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Protection against burn, tourniquet and endotoxin shock by histamine, 5-hydroxytryptamine and 5-hydroxytryptamine derivatives

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Summary

1. 5-Hydroxytryptamine (5-HT), tryptamine, 5-methyltryptamine, 5-methoxytryptamine, N-methyltryptamine, 5-hydroxy-N,N-dimethyltryptamine, and histamine markedly protect mice subjected to burn, tourniquet and endotoxin shock. All of these compounds protect when given 30 min before the production of shock, but not when administered afterwards.

2. The above compounds, as well as purines and purine derivatives have a similar chemical structure. Protection requires the compounds to contain a 5-membered ring with one unsubstituted N atom and a side chain with a basic N atom three atoms from the ring.

3. All other biological amines tested without this chemical structure did not protect.

4. Since the simplest compound containing all the prerequisites for protection is histamine, this compound may play the key role in protection, for both 5-HT and purines release histamine from tissues.

5. Protective doses of 5-HT and histamine prevent swelling of the injured area after tourniquet trauma and produce an increased bleeding volume and lower haematocrit value after burning. These actions of the drugs on the circulation may account for the increased survival after thermal trauma.

Introduction

Many drugs influence mortality after traumatic shock. These include adrenaline, noradrenaline, chlorpromazine and derivatives, phenoxybenzamine, histamine and adrenal hormones. The results have often been contradictory, depending upon the investigator, the type of shock studied, the timing, dose and route of drug administration (for reviews, see Millican & Rosenthal, 1954 ; Paton, 1957 ; Eichler & Farah, 1966 ; Shoemaker, 1967).

Recently we reported that pretreatment of mice with purines and purine derivatives markedly decreased mortality after burn, tourniquet and endotoxin shock (Markley & Smallman, 1970). Because of the effect of these amines, we studied other amines in an effort to find some kind of order in the confusing, but important, problem of drug action in shock.

Our results show that purines, indolealkylamines (that is, 5-hydroxytryptamine (5-HT) and derivatives), and histamine produce a striking improvement in survival

after burn, tourniquet and endotoxin shock when these drugs are given before shock, but not when administered afterwards. The beneficial amines had a similarity in chemical structure that was different from others that were ineffective.

The action of the drugs in improving the circulation after shock may account for their protective effect.

Methods

Animals

Female 18–20 g NIH stock, albino, Swiss Webster mice were used.

Types of shock studied

Burn shock. Mice were anaesthetized with diethyl ether and immersed in water to the axilla (two-thirds body surface area) at 70° C for 8 seconds. If the mice were given no fluid therapy, mortality within 48 h was 82–100%. In the histamine and 5-HT inhibitor experiments, it was necessary to inject 0·6 ml of 0·85% NaCl subcutaneously or intraperitoneally immediately postburn to achieve a 48 h mortality around 50%.

Tourniquet shock. Mice were anaesthetized with diethyl ether for 30–40 seconds. Rubber bands (No. 30, Arrow Rubber Corp., seven twists) were placed high in the inguinal region on both hind legs according to the technique of Rosenthal (1943). The tourniquets were allowed to remain in place for 60–90 min, after which time the bands were cut loose. This degree of tourniquet trauma caused a 49–67% shock mortality 48 h after trauma if there was no fluid therapy after release of the tourniquets.

Endotoxin shock. Mice were given an intraperitoneal injection of 0·5 mg *E. coli* endotoxin (Bacto-lipopolysaccharide *E. coli* 026:B6, Difco). This dose produced a 63–90% mortality in 48 hours.

After production of shock, eight to ten mice were placed together in a plastic shoe box type cage (29 × 18 cm) with sawdust in a constant temperature room at 25° C ± 1° C. Purina laboratory chow and water were allowed *ad libitum.*

Drugs tested

Histamine diphosphate was purchased from National Biochemical Corp.; 5-HT creatinine sulphate monohydrate from Aldrich Chemical Corp., Inc. Other related chemical compounds tested were obtained from the above companies or from Regis Chemical Co., Eastman Organic Chemicals, Sigma Chemical Co., Merck, Sharpe & Dohme, Smith Kline & French Laboratories, and Burroughs Wellcome Company.

