Improvement in the Bioassay of Oxytocin by the Mouse Mammary Gland *in Vitro*[1] (36286)

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Recently we have reported some results on the bioassay of oxytocin by a strip of the lactating mouse mammary gland (1). The most interesting features of this bioassay were its high specificity and sensitivity; the threshold dose usually was 20 μU/ml and frequently 10 μU/ml.

With some modifications here described we have been able to increase the sensitivity about twentyfold and at the same time, to simplify the experimental procedure.

We think that combining this bioassay with an extraction procedure (2, 3) it will be possible to estimate low levels of endogenous oxytocin in small blood samples.

Material and Methods. Lactating mice (Rockland Strain) between the 10th and the 20th day after parturition were used. The animals are killed by a blow on the head and the mammary gland is immediately dissected. A strip of approximately 20 $\times$ 3 $\times$ 2 mm is cut off and kept in Tyrode's solution. To obtain isometric recordings, one end of the strip is attached by a hook to the bottom of a 2 ml capacity microbath. The other end is tied by a cotton thread to a Statham Strain Gauge 0.15 oz connected to a recording system consisting of a Hewlett-Packard, Model 350-1100 M Preamplifier with a Power Supply Model 350-500 B and a Recti Ritter Recorder, Texas Inst. Inc., Model PRRIM-A25. A polyethylene catheter attached to the inside wall of the microbath is used to change fluids. For some experiments the microbath is immersed in a thermostatic bath. Tension applied to the strip is adjusted by a micrometric screw.

Syntocinon Sandoz was used in all experiments.

The influence of various factors on the sensitivity to oxytocin of mammary gland strips was carefully investigated:

1. *Temperature.* In order to study the influence of temperature on the assay, responses elicited by 10 μU/ml of oxytocin were isometrically recorded at varying bath temperatures, from 18 to 37°. Resting tension and composition of Tyrode's solution were maintained constant.

2. *Composition of Tyrode's solution.* The physiologic solution (Tyrode) commonly used in our previous biological assays has the following composition: NaCl 8 g; KCl 0.20 g; $MgCl_2 \cdot 6H_2O$ 0.10 g; $CaCl_2$ anh. 0.17 g; $Na_2HPO_4 \cdot 12H_2O$ 0.0055 g; $NaHCO_3$ 1 g; $C_6H_{12}O_6$ 1 g per liter.

This solution, which we arbitrarily named "Tyrode 100," was diluted adding 10% v/v and 20% v/v of water. These last two solutions were named "Tyrode 90" and "Tyrode 80," respectively.

The influence of Tyrode ionic concentration on the sensitivity of the mammary gland to oxytocin was studied by means of repeated measurements of the hormone at a constant dose (10 μU/ml) using Tyrode 90 and Tyrode 100 as diluents. Temperature and resting tension were maintained constant. Tyrode 80 altered the baseline of the recording; therefore, it was not exhaustively studied.

3. *Resting tension.* Although the influence of the resting tension was investigated in a

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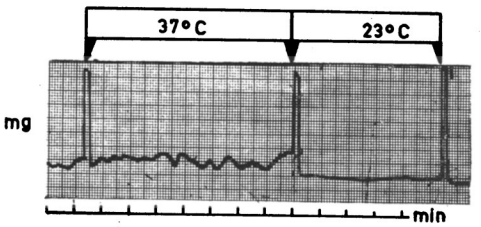

FIG. 1. Influence of temperature on the baseline of the mouse mammary gland bioassay.

previous paper (1), we now studied it under room temperature and using Tyrode 90. Responses to 10 μU/ml of oxytocin at a given tension of 200 to 1500 mg were compared.

4. *Variation of sensitivity during the assay.* There is an important increase in sensitivity along the assay. In order to find out its slope and the period of maximum sensitivity, a constant dose of oxytocin was injected at regular 7.5-min intervals.

Results and Discussion. The following results were obtained:

1. By working at temperatures under 37° the baseline was clearly improved and the sensitivity increased. The highest sensitivity was found around 23° (room temperature) as is shown in Fig. 1. At this temperature the baseline shows no alterations (Fig. 2) even when the recording system is amplified to its maximum (4 mm/mg). Under these conditions the use of a thermostatic bath is no longer necessary.

2. The responses of the mammary gland strips elicited by a given dose of oxytocin are greater when Tyrode 90 instead of Tyrode 100 is used. Analysis of variance for two variables of classification—repeated measurements—was applied showing highly significant results: $F = 852.8$ while $F_{0.95\ (1,2)} = 18.5$.

