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EFFECTS OF ADRENERGIC AGENTS, THEOPHYLLINE AND OTHER DRUGS ON DEXTRAN EDEMA AND HISTAMINE RELEASE IN RATS

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Abstract—Pretreatment of rats with various catecholamines, theophylline and dibutyryl cyclic AMP decreased the reaction to dextran and reduced the associated release of histamine into the plasma. Protection by the catecholamines was inhibited by the β-blocker, propranolol. Some of these protective drugs owed their effectiveness in large part to the production of hyperglycemia. However, it appeared likely that they also acted directly on the mast cells to prevent the release of histamine and other vasoactive factors, as previously observed *in vitro*. Phenoxybenzamine, nicotinamide and ethanol also afforded protection not explainable by effects on blood sugar.

THE RELEASE of histamine from rat mast cells by dextran, like anaphylactic histamine release,[1] was found to be inhibited *in vitro* by drugs that can increase intracellular cyclic AMP.[2] However, there have been few studies to determine if the same types of drugs also prevent release of histamine *in vivo*. We investigated this question by testing the drugs in rats treated with dextran. An intravenous injection of dextran in the rat causes an acute anaphylactoid reaction, characterized by edema, hypotension and collapse.[3] The reaction is caused by the discharge of histamine, serotonin and perhaps other vasoactive factors from the tissue mast cells.[4–7] For the purpose of this study, the protective effect of various drugs was assessed by the ability to suppress histamine release into the plasma and the development of anaphylactoid symptoms after dextran treatment. A further question which was investigated was whether hyperglycemia induced by the drugs contributed to the protection observed *in vivo*.

METHODS AND MATERIALS

The rats, Sprague–Dawley females, about 125 g, were fed a normal pellet diet with water *ad lib*. Germ-free rats (Sprague–Dawley) were obtained from the Division of Research Services, NIH, and were used immediately.

Dextran (Pharmacia, average mol. wt. 2×10^6), 50 mg, and Evans blue dye, 1·5 mg, were administered simultaneously into the vein in 1 ml saline. In some experiments, dextran with a covalently linked dye (Blue Dextran) was used, so that the persistence of dextran in the plasma could be easily detected.

The rats were pretreated with saline or various drugs. The doses and routes of administration are given with the results. The doses refer to the amount administered to a 125 g rat. Drugs were given 10–30 min or, in the case of phenoxybenzamine, 2 hr before dextran. Compound 48/80 was administered four times over 48 hr, with the last dose 4 hr before dextran. Nicotinamide was given in 5% solution

and ethanol in 20% solution. Glucose was given in 5–50% solution by stomach tube (i.g.) 15–30 min before dextran injection. This route was chosen in order to obtain a sustained and stable elevation of blood glucose. A large dose (2·5 g in 5 ml) was adopted in the study of effects of various pretreatments on the dextran reaction. Smaller doses (0·12 to 2·5 g in 5 ml) were used to determine the level of blood glucose required to protect against dextran.

In some experiments rats received L-tryptophan (65 mg) i.p. 15 min before administration of the drugs to inhibit hyperglycemia.[8] A few rats in which hypoglycemia occurred were discarded.

The reaction to dextran after 20 min was assessed visually by noting the degree of swelling and blueing, prostration, and evidence of hemoconcentration (difficulty in obtaining blood from the aorta and decrease in volume of plasma). The usual dose of dextran invariably produced a severe reaction (which is described later) in untreated rats. Because grading the reactions was subjective and difficult to document, the following procedure was adopted to compare reactions in different groups. The reaction in each rat was classified as "no reaction", "moderate reaction" (edema, blueing and prostration considerably less than that produced by dextran alone) or "severe reaction" (not clearly different from the normal dextran reaction). The percentage of rats falling into each category was then calculated. Protection was evident when some or all of the rats fell into the "moderate reaction" or "no reaction" categories. Reactions after shorter times were graded by comparison with similar reactions in control rats and by evaluating the subsequent course.

Blood samples (0·2 ml) were taken from the tail immediately before the dextran injection and at 5–10 min thereafter. Each sample was delivered into 4·0 ml of ice-cold water, and protein-free filtrates were promptly prepared for glucose determinations. At the end of the experiment (20 min after dextran), a larger volume of blood was drawn into a heparinized syringe from the abdominal aorta, after the rat had been anesthetized with Nembutal. The blood was centrifuged at 4 and the plasma was separated and stored at −15 for histamine assay.

Small pieces of skin and subcutaneous tissue were taken from the front feet in some experiments for microscopic examination. The tissue was stained supravitally with neutral red or toluidine blue and examined for degranulation of mast cells. Standard squares of skin were removed from the abdomen for assay of histamine.

