

EFFECT OF NICOTINAMIDE ON IN VITRO HISTAMINE RELEASE AND SLOW-REACTING SUBSTANCE FORMATION IN ANAPHYLACTICALLY SENSITIZED LUNG AND ITS RELATION TO ACID-SOLUBLE NUCLEOTIDES

Elliott Middleton, Jr., M.D., and Anima Devi, Ph.D., New York, N. Y.

IN VITRO histamine release from anaphylactically sensitized guinea pig lung is known to be partially inhibited by nicotinamide.^{1, 2} Nicotinamide is also an inhibitor³ of the enzyme diphosphopyridine nucleotidase (DPNase) which hydrolyzes diphosphopyridine nucleotide (DPN) at the nicotinamide-ribose linkage. It will also be recalled that nicotinamide is an integral part of DPN and TPN (triphosphopyridine nucleotide) molecules. Furthermore, Alivisatos^{4, 4a} has shown that DPNase (from beef spleen) catalyzes a reaction between histamine and DPN, giving rise to a histamine adenine dinucleotide by substituting histamine for the nicotinamide moiety of DPN. This finding has been confirmed.⁵ It was suggested⁶ that this compound might well serve as a precursor of imidazole acetic riboside, an important metabolite of histamine.

These observations suggested to us the possibility that DPN and DPNase may participate in the biochemistry of anaphylaxis and prompted the experiments reported herein. As a first step to test this possibility, studies were made of in vitro histamine release and slow-reacting substance (SRS-A)⁷ formation in the presence of nicotinamide and at the same time measurements were made of DPNase activity and of over-all changes in lung acid-soluble nucleotides, estimated by the orcinol reaction. The effect of nicotinamide on these changes has also been examined.

METHODS

1. *Sensitized Lung Tissue*.—Mixed stock adult guinea pigs of approximately the same weight were sensitized by three subcutaneous injections (50 mg.) of bovine serum albumin (BSA) over a period of 7 to 10 days. Four to 6 weeks after the last injection, the animals were killed by a blow on the head and exsanguinated by bleeding from the carotid arteries. The lungs were removed, finely chopped with scissors into pieces approximately 1 mm. across, and washed

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, N. Y.

Presented at the 18th Annual Meeting of the American Academy of Allergy, Denver, Colorado, February, 1962.

Received for publication Feb. 1, 1963.

three times in Tyrode's solution. Known wet weights of tissue were obtained after blotting on paper toweling. Each aliquot of chopped lung was similar to the others by gross inspection.

2. *Histamine Release and Slow-Reacting Substance Formation.*—The weighed chopped lung was incubated in Tyrode's solution, containing 0.1 per cent BSA, at 37° C. for 20 minutes with frequent agitation. The ratio of wet weight tissue to incubating medium was 1 Gm. in 10 ml. Nicotinamide was dissolved in the BSA-containing Tyrode's solution to give concentrations ranging from 10^{-4} M to 10^{-2} M. Appropriate controls with neither BSA nor nicotinamide were also run. The incubation fluid was collected by filtration through a little cotton and the filtrate clarified by centrifugation. Histamine concentration was determined in the final solution by the spectrophotofluorometric method.⁸

Slow-reacting substance (SRS-A) formation was detected kymographically by testing aliquots of the filtered and centrifuged solution on a 1.5 to 2.0 cm. strip of guinea pig ileum suspended in Tyrode's solution containing pyrilamine 3×10^{-7} M and atropine 1.5×10^{-6} M. The tissue bath (volume 2.5 ml.) was kept at 37° C. and mixing was provided by a constant stream of air bubbles. SRS-A typically produces a slow contraction of the muscle, beginning 5 to 30 seconds after introduction of the sample. Relaxation of the muscle after washout is very prolonged. In the present experiments, the amount of SRS-A in the incubation fluid was not estimated by the semiquantitative method described by Brockelhurst⁷ which employs a standard SRS-A preparation. Instead, the SRS-A activity of a given volume of the solution from tubes containing antigen without nicotinamide was used as the internal standard for each of the three experiments. Equal volumes from the nicotinamide-treated preparations were tested on the muscle strip and the height of the contractions compared with the internal standard. Since the dose-response relationship in the biologic assay for SRS-A is not linear, this method provides only a rough comparison of the amounts of SRS-A in each sample tested and displays a trend rather than absolute quantitative change.

It was established in other control experiments that nicotinamide does not interfere with the spectrophotofluorometric determination of histamine or with the bioassay system for either histamine or SRS-A.