All drugs were dissolved or suspended in sterile distilled water or 10% Tween 80 (Fisher Scientific Co.). Each was injected in 0·1 ml volume intraperitoneally unless otherwise stated. At least four non-lethal concentrations were tested. In the case of tourniquet injury, the injections were made 30 min before the application of tourniquets or immediately after their release. Mortality due to trauma or endotoxin was recorded at 5, 18, 24, 40 and 48 hours.

Changes in the circulation

Groups of five mice were bled into weighed centrifuge tubes on decapitation and the blood weighed. Microhaematocrit values were determined in individual mice by filling capillary tubes and centrifuging in a Drummond centrifuge for 2·5 minutes. Serum proteins were analysed by the copper sulphate method (Phillips, Van Slyke, Hamilton, Dole, Emerson & Archibald, 1950). To determine changes in fluid distribution after tourniquet application, mice were killed with ether and frozen in liquid nitrogen 3 h after tourniquet removal. Wet and dry weights of the viscera and cephalad and caudal halves were determined as described by Tabor, Rosenthal & Millican (1951).

Rectal temperature measurement

Rectal temperature of mice was measured using a physiological probe (Type 402) and a YSI Model 42SC Telethermometer (Arthur H. Thomas Co.) immediately before injection of drug and at 0·5, 1·0, 1·5 and 3·0 h intervals postinjection. Average readings were calculated for ten mice in each group.

Statistical analysis

The probability of 0·05 ($P=0·05$) was chosen as the level of statistical significance. Computations using the Chi square method for mortality and Student's t test for laboratory data were made.

Results

Effect of 5-HT and derivatives on mortality after shock

Table 1 shows that 37·5–640 μmol/kg of 5-HT (17·5–260 mg/kg of 5-HT creatinine sulphate monohydrate) produced a marked beneficial effect against a variety of shock models when injected intraperitoneally 30 min before production

TABLE 1. *Effect of 5-HT on shock mortality*

Type of shock	Therapy	Dose	μmol/kg	No. of mice	Cumulative mortality 24 h %	Cumulative mortality 48 h %
Burn	5-HT	2·2 mg/kg	12·5	30	77	80
	,,	6·6 ,,	37·5	33	33	36‡
	,,	14·0 ,,	80·0	74	24	27‡
	,,	26·0 ,,	147	41	22	24‡
	,,	56·5 ,,	320	64	33	38‡
	,,	113·0 ,,	640	64	28	31‡
	0·3 M NaCl	0·1 ml		74	70	73
Tourniquet	5-HT	2·2 mg/kg	12·5	43	49	56
	,,	14·0 ,,	80·0	42	21	21*
	0·3 M NaCl	0·1 ml		42	38	45
Endotoxin	5-HT	2·2 mg/kg	12·5	40	30	48
	,,	6·6 ,,	37·5	40	20	28†
	,,	14·0 ,,	80·0	40	20	23‡
	,,	26·0 ,,	147	40	13	15‡
	,,	56·5 ,,	320	40	13	28†
	,,	113·0 ,,	640	20	15	15†
	H_2O	0·1 ml		70	46	57

* $P<0·05$; † $P<0·01$; ‡ $P<0·001$. All drugs and solutions were injected intraperitoneally 30 min before production of shock. 5-HT doses higher than those listed were lethal to normal mice.

of shock. 5-Hydroxytryptamine lowered 48 h mortality from 73% to 24% in thermal trauma ($P<0.001$), from 45% to 21% in tourniquet trauma ($P<0.05$), and from 57% to 15% in endotoxin shock ($P<0.001$). When the same doses of 5-HT were injected intraperitoneally up to 30 min after burn, tourniquet or endotoxin shock, there was no significant protection or toxicity.

