ON THE URINARY EXCRETION OF PITUITARY PROLACTIN

BY

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ABSTRACT

Histological studies of the cropsac under some experimental conditions revealed that diffuse cytoplasmic sudanophilia of the epithelial cells could be proposed as another characteristic response of the cropsac for prolactin.

Prolactin can be said to have at least two factors for epithelium-proliferating and fat-mobilizing actions, which are important for production of crop milk. In urine these factors are ont stable and particularly the fat-mobilizing factor can be easily inactivated and modified in quality. It is one of the reasons why urinary excretion of prolactin is supposed to be doubtful. The substance in urine with partial activity may be rather called prolactin-like substance. Its existence could be proved by the systemic assay. The local assay is often disturbed by inflammatory reaction and far from satisfactory, and yet it can be used for determining prolactin-like substance in urine.

As a result of recovery experiment approximately 1/30 to 1/40 of given prolactin can be excreted in urine. In lactating women generally 20 to 30 i.u. of prolactin-like substance per day can be determined in urine. Therefore, it is of considerable interest to note that 600 to 1200 i.u. of prolactin might be released out of anterior pituitary in 24 hours during lactation period.

INTRODUCTION

Since the cropsac test for the lactogenic hormone (prolactin) was established by Riddle, Bates and Dykshorn (1932), there have been published quite a few papers dealing with urinary excretion of prolactin in relation to physiologic and pathologic conditions of women (Lyons and Page, 1935; Meites and Turner, 1941; Coppedge and Segaloff, 1951; Fujii and Shimizu, 1958). However, there was an important contradiction of Bahn and Bates (1956), who denied the presence of prolactin in human urine, confirming that pituitary prolactin always developed histolgically intense cytoplasmic basophylia in the epithelial cells of the pigeon's cropsac, while urinary extract expected to include the hormone maniefsted no such activity. The cytoplasmic basophylia was said to have intimate relationship with ribonucleic acid and protein synthesis.

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It is clinically an important problem, whether pituitary prolactin may be excreted in urine or not. The present paper has to do with this question.

Materials and Method

Used prolactin was a purified preparation out of bovine pituitaries and had an activity of 38 i.u. per mg.

Extraction of urine was carried out by modified kaolin adsorption method designed originally for extracting human gonadotrophin (Bradbury, Brown and Brown, 1949) as Fujii and Shimizu (1958) reported in the previous paper.

Samples to be tested were generally resolved in phosphate buffer solution of pH 8.0, and for local assay equal volume of 0.25 ml. was injected intradermally over the cropsac gland, but for systemic assay varying dose was injected into pectoral muscle of immature Japanese common pigeon less than 8 weeks, weighing 200 to 300 g. The injection was given once a day for four days.

The crop glands were examined on the fifth day by gross inspection to ascertain the minimal proliferation, then dissected and fixed in 10% formalin. In one part paraffin sections were prepared and stained with hematoxylin-eosin, whereas in the other part frozen sections were stained with sudan III.

Samples of urine were obtained from healthy adult men or women in post partum and in menopause. If necessary, bovine prolactin was administered previously.

The dose of prolactin needed for distinct minimal proliferation of the cropsac by gross inspection following intradermal injection was around 0.3 i.u. in used pigeons.

Results

1. Effects of purified prolactin

If prolactin was injected intracutaneously over the crop gland in total dose of 0.25 to 0.3 i.u., a small disk-like thickening of the mucous membrane was observed corresponding to injected area. Histologically it revealed hyperplastic thickening of the epithelial cells often with downgrowth of the basal membrane and at the same time with massive sudanophile granules of irregular size. No inflammatory change was found in the tunica propria. The dose of 0.3 i.u. was sufficient for bringing about minimal thickening of the cropsac by gross inspection (Table 1). If ten times or more dose of prolactin was administered systemically into pectoral muscle of the pigeon,

