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Histidine Decarboxylase Activity in Traumatic Shock[1] (38165)

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(Introduced by R. C. Little)

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Histamine is known to be involved in the response to a variety of noxious stimuli in mammals (1). In inflammation and in anaphylactic shock, there is a liberation of histamine which is stored in mast cells (deposit histamine). This preformed histamine has a powerful vasodilator effect on the arterioles and increases the permeability of the capillaries, thereby contributing to the pathogenesis of shock (2). In other types of shock reactions, such as endotoxin and traumatic shock, the role of histamine is still unclear.

Histamine is also continuously synthesized within the cells of the small blood vessels (induced histamine), and may act as a dilator of the microcirculation (3). Schayer has shown (4, 5) that histamine biosynthesis is regulated by an inducible enzyme, histidine decarboxylase, and has postulated that the control of the enzyme activity is under the influence of local factors (3). Therefore, the rate of synthesis of induced histamine can be adjusted to suit the environment, and histamine may play a physiologically significant role in the maintenance of microcirculatory homeostasis.

Many factors which affect the microcirculation, such as endotoxin, exercise, infection, and tourniquet application have been shown to affect the rate of histamine biosynthesis (6–8). However, in drum shock although significant changes in the microcirculation occur, alterations in histidine decarboxylase activity have not been reported (8). The Reticuloendothelial System (RES) is altered by similar types of stress (9–13) and by histamine (14). Stimulation of the RES in certain instances resulted in an increased tolerance against the development of the shock state (15).

Because of the similarities between drum shock and other shock models, and the possible role of histamine in homeostasis of the microcirculation and in altering RES function, the pattern of induced histidine decarboxylase activity was determined in the lung and spleen of the rat following traumatic shock.

Methods. Young adult female rats of the CF-Nelson Strain (140–175 g), anesthetized with pentobarbital sodium (Nembutal, 2.0 mg/100 g), were used in these experiments. Rats were traumatized by exposing them to drum trauma in a modified Noble–Collip tumbling apparatus as previously described (16). With this procedure, trauma is applied in a controlled graded manner after which a typical picture of shock develops, uncomplicated by infection or hemorrhage (17). Rats were rotated at 40 rpm in a circular drum, 15 in. in diameter and 8 in. in depth. Each animal was exposed to an effective energy of 92,500 J; for a 160 g rat, 500 revolutions were required in the drum. The dose was calculated as the amount of traumatizing energy acting on the body (18), and corresponds to a dose at which 25% of the animals

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succumb. At different times following trauma, 15 min, 4 and 10 hr, rats were sacrificed and tissues analyzed as described below. Controls were handled in an identical manner, but were not exposed to trauma.

Histidine decarboxylase activity was determined by incubating 10 μg ^{14}C L-histidine (Schwarz-Mann) with tissue extracts and assaying ^{14}C histamine by isotope dilution (19). Briefly, this method consists of the following procedures. Rats were sacrificed by decapitation. Lungs and spleens were rapidly removed and immediately placed on ice. The tissues were homogenized at 4° in 0.02 *M* sodium phosphate buffer (pH 7.2) using 5 parts buffer to 1 part tissue. Lung homogenates were prepared by blending for 2 min (16,000 rpm) in a Sorvall Omni-Mixer; and the spleen was homogenized using a Potter-Elvehjem teflon pestle-glass tube homogenizer (10 strokes per spleen). Each homogenate was centrifuged at 20,000*g* for 20 min at 4°, and the supernatant fraction, which contained most of the enzyme, was assayed for histidine decarboxylase. The incubation and subsequent assay procedure for mammalian histidine decarboxylase (EC 4.1.1.22) was essentially the benzenesulfonylhistamine (BSH) method of Schayer (19).