3. *Acid-Soluble Nucleotides.*—Lung content of acid-soluble nucleotides was determined by means of the orcinol reaction.⁹ The nucleotide fraction of living tissue consists mainly of adenosine triphosphate (ATP), diphosphopyridine nucleotide (DPN), triphosphopyridine nucleotide (TPN), and coenzyme A (CoA).⁹ The orcinol reaction measures DPN and TPN in their oxidized state. In this report, the substances measured by the orcinol reaction will be called orcinol-reactive substances. The determination was performed by first homogenizing the blotted chopped lung tissue (40 mg. wet weight/ml.) in tris buffer, 0.1 M, pH 7.6, following incubation. The homogenates were treated with one-tenth volume of concentrated perchloric acid, and, after centrifugation, the acid-soluble nucleotides in the supernatant were measured by the orcinol reaction. About 15 minutes elapsed between the conclusion of the incubation

period and the addition of perchloric acid to the homogenate.

4. *DPNase*.—The DPNase activity in lung tissue after incubation and removal of the supernatant was measured by determining the extent of hydrolysis of DPN in a reaction mixture containing lung homogenate (10 mg. wet weight/ml.) prepared in tris buffer, 0.1 M, pH 7.6, and added DPN, 0.5 micromole). The reaction was run for 15 minutes at 37° C. and the residual DPN measured spectrophotometrically as the cyano-derivative, according to Colowick, Kaplan, and Ciotti.¹⁰

RESULTS

The experimental data are set out in Table I. There it can be seen that nicotinamide is indeed an inhibitor of histamine release from anaphylactically sensitized guinea pig lung in vitro. However, its activity is only apparent at quite high concentrations, 10^{-3} to 10^{-2} M. Austen and Brockelhurst¹ reported that 5×10^{-3} M nicotinamide consistently produced at least 50 per cent inhibition of histamine release, somewhat more effective than the present data indicate.

It is also evident that the formation of SRS is depressed in the presence of the same concentrations of nicotinamide. Although it is impossible as yet to quantitate absolutely the amount of SRS-A liberated into the incubation medium, the reduced degree of contraction of the ileum speaks for a significant inhibition of formation of this agent.

Measurement of orcinol-reactive substances in the three experiments

TABLE I. EFFECT OF NICOTINAMIDE ON HISTAMINE RELEASE AND SRS-A FORMATION: CHANGE IN LUNG CONTENT OF ORCINOL-REACTIVE SUBSTANCES AND DPNase ACTIVITY

EXPER.	ANTI- GEN* (BSA)	NICOTIN- AMIDE, MOLAR CONCEN- TRATION	HISTA- MINE RELEASE (μ G/ML. SUPT.)	PER CENT INHIBI- TION HISTA- MINE RELEASE	SRS-A ACTIV- ITY†	ORCINOL- REACTIVE SUB- STANCES (MG./GM.)	PER CENT OF CONTROL ORCINOL- REACTIVE SUB- STANCES	DPNase ACTIV- ITY‡
1	0	0	0	—	0(0.1 ML.)	1.75	100	85
	+	0	0.181	0	52	1.25	72	88
	+	10^{-4}	0.143	21	54	1.38	79	86
	+	10^{-3}	0.151	17	23	2.03	116	84
	+	10^{-2}	0.101	44	9	2.06	118	86
2	0	0	0§	—	0(0.1 ML.)	1.35	100	86
	+	0	4+	—	38	0.88	65	87
	+	10^{-4}	4+	—	42	1.20	89	87
	+	10^{-3}	3+	—	30	2.20	163	87
	+	10^{-2}	2+	—	14	2.00	148	88
3	0	0	0	—	0(0.3 ML.)	1.50	100	87
	+	0	0.118	0	45	0.80	53	—
	+	10^{-4}	0.118	0	46	1.16	77	—
	+	10^{-3}	0.108	8	40	2.30	153	—
	+	10^{-2}	0.069	42	26	3.30	220	88

*0.1 Per cent BSA in Tyrode's solution, 10 ml. per gram chopped lung.

†Average height of contraction (mm.) on kymograph record. Figures in parentheses indicate volumes of Tyrode's supernatant added to muscle bath, volume 2.5 ml.

‡Per cent degradation of DPN in standard reaction mixture (see Methods).

§Histamine release graded by kymographic technique.

shown in Table I gave values ranging from 1.35 to 1.75 mg. per gram of lung (wet weight), after 20 minutes' incubation in plain Tyrode's solution (plus about 15 minutes handling time for homogenization). Of particular interest is the fact that sensitized lung tissue incubated with antigen, in each of the three experiments, had a decreased amount of orcinol-reactive substances. The decrement amounted to 28 to 47 per cent less than the control without antigen. However, in the presence of nicotinamide this decrease was not seen and, in fact, at the higher concentrations of nicotinamide, there was an apparent increase in the quantity of orcinol-reactive substances in the lung tissue.