Table 1. Effects of locally injected pituitary prolactin on cropsac epithelium

Total does of prolactin (i.u.)	gross thickness	gross crop milk	diffuse sudanophilia	downgrowth of basal membrane
0.125	—	—	++	±
0.250	±	+	++	++
0.300	*++	+	++	++
0.500	+++	++	+++	++
1.000	+++	+++	+++	+++

* gross minimal stimulation

Table 2. Effects of intramuscularly injected prolatin on cropsac epithelium

Total does of prolactin (i.u.)	gross thickness	gross crop milk	diffuse sudanophilia	downgrowth of basal membrane
0.5	±	—	—	—
1.0	+	±	±	±
3.0	*++	+	++	++
5.0	+++	++	+++	+++
10.0	+++	+++	+++	+++

* gross minimal stimulation

the response of the cropsac epithelium was of the same nature and extended over the whole area of bilateral crop gland (Table 2).

Ten i.u. or more of prolactin given systemically or one i.u. or more given locally brought about maximal response of the whole gland, and the more dosage was given, the more production of crop milk resulted.

2. Effect of urine extract on cropsac

When locally given, 1/4 aliquote of 24-hour urine extract from two normal lactating women caused distinct thickening of the crop gland, but no production of crop milk. Histologically proliferation of the epithelial cells with downgrowth was confirmed, being accompanied by slight signs of inflammation of tunica propria. However, cytoplasmic diffuse sudanophilia failed to take place and only larger or smaller droplets of neutral fat were scattered in a mode quite different from diffuse distribution within epithelial cells.

When systemically given, even 48- as well as 72-hour urine extracts obtained from four lactating women could not exert similar effect on the cropsac. But a large amount of urine collected from two lactating women as much as 10 liters could induce histologically slight proliferation of the cropsac, although it was not manifest by gross inspection.

The results so far could not exclude possible non-specific response

caused by unknown substance in urine. It must be studied further whether extraction procedure might have changed activity of prolactin, if the hormone was present in urine.

3. Activity of urine in relation to its extraction procedure

There was a possibility that alcohol treatment might denaturate prolactin in urine so much that some of its activities were lowered or lost. Under such consideraiton three urine samples from lactating women were subjected to kaolin adsorption and then ammonia elution without final alcohol precipitation. When sytemically given, each 1/4 aliquote of 24-hour urine extracted as just mentioned could proliferate the cropsac epithelial cells distinctly with downgrowth of basal membrane, while lipid mobilization was nothing but scattered droplets. It was posturated that urinary hormonal components might be more or less prevented from inactivation by omitting alcohol treatment.

In the experiment above non-specific local stimulation needed not to be taken into consideration and it can be admitted that in urine of lactating women there exists at least prolactin-like substance in spite of absence of characteristic lipid mobilization.

In order to look into inactivation of prolacitn more closely following experiment was designed.

Five i.u. of prolactin was added to every four ml. of phosphate buffer solution (pH 8.0) or every ten ml. of fresh male urine (pH adjusted to 8.0) and after incubation at 37°C for several minutes, 24 and 48 hours was kept each sample in refrigerator. Each test solution was injected into pectoral muscle for four days.

In case of aqueous solution the hormonal activity remained almost unchanged execpt slight decrease of activity in fat mobilization following incubation more than 24 hours.

In case of urinary solution inactivation was quite remarkable. The epithelium-proliferating activity was lowered already within 24-hour incubation, whereas the fat-mobilizing activity was much impaired from the beginning of incubation and far from characteristic for prolactin in effect. Inactivation in vitro was distinctly accelerated in urine and the proliferating activity was more stable than lpid mobilizing activity as far as the crop gland response was concerned.

In the second stage, 40 i.u. of prolactin was added to fresh male urine 30 ml. and absorbed with kaolin. Then, supernatant urine had no activity. The sediment was eluted with N-ammonia quite efficiently. The elution part was effective by the systemic assay method. The crop gland response consisted of epithelial proliferation with downgrowth of basal membrane and more or less characteristic diffuse cytoplasmic sudanophilia. That is to

say, prolactin in urine can be recovered by means of such procedure without much loss of activity characteristic for the hormone. However, when it was precipitated further with 95% alcohol, its activity of lipid mobilizaiton was no more preserved, while epithel-proliferating activity was not so lost.

