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STUDIES ON ANAPHYLACTIC SHOCK

2. INHIBITION OF ANAPHYLACTIC SHOCK WITH VARIOUS DRUGS

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As was reported in the previous paper, during anaphylactic shock, a prominent rise in the serum level of serotonin (5HT) and ATP (Δ10P) is observed. In the present paper, we are going to examine the inhibition of anaphylactic shock with various drugs, and to seek the mechanism of such inhibition.

Materials and Methods

As was described in the lst report, dogs weighing about 10 kg, and guinea pigs weighing about 300 g were sensitized with horse serum. Prior to shock-inducing injection, the following drugs were given to the animals: Atropine sulphate, chlortrimeton, hydrocortisone, reserpine, heparin, artificial hibernation, LSD-25, crude renal ATP-ase, and combination of LSD-25 and crude renal ATP-ase.

With the dogs, blood pressure, respiration, serum 5TH, Δ10P and histamine concentration, platelet count and fibrinolysis were investigated before and after the shock-inducing injection. And with the guinea pigs, lung weight, lung water content, survival rate, and effects of the drugs were investigated.

Results

a. *Effect of atropine sulphate.* It is already an established fact that anti-histaminics and anticholinergics, by suppressing the release of histamine and acetylcholine, respectively can inhibit, to some degree, the development of anaphylactic shock.

Now 0.1 mg/kg of atropine sulphate was given to dogs 5 minutes before the shock-inducing injection. Immediately following the injection, blood pressure fell transiently by 30 mm Hg. with apnoea followed by hyperpnoea. These changes

in blood pressure and respiration returned to the normal in several minutes, and scarcely any shock death occurred. Platelet count and serum 5HT, Δ10P and histamine concentration in peripheral and portal veins and left ventricular blood before and after the shock-inducing injection were shown in Table 1. Atropine

Table 1. Effect of atropine sulphate on anaphylactic shock
(Average of 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	30	30	29.5
After	15	13.5	11.5
Serum 5HT (γ/dl)			
Before	15	20	18
After	30	45	38.5
Serum Δ10P (γ/dl)			
Before	200	130	195
After	480	540	390
Serum histamine (γ/dl)			
Before	3	3	2.7
After	4.5	4.2	3.9

could scarcely inhibit the decrease of platelet count, producing remarkable rise in serum 5HT concentration, and somewhat slighter increase in serum Δ10P concentration in each sample of collected blood (refer to the 1st report).

To guinea pigs, 0.01 mg/100 g of atropine sulphate was given before the shock-inducing injection, and the mortality rate in shock was reduced to 60~80% in the atropine pre-treated group. Further, lung weight and lung water content for dead cases were smaller in the treated group than in the control. Therefore atropine sulphate is considered to exert some inhibitory effect on anaphylactic shock.

b. *Effect of chlortrimeton.* Chlortrimeton is known to have anti-histaminic activity and is believed to relieve the shock syndrome in anaphylaxis.

When 1 mg/kg of chlortrimeton was given to dogs 5 minutes prior to the shock-inducing injection, the blood pressure fall of about 10~20mm Hg. took place. Namely, chlortrimeton was confirmed to have been quite effective in arresting the fall of blood pressure in anaphylaxis. But this drug scarcely prevented the transient apnoea followed by hyperpnoea. Platelet count, and 5HT, Δ10P and histamine concentration in serum before and after the shock-inducing injection are shown in Table 2. Chlortrimeton could scarcely inhibit platelet decrease, producing remarkable rise in serum 5HT and Δ10P. But increase in serum histamine concentration seemed somewhat inhibited. When guinea pigs were pretreated

Table 2. Effect of chlortrimeton on anaphylactic shock
(Average of 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	28	29.5	28
After	11.5	13.4	12
Serum 5HT (γ/dl)			
Before	14	18	16.5
After	31	43.5	38
Serum Δ10P (γ/dl)			
Before	150	150	160
After	430	500	445
Serum histamine (γ/dl)			
Before	3	3.1	2.8
After	2.8	3.5	4.0

with 0.1 mg/100 g of chlortrimeton, the mortality rate after the shock-inducing injection was 60~80%, indicating some degree of inhibitory effect.

c. *Effect of hydrocortisone.* Five mg/kg of hydrocortisone was intramuscularly given to dogs 10 minutes before the shock-inducing injection, and the blood pressure fall of about 20mm Hg. and the slight change in respiration were observed. As seen in Table 3 the fall in platelet count and rise in serum Δ10P and histamine con-