A detailed study of 5-HT derivatives was made in burn shock to determine which part of the molecule was necessary for protective action. Table 2 lists those derivatives of 5-HT that also produced a significant lowering of 48 h mortality. When these derivatives were injected immediately after the burn, no protection occurred.

5-HT derivatives tested that had no protective effect before or after burns included: L-tryptophan (25–635 μmol/kg), 5-hydroxytryptophan (22·5–60 μmol/kg), indole (45–1,365 μmol/kg), 5-hydroxyindole (37·5–300 μmol/kg), 5-hydroxyindole-3-acetic acid (23–185 μmol/kg), N-acetyl 5-HT (21·5–555 μmol/kg), and N,N-dimethyltryptamine (25–350 μmol/kg).

Effect of histamine and derivatives on mortality after shock

Fox & Lasker (1962) reported that histamine and imidazoleacetic acid enhanced survival by pretreatment of mice subjected to burn and endotoxin shock. When we investigated the effect of histamine (Table 3), histamine diphosphate did indeed significantly protect mice against burn, tourniquet and endotoxin shock when injected 30 min before the production of shock. When the 135 mg/kg dose of histamine diphosphate was injected intraperitoneally 5 min after tourniquet release, there was no significant protection or toxicity; the same dose injected intraperitoneally 30 min after a burn was toxic and increased mortality from 23% to 55% ($P<0.01$); the 70 mg/kg dose injected intraperitoneally 15 min after endotoxin injection protected and reduced 48 h mortality from 60% to 33 % ($P<0.02$).

None of the following histamine derivatives tested had any protective effect in burn shock: L-histidine (0·34–6·5 mmol/kg), N-acetylhistamine (32–815 μmol/kg), imidazole (75–515 μmol/kg), imidazoleacetic acid hydrochloride or Na salt (0·02–1·6 mmol/kg), L-histidinol dihydrochloride (23·5–235 μmol/kg), or benzimidazole (2–210 μmol/kg).

TABLE 2. *Effect of 5-HT derivatives on burn shock mortality*

Agent	Optimum dose	μmol/kg	No. of mice	Cumulative mortality 24 h %	48 h %
Tryptamine HCl	103 mg/kg	520	46	61	61†
0·3 M NaCl	0·1 ml		47	87	89
5-Methyltryptamine	55·5 mg/kg	320	60	35	35‡
10% Tween 80	0·1 ml		60	97	97
5-Methoxytryptamine	32·5 mg/kg	170	45	11	11‡
10% Tween 80	0·1 ml		46	91	91
N-Methyltryptamine	91·5 mg/kg	525	41	44	51‡
10% Tween 80	0·1 ml		41	95	95
5-Hydroxy-N,N-di-methyl-tryptamine	16 mg/kg	80	46	31	31‡
10% Tween 80	0·1 ml		46	94	94

† $P<0.01$; ‡ $P<0.001$. All drugs were injected intraperitoneally 30 min before thermal trauma. The following dose ranges were used: tryptamine HCl (0·065–1·05 mmol/kg); 5-methyltryptamine (80–635 μmol/kg); 5-methoxytryptamine (25–685 μmol/kg); N-methyltryptamine (0·065–1·06 mmol/kg); 5-hydroxy-N,N-dimethyltryptamine (80–640 μmol/kg).

Effect of 5-HT and histamine inhibitors on mortality after shock

Various inhibitors of 5-HT and histamine were tested for their ability to influence shock mortality after burns or to reverse the protective effect of either agent. Ganley (1962) has reported that mice sensitized to histamine by *B. pertussis* had an increased survival after thermal trauma when treated before and after injury with cyproheptadine, a potent anti-5-HT and antihistamine drug (Eichler & Farah, 1966). In our non-sensitized mice, however, cyproheptadine did not protect burned mice in doses of 0·25–25·0 mg/kg suspended in 10% Tween 80 and injected subcutaneously 30 min before the burn. When the same doses were given subcutaneously 60 min before the intraperitoneal injection of a protective dose of 5-HT (14 mg/kg) and the animal burned 30 min later, cyproheptadine reversed the protective effect of 5-HT. Forty-eight hour mortality increased from 33% to 85% (control value of 90%). These results are, therefore, compatible with the protection observed with 5-HT.

