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Urinary Kinins and Histamine In Urine of Progressive Muscular Dystrophy

Masao NAKAHARA and Hisashi SAKAHASHI

Department of Orthopedic Surgery, Sapporo Medical College
(*Chief*: *Prof. B. Kawamura*)

Introduction

The study of plasma kinins preceded that of urinary kinins. Plasma kinins are polypeptides derived from plasma which stimulates certain types of smooth muscle. Urinary kinins are closely related to plasma kinins in physiological and biological properties. The excretion of urinary kinins has been found to be fairly constant in both male and female[1], and attempts to purify certain types of kinins have been made[2].

The present study was undertaken in an attempt to investigate urinary kinins and histamine which are excreted in far greater amounts by patients with progressive muscular dystrophy (PMD) than by healthy subjects.

Methods

Urine: Urine samples collected from 10 patients with PMD and 3 healthy subjects were pooled and stored in a refrigerator for 24 hrs. The samples were applied to a Sephadex G–25 Column and eluated with saline-phosphate buffer (μ 0.15, pH 7.4). Protein determination was done on the eluate by absorbancy at 280 mμ. Ninhydrin reaction was carried out by the method of Yemm[3].

Isolated smooth muscle: A preparation of guinea pig ileum was suspended in Tyrode solution in a 10 mℓ bath at 37°C. The Tyrode solution contained atropine sulphate (10^{-5} g/l). The contraction of ileum was recorded by a Magnus apparatus.

Leucine Aminopeptide Activity (LAP): LAP activity was determined by LAP-test of Boehringer.

Other materials: α-chymotrypsin (Eizai Co.) and pronase (Kaken Co.), which were kindly supplied by the Eizai Co. and Kaken Co., were used.

Results

Chromatographic Separation of Urinary Kinins

Ten mℓ of urine from PMD patients was separated by Sephadex G–25 Column into 2 peaks as shown in Fig. 1. Contraction of ileum was recognized in the eluate of the first peak designated by the eluate numbers 15 to 16 in Fig. 1, but failed to appear in the eluate of the first peak from the healthy subjects.

As a result of the eluate by absorbency at 280 mμ two peaks were observed. Ninhydrin reaction was positive in the eluate of the first peak from the PMD patients.

Therefore, it was considered that urinary kinins were contained in the first peak.

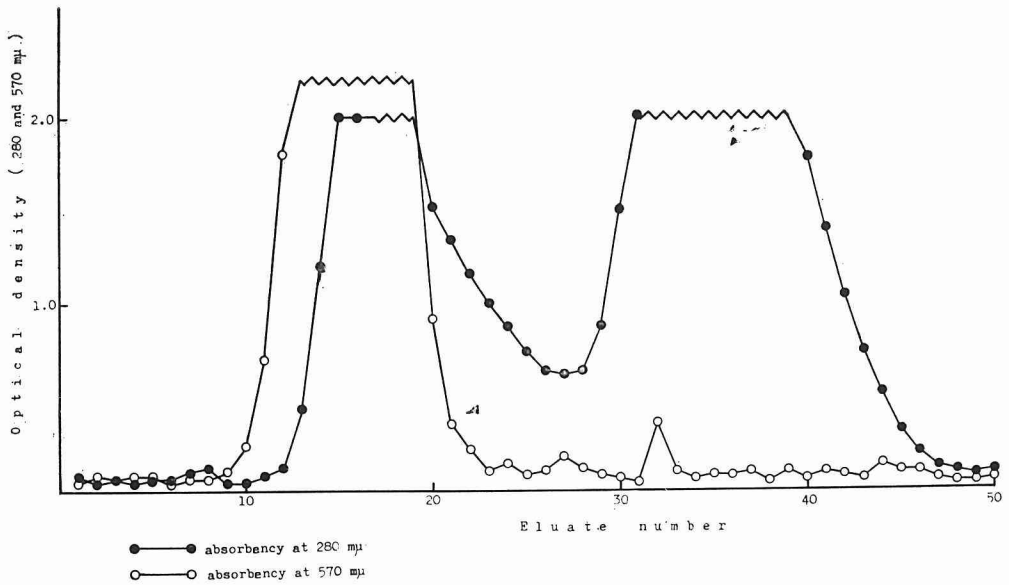
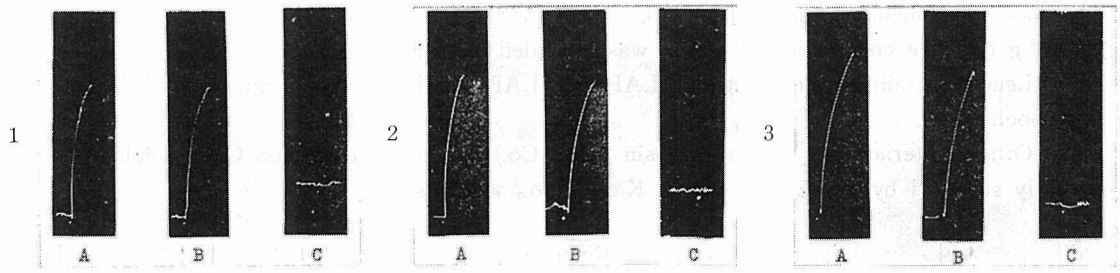


