Endotoxin + MP
IV. Death or 96 h	C 0.9% NaCl	
	E1 Endotoxin (5 mg/kg)	
	E2 Endotoxin (25 mg/kg)	
	E3 Endotoxin (45 mg/kg)	

Study I–III: n = 20 rats/group; E = 45 mg/kg b.w. E. Coli endotoxin intraperitoneally (2 ml); MP = 50 mg/kg b.w. methylprednisolone (Urbason®) intravenously (1 ml); histamine concentration and HDC activity measurements in lung, liver, spleen, and stomach. Study IV: n = 5 rats/group; 5, 25, 45 mg/kg b.w. *E. coli* endotoxin intraperitoneally (2 ml); HDC activity measurements in lung, liver, stomach, heart, small bowel, and kidney.
* Animals for organ explantation with subsequent measurement of tissue-histamine concentration (studies I–III) and HDC activity (studies I–IV).
‡ Animals treated identically to testgroup-animals for measurement of survival time and rate as well as parameters of behavior and condition.

For *measurement of histamine release* a tissue random sample of 0.2 g was stored in 2 ml 1 M $HClO_4$ and frozen at −40°C. On the day of measurement, specimens were thawed to room temperature. They were homogenized for 10–20 s with an Ultraturrax-Homogenizer (Shaft 10N) and centrifuged for 20 min at 6000 × *g* (Laborzentrifuge Typ U32, Christ, Oberroda, Germany) leaving the supernatant for evaluation.

For *measurement of histamine formation* a random tissue sample of 0.8 g (exceptions: heart 0.6 g, spleen 0.3 g) was frozen at −40°C. Organ leftovers were also frozen. On the day of measurement samples and organ leftovers were thawed to 2–4°C. Four samples of 0.8 g organ leftovers were taken for measurement of blank- and recovery values. The rest of organ material was frozen again.

All samples (organ samples as well as blank- and recovery samples) were then put into cooled individual polypropylene centrifugation tubes (10 ml) and mixed with 4 ml of cooled sodium phosphate buffer (0.1 M; pH 7.3) followed by homogenization for 15 s using a Ultraturrax-Homogeniser (Schaft 10 N). The homogenate was centrifugated at 0–4°C and 45,000 × *g* for 10 min (Sorvall Superspeed RC-2B, Hormuth-Vetter, Wiesbaden, Germany). The supernatant (denoted later as a crude enzyme extract) was then used immediately for measuring HDC activity by a modified isotopic dilution method (see below). For each organ and animal double measurements (1.7 ml of crude enzyme extract each) were performed. The pH value was 7.3. Incubation period summed up to 60 min.

Measurement of histamine release—Analysis of tissue-histamine-concentration was performed with a fluorometric-fluoroenzymatic method as described in detail by Hesterberg et al. (13). In addition an internal standard procedure and fluorescence of a blank were determined for each organ.

In brief the supernatant was divided into 0.8 ml aliquots for organ histamine measurement and 0.8 ml aliquots to which exogenous histamine in an appropriate amount was added (0.1 ml histamine standard for lung, liver, and spleen; 0.2 ml histamine standard for stomach; histamine standard 4 μg/ml in 1 M $HClO_4$) for estimation of the internal standard. Both samples were filled up to 2.0 ml with 1 M $HClO_4$. A third sample consisting of only 2.0 ml 1 M $HClO_4$ served as a blank. After adding the 2.0 ml samples to the usual mixture of *n*-butanol, NaOH, and NaCl three extraction steps followed: shaking time for extraction was 20 min in the first, 2 min in the second, and 6 min in the third step of the method.

For fluorometric measurement of the isolated histamine 2 ml of the extract were added to the usual mixture for the condensation reaction with *o*-phtaldialdehyde. Fluorescence intensity of the histamine-*o*-phtaldialdehyde complex was measured in a Zeiss spectrum fluorometer using an excitation wavelength of 360 nm and a fluorescence wavelength of 450 nm. Tissue-histamine concentration was calculated according to a specific formula (see Ref. 13 for details) on the aid of a calibration curve constructed from data obtained on three different days with authentic histamine in 0.1 M HCl.

