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The Influence of Vasoactive Amines on Interferon Production in Mice* (33482)

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As part of a study to better understand the influences of environmental factors on resistance to infectious diseases, we have demonstrated that various stressors alter susceptibility to viral infections (1, 2). In some cases the changes in susceptibility, as well as changes in host–defense mechanisms, were transient and were not associated with the increased adrenocortical secretions induced by stress (3, 4). Because vasoactive amines are also secreted in response to environmental stimuli and in turn induce transient physiologic responses, we are investigating the possible mediating role of these amines in the stress-induced suppression of host–defense mechanisms. A previous report (5) described the transitory impairment of interferon production in mice treated with serotonin. This paper describes primarily the influence of epinephrine on interferon production in mice, with comparative data on the influence of norepinephrine and of histamine.

Materials and Methods. Seven to 8-week-old Swiss-Webster female mice were injected i.p. with either epinephrine hydrocholoride (Parke, Davis & Co.), norepinephrine (Levophed, Winthrop Labs.) or histamine phosphate (Eli Lilly & Co.) in 0.25 ml of saline. Controls received 0.25 ml of saline. At intervals from 1 hr before to 24 hr after the drug injection, mice were stimulated to produce interferon by 0.2-ml i.v. injections of either 5.6×10^7 plaque forming units (pfu) of Newcastle disease virus (NDV), or of 100 μg of *Escherichia coli* lipopolysaccharide endotoxin (Difco). Blood was collected 4 hr after NDV and 2 hr after endotoxin injections; sera from 2 or 3 mice were pooled.

Interferon assays employed secondary mouse embryo tissue cultures in 30-ml plastic flasks which were treated for 3 hr at 37° with serum dilutions, then challenged with about 100 pfu of vesicular stomatitis virus (VSV). An overlay of Tris buffer with 0.6% agar and 5% agamma globulin calf serum was added after 1-hr viral adsorption at 37°. Four flasks were used per dilution and plaques were counted after about 40-hr incubation. Results were expressed as the percentage of plaque reduction from virus control flasks, either at a single predetermined interferon dilution or, when necessary, as the dilution inducing 50% pfu reduction. Levels of significance were determined by the Student *t* test.

Experiments and Results. Epinephrine. Figure 1 shows the influence of different

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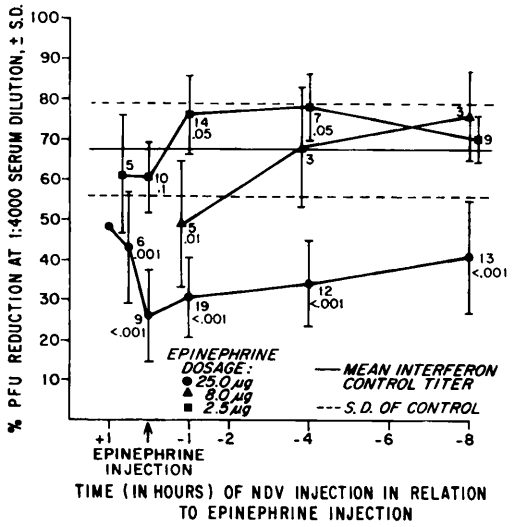

FIG. 1. Interferon levels induced in mice by NDV given at intervals in relation to injections of epinephrine. Points on the graph represent the mean deviation of experimental groups from their controls. The numbers by each point indicate the number of serum pools tested and, where appropriate, the p value, determined by the Student t test.

doses of epinephrine on NDV induced interferon production. Doses of 25 μg induced an immediate impairment of the ability to produce interferon, followed by a gradual return to normal over the following 24 hr. The reduction at 16 hr, not shown in Fig. 1, was −14% ($p = 0.05$). Doses of 2.5 μg induced a slight initial impairment followed by a moderate enhancement of interferon production. The effect of an 8-μg dose was midway between the 2.5 and 25 μg doses.

The release of endotoxin-induced interferon also was impaired by a 25-μg dose of epinephrine, but only when the endotoxin was given after the epinephrine injection. In mice injected with endotoxin 30 min before receiving a 25-μg dose of epinephrine, a moderate enhancement of interferon release was noted, while a slight impairment of interferon release was seen when endotoxin was injected 30 min before a 2.5-μg dose of epinephrine. A 2.5-μg dose of epinephrine had no influence on the release of endotoxin induced interferon when given before the endotoxin injection (Fig. 2).

A series of experiments was next conducted to determine the possible mechanisms involved in the epinephrine induced impairment of the virus stimulated interferon production. Splenectomized mice were subjected to experiments similar to those above using 25-μg doses of epinephrine, to determine if the impairment was related to contraction or other possible influences on the spleen. These mice responded much like the intact animals (Fig. 3). Adrenalectomized mice were next employed to determine if endogenous secretions of adrenocortical or medullary hormones played a role in this response. Epinephrine also induced an impairment in the ability of adrenalectomized mice to produce interferon (Fig. 3). Control levels of interferon were higher in adrenalectomized and lower in splenectomized mice, as also observed by others (6, 7).