Unlike tourniquet shock (Millican & Rhodes, 1958), pretreatment with the antihistaminic and anti-5-HT drug, chlorpromazine hydrochloride, had no protective effect in thermal trauma in doses of 0·5–20 mg/kg; however, a dose of 2·5 mg/kg or higher could reverse the beneficial effects of 135 mg/kg of histamine diphosphate.

The monoamine oxidase inhibitor, 2-phenylcyclopropylamine (2·5 mg/kg) lowered 48 h mortality from 69% to 40% ($P<0·02$). Ergonovine maleate (2·5 mg/kg), which has anti-5-HT as well as antimonoamine oxidase activity, also significantly decreased 48 h mortality from 77% to 45% ($P<0·01$). These results are compatible with those observed with the exogenous administration of 5-HT and histamine, since the monoamine oxidase inhibitors can produce an accumulation of endogenous amines, including 5-HT histamine (Eichler & Farah, 1966).

Effects on mortality of other biological amines

None of the following amines showed any protection in the burn shock model when given 30 min before or immediately after the burn: L-adrenaline hydrochloride (0·23–9·1 μmol/kg), noradrenaline (L-noradrenaline, 1·2–14·8 μmol/kg), acetylcholine

TABLE 3. *Effect of histamine on shock mortality*

Type of shock	Therapy	Dose	μmol/kg	No. of mice	Cumulative mortality 24 h	Cumulative mortality 48 h
					%	
Burn	Histamine $diPO_4$	5 mg/kg	16	20	85	85
	,,	35 ,,	114	49	47	49‡
	,,	70 ,,	228	26	19	31‡
	,,	135 ,,	440	48	25	27‡
	,,	250 ,,	815	29	48	48†
	H_2O	0·1 ml		50	82	82
Tourniquet	Histamine $diPO_4$	5 mg/kg	16	66	41	44
	,,	135 ,,	440	67	31	33*
	H_2O	0·1 ml		66	47	55
Endotoxin	Histamine $diPO_4$	5 mg/kg	16	66	26	43
	,,	35 ,,	114	66	11	24‡
	,,	70 ,,	228	66	9	17‡
	,,	135 ,,	440	66	6	9‡
	H_2O	0·1 ml		96	39	54

* $P<0·02$; † $P<0·01$; ‡ $P<0·001$. All drugs were injected intraperitoneally 30 min before production of shock. Histamine doses higher than those listed were lethal to normal mice.

chloride (0·3–55 μmol/kg), dopamine (2·6–3,950 μmol/kg) or spermine tetrahydrochloride (14·4–144 μmol/kg).

Phenoxybenzamine, a non-biological amine reported by others to protect in tourniquet and haemorrhagic shock, showed no protection when given in doses of 0·25–2·5 mg/kg 30 min before thermal injury.

Effects of 5-HT and histamine on the circulation

Figure 1 illustrates the effects of 5-HT and histamine injected 30 min before burning on the bleeding volume, haematocrit values and total serum protein concen-