Table 3. Effect of hydrocortisone on anaphylactic shock
(Average of 5 dogs

	Peripheral vein	Portal vein	Left ventricle
platelet count (10^4/cmm)			
Before	29	27	30
After	18	18.5	21
Serum 5HT (γ/dl)			
Before	15	20	17.5
After	25	30	30.5
Serum Δ10P (γ/dl)			
Before	250	200	180
After	350	380	370
Serum histamine (γ/dl)			
Before	2.7	3	2.8
After	3.3	3.3	3.9

centration were slight, and fibrinolysis scarcely developed. As reported previously, prominent fall in blood pressure took place following the incompatible transfusion. Now, no shock was induced by incompatible transfusion when animals

were pretreated with 5 mg/kg of hydrocortisone. The drug also reduced the rate of shock death in guinea pigs to 80%. Thus hydrocortisone seemed to suppress the release of histamine and to prevent rise in Δ10P concentration. However, the release of 5HT in serum was scarcely inhibited, assumedly because ACTH, cortisone, hydrocortisone and prednisolone have activity to accelerate the release of 5HT.

d. *Effect of reserpine.* When 0.1 mg/kg of reserpine was intramusculary administered to dogs, serum level of 5HT rose to 20~30 γ/dl in several hours after the shock-inducing injection, and then gradually dropped, returning to the pre-injection level after 10 hours. When a shock-inducing injection was made 10 hours after the administration of reserpine, the fall in blood pressure and the changes in respiration were extremely slight in the majority of the cases. And as seen in Table 4, serum concentration of 5HT showed scarcely and serum concentration of histamine only slight elevation, while serum level of Δ10P attained 3~4 times the pre-injection level, almost the same as in the control. Also the decrease in platelet count seemed to be semewhat inhibited. When 0.01 mg/100 g of reserpine was intramuscularly administered to guinea pigs 10 hours before the shock-inducing injection, the rate of shock death was 70%. In this way, reserpine, by arresting increase in serum 5HT and histamine, tended to inhibit somewhat the development of shock in anaphylaxis.

Table 4. Effect of reserpine on anaphylactic shock (Average of 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	32	30	30
After	15	15.5	13.5
Serum 5HT (γ/dl)			
Before	20	25	20.5
After	25	30	29.5
Serum Δ10P (γ/dl)			
Before	180	185	195
After	490	500	530
Serum histamine (γ/dl)			
Before	3	2.8	2.7
After	4.5	4.0	5.0

e. *Effect of heparin.* It is well known that heparin, through its anti-thrombin action, can inhibit blood coagulation. On the other hand, thrombus is often observed in various organs during anaphylactic shock.

In the present experiments, 10 mg/kg of heparin was intravenously given to dogs, and after 10 minutes, the shock-inducing injection was performed. Blood pressure fell about 20 mm Hg., and returned to initial level in several minutes in the majority of the cases. Changes in respiration were also slight. As shown in Table 5, decrease in platelet count and hence increase in 5HT concentration were

Table 5. Effect of heparin on anaphylactic shock
(Average of 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	29.5	30	30
After	23	24	25
Serum 5HT (γ/dl)			
Before	15	16	15
After	20	24	24
Serum Δ10P (γ/dl)			
Before	230	200	195
After	480	500	600
Serum histamine (γ/dl)			
Before	3	2.5	2.8
After	5.5	4.8	5.5

extremely small in each site of blood sampling. However, serum Δ10P concentration was remarkably elevated almost the same as in the control. Namely, despite the evident suppression of the release of 5HT, the release of ATP still persisted. Pre-injection of heparin in a dose of 5 mg/100 g could not sufficiently inhibit platelet decrease and 5HT increase. The intravenous administration of heparin to guinea pigs in a dose of 1 mg/100 g reduced shock death to 70%. Heparin thus tended to inhibit the development of shock by arresting platelet decrease and the release of 5HT in anaphylaxis.

f. *Effect of artificial hibernation.* Dogs were subject to artificial hibernation by intramuscularly giving each 5 mg/kg of chlorpromazine, pyrethiazine and opystan and cooling them as low as 30°C of rectal temperature, and then the shock-inducing injection was performed. Blood pressure fall of 20~30 mm Hg. was induced by the cocktail injection, but further fall was scarcely caused by the shock-inducing injection. Respiration was quiet, and decrease in platelet count and rise in serum 5HT, Δ10P and histamine concentration were extremely slight (Table 6). As was described in the 1st report, infusion of 5HT and ATP into the left ventricle induced shock-like blood pressure fall. When, however, the same concentration of 5HT and ATP were infused into the left ventricle of animals which underwent