Measurement of histamine formation—HDC activity was determined with the modified isotope dilution method described in detail by Neugebauer and Lorenz (14). In brief the method is divided into five steps: (*a*) incubation with crude enzyme extract; (*b*) extraction of [^{14}C]histamine formed; (*c*) conversion of histamine to dibenzene sulfonyl-histamine derivative and crystallization [^{14}C]histamine formed; (*e*) calculation of enzymatic activity. Furthermore reliability tests regarding sensitivity, specificity, precision, and accuracy of the isotopic dilution method for HDC measurement (applying criteria recommended by the International Federation of Clinical Chemistry) were performed in addition to investigation of blank suitability and reaction kinetics. For more details see Refs. 14 and 15.

Clinical observation and survival rates—For documentation of clinical observations during endotoxic shock behavior (normal to lethargic), flight reaction (normal or slow after a fast blow of 20 ml of air onto the head of the animal) and pilo-arrection (normal to bristle) were measured in all animal groups every half h within the first 12 h then every hour until 24 h followed by every 4 h up to 96 h. Survival time and rates (mortality) were ascertained by continuous animal observation up to 96 h (long-time survival).

Statistical analyses—Single values (n = 20) of histamine contents and histidine decarboxylase activities of each organ and group of studies I–III were estimated and graphically displayed in Figs. 1 and 3. For calculation of differences between study groups in each study the nonparametric Mann-Whitney test was used (Hewlett Packard Modell 9815A, statistic Vol. I). Figs.

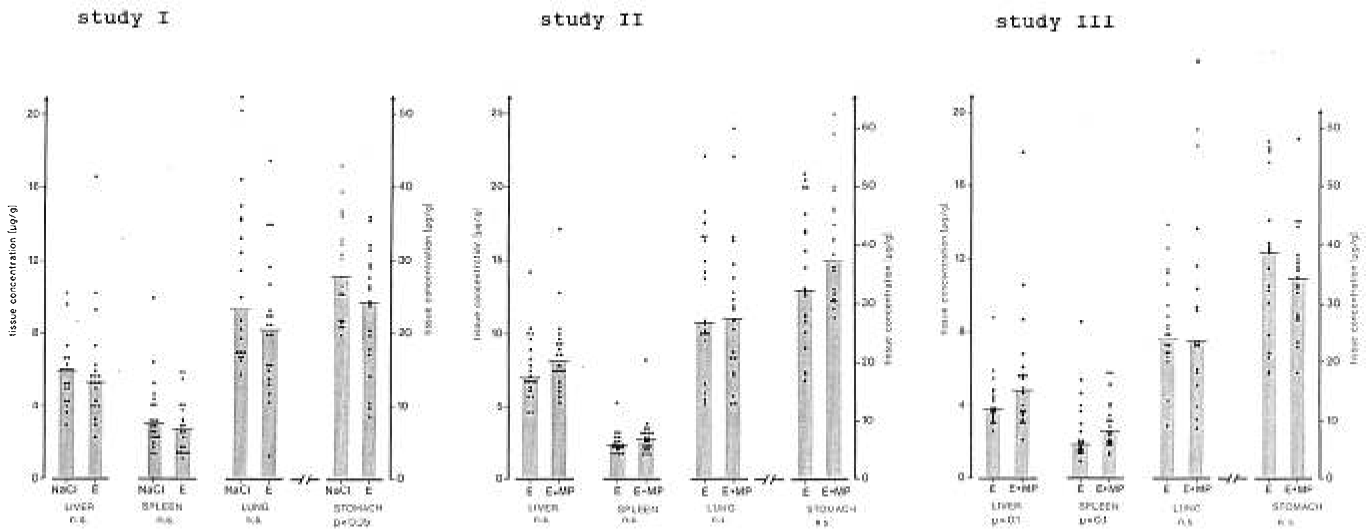

FIG. 1. **Changes of tissue-histamine-concentration in four organs 4 h (study I and II) and 8 h (study III) after endotoxin (E = 45 mg/kg b.w.) application compared to a NaCl control group (study I) and a MP (50 mg/kg b.w.)-treated group (studies II and III).** Single dots = single values/organ of each animal; n = 20 animals/group; columns = median; test statistics: Mann-Whitney; n.s. = non significant.

2 and 4 show median values (1–3 percentile) of histamine contents and HDC activities compared to NaCl controls of study I. Differences were calculated using the Mann-Whitney test. Because of multiple testing the probability of the α error ($p < .05$) had to be protected by using a simple sequentially rejective multiple test procedure of Holm (37). Survival curves of the observation groups in studies I–III are displayed using the Kaplan-Meier method of life table estimation.