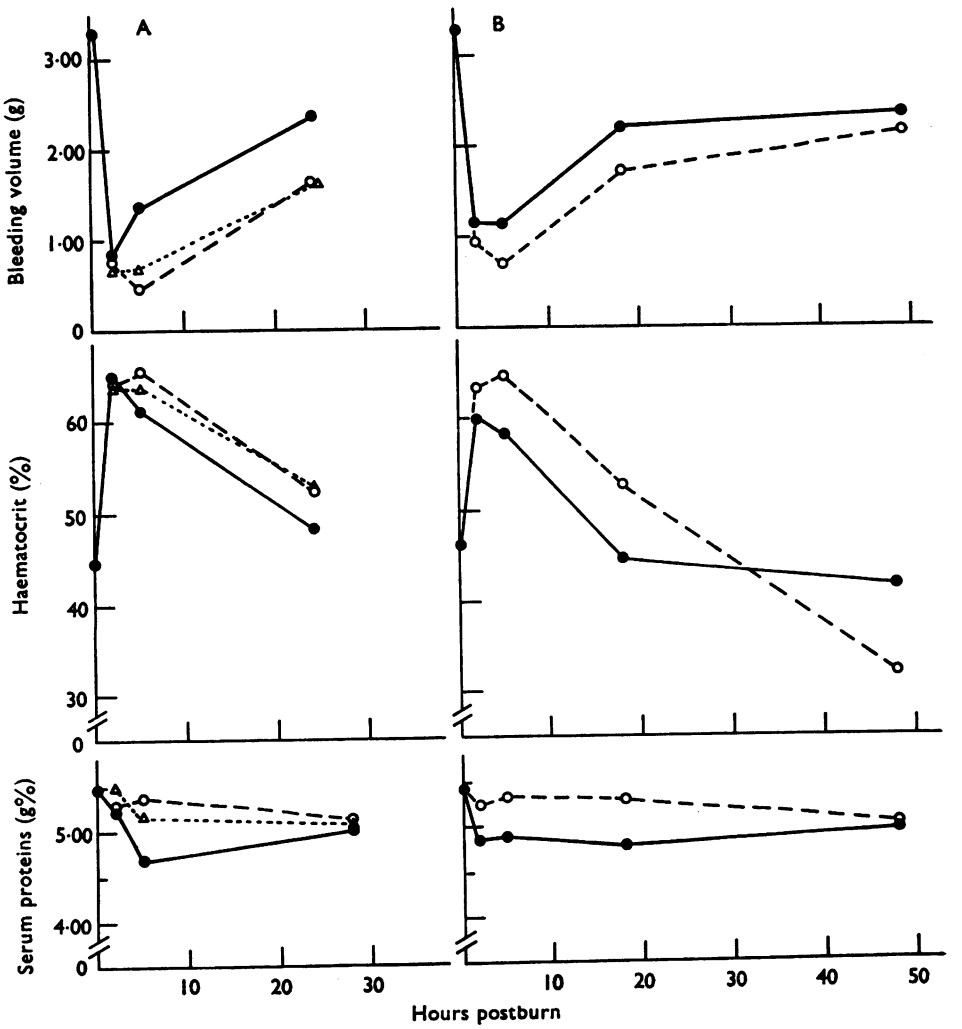

FIG. 1. Effect of 5-HT and histamine on bleeding volume, haematocrit values, and serum protein concentrations in burned mice. Animals received a 2/3 body surface area burn at 70° C for 8 seconds. Each group received 0·1 ml of specified solution intraperitoneally 30 min before thermal trauma. Each point represents the average of five–twelve experiments with five mice combined for each value of bleeding volume, of ten–twenty-six values of individual mice for haematocrit value and six–thirteen experiments with three–five mice combined for each value of serum protein concentration. Column A: ●——●, 14 mg/kg 5-HT; △- - -△, 2·2 mg/kg 5-HT; ○- - -○, 0·3 M NaCl. Column B: ●——●, 135 mg/kg histamine diphosphate; ○- - -○, H_2O.

trations. In Figure 1A, a protective dose of 14 mg/kg of 5-HT produced a significantly increased bleeding volume at 5 and 24 h postburn compared with the 0·3 M NaCl controls ($P<0·01$). 5-HT at a dose that was non-protective (2·2 mg/kg) produced no significant change in bleeding volume compared with the control. The haematocrit value was significantly decreased at 5 and 24 h postburn ($P<0·05$) in mice that had received the protective dose of 5-HT. The serum protein concentration was significantly lower only at 5 h postburn with the protective dose.