Table 6. Effect of artificial hibernation on anaphylactic shock
(Average of 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	28.5	30	30
After	27.0	25.5	26.5
Serum 5HT (γ/dl)			
Before	15	14	15
After	18	25	23
Serum Δ10P (γ/dl)			
Before	210	200	210
After	350	300	330
Serum histamine (γ/dl)			
Before	2	2.4	2.5
After	2.8	3.3	3.7

hibernation, fall in blood pressure scarcely developed. Thus hibernation seemed to inhibit not only platelet decrease and the release of 5HT, Δ10P and histamine during anaphylaxis, but also responses of the living body to these substances. Hibernation also reduced the rate of shock death in guinea pigs to 20%.

g. *Effects of LSD-25.* It is widely known that 1/3~2 volumes of LSD-25 (lysergic acid diethlyamide) well antagonize with 5HT. In the present experiments, 10 γ/kg of LSD-25 was intramuscularly administered to dogs, and after 10 minutes, a shock-inducing injection was performed. As shown in Fig. 1, blood pressure began to fall immediately after the development of shock, attaining 30~40 mm Hg, and after 30 minutes returned to the initial level. But no bradycardia was observed in the electrocardiogram, and there was scarcely any shock death. Respiration was relatively quiet. After the shock-inducing injection, the platelet count fell to 10×10^4~20×10^4/cmm in average at each site of blood collection, and serum Δ10P and histamine concentration were elevated remarkably almost the same in the control. Only the rise in 5HT concentration was extremely slight. LSD-25 thus suppressed the release of 5HT, but could not perfectly arrest the

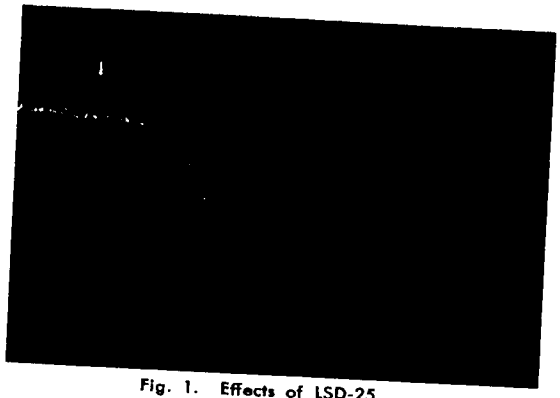

Fig. 1. Effects of LSD-25
↓ indicates challenging injection of horse serum

development of shock, and caused anaphylactic shock death of guinea pigs nearly at the same rate as for the control.

h. *Effect of crude renal ATP-ase.* As reported in the previous paper, ATP was released in serum in anaphylaxis. Crude-ATP-ase was prepared from the kidney of the dog, and administered in 1 cc/kg to animals intravenously or intramuscularly 5 minutes before the shock-inducing injection. This pretreatment could not perfectly inhibit the development of shock. Rise in serum ΔIOP was hardly or only slightly observable, but platelet decrease and 5HT and histamine increase were the same as in the control. APT-ase could in this way inhibit an increase in ΔIOP concentration, but not perfectly arrest the development of shock.

Fig. 2. Effect of combined use of LSD-25 and ATP-ase
↓ indicates challenging injection of horse serum

i. *Effect of combined use of LSD and APT-ase.* Both LSD-25 and ATP-ase respectively could suppress the release of 5HT and ATP, but neither of them could perfectly suppress the development of shock. In view of this, 10 γ/kg of LSD and 1 cc/kg of ATP-ase were concurrently given intramuscularly or intravenously, and 10 minutes later, a shock-inducing injection was performed. As shown in

Table. 7. Effect of combined use of LSD-25 and ATP-ase on anaphylactic shock (Average 5 dogs)

	Peripheral vein	Portal vein	Left ventricle
Platelet count (10^4/cmm)			
Before	30.5	29.0	30.0
After	13.0	12.5	12.4
Serum 5HT (γ/dl)			
Before	13.0	14.5	13.0
After	15.0	15.5	14.5
Serum ΔIOP (γ/dl)			
Before	225	250	250
Afte,	240	250	245
Serum histamine (γ/dl)			
Before	3.0	2.5	2.5
After	4.5	3.6	4.0

Fig. 2, scarcely any changs in blood pressure and in respiration were observed. Nor was seen any bradycardia in the electrocardiogram. This treatment was thus proved to have successfully inhibited the development of shock. Platelet decrease

at each site of blood collection was observed in the same way as in the control, but there was scarcely any rise in serum 5HT and ΔIOP concentration. When, however, 5 γ/100 g of LSD and 0.1 cc/100 g of ATP-ase were concurrently given to guinea pigs and then a shock-inducing injection was performed, shock death occurred in several minutes in 60% of them. In this way, the pre-injection of sufficient doses of LSD-25 and ATP-ase could well arrest the development of anaphylactic shock in dogs.

