*Brit. J. Pharmacol.* (1962), **19**, 136–141.

# ASSAY OF OXYTOCIN BY RAT MAMMARY GLAND IN VITRO

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(*Received March* 15, 1962)

A new method for quantitative assay of oxytocin using strips from rat mammary gland *in vitro* has been investigated. The method is very suitable for determination of small amounts of oxytocin. Doses down to 2 $\mu$-u. oxytocin have been estimated. The dose-response curve is shown to be linear, and the precision of the method is good ($\lambda = 0.10$ to 0.14). The effect of adrenaline, noradrenaline, acetylcholine, histamine and 5-hydroxytryptamine on the rat mammary gland has also been tested.

In 1960 Mendez-Bauer, Cabot & Caldeyro-Barcia reported a new method for quantitative estimation of oxytocin. Using strips from rabbit mammary gland *in vitro*, they were able to demonstrate that oxytocin caused an increased tension of the strips and that, within a certain range, the response was proportional to the dose of oxytocin given.

In preliminary studies with rat mammary gland *in vitro*, we were able to show the same effect of oxytocin as obtained on the rabbit mammary gland (Sjöholm & Rydén, 1962). Similar results have been reported by Smith (1961).

In order to investigate if this method can be suitable for quantitative estimation of oxytocin, we have performed a statistical analysis of the method. We have also studied the effect of some other agents on strips from the mammary gland.

## METHOD

Lactating rats, 3 to 14 days after delivery, having litters of more than 4, were used. The rats were killed by a blow on the neck and the lower mammary glands were dissected from the skin and the underlying abdominal fascia. Strips, measuring about 2×4×30 mm, were cut out and suspended in an organ bath (4 to 15 ml.) containing Tyrode solution. (8 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2.6H_2O$, 0.2 g $MgCl_2.6H_2O$, 1 g $NaHCO_3$, 5 g $Na_2HPO_4.12H_2O$ and 1 g glucose to 1,000 ml. All reagents were of analytical grade.) The solution was oxygenated by a gas mixture containing 95% $O_2$ and 5% $CO_2$. The temperature of the bath was 38° C. The suspended strips were attached to a strain gauge of high sensitivity (TLK 5/0.01, Torsten Ljungström AB, Ringvägen, Sollentuna, Sweden). With this strain gauge and a carrier frequency bridge (Ljungström, TL 21 H) isometric contractions were measured and recorded by a potentiometer recorder (Ljungström, TL PS-250). From each gland two strips could usually be obtained. The glands and the strips were sometimes stored in Tyrode solution at 4° C for 24 hr before use without any loss of activity.

Before the experiment began, the tension of the strip was adjusted to give a resting tension of about 160 mg. The agents administered were allowed to act on the strip until maximum tension was developed, which usually occurred within 1 to 3 min. After prolonged use and

after higher doses, the maximum tension was reached more slowly (2 to 5 min). When the maximum was passed, the Tyrode solution was changed 3 to 4 times. Before further addition of the drug, the strip was allowed to regain its resting tension, which usually required 3 to 5 min. In most cases the proper resting tension of the strip was reached automatically, but sometimes it had to be adjusted by altering manually (lowering or raising) the height of the strain gauge; 6 to 8 determinations could be performed every hour, and each strip could be used for at least 6 to 8 hr with only slight changes of sensitivity.

*Drugs used.* Oxytocin, synthetic, "Syntocinon," Sandoz AG., Basle; acetylcholine chloride, crystalline, F. Hoffman-La Roche & Co. AG., Basle; adrenaline, 0.1% solution according to the Swedish pharmacopoeia Ed. XI; histamine dihydrochloride, F. Hoffmann-La Roche & Co. AG., Basle; noradrenaline, 0.1% solution, "Nor-Exadrin conc.," Astra, Södertälje, Sweden; 5-hydroxytryptamine creatinine sulphate, F. Hoffmann-La Roche & Co. AG., Basle.