As shown in Fig. 1B a protective dose of 135 mg/kg of histamine diphosphate produced a significantly increased bleeding volume at 2, 5 and 18 h postburn ($P<0·02$) and a decreased haematocrit value at the same time intervals ($P<0·02$). The serum protein concentration was also significantly lower at 2, 5 and 18 h postburn ($P<0·01$) with the protective dose.

Changes in fluid weight were studied in the cephalad and caudal halves of the body as well as in the viscera in mice 3 h after release of bilateral hindleg tourniquets. No significant changes were found in the cephalad half and the viscera. Table 4 shows the effects of 5-HT and histamine on fluid changes of the caudal half of the body. After injury by tourniquet there was an increased amount of fluid in the caudal half of the control groups, rising from 11·29 g to 14·48–14·57 g. A protective dose of 14 mg/kg of 5-HT injected 30 min before application of tourniquets significantly lowered the amount of fluid lost into the injured caudal half when compared with the control group given 0·3 M NaCl. The same phenomenon was observed with the protective dose of histamine. These fluid changes in the injured half were not obtained when either 5-HT or histamine were given immediately after release of the tourniquets.

Effect of drugs on body temperature and bleeding volumes of normal mice

Since it is well known that histamine, 5-HT and ATP produce a state of shock in normal mice at the high dose administered in these experiments, rectal temperature and bleeding volumes were measured as an indication of the shock state. Table 5 shows the results of these compounds as well as related purine compounds previously found to be protective (Markley & Smallman, 1970). As controls, some non-effective drugs, such as azathioprine, adrenaline HCl and phenoxybenzamine, are included. In general, drugs that give good protection produced a significantly decreased body temperature and bleeding volume, whereas some non-protective

TABLE 4. *Effect of 5-HT and histamine on fluid accumulation in caudal half of mice after tourniquet trauma*

Drug	Dose mg/kg	Dose μmol/kg	No. of mice	Average water content in caudal half (g)	△ Weight (drug control group) (g)
Untreated	–	–	30	11·29	
0·3 M NaCl	–	–	15	14·57	
5-HT	14	80	15	13·73	−0·83*
,,	2·2	12·5	15	14·22	−0·34
H_2O	–	–	15	14·48	
Histamine	135	440	15	13·79	−0·69†
$diPO_4$	5	16	15	14·58	+0·09

* $P<0·02$; † $P<0·01$. Water and drug solutions (0·1 ml) were injected intraperitoneally 30 min before application of tourniquets. Tourniquets were removed after 90 minutes. Three hours later the mice were killed with ether, frozen in liquid N_2 and dissected.

TABLE 5. *Effect of amines on body temperature and bleeding volume of normal mice 30 min postinjection*

	Protective agents					Non-protective agents			
	Dose		Temp. °C	Bleeding volume g		Dose		Temp. °C	Bleeding volume g
	mg/kg	μmol/kg				mg/kg	μmol/kg		
0·3 M NaCl			37·4	2·87	0·3 M NaCl			37·4	3·67
ATP	500	800	33·6†	2·11†	ATP	50	80	37·3	2·89†
H_2O			37·4	3·18	H_2O			37·4	3·18
5-HT	14	80	35·0†	1·98†	5-HT	2 2	12·5	37·0*	2·81†
H_2O			37·6	3·26	H_2O			37·6	3·26
Histamine $diPO_4$	70	228	34·7†	2·07†	Histamine $diPO_4$	5	16	37·5	3·14
H_2O			37·8	3·33	H_2O			37·8	3·33
Hypoxanthine	500	3675	37·8	3·20†	Hypoxanthine	50	367	37·9	3·26
H_2O			37·8	3·18	H_2O			37·8	
DPN	350	550	34·8†	2·95	DPN	50	75	37·6	
H_2O			37·9	3·39	Tween 80			37·7	3·16
Uric acid	500	2975	36·3†	2·79†	Azathioprine	275	1000	35·0†	2·90†
						25	91	37·8	3·21
					0·15 M NaCl			37·5	3·35
					Adrenaline	1·3	6	36·2†	2·58†
					HCl	0·05	0·2	37·7	3·55
					H_2O			37·6	3·15
					Phenoxy-benzamine	0·5	1·6	37·2*	2·38†
						0·05	0·2	37·4	2·89