RESULTS

Histamine release

Fig. 1 summarizes the results of histamine concentration measurements in different organs of studies I–III, while Fig. 2 compares the endotoxin groups of all three studies to the NaCl con-

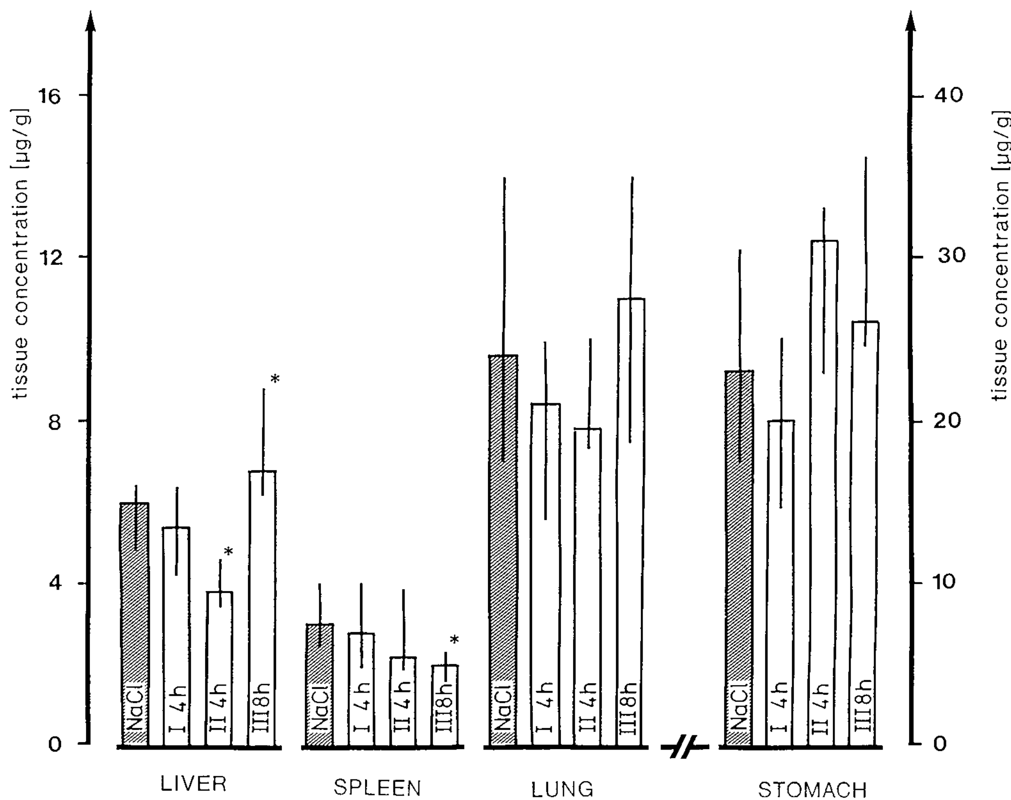

FIG. 2. **Changes of tissue-histamine concentration during development of endotoxic shock in rats: Summary of endotoxin shocked animals (study I–III) compared to a NaCl group (study I).** The median with its first and third quartil (n = 20 animals/group) is demonstrated. The hatched columns represent the NaCl group of study I. *Significant ($p < .05$) compared to NaCl group (Mann-Whitney).

trol group: 4 h after endotoxin application a decrease in liver histamine concentration occurred; 8 h after endotoxin application an inverse significant effect resulted with increased histamine levels in this organ. In addition to the liver only the spleen showed a decrease in histamine concentration during shock development; 8 h after endotoxin application significance was achieved compared to the NaCl group. Lung and stomach showed no significant changes throughout shock development up to 8 h after endotoxin application. The lung at best demonstrated a slight tendency with $p < .1$ at 4 h. The results of all organs 4 h after endotoxin application were reproducible in their tendency in both studies I and II except for the stomach.

Histamine formation

Fig. 3 summarizes the results of studies I–III for testgroups A and C (see Table 1) in liver, spleen, lung, and stomach. The endotoxin groups of all three studies are compared to the NaCl group in Fig. 4. Figs. 3 and 4 correspond to Figs. 1 and 2 of histamine release.