## RESULTS

### *Oxytocin*

Administration of oxytocin caused an increased tension of the mammary gland. Usually the registered tensions varied between values corresponding to 2 to 15 mg.

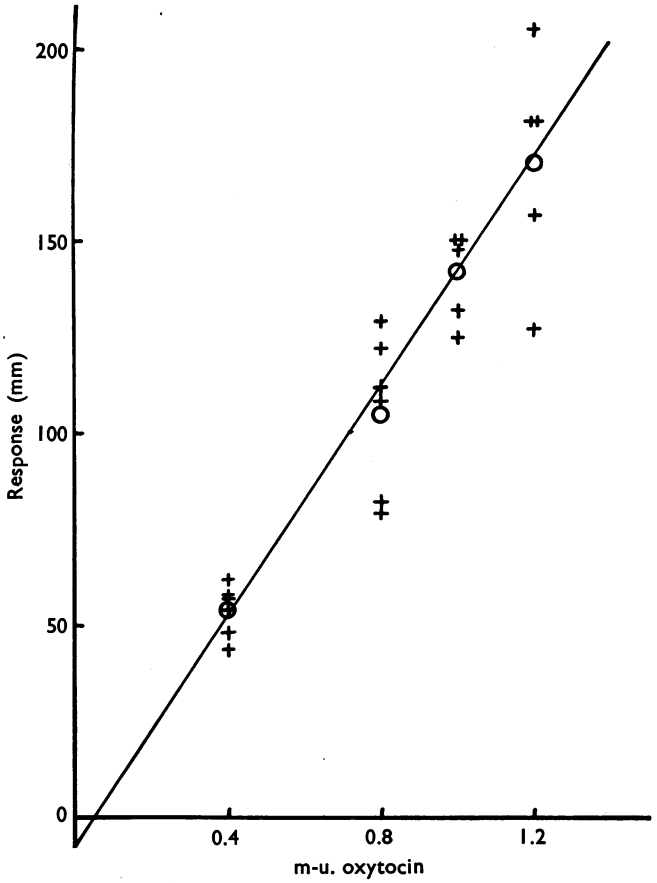


Fig. 1. The effect of oxytocin on a strip from rat mammary gland *in vitro*. The single responses (+) and the mean responses (○) are plotted against the dose. The line drawn is the regression line statistically estimated.

The lowest concentration of oxytocin which caused a measurable increase in tension was 0.0005 m-u./ml. The volume of the bath was in some experiments 4 ml. As a rule, however, a larger bath was used (10 to 14 ml.) and the concentration of oxytocin estimated was in the range of 0.005 to 0.1 m-u./ml.

*Dose-response curve.* To study the usefulness of the method for quantitative estimation of oxytocin, the linearity of the dose-response curve has been investigated and the statistical error and precision of the method has been estimated. The response of 4 different doses of oxytocin mutually randomized was determined and collected. Fig. 1 shows the results of a typical experiment together with the mean responses and the regression line statistically calculated.

The slope of the regression line was estimated according to

$$b = \frac{S(x-\bar{x})(y-\bar{y})}{S(x-\bar{x})^2} \pm \sqrt{\frac{\overline{[S_{(y-\bar{y})}]^2}}{S(x-\bar{x})^2}} = 149.2 \pm 12.2.$$

The slope is significantly different from zero ($P<0.001$ ; d.f.: 22).

A variance analysis was carried out and the results are summarized in Table 1.