*$P<0·05$; † $P<0·01$. Water and drug solutions (0·1 ml) were injected intraperitoneally 30 min before the measurements above.

drugs produced no significant changes in these parameters. But the correlation was not perfect in that (a) small doses of ATP and 5-HT, which showed no protection, also lowered one or both of these parameters ; (b) some of the drugs that protected had no effect on body temperature and only a slight effect on bleeding volume (hypoxanthine) or lowered body temperature only (DPN) ; and (c) non-protective drugs such as azathioprine, adrenaline and phenoxybenzamine, usually lowered both parameters significantly.

Discussion

These experiments demonstrate that pretreatment of mice with histamine, 5-HT and 5-HT derivatives markedly protected against burn, tourniquet and endotoxin shock. Previous studies in our laboratory also showed that purines and purine derivatives are protective (Markley & Smallman, 1970). On the other hand, pretreatment with many other biological amines produced no beneficial effect, at least not in burn shock.

A host of derivatives or related compounds was also tested and Fig. 2 shows the similarity of structure among the beneficial compounds. The basic structure for protection includes a 5-membered ring containing one unsubstituted N atom and a side chain with a basic N three atoms from the ring.

Among the 5-HT derivatives, the following deviations from the basic structure resulted in a loss of protective activity: (a) a decrease in the basicity of the N atom of the side chain by adding a COOH group (L-tryptophan and L-hydroxytryptophan) or by an acetyl group (N-acetyl-5-HT) ; (b) removal of the N atom from the side chain and a decrease in the number of atoms in the side chain (5-hydroxyindole-3-acetic acid) ; or (c) removal of the side chain entirely (indole or 5-hydroxyindole). Contrary to predictions, N,N-dimethyltryptamine was not effective, despite the fact that the same compound with a 5-hydroxy group was effective.

None of the derivatives of histamine tested protected against shock. These deviations from the basic structure required for protection produced a loss of activity: (a) a loss of the N atom in the side chain (imidazolacetic acid or benzimidazole) ; (b) decrease in the basicity of the side chain N atom by adding

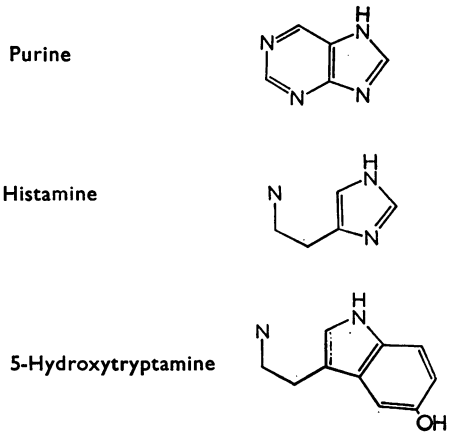

FIG. 2.

a COOH group (L-histidine), an acetyl group (N-acetylhistamine), or an OH group (L-histidinol); or (c) entire removal of the side chain (imidazole).