Changes in HDC activity throughout endotoxic shock developed differently in all four organs. Except for the liver, a significant decrease of enzymic activity was measured in spleen, lung, and stomach. In the liver the HDC activity increased more than 300% at 4 h compared to the NaCl control group; 8 h after endotoxin application HDC activity increased four to five times as compared to the NaCl group (Fig. 4). Stomach values changed in opposite direction. 4 and 8 h after endotoxin application less than half of the activity was measured compared to controls. Lung and spleen HDC activities showed a different pattern. In both organs enzymic activity decreased early (4 h) and rapidly by 40–60% and increased during the time up to 8 h. Eight h after endotoxin application no significant changes were measured any more compared to controls.

Fig. 5 demonstrates the results of study IV. Of all six organs only lung, liver, and stomach showed measurable changes in HDC activity. In the *liver*, as in all former studies, HDC activity increased following endotoxin application (25 and 45 mg/kg). There was no measurable increase of HDC activity after 5 mg/kg endotoxin injection, neither at death nor in survival animals. In the *lung* a significant increase of HDC activity was achieved in the "high dose" groups (25 and 45 mg/kg). This confirms the increased HDC activity trend at > 8 h of study III. As in liver there was no measurable increase in HDC activity after 5 mg/kg endotoxin application. In the *stomach*, in contrast to liver and lung, all three endotoxin doses showed strong inhibition of enzyme activity compared to NaCl groups. HDC activity stayed significantly decreased even in animals dying later in shock (group E1). Only until 96 h (long-time survival) after endotoxin application enzymatic activities were detected again in NaCl groups (two survivors of E1 endotoxin group).

Changes in HDC activity of all four randomized controlled studies in the rat endotoxic shock model can be summarized as follows:

1) In only two of seven organs (lung and liver) an increased histamine formation was measured during endotoxic shock development. In contrast a strong inhibition of the enzymic activity was measured in the stomach. A short inhibition was also measured in spleen (after 4 h). Heart, kidneys, and small bowel showed no changes in HDC activity.

2) While HDC activity gradually increased up to maximum values between 8 and 15 h in the liver, compared to NaCl groups, a decrease occurred in the stomach. The HDC activity in the later was found to be normal again 96 h after endotoxin application (long-time survivors), while the liver enzyme normalized after 21.5 h.

