CONTRACTION OF THE RABBIT MAMMARY STRIP IN VITRO IN RESPONSE TO OXYTOCIN

By

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ABSTRACT

The isometric contractile response to oxytocin has been studied in vitro utilizing strips of tissue from rabbit mammary glands. Responses were maximal at resting tensions of 200–400 mg/mm². The force of contraction increased from the beginning of lactation to about 9 days post partum and then leveled off. Little or no response is noted below 15°C and the maximum response is obtained in the range of 32 to 36°C. Irreversible changes begin above 41°C. As little as 0.1 mU oxytocin/ml could be detected and the dose-response curve was demonstrated statistically to be linear between the dosages of 0.5 to 10 mU oxytocin/ml. The dose-response curve reached a plateau at about 8–11 mU/ml. Replacing sodium with potassium resulted in a logarithmic decrease of contractile force with time. This decrease was partially reversible. The strip became inexcitable in calcium free Tyrode’s solution. Excitability was again established by adding calcium. Other than to oxytocin, the mammary strip responded only to acetylcholine. The effect of acetylcholine could be blocked by atropine and this treatment did not affect the response to oxytocin. The site and mechanism of action of oxytocin are discussed.

Mendez-Bauer et al. reported in 1960 the use of strips of rabbit mammary tissue for the estimation of oxytocin in vitro (Mendez-Bauer et al. 1960). They were able to show that oxytocin caused the development of contractile force

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in such strips. Doses within the range of 1 to 7 milliunits/ml (mU/ml) gave an approximately linear dose-response curve; however, the linearity was not statistically investigated. Smith (1961) and Sjöholm & Rydén (1962) were able to show that oxytocin caused the development of contractile tension in rat mammary strips in vitro. Rydén & Sjöholm (1962) demonstrated that the response of the rat mammary strip was linear over the range of 0.005 to 0.100 mU/ml oxytocin. They also reported that the rat mammary strip was very sensitive to acetylcholine, adrenaline, nor-adrenaline and histamine. Serotonin (5-hydroxy-tryptamine) was effective in higher doses. The results suggest that the mammary strip might provide a more specific assay for oxytocin than the assays previously used.

As reported by Rydén & Sjöholm (1962) the rat mammary strip, although giving a linear response over the tested range, suffers from lack of specificity. For this reason, it was considered desirable to determine statistically if the response curve of the rabbit strip is linear over the entire range and to test the specificity of the rabbit preparation.

MATERIALS AND METHODS

Lactating rabbits of a Dutch-Belted strain weighing 2 to 2.5 kg were used. The rabbits were fed Wayne Rabbit Ration and water ad libitum. After mating, the rabbits were kept in individual cages until approximately 1 week before delivery, at which time they were put in a larger cage which contained a nesting box. Immediately prior to use, the animals were removed from their young and anaesthetized by injection of 30 to 35 mg Nembutal/kg body weight into an ear vein.

After shaving the abdomen, the outline of the mammary gland could be seen through the skin and the best developed area, usually just below the costal margin, was selected. A longitudinal incision extending between two teats was then made through the skin and the gland freed from the skin by blunt dissection. Beginning at the lateral edge of the gland, the mammary tissue was readily separated from the underlying abdominal wall. Lifting up the lateral edge of the mammary tissue exposed the veins which run in an anterior-posterior direction along the dorsal aspect of the glands. Two haemostats (mosquito type) were then used to clamp the mammary tissue including its blood vessels to control haemorrhage. The haemostats were applied parallel to each other about 6 cm apart to isolate a band of mammary tissue running from the lateral edge of the gland to the middle and extending from one teat to the next. This band measured approximately 4 to 6 cm and included tissue from two mammary glands, but did not include the teats. The tissue was then removed with scissors and immediately placed in Tyrode’s solution.

A concentrate of Tyrode’s solution was stored in the frozen state and was thawed and diluted on the day of use. The Tyrode’s solution was then gassed with a mixture of 5% CO\textsubscript{2} and 95% O\textsubscript{2} for 5 to 10 minutes to bring the pH to approximately 7.2 at 32°C.