The lungs of the guinea pigs in these experiments all contained readily measurable DPNase. It can also be seen that no change occurred in DPNase activity in any of the experiments. It must be recalled, however, that DPNase activity was determined in lung homogenates prepared after the nicotinamide-containing supernatant was removed following incubation. In this situation, of course, there would be little or no nicotinamide remaining in the environment of the lung DPNase when its activity was estimated by the standard technique stated in Methods. But it must be noted that nicotinamide, added to the lung homogenate in concentrations comparable to those existing when chopped lung, antigen, and nicotinamide are incubated together, is a potent inhibitor of the lung DPNase (Table II). Thus, in the lung-antigen-nicotinamide incubation experiments, nicotinamide was present in concentrations which could readily inhibit lung DPNase. These data are consistent with the fact that DPNase is reversibly inhibited by nicotinamide.³

TABLE II. EFFECT OF NICOTINAMIDE ON DPNase ACTIVITY OF GUINEA PIG LUNG HOMOGENATES

MOLAR CONCENTRATION OF NICOTINAMIDE IN DPNase REACTION MIXTURE*	DPNase ACTIVITY†	PER CENT INHIBITION OF DPNase
0	85	—
3.8×10^{-3}	57	33
7.5×10^{-3}	37	57
1.5×10^{-2}	26	70
3.1×10^{-2}	15	83

*DPNase reaction mixture: see Methods.

†DPNase activity expressed as per cent degradation of DPN in reaction mixture.

DISCUSSION

In the present experiments it has been reaffirmed that nicotinamide (in high concentration) is effective in partially inhibiting the release of histamine *in vitro* from anaphylactically sensitized guinea pig lung. This observation confirms the previously reported finding of Austen and Brockelhurst¹ and Yamasaki and his co-workers.² It has also been demonstrated that SRS-A formation is markedly reduced in the presence of nicotinamide. In this situation there appears to be a coupled relation between histamine release and SRS-A formation in *in vitro* anaphylaxis. Based on a series of experiments studying the effect of peptidase substrates and inhibitors on *in vitro* anaphylaxis, Austen and Brockelhurst¹ concluded that the antigen-antibody reaction resulted in activation of a chymotrypsin-like enzyme. Such an activated enzyme

may then bring about the subsequent events of anaphylaxis, such as histamine release. In this regard it is significant that nicotinamide is a monofunctional chymotrypsin inhibitor,¹ and its activity may indeed reside in its ability to inhibit the chymotrypsin-like enzyme.

No change in DPNase activity in lung was noted in the present experiments. This is in keeping with the results of Yamasaki and his associates¹² who also showed that beef spleen DPNase was incapable of releasing histamine from guinea pig lung. These workers also concluded that nicotinamide did not prevent histamine release by inhibition of DPNase.

The lung content of orcinol-reactive substances in the control preparations ranged from 1.35 to 1.75 mg. per gram of wet tissue. The decrease of 28 to 47 per cent of orcinol-reactive substances in lung after *in vitro* anaphylaxis represents a real change and must now be considered as an event which accompanies or follows *in vitro* anaphylaxis. Which of the acid-soluble nucleotides measured by the orcinol reaction is affected by the anaphylactic reaction, however, remains to be determined by specific analytical techniques. It is of interest, nevertheless, that Alivisatos¹¹ claims that there is a reduction in lung DPN content after *in vitro* anaphylaxis. In the presence of nicotinamide there was either less decrease or an apparent increase in the lung orcinol-reactive substances. This observation is probably accounted for by the fact that nicotinamide inhibition of DPNase prevented the degradation of DPN during the period of incubation and homogenization of the tissue. Therefore, the control values for lung orcinol-reactive substances are probably low by an amount approximately equal to the amount of DPN destroyed during incubation and homogenization.

Although the fate of orcinol-reactive substances after *in vitro* anaphylaxis has not as yet been elucidated, it is attractive to speculate that one of them, DPN, is converted to histamine adenine dinucleotide on reaction with the released histamine under the catalytic influence of lung DPNase, as suggested by Alivisatos.⁶ The validity of this hypothesis rests on detection of the histamine adenine dinucleotide in the reaction products of anaphylaxis, and remains to be shown. Since imidazole acetic acid riboside is an important metabolite of histamine, it is clear that this reaction may be of significance in the metabolism of the released histamine. If substitution of histamine for nicotinamide in DPN occurs to any significant degree, then the released nicotinamide would be free to inhibit DPNase and the enzyme activated by the antigen-antibody reaction. This mechanism could serve as a factor limiting the extent of histamine release. Also, if the removal of histamine from the vicinity of its release (by formation of histamine adenine dinucleotide) was inhibited by interference with the activity of DPNase, the total amount of released histamine might be reduced.

SUMMARY

The present experiments confirm that nicotinamide partially inhibits the *in vitro* release of histamine from anaphylactically sensitized guinea pig lung

on incubation with antigen. It has also been demonstrated that formation of SRS-A is reduced by nicotinamide.

No change in DPNase activity of lung tissue was demonstrable after in vitro anaphylaxis.

In vitro anaphylaxis caused a 28 to 47 per cent reduction in lung content of acid-soluble nucleotides as measured by the orcinol reaction. The possible significance of this change is discussed.

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