To determine if epinephrine impaired the total amount or just delayed interferon production, serum samples were collected at regular intervals over a 16-hr period from mice stimulated with NDV 1 hr after a 25-μg epinephrine injection. With the exception of the samples collected at 6 hr, a substantial impairment of interferon production was seen over the entire period (Table I), thus, indicating the differences seen at 4 hr were not

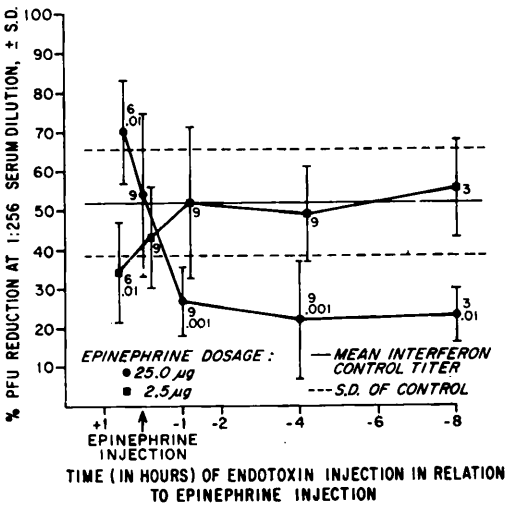

FIG. 2. Interferon levels induced in mice by endotoxin given at intervals in relation to injections of epinephrine. The numbers by each point indicate the number of serum pools tested and, where appropriate, the p value, determined by the Student t tests.

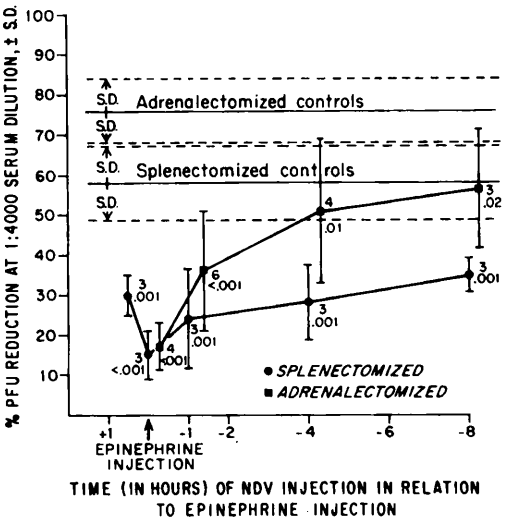

FIG. 3. Interferon levels induced in adrenalectomized and in splenectomized mice by NDV given at intervals in relation to injections of 25 μg of epinephrine. The numbers by each point indicate the number of serum pools tested and, where appropriate, the p value, determined by the Student t test.

due simply to a shift in the time of maximal interferon production.

To determine the influence of epinephrine on the development of tolerance to induction of interferon, normally seen after a primary stimulation with virus (8), mice were initially stimulated with NDV 1 hr after either a 2.5- or 25-μg dose of epinephrine, followed in 48 hr by a second NDV injection. Serum was collected 4 hr after the second NDV injection. No modification was noted in the tolerant state in the epinephrine treated mice (Table II).

Epinephrine also impaired interferon production *in vitro*. Tubes containing 2×10^6 freshly harvested mouse peritoneal leukocytes in 1 ml of Eagle's medium were treated with either 1 or 10 μg of epinephrine followed in 1 hr by about 2×10^7 pfu of NDV. After overnight incubation at 37° in a roller drum the fluid was collected, the virus was inactivated by treatment at pH 2, and the 50% plaque reduction interferon titers were determined. The average titer in tubes receiving 10 μg of epinephrine was 1:41 compared to 1:154 in those receiving 1 μg and to 1:167 ($p = 0.02$, compared to 1:41) in the control tubes which received only NDV.

TABLE I. Influence of Injection (25 μg) of Epinephrine on the Rate of Interferon Production.

After NDV injection (hr)	Serum[a] dilutions inducing 50% pfu reduction	
	Epinephrine treated	Controls
2	1:995	1:2451
4	1:5039	1:7001
6	1:12,650	1:12,690
8	1:12,670	1:15,500
10	1:6560	1:8920
12	1:6185	1:9513
16	1:3100	1:4620

[a] One serum pool from 7 mice for each sample.

TABLE II. Effect of Epinephrine on the Induction of Tolerance to Interferon Production.