3) In the lung HDC activity decreased for a short period of

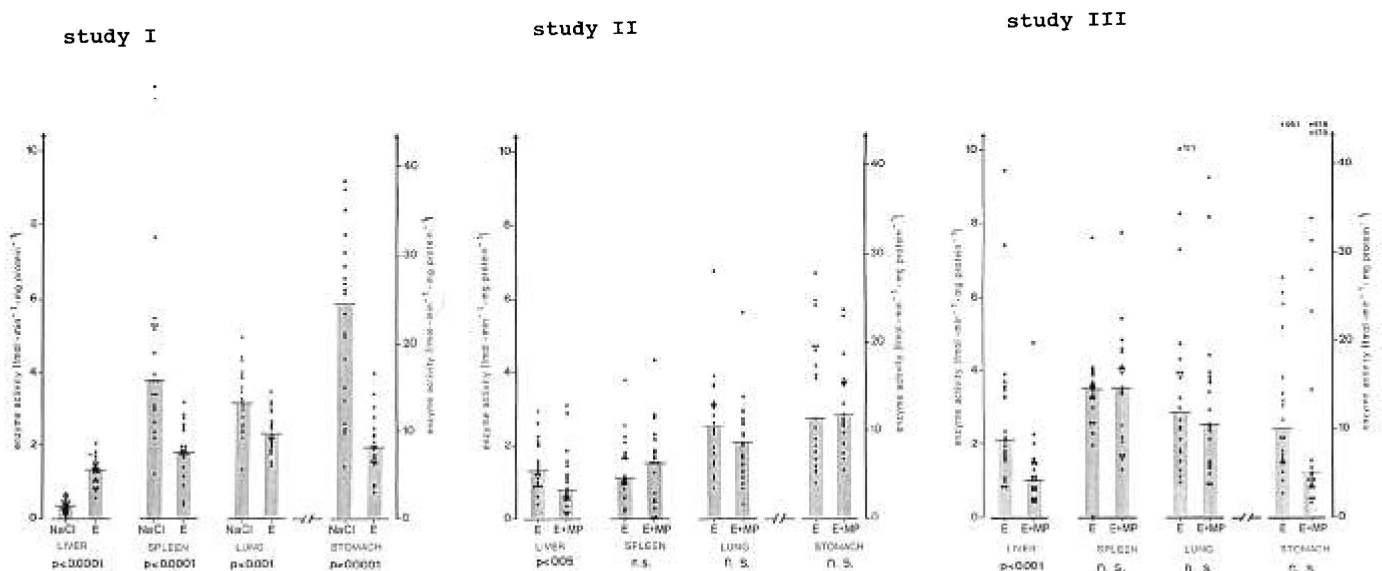

FIG. 3. **Changes of histidine decarboxylase activity in four organs 4 h (study I and II) and 8 h (study III) after endotoxin (E = 45 mg/kg b.w.) application compared to a NaCl control group (study I) and a MP (50 mg/kg b.w)-treated group (studies II and III).** Single dots = mean of double measurements per organ of each animal; n = 20 animals/group, columns = median; test statistics: Mann-Whitney; n.s. = non significant.

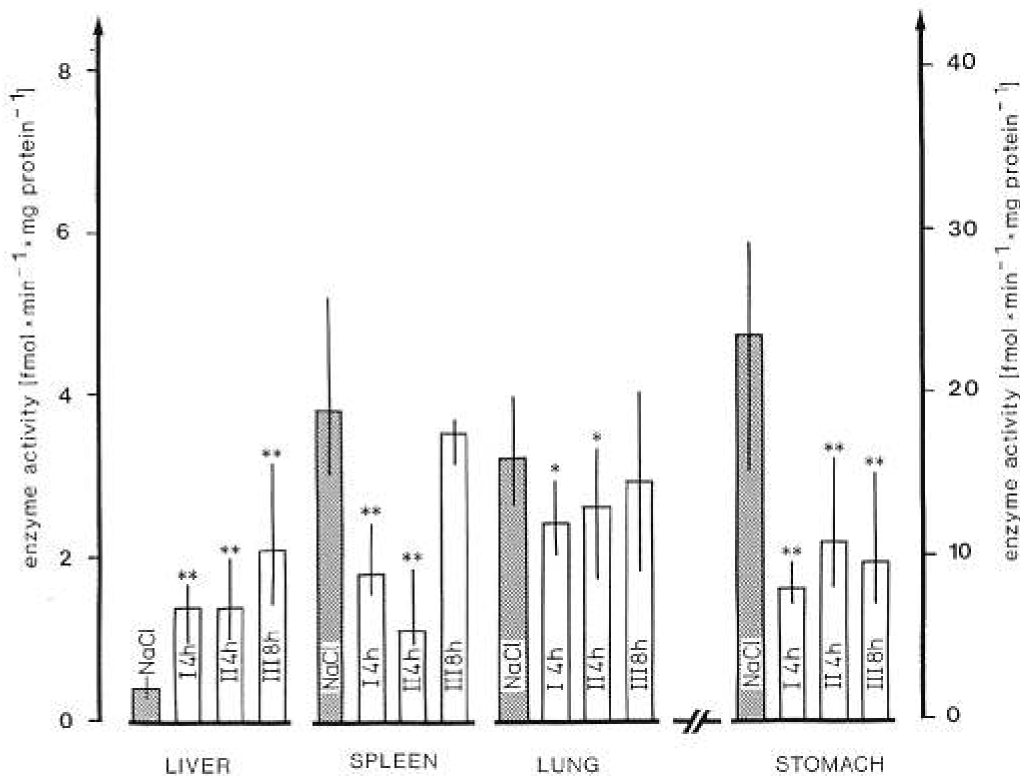

Fig. 4. **Changes of histidine decarboxylase activity during development of endotoxic shock in rats: Summary of endotoxin shocked animals (study I–III) compared to a NaCl group (study I).** The median with its first and third quartil (n = 20 animals/group) is demonstrated. The hatched columns represent the NaCl control group of study I. *Significant ($p < .05$), **significant ($p < .0001$) compared to control group (Mann-Whitney).