After removal of the gland, the wound was sutured and the rabbit allowed to recover. Such animals could be used a second or third time, but for the results reported here, only primaparous mothers were used.
After removal, the mammary tissue was stored in Tyrode's at 4° C for up to 72 hours. During this time, strips measuring (2-4) × (2-4) × (15-25) mm were cut radially from the gland and used.

Two strips were run simultaneously, using two chambers and two complete amplification and recording channels. The glass chambers could accommodate the mammary strip plus 1.00 ml solution. Water at the desired temperature was circulated through a glass water jacket surrounding each chamber. The chamber had the shape of an inverted »T« (see Fig. 1). In one arm of the »T«, was placed a cork which held an »L« shaped piece of 2 × 100 mm capillary glass tubing. The free arm of the tubing extended above the level of liquid in the chamber. By rotating the tubing in the cork, the free arm could be turned down to empty the solution through the tube with a siphoning action pulling the last drops from the chamber. The Tyrode's solution in the chamber was gassed continually with a mixture of 95 % O₂ and 5 % CO₂ admitted through fritted glass. The bubbles of gas were less than 1 mm diameter and produced no observable »noise« on the recording due to mechanical disturbances of the mammary strip.

All doses of oxytocin were dissolved in 0.05 ml of solution and injected from a 0.25 ml tuberculin syringe. The solution was injected into a volume of 0.95 ml in the water bath to give a final volume of 1.00 ml. The test solutions were always injected vigorously to insure good mixing.

After each response had reached a maximum, the Tyrode's solution in the bath was removed and three washes were made. After ten minutes, the bath was again emptied and washed with Tyrode's solution two more times. An additional period of five minutes was then allowed to elapse before injection of the test-dose. Thus, just slightly over fifteen minutes elapsed between each dose. If less time and/or fewer washings were allowed, tachyphylaxis was observed.

During washings, the strip of mammary tissue was exposed to air for a maximum of 2 to 3 seconds. It is doubtful that the surface layer of solution could evaporate during this time interval and in any case, the resting tension returned to normal immediately upon the addition of Tyrode's solution, and no interference due to this treatment was detected.

The strips were anchored at the bottom of the chamber with 4-0 surgical silk by clamping the thread in place with the cork. The upper end of the strip was attached by 4-0 silk to a Grass FT-03 bridge type transducer with no loading springs in place.
The output (30 millivolts/kg/volt) from this transducer was then fed into a Grass Model 5P1H low-level D.C. pre-amplifier which then fed into a Grass Model 5E Polygraph. Both channels were recorded simultaneously. By using two channels, two determinations could be done every fifteen minutes, i.e. six to eight determinations per hour were possible.

Strips were allowed to equilibrate 30 minutes to one hour in Tyrode's solution after applying resting tension. The tension asymptotically approached a lower level during this time. Once the resting tension reached equilibrium, the strip was tested with a known dose of oxytocin* for reproducibility. Sometimes it was necessary to wait up to three hours after establishment of resting tension before the response would stabilize. The strip could then be used for 6 to 8 hours with only a slight decrease in sensitivity. When failure did occur, it was rapid.

Blood samples which were to be assayed for their oxytocin content were brought to a pH of 11 to 12 by addition of NaOH immediately upon withdrawal. This was done to inactivate any oxytocinase which might be present. The samples were then stored in the frozen state. Just prior to assay the samples were rapidly thawed and the pH adjusted to 7.2 using HCl. Allowance was made for dilution in calculations.

At 37°C and a pH of 11.5, the nomogram given by Gaddum (1930) shows that in 5 to 10 minutes, which was the approximate time required for freezing of the samples, only 1-2% of the oxytocin would be destroyed. This small per cent would not be detected by the assay method and indeed known aliquots of oxytocin showed no change in activity after this treatment.