Pretreatment[a]	Serum[c] dilutions (± SD) inducing 50% pfu reduction
Epinephrine (25 μg) + NDV[b]	1:641 ± 122
Epinephrine (2.5 μg) + NDV[b]	1:608 ± 91
Saline + NDV[b]	1:610 ± 36
Epinephrine (25 μg) only	1:6373 ± 130
Saline only	1:6490 ± 294

[a] Given 48 hr before second NDV injection.

[b] Given 1 hr after epinephrine or saline.

[c] Collected 4 hr after second NDV injection, 3 pools/group.

Experiments were next conducted to determine if the suppression of interferon production was mediated via either the alpha or beta adrenregic receptors. Mice were treated with either the alpha blocking agent phenoxybenzamine (Dibenzyline, Smith, Kline and French Labs.) in 100-μg doses or the beta blocking agent propranolol (Inderal, Ayerst Labs.) in 20-μg doses 1 hr before, same time as, or 15 min after a 25-μg epinephrine injection. Mice were injected with NDV 1 hr after the epinephrine injection. Phenoxybenzamine when given before epinephrine successfully blocked the suppressing effect of epinephrine on the interferon production; no significant influence was seen when this blocking agent was given with or after the epinephrine (Table

TABLE III. Effect of Alpha and Beta Adrenergic Blocking Agents on Epinephrine Induced Suppression of Interferon Production.

Time (min) of injection of blocking agent in relation to to injection of epinephrine[a]	Blocking agent	% pfu reduction (± SD) at 1:4000 serum dilution[b]
+60	Phenoxybenzamine	58 ± 6
0	Phenoxybenzamine	33 ± 18
−15	Phenoxybenzamine	26 ± 8
+60	Propranolol	34 ± 15
0	Propranolol	36 ± 13
−15	Propranolol	16 ± 8
Controls		
Epinephrine + NDV		24 ± 4
Saline + NDV		66 ± 4
Phenoxybenzamine + NDV		58 ± 7
Propranolol + NDV		57 ± 6

[a] NDV was injected 1 hr after epinephrine or control injections.
[b] Three serum pools/group.

III). Propranolol did not block the effect of epinephrine on interferon production; there was some indication of an additive effect when given 15 min after epinephrine. Neither blocking agent alone had a significant influence on interferon production.

To determine if epinephrine impaired interferon production in cells other than those of the blood–vascular system, mice were injected i.p. with NDV at different intervals in relation to an injection of epinephrine. Peritoneal washings were collected after 4 hr and assayed for interferon. The 50% pfu reduction titer for the control samples was 1:344 ± 73. The marked prolonged interferon suppression seen in blood serum was not observed in the peritoneal cavity. However, virus injected from 30 min before to 1 hr after epinephrine induced erratic interferon responses which were not significantly different from controls, although a slight transitory impairment with 25μg and a slight enhancement with 2.5-μg doses of epinephrine were suggested. By 2–3 hr after injection of epinephrine, the mice responded the same as controls.

Norepinephrine. In 25-μg doses, norepinephrine also suppressed the production of NDV induced interferon (Table IV). The effect was greatest immediately after injection, but the overall influence was of shorter duration (less than 8 hr) than with epinephrine. Doses of 2.5 μg induced no immediate changes, however, 4–8 hr after injection a slight (10%) enhancement was usually noted. Phenoxybenzamine given 1 hr before 25 μg of norepinephrine blocked its suppressive influence on interferon production, while propranolol enhanced the suppressive influence. Interferon levels (% reduction at 1:4000 dilution of serum) were 79 ± 16 in propranolol ($p = 0.02$), and 61 ± 4 ($p = 0.05$) in norepinephrine-only treated mice.

Histamine. An extended (up to 24 hr) suppression of the ability of mice to produce NDV induced interferon was seen following a 0.46-mg injection of histamine (Table V). Histamine had no significant effect on the

TABLE IV. Deviations of NDV-Induced Interferon Levels in Norepinephrine-Treated Mice.

Time[a] (hr)	No. of serum samples assayed	Deviation (%) from control values[b] ± SD	p value
+ .5	3	−25 ± 11	0.05
0	4	−28 ± 9	0.01
−1	10	−12 ± 7	0.02
−4	4	−14 ± 5	0.02
−8	1	+12	

[a] Time of NDV injection in relation to norepinephrine injection.
[b] Mean control value was 73 ± 6% pfu reduction at 1:4000 serum dilution.

TABLE V. Deviation of NDV-Induced Interferon Levels in Histamine-Treated Mice.

Time[a] (hr)	No. of serum samples assayed	Deviation (%) from control values[b] ± SD	*p* value
+ .5	3	+13 ± 8	0.10
0	14	−15 ± 15	0.01
− 1	11	−16 ± 12	0.001
− 2	9	−26 ± 11	0.001
− 4	8	−13 ± 12	0.02
− 8	6	−14 ± 10	0.05
−16	3	− 9 ± 4	0.20
−24	3	−16 ± 5	0.06

[a] Time of NDV injection in relation to histamine injection.