Two ml of supernatant were incubated with 1 μCi (0.1 ml) of ^{14}C-histidine (sp. act. 312 mCi/mmole), 50 μg (0.1 ml) of pyridoxal-5^{1}-phosphate and a diamine oxidase inhibitor, aminoguanidine (10^{-4} *M*), under nitrogen at 37° for 1 hr. The rate of production of ^{14}C-histamine was linear over the time studied, and was proportional to the concentration of histidine decarboxylase. Carrier histamine was added to the newly formed ^{14}C-histamine which was extracted and converted to benzenesulfonylhistamine (BSH). Blanks were prepared for each sample by adding 0.1 ml of 10^{-3} *M* bromcresin (NSD-1055), gift from Lederle, to an identical reaction mixture. After crystallization, BSH was weighed in a glass scintillation vial, dissolved in 1 ml of acetone and 10 ml of liquid scintillation fluor (0.4% PPO and 0.01% POPOP in toluene) was added. Samples were counted in a Nuclear-Chicago liquid scintillation spectrophotometer for which the counting efficiency was 75%. Unless otherwise specified, histidine decarboxylase activity was expressed as counts per min (cpm) per 100 mg BSH per 100 mg of tissue (wet wt).

The data was analyzed statistically using the Duncan's test to determine the significance between the means. *P* values less than 0.05 were considered significant. Means and standard errors of the mean (SEM) were calculated by conventional statistical methods and are shown in the tables.

Results. Significant changes in the lung histidine decarboxylase (HD) activity occurred after 500 revolutions and are listed in Table I. Group 2, which was sacrificed 15 min after trauma, showed an increase of 170% above controls from 165 to 439 cpm/100 mg BSH/100 mg tissue ($P < 0.05$). Group 3, sacrificed 4 hr after trauma, increased over controls by 280% from 165 to 621 cpm ($P < 0.01$). Group 4, 10 hr after exposure to trauma, dropped to 176

TABLE I. Histidine Decarboxylase Activity After Trauma.[a]

Group number	Time after trauma	Lung activity[b]	Spleen activity[b]
1	Controls	152 ± 32[c] (15)	285 ± 63[d] (16)
2	15 min	439 ± 83 (11)	331 ± 65 (13)
3	4 hr	621 ± 111 (13)	845 ± 128 (13)
4	10 hr	176 ± 57 (7)	213 ± 28 (7)

[a] Rats received an effective energy of 92,500 J in Noble–Collip Drum.

[b] Mean activity ± SE in counts/min/100 mg BSH/100 mg tissue. The numbers in parentheses indicate the number of animals studied in each group.

[c] Lung: groups 2,3 > 1,4 $P < .01$: Group 3 > 2 $P < .05$.

[d] Spleen: group 3 > 1,2,4 $P < .01$: Groups 1,2,4 NSD.

cpm and did not differ from the control value.

Spleen HD activity also changed after exposure to trauma. The changes in HD activity are shown in Table I for controls and Groups 2, 3, and 4. Fifteen min after trauma, the HD activity did not increase but remained at baseline level, 285 vs 333 cpm/100 mg BSH/100 mg tissue ($P < 0.1$). However, Group 3, 4 hr after trauma had a 200% increase in activity, from 285 to 845 cpm ($P < 0.01$). Group 4, 10 hr after trauma, decreased to 214 cpm and did not differ from the control value.

In Table II, the effect of exposure to trauma in the whole organ of the lung and spleen is shown. The resting lung and spleen activities were approximately equal. The activity in the lung was greatly increased at fifteen minutes after trauma whereas the activity in the spleen remained essentially unchanged. By 4 hr both organs showed significant increases, but the total activity of the spleen remained less than the lung.

Because the calculation of enzyme activity for the whole organ depends on the weight of the tissue, changes in this weight after trauma could influence the results. Thus, the wet weight of the lung and spleen was analyzed for differences which might occur due to trauma. However, neither the lung nor the spleen showed any significant increase in weight following trauma.

TABLE II. Histidine Decarboxylase Activity in the Lung and Spleen After Trauma: Whole Organ Analysis.[a]

Group number	Time after trauma	Lung activity[b]	Spleen activity[b]
1	Controls	1680[c]	1312[d]
2	15 min	5310[e]	1526
3	4 hr	7200[e]	3887
4	10 hr	2020	1024

[a] Rats received an effective energy of 92,500 J in Noble–Collip Drum.

[b] Mean activity in counts/min/whole organ.

[c] Lung: groups 2,3 > 1,4 $P < .01$: group 3 > 2 $P < .05$.

[d] Spleen: group 3 > 1,2,4 $P < .01$: groups 1,2,4 NSD.