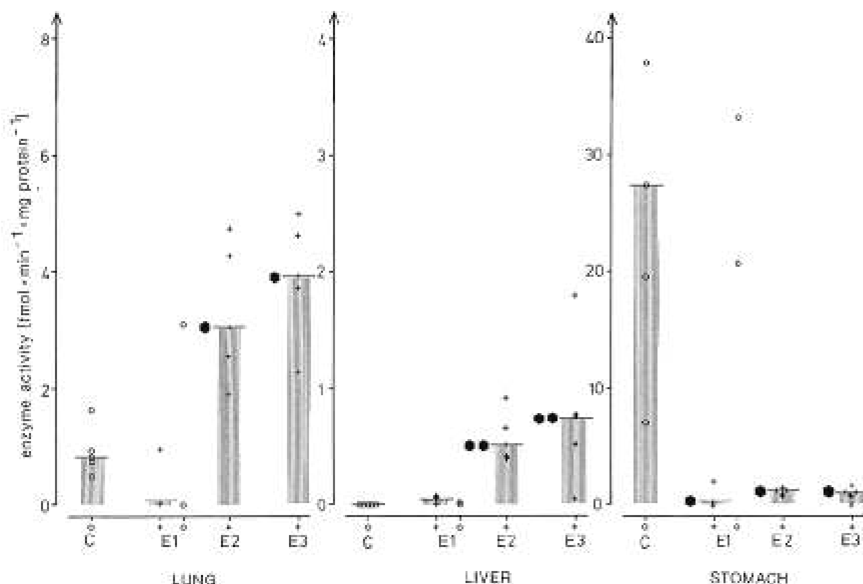

Fig. 5. **Changes of histidine decarboxylase activity in rat endotoxic shock (study IV). n = 20 animals (five animals/group); E1 = 5 mg, E2 = 25 mg, E3 = 45 mg endotoxin/kg b.w. i.p. (2 ml) C = .9% NaCl i.p. (2 ml).** Determination of HDC activity at animal death (+) or long time survival (96 h) after injection (○). Columns = median of individual values, significance: $^{*}p < .05$, $^{**}p < .01$ (Mann-Whitney) compared to NaCl group (C).

time at 4 h after endotoxin application; after 8 h normal values were measured again. Later on (up to 14.5 h, study IV) histamine formation was again increased. After 21.5 h a measurable difference in the enzymic activity as compared to the NaCl group did no longer exist even in the animals who died (study IV).

Clinical observations and survival rates

Clinical observations of behavior and condition (spontaneous behavior, flight reaction, pilo-arrection) were documented regularly throughout the studies. Pilo-arrection seemed to be an early detrimental prognostic sign. Shortly before death most animals were extremely lethargic. 8 h after endotoxin applica-

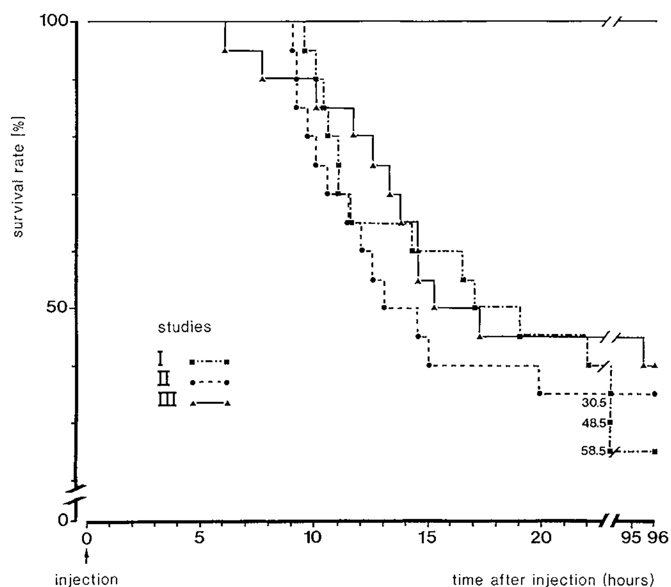

FIG. 6. **Survival curves in the standardized rat endotoxic shock model: untreated endotoxin groups of studies I–III compared to the NaCl control group of study I (100% survival).** The MP-treated observation groups (group D) in study II and III survived to 100% and 95% (19/20 animals), respectively. Survival curves were obtained from the observation groups (n = 20 rats/group). All animals were observed up to 96 h (long-time survival). Injection = application of endotoxin or NaCl.

tion most untreated animals in all three groups showed similarity in parameters of spontaneous behavior. The survival curves of studies I–III are summarized in Fig. 6 showing the excellent reproducibility of the endotoxic shock model. Observation time was 96 h. Survival of untreated endotoxin animals was 25% in study I, 35% in study II and 40% in study III. Most untreated animals died in a relatively narrow time interval between 9 and 17 h after endotoxin application. All animals of the NaCl control group survived (study I). MP-treated control animals survived to 100% in study II and to 95% in study III (not shown).