Fig. 1 Column chromatographic separation of urinary kinins.
Ten mℓ of crude urine from patient with PMD was applied to Sephadex G-25 column (2.0×80 cm) and eluted with saline phosphate buffer. Each eluate was 5 mℓ in volume. 0.5 mℓ of eluate was used for the assay of ninhydrin reaction.

Effect of Boiling and Dialysis on Separated Urinary Kinins

The eluate of urine from PMD patients separated by Sephadex G-25 were used in this experiment. After boiling the eluates of 3 cases at 100°C for 10 min., their effect on the ileum showed no changes as seen in Fig. 2-B. But when these 3 eluates were dialized through a cellophane membrane, no contraction was observed as shown in Fig.



1 : 5 year old, male, Duchenne type
2 : 31 year old, male, Limb-girdle type
3 : 24 year old, male, Limb-girdle type

A : 1 mℓ of condensed eluate of urinary kinins
B : 1 mℓ of condensed eluate of urinary kinins after boiling at 100°C for 10 min
C : 1 mℓ of condensed eluate of urinary kinins after dialysis with cellophane membrane
Eluate of urinary kinins was condensed to one tenth of the original amount

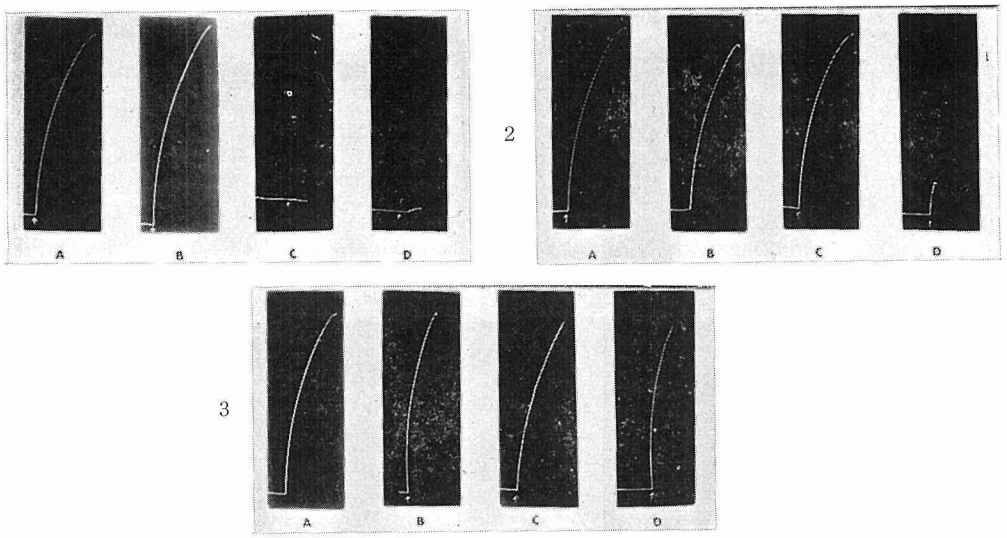
Fig. 2 Action of urinary kinins taken from patient with PMD on guinea pig ileum.