In those experiments where it was necessary to compare one strip with another, it was necessary to correct for variations in the cross-sectional area of the strips. This was done by dividing both the contraction force and the resting tension in mg** by a figure representative of the cross-section of the strip; the result being called the index and expressed in mg-mm/mg. As the cross-section area could not be directly measured, it was approximated by dividing the weight (in mg) of the strip by its length in mm. If the specific gravity of the strip were one, this latter number would represent the average cross-section area of the strip in mm².

RESULTS

Fig. 2 shows the effect of resting tension upon the force of contraction. The response reaches a maximum at a resting tension index of about 200 mg–mm/mg and remains relatively constant to a resting tension index of about 400 mg–mm/mg. Depending on the cross-section figure, this represents a resting tension of from about 1300 to about 3400 mg for the size strips we were using.

Fig. 3 shows the effect of days of lactation upon the response of the strip. Initially the strip responds poorly, but with continued lactation, the response increases and seems to remain relatively constant from approximately 9 days

* The oxytocin used in these experiments was the synthetic Syntocinon. It and the 8-arginine vasopressin were obtained through the courtesy of Dr. John Der Hovanesian, Sandoz Pharmaceuticals.

** For convenience, force is expressed in mg. The authors are aware that one mg of mass produces approximately 0.98 dynes of force with gravity equal to 980 cm/sec².
The effect of resting tension upon the force of contraction. The force of contraction and the resting tension are both plotted in terms of an index which is obtained by dividing the force in mg by a figure proportional to the cross sectional area of the muscle. The plotted data are from four separate strips obtained from two rabbits. All responses are to 10 mU oxytocin/ml.

Fig. 2.

The maximal response is plotted against days lactation. The first two points, at 24 hours and at 36 hours, are single determinations. The other points represent the average of 4 to 8 determinations from a single rabbit and the lines extending from each point represent the standard error. All responses are to 10 mU oxytocin/ml.

Fig. 3.

Fig. 4 shows the effect of temperature upon the response to oxytocin. These results were obtained from 4 different strips obtained from 4 different rabbits and the curve was estimated visually. The maximum response occurs in the approximate range of 32 to 36 degrees. The response falls off rapidly below approximately 30° C and above approximately 40° C. Above about 41° C, indicated by the arrow in Fig. 4, the drop in response is irreversible. Temperature changes below 40–41° C apparently were reversible.

to 44 days post-partum. These determinations were made on primaparous rabbits. From this data, it was decided to use strips taken from 9 to 40 days after delivery.
The effect of temperature upon the response is shown. The data is obtained from four separate strips and expressed in terms of contraction index so that results may be compared. The curve was estimated visually. The arrow indicates the approximate temperature above which irreversible changes began. All responses were to 10 mU oxytocin/ml.

The response (Y) of the mammary strip *in vitro* is plotted against the dose (x) of oxytocin. The line follows the equation given and was determined by the linear regression method of least squares.

Depending on the particular strip, the lowest detectable concentration of oxytocin was 0.1 mU/ml and the response was no longer incremental above about 8–11 mU/ml. In Fig. 5 the contraction force is plotted over the entire range of response for a strip from one particular animal and a linear regression line calculated from the data by the method of least squares is plotted. Doses were given in a random order. The standard deviation (s), the precision (s/b), and the correlation coefficient (r) are shown above the equation of the line.

The slope of the line was estimated by:

\[
b = \frac{n \Sigma x_i y_i - \Sigma x_i \Sigma y_i}{n \Sigma x_i^2 - (\Sigma x_i)^2}
\]
and the y intercept is given by
\[ a = \frac{\Sigma y_i - b \Sigma x_i}{n} \]

An analysis of variance was carried out to determine if the scatter about the regression line could be attributed entirely to random scatter or if some non-linear component must exist to contribute to the scatter.

The model for this analysis was:
\[ Y = a + bx_{ia} + E_i + E_{ia} \]

where the deviation from the regression line, \( E_i + E_{ia} \), is composed of two parts; \( E_{ia} \), the error due to randomness and \( E_i \), the non-random error due to any non-linear components.