3. Changes in the resting tension when working at room temperature do not significantly modify the sensitivity. Nevertheless, over 1000 mg tension baseline becomes quite irregular and responses are difficult to control. On the other hand, at low tensions (around 200 mg) the strip of mammary gland loosens and may touch the wall of the microbath. If this occurs the baseline is distorted thus making it very difficult to accurately estimate the responses.

4. As shown in Fig. 3 a linear regression curve is obtained until about 8 hr of work with one strip of mouse mammary gland; at this time the maximum sensitivity is reached. By means of the four point or any other method, it is possible to make very accurate estimations of oxytocin at low cencentrations throughout the assay. Only when extremely small quantities of oxytocin—around 1 μU or less—wish to be measured it is necessary to wait for some time before this can be achieved.

It is worthwhile to point out that one strip can give useful recordings during 12 or more hours.

5. After more than two hundred assays we may state that mammary glands of lactating mice, 12 to 15 days after parturition give the best results.

The above results allow us to conclude that a good sensitivity is obtained under the following conditions:

a. Strips should be obtained from lactating mice 12 to 15 days after parturition.

b. Tyrode 90 should be used as physiologic solution.

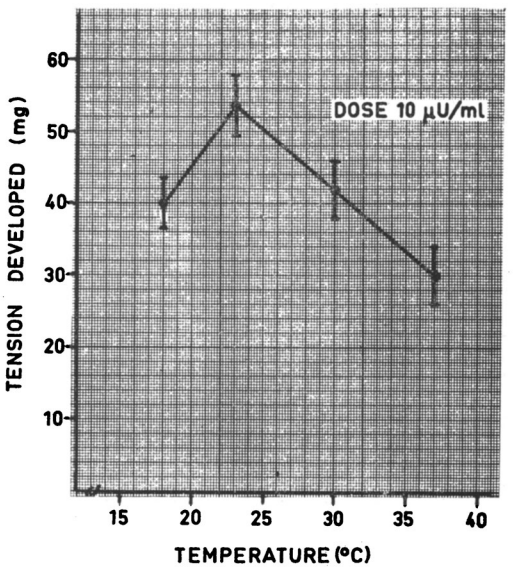

FIG. 2. Influence of temperature on the sensitivity of the mouse mammary gland *in vitro*. Each point is the mean of six observations; the bars represent $\overline{X} + SE \times t_5$.

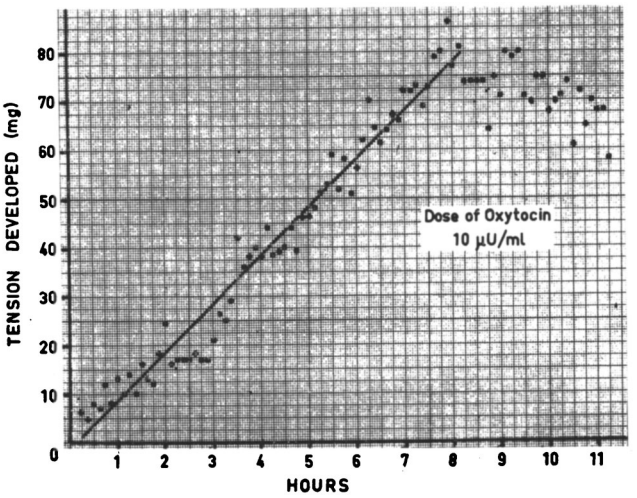

FIG. 3. Time-response curve at a constant dose of 10 μU/ml. A linear regression is obtained in the first 8 hr.

c. Room temperature should be around 23°.

d. The most adequate resting tension lies in the range of 400 to 700 mg.

e. When maximum sensitivity is required (1 μU/ml or less) the determinations must be performed 5 to 7 hr after starting the assay.

In 27 bioassays carried out under these conditions, we were able to detect 5 μU/ml in 93%, 1 μU/ml in 57%, and 0.1 μU/ml in 14% of the assays.

According to these results and those previously reported (1) it seems that the bioassay of oxytocin with the lactating mouse mammary gland *in vitro* is more specific (1) and sensitive than that of the lactating rat either *in vitro* (4–7) or *in vivo* (7, 8).

Summary. Some simple changes in the composition of Tyrode's solution, temperature, and resting tension, were added to our method for bioassay of oxytocin by the isolated strip of lactating mouse mammary gland. The increase of sensitivity was the main improvement obtained, thus enabling the estimation of 1 or less μU/ml of oxytocin, in 50% of the assays.

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