TABLE 1

THE EFFECT OF OXYTOCIN ON RAT MAMMARY GLAND *IN VITRO*

Variance analysis of the results plotted in Fig. 1; 6 groups of 4 different doses were collected (1 value was lost)

| Variance reason | Degrees of freedom | Mean square | Variance ratio | Significance |
|---|---|---|---|---|
| Regression part | 1 | 46,771·22 | | |
| Deviation from the regression | 2 | 136·89 | 1·95 | $P>0·05$ |
| Between doses | 3 | 15,678·33 | 58·78 | $P<0·01$ |
| Between groups | 5 | 572·5 | 2·15 | $P>0·05$ |
| Residual variation | 14 | 266·75 | | |
| Total | 22 | | | |

The variance of the deviation from the regression line is less than the residual variation. Thus, the line can be regarded as straight and its equation is

$y = 149.2x - 8.35.$

The experimental error: $s = \pm\sqrt{266.75} = \pm 16.33.$

$s\% = \pm 13.78 \pm 2.60\%.$

The index of precision $\lambda = \frac{s}{b} = 0.109.$

When the solution of oxytocin was assayed against itself, the limits of error were calculated using the equation:

$$s_Y^2 = \frac{s^2}{b^2}\left[\frac{1}{N_{St}} + \frac{1}{N_T} + \frac{\left(\bar{y}_{St} - \bar{y}_T\right)^2}{S(x-\bar{x})^2}\right]$$

where $N_{St}$ and $N_T$ are the number of doses given and $\bar{y}_{St}$ and $\bar{y}_T$ are the mean responses of the "standard" and "test" respectively. The limits of error are then $\pm t.s_Y.100\%$. In this case the limits of error were $\pm 10.2\%$, at $P=0.05$.

In Table 2 the results from 5 different experiments are summarized.

TABLE 2

THE EFFECT OF OXYTOCIN ON RAT MAMMARY GLAND *IN VITRO*

Summary of the statistical analysis of the dose-response curve from 5 different estimations of a standard solution of oxytocin

| Expt. no. | No. of doses given N | Experimental error s | Experimental error s% | Slope of the regression line b | Index * of precision λ | Limits of error % |
|---|---|---|---|---|---|---|
| 1 | 20 | 15·45 | 16·05±3·28 | 107·3±15·1 | 0·144 | ±14·0 |
| 2 | 24 | 16·33 | 13·78±2·60 | 149·2±12·2 | 0·109 | ±10·2 |
| 3 | 28 | 21·19 | 24·02±4·12 | 196·2±20·7 | 0·108 | ±8·6 |
| 4 | 28 | 18·61 | 22·53±3·98 | 180·0±21·0 | 0·103 | ±8·3 |
| 5 | 24 | 14·21 | 23·68±4·48 | 125·9±11·5 | 0·113 | ±12·4 |
| | | | | Mean | 0·115 | |

### *Other agents*

To study the specificity of the mammary gland for oxytocin the effect of acetylcholine, adrenaline, noradrenaline, 5-hydroxytryptamine and histamine has been determined. The gland was very sensitive for all agents tested except of 5-hydroxytryptamine. The gland has been tested with gradually increasing doses of the drugs, and the lowest dose which gave an increased tension has been determined. The effect on the response of repeated additions of the drugs as well as the effect of very small doses of the drugs on the sensitivity of the gland to oxytocin has been studied. The results are summarized in Table 3.

TABLE 3

THE EFFECTS OF SOME AGENTS ON RAT MAMMARY GLAND *IN VITRO*

| Agent tested | Minimum effective concentration at the first addition (μg/ml.) | Effect of repeated addition on the sensitivity of the gland to the agent | Effect on the oxytocin response of the gland |
|---|---|---|---|
| Acetylcholine | $1\cdot10^{-6}$–$1\cdot10^{-5}$ | Decreasing sensitivity | Decreasing |
| Adrenaline | $1\cdot10^{-5}$–$1\cdot10^{-4}$ | Decreasing sensitivity | None or decreasing |
| Noradrenaline | $1\cdot10^{-5}$ | Decreasing sensitivity | None |
| Histamine | $1\cdot10^{-5}$–$1\cdot10^{-2}$ | Decreasing sensitivity | None |
| 5-Hydroxy-tryptamine | 0·01–1 | Decreasing sensitivity | None |
| Oxytocin | $1\cdot10^{-6}$ | None | — |

*Log-dose-response curve.* If the dose-response curve is studied over the whole of the dose-interval used with x = log-dose instead of the dose, a curve is obtained, the deviation of which from a straight line is significantly different from the residual variation.