Blood glucose was determined by the enzymatic Glucostat method (Worthington Chemical Co., Freehold, N. J. 07738), after precipitation of proteins with barium hydroxide and zinc sulfate. Values were verified in some samples by the copper reduction method of Nelson.[9]

Plasma and tissue histamine was determined by the enzymatic procedure of Snyder *et al.*[10] as modifed by Beaven *et al.*[11] In this procedure, histamine is converted to [^{14}C]-methylhistamine by incubation with ^{14}C(methyl)-*S*-adenosylmethionine and histamine-*N*-methyltransferase and the labeled amine extracted for assay of ^{14}C. The method is specific and permits assay of nanogram amounts of histamine. The presence of Evans blue or the various drugs in plasma did not interfere with the assay.

Crystalline *l*-isoproterenol HCl was obtained from Sterling Winthrop Laboratories, phenoxybenzamine HCl from Smith Kline & French Laboratories, N^6, $O^{2'}$-dibutyryladenosine monophosphate Na salt (dibutyryl cyclic AMP) from Boeh-

ringer Mannheim Corp., and 48/80 (lot 46664) from Burroughs Welcome & Co. Propranolol HCl was from Imperial Chemical Industries, Ltd., *l*-epinephrine HCl from Parke, Davis & Co., and *l*-norepinephrine bitartrate from Winthrop Laboratories.

RESULTS

Dextran reaction in normal rats. After dextran injection, the classical reaction developed. Uneven swelling of the feet and other parts of the body began within 3–5 min and confluent swelling of the feet was evident by 10 min. Diffusion of the Evans blue into tissues accompanied the edema. The reaction reached a peak in about 20 min, at which time there was hypotension, hemoconcentration and prostration. A slow recovery of the rats then ensued, although dextran was still present in the circulation. Plasma histamine levels also increased to a maximum by 20 min and then declined as the rats were recovering (Fig. 1). Intact mast cells were still present in the skin after the reaction reached a peak, and abdominal skin histamine levels ranged from 29 to 66 μg/g ($n = 4$) compared with 24–48 μg/g ($n = 4$) in untreated rats. These data indicate that a substantial part of the mast cell histamine stores in certain areas remained intact.

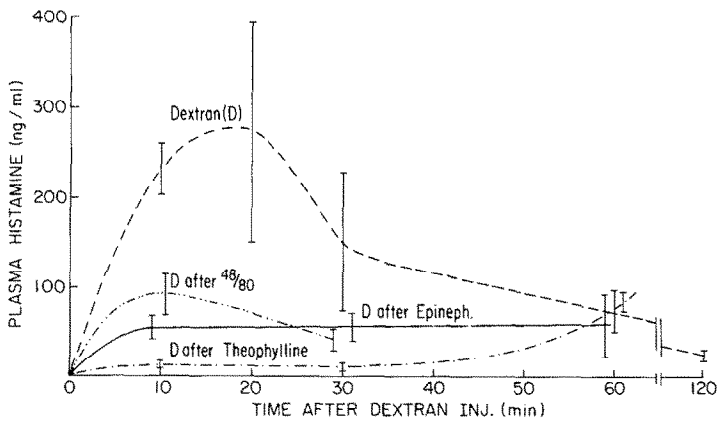

FIG. 1. Time course of plasma histamine level after intravenous injection of dextran in control rats and in rats that had been pretreated with epinephrine, theophylline or compound 48/80.

Germ-free rats reacted to dextran in the same manner and degree as did normal rats. The dextran reaction therefore did not appear to depend on prior sensitization by bacteria.

Effect of drugs on the dextran reaction. In the course of this study, various doses and times of pretreatment were tested. To simplify presentation, some of the data have been pooled in Table 1, since the drugs appeared to be equally effective over the range included. The average interval before dextran was 15–20 min for theophylline, dibutyryl cyclic AMP, epinephrine (0·1 mg) and isoproterenol (0·1 to 0·2 mg) and about 25 min for the other drugs and doses with the exceptions already stated.

The incidence and severity of the dextran reaction as well as release of histamine into the plasma were greatly reduced after pre-treatment with epinephrine (Table 1, Fig. 1). Isoproterenol was much less effective and, even after large doses, moderate reactions often occurred (Table 1). Norepinephrine in large doses was also effective.