[b] Mean control value was 57 ± 13% pfu reduction at 1:4000 serum dilution.

production of endotoxin-induced interferon; deviations from control values (mean 38 ± 5% pfu reduction at 1:256 dilution) in mice injected with endotoxin at 0, 2, 4, and 8 hr after histamine were +5 ± 10, −2 ± 11, 0 ± 9, and +14 ± 19%, respectively.

Discussion. Although these data clearly show that epinephrine influences the capacity of mice to produce interferon, the mechanisms responsible are not fully explained. Possible mechanisms are: (i) changes in circulation dynamics, primarily vasoconstriction, which reduces the number of cells exposed to the interferon inducer; (ii) changes in metabolic functions which impair the cellular interferon producing capabilities, or (iii) a combination of both in which vasoconstrictive ischemia impairs metabolic functions. Findings which favor the vasoconstrictive mechanisms are: the ability of the alpha blocking agent, which prevents vasoconstriction, to block the effect of epinephrine on interferon production (Table III) and the impairment of endotoxin induced interferon (Fig. 2) which is thought to be preformed and not dependent on cellular metabolism for release (9). However, failure of epinephrine to modify the induction of a state of tolerance to interferon inducers (Table II), suggested that all interferon producing cells were stimulated during the period of impaired interferon production. The *in vitro* impairment of interferon production by relatively high levels of epinephrine gives support to the metabolic mechanisms, however, comparison with *in vitro* systems may not be valid (10). Regardless of the mechanisms involved, the impaired ability to produce interferon is not adrenal or splenic mediated (Fig. 3), nor is it due to a shift in the rate of production (Table I). The effect is, however, a function of dosage; impairment resulting from larger, and enhancement from smaller doses of epinephrine (Fig. 1).

The mode of action of epinephrine on interferon production differs in several respects from that of serotonin (5). Serotonin also induced a transient impairment of viral-induced interferon production, but the duration was much shorter, 1- to 2- vs 16- to 24-hr. Furthermore, serotonin had no effect on endotoxin induced interferon, but did alter the degree of induction of the tolerant state. In limited studies, norepinephrine seemed to induce a response similar to that of epinephrine, except the duration was shorter (Table IV). Histamine also induced impairment in the NDV induced interferon producing abilities of mice (Table V). The failure of histamine to impair endotoxin-induced interferon production, suggests that its influence resembles more closely the response obtained with serotonin (5). These results were all seen following i.v. injections; interferon production in the peritoneal cavity was not significantly influenced by epinephrine. On the other hand, Tokumaru (11) reported slightly lower interferon levels in the skin of guinea pigs 24 hr after herpes simplex virus injections which were given concurrently with either epinephrine or histamine.

While these studies show an influence on interferon production, the possible roles of vasoactive amines on host resistance to infections is not fully understood. Several studies (12, 13) have demonstrated the activation of latent herpes simplex infections following repeated injection of epinephrine. Other studies have shown a very transient alteration in passage of viruses across the blood–brain barrier immediately after injection of various amines (14). My preliminary results indicate mice challenged with virus during the period of impaired ability to produce interferon,

which follows epinephrine injection, are more resistant to fatal infection. This suggests that the mechanisms that impair production of interferon also impair viral pathogenesis. The above responses were induced by epinephrine doses which exceeded physiological levels. However, other studies (4) have demonstrated a similar transient impairment of interferon production during exposure of mice to stress, thus indicating that endogenous mechanisms are capable of inducing such a response; this stress response was not adrenal mediated, whether vasoactive amines were involved is not known.

Although the impairment of interferon production has been emphasized in this study, the enhanced response following 2.5-μg doses (Fig. 1) and the biphasic responses with endotoxin (Fig. 2) must also be considered in the analysis of the influences of epinephrine on host–parasite relationships. The transitory and multidirectional responses of animals to vasoactive amines make precise interpretation of studies of this type difficult. Such responses may increase resistance to one infection and decrease it to another, depending on the pathogenesis and the temporal relationships involved. Such a consideration may help explain differences in the interferon responses of stressed mice reported in different studies (4, 7).

Summary. The ability of mice to produce virus-induced interferon was impaired by 25-μg doses of epinephrine and enhanced by 2.5-μg doses. Endotoxin-induced interferon production was also impaired by epinephrine. The impairment was not mediated via the adrenal glands or the spleen, but was prevented by an alpha adrenergic blocking agent. Norepinephrine induced a response similar to that seen with epinephrine. Histamine impaired the production of virus-induced but not of endotoxin-induced interferon.

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