Discussion

It is already a well known fact that anti-histaminics and anti-cholinergics, by suppressing the release of histamine and acetylcholine, respectively, can, to some degree, arrest the development of anaphylactic shock. In this series of experiments, various drugs were administered prior to shock-inducing injection, and their effects on the development of anaphylactic shock and on the release of serotonin and ATP were investigated. Neither atropine nor chlortrimeton, which have respectively anti-cholinergic and anti-histaminic activity, could perfectly inhibit the development of shock, the decrease in platelet count and release of serotonin and ATP in serum being observed remarkably despite their use. The release of ATP was somewhat inhibited, assumedly because atropine has some activity to inhibit fibrinolysis, and consequently the release of ATP from other source than platelets may have been suppressed.

Heparin is known to inhibit well platelet decrease and thrombus formation. But in order to inhibit platelet decrease in anaphylaxis, it seems necessary to administer in dosis of 10 mg/kg or more. When 5 mg/kg was pre-injected, platelets still decreased, and the release of serotonin was not sufficiently inhibited. And even when the release of serotonin was evidently inhibited, the release of ATP seemed to continue still.

As to reserpine, Fink (1956) reported that it could inhibit the contraction of the uterus by serotonin. Fletscher, Shore & Brodie (1956) and their collaborators entertained view that reserpine would activate the release of serotonin, and that thruogh the mediation of this mechanism, reserpine would exert its effect upon the central nervous system. According to Shore, Fletscher, Tomich, & Brodie (1956), reserpine released 5HT from platelets. And Haverback, Dutcher, Shore, Tomich, Terry & Brodie (1957) reported that when 0.015 mg/kg of reserpine was daily given to guinea pigs, serotonin content in the platelet became zero in a week. In our experiments, the release of serotonin into serum and plasma was thoroughly effected by giving reserpine, and then the shock-inducing injection was

performed. Although no further increase in serotonin concentration was caused, the development of shock was nevertheless observed to some degree.

Pallota & Ward (1957) reported that the administration of LSD-25 could prevent shock death in guinea digs. And Fink (1956) reported that it could arrest the contraction of the uterus of a sensitized mouse. In our experiments, the single administration of either LSD-25 or renal ATP-ase could not perfectly prevent the development of shock. But when administered concurrently, they coud considerably well arrest the development of shock in dogs, though not prefectly in guinea pigs.

From all the above described results, it may be assumed that such severe reflexes (or collapse thereon) as causes shock death can not be attributed to the single action of either ATP or serotonin. Probably it will be more adequate to ascribe it to combined action of a number of such active substances. Further, instead of single reflex system, a combination of plural reflexes should be considered to participate in inducing severe shock. That antihistaminic and anti-cholinergic drugs, LSD, heparin and ATP-ase, when used singly, all exhibited often weak effect seems to indicate the plurality of the reflex-activating substances. From this point of views, it is very natural that artificial hiberantion and hydrocortisone administration, which depress the antigen-antibody reaction itself, are effective in inhibiting the development of shock.

Summary

Anaphylaxis was induced in dogs and guinea pigs, which were pretreated with various drugs, and the degree of its development, and serotonin, Δ10P and histamine concentration in serum and platelet count were investigated with the following results: (1) Atropine sulfate inhibited rise in Δ10P concentration in serum, and to some degree the development of shock. (2) Chlortrimeton inhibited rise in histamine concentration in serum, and to some degree the development of shock. (3) Hydrocortisone and artificial hibernation significantly inhibited the development of shock by depressing antigen-antibody reaction. (4) When reserpine was pre-administered, increase in serum serotonin and histamine, caused by the shock-inducing injectson, was prevented, and the development of shock was slight. (5) Heparin remakably arrested the decrease in platelet count, and the development of shock was slight. (6) The concurrent administration of LSD-25 and ATP-ase remarkably inhibited the development of shock, while the single administration of either of them did not appear to inhibit it perfectly.

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