2-C. Identical results were obtained in samples from other patients with PMD.

Effect of Urine Treated with α-Chymotrypsin, Pronase and NaOH on Guinea Pig Ileum

To 1 mℓ of crude urine of PMD, 0.5 mg of α-chymothypsin or 2 UPK of pronase was added respectively and incubated at 37°C for 60 min. Next to another 1 mℓ of crude urine from a PMD patient, 0.5 mℓ of 2N-NaOH was added and boiled at 100°C for 30 min, then neutralized with HCl.

As shown in Fig. 3, the urine sample from case 1 was resistant to α-chymotrypsin (B), and was completely destroyed by pronase (C) and NaOH (D). Urine from case 2 was resistant to both α-chymotrypsin (B) and pronase (C) and destroyed by NaOH (D) to a considerable extent. Urine from case was resistant to all treatments and stimulated the ileum as extensively as crude urine.



1 : 28 yers old, male, Limb-girdle type
2 : 10 year old, male, Duchenne type
3 : 34 year old, male, Limb-girdle type

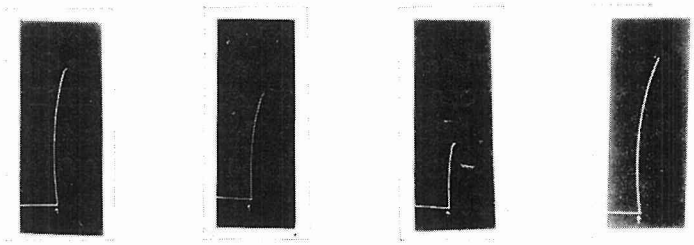
A : crude urine 1 mℓ
B : crude urine 1 mℓ incubated with α-chymotrypsin 0.5 mg at 37°C for 60 min
C : crude urine 1 mℓ incubated with pronase 20 UPK at 37°C for 60 min
D : crude urine 1 mℓ incubated with 2N-NaOH 0.5 mℓ at 100°C for 30 min

Fig. 3 Action of α-chymotrypsin, pronase and alkaline treated urine on guinea pig ileum.