The sum of squares \( (Y_{ia} - \bar{Y})^2 \) can be expanded to:

<table>
<thead>
<tr>
<th>Source of Estimate</th>
<th>Sum of Squares ( S )</th>
<th>D. F.</th>
<th>Mean Square ( S )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity</td>
<td>( S_1 = \Sigma n_i (a + bx_{i} - \bar{Y})^2 )</td>
<td>1</td>
<td>( S_1 )</td>
</tr>
<tr>
<td>Deviation of means from regression</td>
<td>( S_2 = \Sigma n_i (Y_{i} - a - bx_{i})^2 )</td>
<td>( k-2 )</td>
<td>( S_2 ) ( k-2 )</td>
</tr>
<tr>
<td>Within class (primitive error)</td>
<td>( S_3 = \Sigma (Y_{ia} - \bar{Y})^2 )</td>
<td>( N-k )</td>
<td>( S_3 ) ( N-k )</td>
</tr>
<tr>
<td>Total</td>
<td>( S = \Sigma (Y_{ia} - \bar{Y})^2 )</td>
<td>( N-1 )</td>
<td></td>
</tr>
</tbody>
</table>

If non-linear components are absent, there should be no non-random error contributing to the deviation of class means from the regression, and an F test should fail to distinguish between the two mean squares, \( S_2/k-2 \) and \( S_3/N-k \). In such a case, the deviation from the linear regression could be entirely explained by random effects.

An analysis of variance on the data shown in Fig. 5 is summarized in the first part of Table 1. This table includes the results of two other experiments.

The variance due to the deviation of the means from the linear regression line is not significantly different (even at the 0.25 level) from the primitive error and thus the equation:
\[ Y = -39.05 + 66.663 \cdot X \]
can be considered linear within experimental error over the range of the data, which represents at least three-quarters of the whole range of graded response.

Table 2 shows the effect of some compounds upon the mammary gland strip. 100 mU arginine vasopressin was found to have the activity of 15 mU of oxytocin. Of the compounds tested, the only other one which was effective was acetylcholine and its effect could be blocked by atropine. Atropine did not interfere with the response to oxytocin.

Fig. 6 shows the effect of replacing sodium with potassium. The response
### Table 1.
Analysis of Variance of Results of

\[ r = 0.964 \quad s/b = 0.993 \quad Y = -39.05 + 66.663 \cdot X \quad s = 66.20 \]

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>linearity</td>
<td>1,538,631</td>
<td>1</td>
<td>1,538,631</td>
<td>308.5</td>
<td>0.01</td>
</tr>
<tr>
<td>non-linearity</td>
<td>10,696.4</td>
<td>3</td>
<td>3,565.5</td>
<td>0.7505, (1/F = 1.3985)</td>
<td>0.25</td>
</tr>
<tr>
<td>error</td>
<td>74,794</td>
<td>15</td>
<td>4,986.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total | 1,624,121 | 19 |

\[ r = 0.980 \quad s/b = 0.813 \quad Y = -28.90 + 133.99 \cdot X \quad s = 108.9 \]

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>linearity</td>
<td>6,772,723</td>
<td>1</td>
<td>6,772,723</td>
<td>26.95</td>
<td>0.01</td>
</tr>
<tr>
<td>non-linearity</td>
<td>27,935.64</td>
<td>4</td>
<td>6,983.9</td>
<td>1/F = 1.786</td>
<td>0.25</td>
</tr>
<tr>
<td>error</td>
<td>249,400</td>
<td>20</td>
<td>12,470</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total | 7,000,058 | 25 |

\[ r = 0.990 \quad s/b = 0.450 \quad Y = -7 + 37.50 \cdot X \quad s = 16.8 \]

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>linearity</td>
<td>340,439</td>
<td>1</td>
<td>340,439</td>
<td>12.04</td>
<td>0.01</td>
</tr>
<tr>
<td>non-linearity</td>
<td>1,020.77</td>
<td>4</td>
<td>255.194</td>
<td>0.903</td>
<td>0.25</td>
</tr>
<tr>
<td>error</td>
<td>2,827</td>
<td>10</td>
<td>282.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total | 344,288 | 15 |

To a constant dose of oxytocin decreases exponentially with time. The regression curve of the log of the contractile force response plotted against time was shown by analysis of variance to be compatible with a straight line and thus the response may be said to decrease exponentially with time. The response increases again following reintroduction of Tyrode's solution containing normal concentrations of potassium and sodium.