TABLE 1. REACTION OF RATS TO DEXTRAN AFTER PRETREATMENT WITH SELECTED DRUGS

Pretreatment			Response to dextran (after 20 min)					
			Reaction				Plasma histamine*	
Drug	Route	Dose (mg)	No. of rats	None (%)	Moderate (%)	Severe (%)	No.	Concn (ng/ml)
None (no dextran)			8	100	0	0	8	8 ± 7
None			68	0	0	100	25	243 ± 144
Epinephrine	s.c.	0·025	8	25	62	13†	8	43 ± 23†
Epinephrine	s.c.	0·1	25	72	24	4†	10	12 ± 11†
Isoproterenol	s.c. ;i.p.	0·1 0·2	15	7	33	60†	14	167 ± 80
Isoproterenol	s.c. ;i.p.	5·0	9	33	56	11†	6	92 ± 33†
Norepinephrine	s.c.	0·15–0·2	7	57	29	14†	6	50 ± 36†
Theophylline	i.p. ;i.v.	10	21	57	33	10†	7	14 ± 14†
Dibutyryl-cyclic-AMP	i.v.	6–8	22	41	14	45†	12	45 ± 30†
Phenoxybenzamine	i.v.	0·8	7	100	0	0†	7	32 ± 20†
Nicotinamide	s.c.	150–200	6	83	17	0†	5	25 ± 18†
Ethanol	i.g.	460 960	6	0	67	33†	5	92 ± 26
Compound 48/80	i.p.	4 × 0·125	4	0	0	100	4	18 ± 4†
D-Glucose	i.g.	2500	12	58	42	0†	5	12 ± 8†
Insulin	s.c.	6–8 units	4	0	0	100	3	704 ± 121†

* Values are mean ± S.D. Plasma histamine was not measured in all experiments, but the animals in which it was measured appeared to be representative of all the animals treated similarly.

† Significantly different ($P < 0.03$) from dextran result without pretreatment.

The β-adrenergic blocker, propranolol (0·5 mg, s.c.), virtually abolished the protective effect of epinephrine, whereas the α-blocker, phenoxybenzamine (0·5 to 1·5 mg, i.g.), did not (not shown). The blockers alone, as employed with the catecholamines, may have had some effect on the dextran reaction, and phenoxybenzamine, 0·8 mg intravenously, completely prevented reactions and histamine release.

Theophylline in doses of 10–12 mg/rat prevented severe reactions and release of histamine (Table 1, Fig. 1). Dibutyryl cyclic AMP also exerted a significant protective effect, although its effectiveness was variable. Nicotinamide in doses of 150–200 mg afforded almost complete protection, and large doses of ethanol gave partial protection. Among other tested drugs not included in Table 1, glucagon, hydrocortisone and ACTH appeared ineffective.

Pretreatment of the rats with compound 48/80, which lowered the skin histamine to 13 μg/g (range 8–16), did not prevent the reaction to dextran, but did abolish the rise in plasma histamine levels (Table 1, Fig. 1).

Glucose in large doses (2500 mg) prevented severe reactions and histamine release. Insulin, in contrast, enhanced release of histamine (Table 1).

Role of hyperglycemia. The studies with large doses of glucose (2500 mg) indicated that hyperglycemia alone could inhibit the dextran reaction. Experiments with smaller doses of glucose (120–2500 mg) resulted in variable increases in blood glucose (Fig. 2), and only rats with levels of 150 mg/100 ml or greater were protected against dextran. As shown in Fig. 2, blood glucose rose above 150 mg/100 ml in certain rats after treatment with epinephrine, dibutyryl cyclic AMP and theophylline. However, in some of the animals receiving each of these drugs as well as the other drugs shown in Fig. 2, blood sugar did not exceed 150 mg/100 ml and the animals were fully pro-

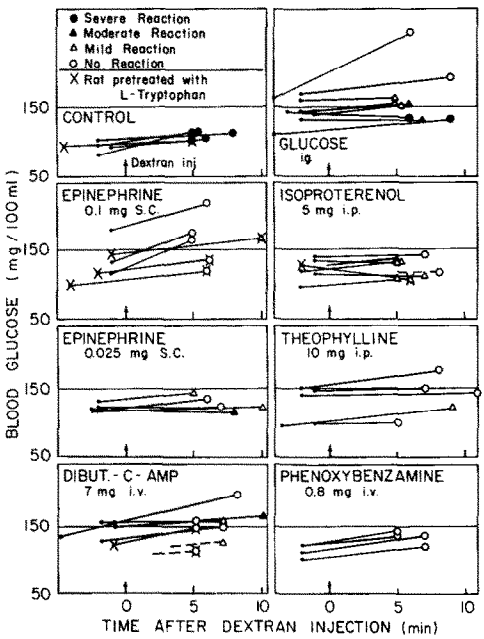

FIG. 2. Changes in blood glucose and the response to dextran in individual rats pretreated with glucose (0·12 to 2·5 g) and various protective drugs. The horizontal line, 150 mg/100 ml, depicts the level of blood sugar which appeared to prevent severe anaphylactoid reaction in the group of glucose-treated rats. The assumption was made that in animals whose blood sugar remained below this level, the protection after drug treatment was not through changes in blood glucose. Several rats received drug doses which differed from the usual doses shown.

tected. In other experiments (not shown) rats received theophylline, 10–12 mg, and were protected, but the blood glucose level when dextran was injected was only 126 ± 15 mg/100 ml ($n = 6$). Ethanol produced no increase in blood sugar, and nicotinamide caused little increase.