4. Urinary excretion of administered prolactin

When purified prolactin 600 i.u. was injected to men or menopausal women and 1/2 aliquote of 24-hour urine was subjected to kaolin absorption and then ammonia elution and assayed by the systemic method, gross and histologic response of the crop gland was substantially the same with that by urine extract from lactating women as just described above. Under such conditions any local non-specific stimulation could be neglected, in all four instances. That is why endogenous pituitary prolactin was supposed to be metabolized and excreted into urine, although its biological activity was partially lowered and modified in quality. The activity for proliferation of the cropsac epithelial cells could be retained to a greater extent following concentration with the ordinary kaolin absorption method. In this connection it might be possible to estimate urinary excretion if kaolin adsorption method is applied for extraction and the local assay is carefully carried out.

5. Clinical experiences with urinary excretion of prolactin

Urine samples were extracted with kaolin absorption method and subjected to local assay taking the minimal thickening of cropsac at injected area as a criterium by gross inspection.

When two men injected with purified prolactin 200 and 600 i.u. respectively at a time, urinary prolactin was elevated on the next day from 1.6 to 4.8 i.u. and from 1.2 to 19.2 i.u. respectively, being lowered to previous values in two or three days.

In a menopausal women given 800, 1000 and 1000 i.u. prolactin consecutively the maximal level was 19.2 i.u. per 2 4hours, and another menopausal woman who was given 1600 i.u. at a time excreted prolactin as high as 38.4 i.u. per 24 hours.

These results show that only 1/30 to 1/40 out of given prolactin may be putout in urine as modified prolactin.

Discussion

In general, every endogenous hormone are excreted in urine as itself or its metabolite, which has not always the same biological activity as the original hormone. As for the pituitary prolactin it is not yet established, whether it could pass through kidney into urine or not. Notwithstanding there have been quite a few reports on determination of urinary prolactin

in relation to physiologic and pathologic conditions (Lyons and Page, 1935; Meites and Turner, 1941; Copedge and Segaloff, 1951; Fujii and Shimizu, 1958).

So far as the local cropsac assay method is used, proliferation of epithelial cells alone is not strictly specific for prolactin. Ethanol, tannic acid, pseudomonas polysaccharide and mustar (Bahn and Bastes, 1956) or Buthanol and benzene (Uruta, 1964) could cause varying degree of gross and microscopic thickening of the epithelium accompanied by distinct edema and inflammation of the tunica propria. Also that is the case with urine extract and the more impure is the extract, the more remarkable is inflammatory thickening of the epithelium. Therefore, purification of urine extract is quite necessary, in spite of the fact that purification lowers its activity, especially for fat mobilization. If impure substance is to be tested on prolactin activity, the systemic assay method is an inevitable procedure.

On the other hand, qualitative evidence of prolactin activity must be taken into consideration. As characteristic responses for prolactin downgrowth of the basal membrane (Prince Masahito and Fujii, 1959) and diffuse cytoplasmic basophilia (Bahn and Bates, 1956) of the cropsac epithelial cells were proposed. In the present paper the diffuse cytoplasmic sudanophilia is to be added as another characteristic response for prolactin. The diffuse cytoplasmic basophilia is said to have bearing on ribonucleic acid and protein synthesis. The diffuse cytoplasmic sudanophilia is nothing but massive mobilization of neutral fat into epithelial layer of cropsac. Both histological changes seem to be closely concerned with production of crop milk of the pigeon, which contains 15% protein and 15% fat (Carr and James, 1931).