Effect of Histamine and Guanidine Sulphate on Guinea Pig Ileum

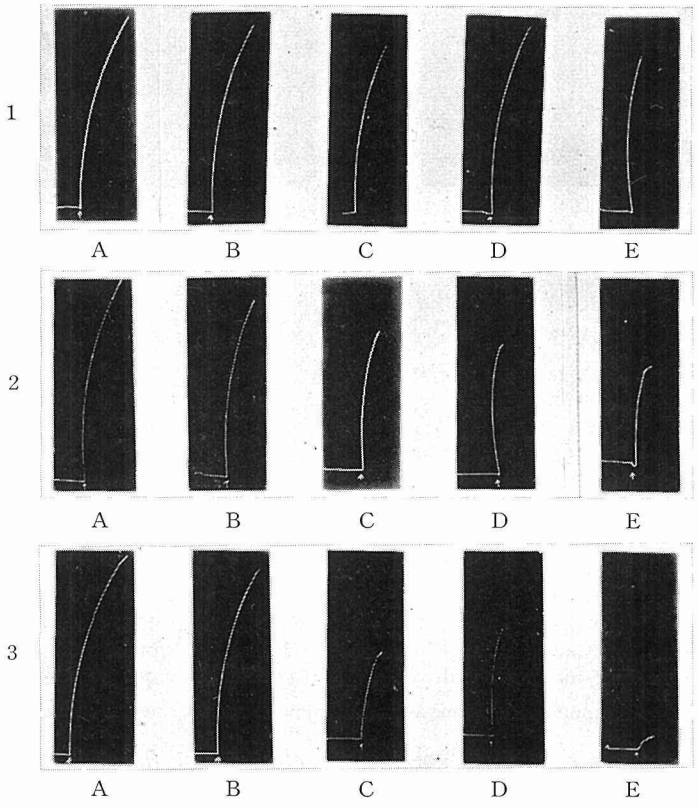
Ileum contraction was seen by adding 0.5 γ of histamine to 10 mℓ of Tyrode solution as shown in Fig 4-A. By an addition of 5 mg of guanidine sulphate, antihistamine, to 0.5 γ of histamine, the effect of histamine on the ileum was slightly inhibited as shown in Fig. 4-B. By an addition of 15 mg of guanidine sulphate, a fairly strong inhibition was seen as shown in Fig. 4-C. Then, with 0.5 γ of histamine without guanidine sulphate, again the same degree of contraction as seen in Fig. 4-A was obtained as shown in Fig. 4-D.

Therefore, it may be considered that the ileum is not affected by guanidine sulphate.



A : histamine 0.5 γ
B : histamine 0.5 γ+guanidine sulphate 5 mg
C : histamine 0.5 γ+guanidine sulphate 15 mg
D : histamine 0.5 γ

Fig. 4 Effect of urine taken from patient with PMD on guinea pig ileum against guanidine sulphate.



1 : 28 year old, male, Limb-girdle type
2 : 10 year old, male, Duchenne type
3 : 34 year old, male, Limb-girdle type

A : crude urine 1 mℓ
B : diluted crude urine 1 mℓ (1 : 10)
C : diluted crude urine 1 mℓ (1 : 100)
D : crude urine 1 mℓ+guanidine sulphate 15 mg
E : diluted crude urine 1 mℓ (1 : 100)+guanide sulphate 15 mg

Fig. 5 Effect of urine from patient with PMD on guinea pig ileum against guanidine sulphate.

Effect of Urine from PMD Patients on Guinea Pig Ileum Against Guanidine Sulphate

Crude and diluted urine (1 : 10, 1 : 100) were used in this experiment. As show in Fig. 5-A, B, and C, the effect of urine on the ileum was slightly diminished in the (1 : 100) urine sample.

When 15 mg of guanidine sulphate was added to crude and diluted urine (1 : 100) respectively, as shown in Fig. 5-D and E, the effect of urine from case 1 was not inhibited by guanidien sulphate. Urine from case 2 was slightly inhibited not only in crude but also in diluted urine (1 : 100) and urine from case 3 was markedly inhibited in diluted urine (1 : 100). These findings suggest that histamine was excreted in a higher amount in some cases of PMD.

Activity of Urinary LAP

No difference in urinary LAP activity was observed among samples from patients with PMD and 3 samples from healthy subjects as shown in Table 1.

Table 1. *Leucine Aminopeptidase Activity in Urine*

	leucine aminopeptidase activity (LAPU/day)
28 year old, male, Limb-girdle type	376
10 year old, male, Duchenne type	470
34 year old, male, Limb-girdle type	408
27 year old, female, Limb-girdle type	692
29 year old, female, Limb-girdle type	560
32 year old, male, healthy subject	343
54 year old, male, healthy subject	605
10 year old, male, healthy subject	969

Discussion

Werle and Erdos[4] studied systematically the actions and properties of a urinary depressor substance and named this substance Z in 1954. Gomes[5] reported that its active component was a polypeptide similar to bradykinin. Walaszek[6] separated two oxytocic fractions from extracts of human urine by paper chromatography and referred to them as substance Z_1 and substance Z_2. Jensen[7] also confirmed the results of Walaszek by using IRC-50. Horton[8] reported that the excretion of urinary kinin in human beings was fairly constant and was not affected by the rate of formation of urine, urinary pH, or time of day.