DISCUSSION

Two different hypotheses for a causal role of histamine in the pathogenesis of septic/endotoxic shock have been described: (*a*) an increased histamine release from its mast cell storage sites and (*b*) an increased histamine formation by induction of histidine decarboxylase activity from non-mastcell stores. Both hypotheses were investigated by a sequelae of randomized controlled studies in a standardized rat endotoxic shock model. In studies I–III shock was induced by intraperitoneal application of 45 mg of endotoxin/kg body weight. It might be argued that this model is of dubious clinical relevance. The reasons for choosing this model are:

(*a*) That the rat reacts to histamine releasing agents very similar to humans except in experiments with the plasma substitute dextran (12).

(*b*) That Schayer's hypothesis (see below) which should be proved in our experiments are mainly based on experiments in rat endotoxic shock. We rejected models with bacteria in these initial investigations because of unknown effects of necessary antibiotics on histidine decarboxylase activities and histamine metabolisms.

(*c*) That this model fulfills a number of criteria described by Wichtermann (38) for an appropriate study model such as sufficient reproducibility with high mortality rates, sufficient duration of sepsis to induce host defense mechanisms, and visible signs of sepsis. The excellent reproducibility of our model is shown in Fig. 6. The median survival time is 12 ± 3 h; untreated animals showed signs of sepsis also seen under clinical conditions. However, it is obvious, that the results obtained need verification in further models of sepsis especially because this model cannot mimic the hyperdynamic sepsis seen under clinical conditions.

Histamine release

For a long time the role of histamine release in septic/endotoxic shock was assessed by the global results of the different studies and not by their methodology. Neither the magnitude of the effects found, nor the quality of the method used, nor the validity of the research designs were evaluated adequately. This unfortunate situation, with many contradictory views, has confused this research field.

Meta-analysis in combination with a decision tree led to a new approach of critically investigating the role of histamine in septic/endotoxic shock (12). After meta-analysis on the first two KOCH-DALE criteria (selection of 27 studies from more than 45.000 publications) and evaluation of study deficiencies, only six studies could be recommended as validly demonstrating histamine release in septic/endotoxic shock. One study performed in patients with confirmed sepsis (16), however, did not differentiate between distinct phases of shock development. From the two studies in dogs (17, 18), Hinshaw et al. demonstrated histamine release more as an effect than as the cause of shock, with hypotension as a shock symptom beginning much earlier than histamine release. Weil and Spink documented the close relationship of histamine release to hypotension, increase of portal vein pressure and pooling of blood in the viscera sufficiently but in only one case. The two studies in rabbits (19, 20) can be criticized because of the dubious clinical relevance of the species (main storage site of histamine are platelets and mot mast cells as in humans). Finally the study in rats (21) confirmed histamine release only in the late phase of shock development with the highest histamine levels prior to death. This unsatisfactory situation and these obvious methodological shortcomings led us to perform a series of experimental studies to prove histamine release in endotoxic shock.

Histamine release was measured *indirectly* as decrease of tissue-histamine concentration in different shock organs such as lung, liver, spleen, and stomach. When using this method it must be kept in mind that the histamine content of a given tissue is always the result of histamine release and formation. A significant decrease in tissue-histamine contents was achieved only in the liver 4 h after shock induction. The same trend was measured in lung and spleen. 8 h after endotoxin application, however, the histamine concentration increased in liver (significantly $p < .05$) and lung compared to the NaCl control group. Plasma histamine

measurements, although possible, would not be able to distinguish between changes in specific organs. Organ outflow measurements were considered too difficult to perform in rats.

Numerous examples of the method of indirect evaluation are known throughout the literature and were widely accepted: for instance, decreased histamine levels in gastric mucosa of patients with ulcus duodeni compared to controls were interpreted as increased histamine release (22) especially as levels normalized following operation or H_2-antagonistic therapy and decreased again following ulcer recidivation (23). However, it must always be kept in mind, that all indirect measurements can also lead to misinterpretations.