According to the present investigation pituitary prolactin has, at least, two important actions on the cropsac. One is gross and microscopic thickening of the epithel layer with downgrowth of the basal membrane and the other is diffuse cytoplasmic sudanophilia of the epithelial cells. Of the two activities the former is not so easily influenced by extraction process, while the latter strikingly modified or inhibited following chemical treatment. When prolactin was added to fresh male urine and subjected to kaolin absorption, ammonium elution and finally alcohol precipitation and tested by the systemic assay, no difinite response took place. However, if alcohol treatment was omitted in the extraction procedure, the two kinds of crop response were retained and the mode of fat mobilization was consistent with that characteristic for pituitary prolactin, at least partially.

When purified prolactin was added to male fresh urine and incubated at 37°C in vitro, the hormonal activity soon began to be lowered, particularly for fat mobilization. Even if pituitary prolactin were excreted in urine

as itself, its activity might be rapidly lowered and modified in a few hours. Under this condition crop-proliferating factor was more stable than fat-mobilizing factor, too.

As for excretion of administered prolactin men or menopausal women injected with prolactin could putout in urine similar substance to that by lactating women, which was active systemically if alcohol treatment was omitted in kaolin method of extraction.

The experimental facts above mentioned support the concept that pituitary prolactin can pass through kidney into urine as itself or unknown metabolite, but it is not stable in urine and in extraction procedure and difficult to catch as similar hormone entity.

On the other hand, there is correlation between administered prolactin dosage and cropsac thickening ability of urine, and reasonable parallelism between quantity of milk secretion and urinary excretion of substance which stimulates the cropsac. Approximately 1/30 to 1/40 of administered prolactin could be excreted in urine as prolactin-like substance. Generally speaking, lactating women excrete daily around 20 to 30 i.u. of prolactin-like substance (Fujii and Shimizu, 1958) and release of prolactin out of anterior pituitary might be roughly estimated to be in range 600 to 1200 i.u. per 24 hours during lactation.

As to prolactin in blood plasma very few are known at present, and it is obscure whether pituitary prolactin might be changed in nature and activity before or after passage through kidney.

It is accepted that impure urine extracts in strict sense could cause non-specific thickening of cropsac by the local assay method and yet the existence of prolactin-like substance in urine cannot be denied, for it was proved by the systemic assay under modified extraction of urine.

At any rate prolactin may appear in urine as modified prolactin-like substance, which can be determined by the local assay following kaolin treatment, and it is of clinical importance.

Summary

Experiments were atempted to approach the problem, whether pituitary prolactin might be excreted in urine or not.

In pigeons purified bovine prolactin seemed to have at least two activities of thickening the cropsac with proliferation of the epithelial cells and of mobilizing neutral fat in their cytoplasma as diffuse sudanophilia. The epithelium-proliferating activity was more stable than the fat-mobilizing one. When out of prolactin solution in urine incubated at 37°C recovery was attempted by kaolin method (kaolin absorption, ammonia elution and

alcohol precipitation), the fat-mobilizing activity, in particular, could be so easily lowered and modified that only a few of larger or smaller fat droplets were mobilized scattering in the epithelial layer.

When prolactin was added to fresh male urine and extracted by incomplete kaolin method (alcohol precipitation omitted) and its activity was tested by the systemic assay, cropsac response revealed two activities of prolactin.

When urines obtained from lactating wmen and men, who were injected with prolactin, were treated with the incomplete kaolin method and tested systemically, their activity was quite similar, although fat mobilization was modified as just mentioned. These facts suggested that prolactin might be excreted in urine with some metabolic modification as prolactin-like substance. Such urines extracted by complete kaolin method brought about only proliferating response of cropsac by the local assay, but in good proportion to clinical milk secretion or given dose of prolactin taking into consideration non-specific response.

As a result of recovery experiment approximately 1/30 to 1/40 of given prolactin could be excreted in urine. In lactating women generally 20 to 30 i.u. of prolactin-like substance can be putout in urine and it is of interest to note that prolactin elaboration out of anterior pituitary might be estimated in range from 600 to 1200 i.u. per day.

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