In this experiment, excretion and properties of urinary kinins and excretion of urinary histamine were investigated on the following points :

Excretion of urinary kinins : Urinary kinins were separted by Sephadex G-25 column and were found to be contained in the first peak of Fig. 1. The eluate in the first peak obtained from PMD patients contracted the guinea pig ileum, while the eluate from healthy subjects did not show any effect unless condensed. Therefore, it is possible that in patients with PMD (1) an increase of urinary kinin formation is present, though the cause is unknown, (2) a decrease of its destruction by kininase may occur (3) and an

increase of excretion of plasma kinins into urine may be present, Among these three points (1) might be considered as the main factor responsible for an increased amount of urinary kinins. Because, in regard to (2) LAP activity, a kind of kininase found in urine from patients with PMD was almost the same as in healthy subjects, and as regards (3), free plasma kinins are not in excess in patients with PMD as compared with healthy subjects[13].

Boiling: Beraldo[9] has shown that boiling the neutralized urine for 30 min resulted in a partial inactivation amounting to about 50 per cent. But in this experiment a 10 minute boiling of the eluate, failed to produce a contraction of the guinea pig ileum. The difference in results is probably due to the amount of urinary kinins rather than by the duration of boiling.

Dialysis: It has been reported that urinary kallikreine is nondialyzable, but urinary kinins[10], such as substance U, are dialyzable through cellophane membrane[11]. Since no effects of urinary kinins of PMD Patients on the ileum were observed after dialization as shown in Fig. 2, urinary kinins of PMD patients were considered to be dialyzable.

Treatment with alkaline: It has been reported that boiling for 30 min in 0.5 N-NaOH is sufficient to destroy the substance U[10]. However, the smooth muscle stimulating substance in urine from patients with PMD was partially destroyed in some cases by boiling in NaOH as shown in Fig. 3-D. On the other hand it seems possible that other smooth muscle stimulating substances are present in the urine of PMD patients besides urinary kinins.

Treatment with proteolytic enzyme: It has been shown that when 0.2 mg of α-chymotrypsin was added to 1 mg of dried urinary extracts[5] or when 1 to 2 mg of α-chymotrypsin were added to 2 mℓ of neutralized urine[10] and the mixture was incubated at 37°C for 60 min, the activity disappeared completely. In the prsent investigation 0.5 mg of α-chymotrypsin was added to 1 mℓ of crude urine, but the treatment did not alter the effect of urinary kinins. It may be that the amount of α-chymotrypsin used in this experiment was not enough to destroy the large amount of urinary kinins in the urine of PMD patients. On the other hand in some cases the activities were completely destroyed by pronase.

Histamine: It has been reported that only traces of histamine are sufficient to stimulate the ileum and it is known that histamine is excreted in urine of PMD patients[12]. Now the smooth muscle stimulating substances in urine of PMD patient were observed to be fairly well inhibited by guanidine sulphate in Fig. 5. This fact may suggest that histamine is excreted into urine from patient with PMD together with urinary kinins. The smooth muscle stimulating substances in urine from case 3 in Fig. 3 which were resistant to α-chymotrypsin, pronase and NaOH might support the above suggestion. And the smooth muscle stimulating substances in urine from case 1 in Fig. 3 were destroyed by pronase and were resistant to guanidine sulphate. Iu this case a predominance of urinary kinins over histamine is suggested.

It is suggested from Fig. 3 and 5 that the excretion of urinary kinins and histamine might very among patients with PMD.

Conclusion

Urinary kinins and histamine were studied in 6 cases of Limb-girdle type, 4 cases of Duchenne type of progressive muscular dystrophy and 3 healthy subjects. It was shown that urinary kinins and histamine were excreted in urine from patients with progressive muscular dystrophy.

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