A decrease of tissue-histamine concentration demonstrated 4 h after shock induction in liver, spleen, and lung supports the hypothesis of an early histamine release from mastcell storage sites. Increases in histamine concentration after 8 h, especially in liver, however, possibly demonstrated an increased histamine formation at this point of time (see below).

These results are partially controversial to Endo (24), who found an increased histamine level in lung, liver, spleen, and kidneys (not thymus or brain) already 4 h after endotoxin application (0.5 mg/kg) in mice. He achieved a maximum histamine level after 6 h with normalization after 10–16 h. These results were interpreted as an increased histamine formation throughout all organs (maximum after 4.5 h) which could only be verified for the liver in our own studies. Species differences may explain these contradictory findings.

Histamine formation

Schayer postulated a causal relationship between increased histamine formation by induction of histidine decarboxylase and lethal outcome of endotoxic shock. Cells in or near the microcirculation were thought to be the origin of increased histamine formation. Schayer even extended his hypothesis by giving histamine formation a physiological control function in the microcirculation adjusting local disbalances between vasoconstriction and vasodilation in different states of shock (25, 26). For irreversible shock and death a maximum organ HDC activity was assumed (27). This hypothesis was generalized and postulated for all organs and all species (27, 28). Several other data also exist to support this idea. Especially Hollis and coworkers made endothelial cells responsible for histamine formation (29, 30). They demonstrated histidine decarboxylase activity in endothelial cells and formulated a causal relationship between increased histamine synthesis and increased transmural albumin permeability in rabbit and rat aorta (31, 32). Several groups confirmed findings of non-mastcell histamine formation in vascular walls (33, 34). Also the presence of histamine-metabolizing enzymes, the histamine-methyltransferase and diamine-oxidase (35), in rat and guinea-pig microvascular endothelial cells, as well as proof of endothelial cell H_1- and H_2-receptors (36) hint at histamine involvement in endothelial cell function.

It was the aim of this study to reinvestigate Schayer's hypothesis of a causal role of histamine formation in endotoxic shock by verifying KOCH-DALE criteria 1 and 2 in a standardized rat model. Increased histamine formation was demonstrated by reliable measurements of HDC activity. In all four studies performed, levels of HDC activity were determined in lung, liver, spleen, and stomach 4 and 8 h after shock induction; in addition, the level of HDC activity was measured at the time of death in lung, liver, stomach, heart, kidney, and small intestines. At 4 and 8 h after shock induction, only the liver showed a strong increase in HDC activity; in lung, spleen, and stomach, a significant decrease occurred.

In Schayer's hypothesis, the time between 3.5 and 5 h after endotoxic challenge was considered as the period of histamine predominance followed by irreversibility, capillary destruction, and death. Retrospectively our point of measurement at 4 and 8 h was well chosen in this model: after 4 h progredient shock development started; after 8 h an irreversible state was established. Results of our studies are partially contradictory to Schayer's hypothesis: 4 and 8 h after shock induction the HDC activity only increases strongly in liver. In lung and spleen values decreased to 30–60% after 4 h followed by normal values compared to NaCl control groups after 8 h. Even more: gastric HDC activity decreased continually and drastic starting with shock induction.

HDC activity changes until long-time survival or death of animals (study IV) are also contradictory to Schayer's hypothesis: In liver HDC activity increased up to about 14 h, but then decreased to normal within the 20th h. Also animals that died from shock later showed no increase in HDC activity. Lung enzymatic activity followed parallel to liver enzymatic activity after 8 h. Gastric enzymatic activity (probably acid-specific HDC localized in enterochromaffine cells) was always decreased. Only after long-time survival normal values were measured again. In other organs such as in kidneys, heart, and small bowel no HDC activity changes were measured whatsoever.

In summary the manifestation of changes in HDC activity in different shock organs was selective (i.e., not all organs showed alterations), nonuniform (decreased as well as increased activities were measured), and time-dependent (there was no increase in HDC activity in animals dying > 20 h after shock induction).

Our results on histamine release and formation let us conclude, that histamine is in fact involved in the development of endotoxic shock (fulfillment of KOCH-DALE criteria 1 and 2). Previous results with H_1- and H_2-antagonists (KOCH-DALE criterium 4) with the same endotoxic shock model let us assume that histamine cannot be considered solely as a shock toxin (9). The different actions of histamine via H_1- and H_2-receptors need to be investigated more thoroughly. Further studies are in progress.

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