Some rats were given L-tryptophan without loss of protection. Blood glucose levels in these rats remained relatively low.

Glucose levels in blood taken from the aorta at the end of the experiments were considerably higher than those in the earlier tail-vein samples, even in rats not pretreated with any drug. These high values may have been due to the removal of large amounts of blood and to anesthesia, and for this reason have not been included in Fig. 2. The correlations of dextran reactions and blood glucose levels involved a degree of uncertainty, because of the dynamic aspects and the necessity of grading early lesions or assuming stable glucose levels.

It is of interest that many of the rats that were protected by drugs never developed definite reactions to the dextran, even after effects of the drugs had worn off and despite the fact that dextran could still be demonstrated in the plasma.

DISCUSSION

The basic mechanism by which dextran acts on the mast cells is still unclear, but its action is reminiscent of anaphylaxis,[2,12] and the presence of antibodies to dextran is one possible mechanism. Antibodies to dextran have not been found,[13] however, and, as the present study showed, the reaction occurs in rats that have not been

exposed to bacteria. The dextran-induced histamine release and anaphylactoid reaction are prevented by pretreatment with glucose and are enhanced by pretreatment with insulin, as shown by this and earlier studies by Goth *et al.*[14] and by Adamkiewicz and Sacra.[15] Dextran is a glucose polymer and glucose is thought to protect by competing with dextran for mast cell receptors.[16] However, there is some evidence that glucose may inhibit various immune reactions *in vivo*.[15,17–19]

The present studies show that the same types of drugs that inhibit histamine release by dextran *in vitro*[2] also inhibited histamine release and development of anaphylactoid symptoms *in vivo*. The effectiveness of epinephrine has been observed previously *in vivo*,[20] but its mode of action was not determined. It is likely that these drugs protect by preventing the discharge of histamine and other vasoactive substances from tissue mast cells. Although these drugs are capable of acting directly on mast cells *in vitro*, possibly through the cyclic AMP system, other factors may come into play *in vivo*. Because many of the drugs are hyperglycemic agents, effects on blood sugar were specifically investigated. By comparison with effects of glucose, the increases in blood sugar produced by the drugs appeared to be insufficient, in various degrees, to account for the level of protection observed. It was impossible, however, to assess precisely the role of hyperglycemia and of other possible indirect effects of the drugs *in vivo*.

Although the same drugs were effective *in vitro* and *in vivo*, quantitative differences were noted. *In vitro*, inhibition of histamine release by the catecholamines was never complete (usually less than 50 per cent), whereas, *in vivo*, complete protection was observed in some instances. Another discrepancy was that isoproterenol was more effective than epinephrine *in vitro*, but much less effective *in vivo*. These discrepancies were undoubtedly explained in part by the presence of glucose in various amounts *in vivo*. Distribution and metabolism could also alter the effectiveness of these compounds *in vivo*. Dibutyryl cyclic AMP was less effective *in vivo* than might have been expected from results *in vitro*, despite its strong hyperglycemic action.

Of the catecholamines investigated, epinephrine was particularly effective. Studies with adrenergic blocking agents suggested that the protection was through a β-effect, as previously suggested.[21]

The protection which in some cases persisted after pharmacological effects of the drugs had subsided, presumably resulted from desensitization of the mast cells to dextran during the time that the drugs were effective, as noted *in vitro*.[22]

Although the present results are consistent with the concept that histamine release is regulated through intracellular cyclic AMP, this may not be the only mechanism. Drugs which have no known action on the cyclic AMP system, such as ethanol and nicotinamide, achieved considerable protection, as they also did *in vitro*.[2]

Factors other than histamine have been implicated in the anaphylactoid reaction,[5,7] and the present observation that dextran produced reactions accompanied by little histamine release in rats pretreated with 48/80 supports this concept. Most of the other drugs, however, changed histamine release and anaphylactoid reactions in parallel fashion, showing that histamine and the other factors are controlled by common mechanisms and that histamine release generally reflects release of all the factors.

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