Fig. 7 shows the effect of placing the strip in Tyrode's which contains no Ca. The response of the experimental strip is divided by the response of a control strip in Tyrode's solution and containing normal Ca and this is plotted against time. The solution was changed once before and three times after each
**Table 2.**

The Effect of Various Compounds on the Rabbit Mammary Gland *in vitro.*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosage</th>
<th>Effect</th>
<th>Effect upon Oxytocin Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine</td>
<td>0.125 mg/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>5 mg/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td>$2.5 \times 10^{-2}$ mg/ml</td>
<td>10 mU oxytocin</td>
<td>none</td>
</tr>
<tr>
<td>atropine</td>
<td>$1.5 \times 10^{-4}$ mg/ml</td>
<td>no effect</td>
<td>none (completely blocked ACTH)</td>
</tr>
<tr>
<td>8-arginine vasopressin</td>
<td>50 mU/ml</td>
<td>7.5 mU oxytocin</td>
<td>no</td>
</tr>
<tr>
<td>Serotonin</td>
<td>$5 \times 10^{-5}$ mg/ml</td>
<td>none</td>
<td>blocked 1 mU oxytocin</td>
</tr>
<tr>
<td>Serotonin</td>
<td>$5 \times 10^{-2}$ mg/ml</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>Epinephrine</td>
<td>$10^{-9}$</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>$10^{-7}$</td>
<td>none</td>
<td>no</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>$10^{-5}$</td>
<td>none</td>
<td>$42%$ decrease</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>$10^{-4}$</td>
<td>none</td>
<td>-</td>
</tr>
<tr>
<td>Ergonovine maleate</td>
<td>$5 \times 10^{-4}$ mg/ml</td>
<td>none</td>
<td>-</td>
</tr>
<tr>
<td>Ergonovine maleate</td>
<td>$2.5 \times 10^{-2}$ mg/ml</td>
<td>none</td>
<td>-</td>
</tr>
<tr>
<td>Insulin (regular)</td>
<td>2 units/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Relaxin</td>
<td>4 units/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Acetyl strophanthidin</td>
<td>$1.5 \times 10^{-3}$ cat</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Progesterone</td>
<td>$10^{-2}$ mg/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>DOCA</td>
<td>$1.4 \times 10^{-3}$ mg/ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>Haemolyzed human blood from:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 year old male</td>
<td>0.1 ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>2 non-pregnant females</td>
<td>0.1 ml</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>female during delivery</td>
<td>0.1 ml</td>
<td>.5-.6 mU oxytocin</td>
<td>-</td>
</tr>
</tbody>
</table>

contraction. A standard stimulus of 10 mU of oxytocin was used. The strip eventually became completely inexcitable. Excitability was regained by reintroduction into Tyrode's containing normal calcium.

**DISCUSSION**

A truly linear function is a rather rare phenomenon in nature. However, a reasonable model may be postulated in which each receptor site for oxytocin activates approximately the same amount of contractile mechanism. Working on this assumption, it has been possible to construct a model giving a linear
The contractile force response is plotted on a logarithmic scale against the minutes the strip has been in Tyrode's in which potassium has replaced the sodium. The arrow indicates the point at which the strip was introduced into Tyrode's containing normal concentrations of potassium and sodium. All responses were to 10 mU oxytocin/ml.

**Fig. 6.**

The response of a strip is plotted against the time the strip was in a solution of Tyrode's containing no calcium. The solution was changed once before and three times after each contraction. The arrow indicates the point at which the experimental strip was replaced in Tyrode's containing calcium. All responses were to 10 mU oxytocin/ml.
response only if the assumption is made that the hormone molecule occupies the receptor site for an extremely short period of time. Further attempts are being made to define such a model.