## DISCUSSION

Our method for assay of oxytocin by strips from rat mammary gland is suitable for the determination of small amounts of oxytocin. A complete 4-point assay with 4 to 5 groups demands in general less than 3 hr work. As the sensitivity of the

gland is reasonably constant, it is possible to determine simultaneously two or more unknown samples without any loss of accuracy.

The mammary gland is very sensitive to oxytocin. Using the milk ejection test in the rabbit, Fitzpatrick (1959) was able to estimate doses of 0.08 to 0.20 m-u. of oxytocin after intra-arterial injection. As a rule we have been working with a bath of 10 to 15 ml. volume and with doses of 0.05 to 1.0 m-u. of oxytocin. We have, however, also used a bath of 4 ml. volume, and with increased sensitivity of the apparatus we have estimated doses down to 2 $\mu$-u. with considerable accuracy, and it should be possible to detect still smaller doses of oxytocin. The rat mammary gland *in vitro* is much more sensitive than the rabbit mammary gland *in vitro* (Mendez-Bauer *et al.*, 1960). It seems to be as sensitive as the superfused rat uterus. Using the superfusion technique (Gaddum, 1953) with strips from rat uterus in pro-oestrus, Fitzpatrick (1959) was able to determine in general doses of 2 to 8 $\mu$-u. with considerable accuracy. However, the rat mammary gland *in vitro* never exhibits a spontaneous activity during the oxytocin assay.

When the tonus increase of the rat mammary gland is plotted against the dose of oxytocin, a straight line is obtained within the dose-interval generally used. This means that the log dose-response curve is not linear over the dose-interval in question. After testing our technical equipment with known weights, we conclude that this unexpected observation is not due to distortion by the recording apparatus. This fact will be a subject for further study.

The index of precision of the method, $\lambda = \frac{s}{b}$, is good, and in general $\lambda$ is 0.10 to 0.14. However, it is not as good as the rat uterus method of Holton, the precision of which is 0.022 to 0.14 (Holton, 1948). In most of the control assays of oxytocin, $\lambda$ was about 0.11. With this high precision one can get theoretical limits of error of 8.7 to 9.6% at a 4-point assay with 28 to 24 determinations. With less determinations (16 to 12) one can still get rather good limits of error (12.4 to 15.5%). With the superfused rat uterus, Fitzpatrick calculated the theoretical limits of error to be $\pm 7.5\%$ with 8 to 12 determinations (Fitzpatrick, 1959).

Mendez-Bauer *et al.* (1960) observed that the rabbit mammary gland *in vitro* was very insensitive to common pharmacological agents other than oxytocin. Unfortunately, we have not found this valid for the rat mammary gland, which is highly sensitive to acetylcholine, adrenaline, noradrenaline and histamine. When oxytocin assay of tissue extracts is carried out, these compounds may influence the estimation, if specific inhibitors are not added. The gland also reacts to 5-hydroxytryptamine, but the sensitivity is much less than to the other drugs. It was also observed that repeated administration of the drugs caused gradually decreasing response. Small doses of the drugs tested, as a rule, did not change the sensitivity of the gland to oxytocin. However, acetylcholine in most experiments caused a considerably decreased response to oxytocin.

We are very much indebted to Dr Stellan Ljungberg for valuable discussions about the statistics, and to Miss Erika Gerhardt and Miss Ulla Jansson for technical assistance. The work has been supported by grants from " Anslaget för främjande av ograduerade forskares vetenenskapliga verksamhet " (I.S.) and from " Karolinska institutets reservationsanslag " (G.R.).

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