[e] Lung > spleen: groups 2,3 $P < .01$.

Plasma HD activity was also evaluated after trauma. The mean HD activity of the plasma for controls and traumatized groups is listed in Table III. There was no significant difference between the controls and Group 3, which exhibit peak tissue activity following trauma. In the other 2 groups, samples varied widely and statistical treatment was not possible.

Discussion. The present study, in conjunction with reports of endotoxin and tourniquet shock (6, 8) suggests that alterations in the microcirculation during shock are associated with increased histidine decarboxylase activity. This enzyme, which converts histidine to histamine, increases in activity in highly vascularized organs such as the spleen and lung and may cause an increase in histamine release at local sites.

In the spleen, 15 min after trauma, there was no alteration in histidine decarboxylase activity (Table I). However, 4 hr after trauma the enzyme activity had significantly increased over the control levels and then returned to normal at 10 hr. Gecse has described increased histidine decarboxylase activity in the digestive tissues in traumatic shock (20, 21), but such an increase may be due to nonspecific enzymes present in these tissues which may be influenced by dietary factors. It is interesting to note that Schayer (8) observed an increase in spleen histidine decarboxylase activity in endotoxin shock but did not in tourniquet shock. This might reflect differences in the shock inducing mechanism following various stresses.

Unlike the spleen, there was an immedi-

TABLE III. Plasma Histidine Decarboxylase Activity After Trauma.[a]

Group number	Time after trauma	Number per group	Enzyme activity[b]
1	Controls	6	18.6 ± 15[c]
2	15 min	6	22.0 ± 20
3	4 hr	7	55.8 ± 22
4	10 hr	4	54.2 ± 27

[a] Rats received an effective energy of 92,500 J in Noble–Collip Drum.

[b] Mean Activity ± SE in counts/min/100 mg BSH/ml of plasma.

[c] Groups 1,3 NSD.

ate increase in the histamine forming capacity of the lung following trauma (Table I). This increased further at 4 hr, and, as in the spleen, the enzyme activity returned to normal at 10 hr. The induction time of histidine decarboxylase in traumatic shock is shorter than that seen in endotoxin and tourniquet shock (8), and may again reflect differences in the pathway producing shock.

The histidine decarboxylase activity on a unit weight basis was greater in the spleen than in the lung in controls and in animals exposed to trauma. However, the pattern of activity displayed by the whole organs was different. In the controls the levels of enzyme activity in the lung and spleen were equivalent. Following exposure to trauma, the total lung activity increased significantly over that seen in the spleen. These changes are apparent from the total organ activities listed in Table II. The fact that enzyme formation is more rapid in the lung and that total activity is greater in the lung than in the spleen suggests the importance of this organ in the shock syndrome. Reilly and Schayer (22) have shown that the histidine decarboxylase assay as used in this study gives an accurate reflection of histamine formation *in vivo*. Other studies have shown that the lung plays an important role in the production and release of histamine (23, 24). Thus, histamine produced during trauma may act locally; but in addition, histamine may be released from the injured lung and affect the systemic circulation as suggested by Aviado (25).

The low plasma activity in the controls and in the traumatized rats (Table III) supports the concept that the enzyme is not present in the circulation, but restricted primarily to the organs where it acts locally.

Reichard (14, 16) has suggested that after trauma, activation of the reticuloendothelial system (RES) is important in the maintenance of homeostasis. Other studies have shown that exogenous histamine stimulates RES activity (26). Schayer also reports the activation of histidine decarboxylase in the reticuloendothelial organs by agents that stimulate phagocytosis (4). He suggests that these effects may be due to the circulatory action of histamine which regulates blood flow to the phagocytic cells. In this regard, it is significant that at 2–4 hr after trauma, RES activity in the spleen is increased (27) in conjunction with increased histidine decarboxylase activity.

Summary. Histidine decarboxylase activity was studied in the rat at various times following exposure to trauma. The enzyme activity in the lung increased 15 min after exposure to trauma, reached a peak at 4 hr, and returned to normal by 10 hr. In the spleen, the activity increased at four hr and returned to normal by 10 hr. No changes in the activity were found in the plasma. These results support the concept that in shock induced histamine is formed locally and may contribute to the changes in the microcirculation.

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