Of the purines tested by Markley & Smallman (1970), DPN, TPN, ATP, ADP, AMP, cyclic 3′,5′-AMP, adenosine, guanosine, deoxyadenosine, inosine, hypoxanthine, and uric acid protected against the same three types of shock. The following compounds had the basic structure required for protection, but did not protect: (a) xanthine, perhaps due to extreme insolubility; (b) allopurinol, because of its atypical 5-membered ring; (c) azathioprine, perhaps due to the S atom in the compound, and (d) allantoin, for no obvious reason. Other compounds that did not have the basic requirements and did not protect were: (a) caffeine and 7-methylxanthine because there was no unsubstituted N atom on the 5-membered ring; (b) theophylline because of the methyl group on the 1 position of the purine ring; and (c) 4-amino-5-imidazolecarboxamide because of a configurational change in the side chain containing the basic N group.

Pyrimidines (Markley & Smallman, 1970), as well as other biological amines such as L-adrenaline, noradrenaline, dopamine, acetylcholine, and spermine had no beneficial effects and did not have the structural requirements for protection.

The simplest compound containing all the prerequisites for protection is histamine. Indeed, histamine may be the key compound since it is well known that 5-HT (Feldberg & Smith, 1953) and purines (Moulton, Spector & Willoughby, 1957; Spector & Willoughby, 1957) liberate histamine. The fact that histamine is toxic when given after burns supports the idea of the central importance of histamine. In addition, chlorpromazine, which has antihistaminic properties, had no protective effect *per se* and could reverse the beneficial effects of histamine. Against the histamine hypothesis, however, are the data demonstrating that large doses of histamine are required and that histamine causes increased permeability (Spector & Willoughby, 1957), which would be deleterious in shock. The fact that large doses of histamine are required for protection in these experiments does not necessarily rule out its key role since it is possible that smaller endogenous doses at a specific receptor site may accomplish the same end. In the past, histamine was believed to cause shock (Schayer, 1960), but this theory has not been widely accepted. Our experiments would raise another interesting and paradoxical question: can histamine both cause and prevent shock?

The three groups of compounds were only effective when given before the production of shock but never afterwards, with the exception of histamine in endotoxin shock. It is possible to speculate that products released by injured tissues attach to certain cellular receptor sites and produce deleterious effects leading to death. Histamine, indolealkylamines and purines may compete for the sites and protect them. Consequently, these agents must be given before release of the natural products due to shock in order to protect. This hypothesis would explain why large doses are required for protection, since competitive blocking action may demand high concentrations.

One possible objection to this hypothesis might be that the shock produced by these compounds themselves reduced the amount of trauma during the production of injury secondarily by their effect on vital body functions. This objection does not seem likely since there was no absolute correlation between protection and lowering of body temperature or bleeding volume. For example, ATP and 5-HT (in small doses) or azathioprine, adrenaline and phenoxybenzamine (in large doses)

produced significant changes in these functions, yet caused no protection ; on the other hand, certain protective compounds, such as hypoxanthine and DPN, did not produce significant alterations, at least in one of the two parameters.

Various physiological parameters of the circulation were studied to elucidate the mechanism of action of all three classes of compounds. All reduce the amount of fluid lost into the injured area after tourniquet trauma. In a slightly different experimental model Rosenthal (1969) found that 5-HT (25 mg/kg), but not histamine at a smaller dose of 10 mg/kg, decreased tail swelling after injury by tourniquet or burning. The reduction of fluid loss can explain the higher bleeding volume, and haemodilution the lower haematocrit values and serum protein concentrations observed in burned animals. The better circulation achieved with pretreatment by compounds that protect is meaningful, since a non-protective amine such as thymidine-5′-monophosphate did not significantly alter bleeding volume, haematocrit value or serum protein concentrations, even though it produced a state of prostration similar to that seen with the protective compounds (Markley & Smallman, 1970).

The mechanism by which the protective drugs improve the circulation still remains obscure. At least two possibilities seem feasible. First, vasoconstriction produced by the drug could decrease fluid loss into the area of injury. Second, the protective drug could increase fluid loss at the site of injection, where it is held while fluids are lost at the site of injury, and then return it to the circulation after fluid loss due to injury has passed its peak. Further studies are now in progress to delineate the mechanism of action of these drugs.

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