It must be kept in mind that the theoretical curve need only closely approximate a linear function as a non-linear component smaller than the random error cannot be experimentally detected. It is our opinion that the dose-response curve is not linear, but some function which approximates linearity. If the dose response curve had been a log-dose curve, the log term would have showed up in the model in $E_i$. This would have introduced a non-random deviation of the class means from the linear regression. Thus the variation of the deviation of the means from the linear regression would have been greater than the variation expected from random effects (i.e. the within class, or primitive error) alone. If this had been the case, the difference between the two mean squares $S_2/k-2$ and $S_2/N-k$ would have been sufficient to have been detected by the F-test.

In any case, our work suggests that oxytocin acts upon the membrane of the myoepithelial cells. Richardson (1949) and Linzell (1952) have provided good evidence that, although the mammary gland contains smooth muscle, oxytocin must act upon the myoepithelium. And our observation that high potassium decreases the response and that calcium-free Tyrode's blocks the response support the concept that the action is upon the cell membrane.

There is considerable evidence that calcium is a link in the excitation-contraction coupling in cardiac muscle (Niedergerke 1956; Luttgau & Niedergerke 1958), skeletal muscle (Bianchi & Shanes 1959; Frank 1960), and smooth muscle (Durbin & Jenskinson 1961; Edman & Schild 1961, 1962). Winegrad & Shanes (1962) showed that the calcium influx through the cell membrane per beat was directly proportional to the strength of contraction. If oxytocin were to mediate its effects via the cell membrane, controlling calcium influx, it would be expected that calcium-free Tyrode's would abolish the contractile response to oxytocin. Coutinho & Csapo (1959) have shown that the rat and rabbit uterus became unresponsive to oxytocin in calcium-free media. This is compatible with our observation that the mammary strip became unresponsive to oxytocin in calcium-free media.

High concentrations of potassium usually depolarize cell membranes and such treatment might be expected to abolish the response to a compound which acted via the cell membrane. Csapo (1954, 1960) has reported evidence that oxytocin does not directly stimulate the contractile system of uterine muscle, but only regulates membrane function. Oxytocin failed to elicit a contractile response in uteri depolarized by 120 mM potassium. The contractile system was intact as was shown by causing a close to maximum contraction by a longitudinal electric field. Kuriyama & Csapo (1961) used microelectrodes and Marshall & Csapo (1961) used the sucrose gap to show that oxytocin caused
a lowering of the membrane potential of the parturient uterus of rabbits and rats.

Our observations showed an exponential decrease in response with time in Tyrode's containing high potassium. We are unable to explain why high potassium did not produce complete inexcitability. Similar results were observed in the rat uterus by Evans et al. (1958) who found that K₂SO₄ Ringer failed to produce unresponsiveness to oxytocin.

The explanation for the exponential decrease in responsiveness after exposure to high potassium may possibly be related to the fact that cells exposed to high potassium take up water (Harris 1960). The explanation for this comes from an examination of the Donnan effect. The resting membrane potential keeps the internal chloride concentration at a low level. When the membrane is depolarized by a high potassium solution, chloride ions are able to enter the cell. As this entry is approximately a first order reaction (depending only upon the concentration gradient), the influx of chloride follows an exponential time course. As chloride enters the cell, it is accompanied by potassium in order to maintain electrical neutrality. An osmotic equivalent of water follows the potassium and chloride to maintain osmotic equilibrium.

In our experiments, mammary strips were tested at 32°C. Mendez-Bauer and his colleagues (Mendez-Bauer et al. 1960), Rydén & Sjöholm (1962) and Sjöholm & Rydén (1962) used 38°C. We felt this temperature was too near the steep part of the curve and the point (around 41°C) where irreversible changes begin. The strips became inactive below approximately 15°C. This is interesting in view of the suggestion that actomyosin undergoes a change in configuration at approximately 16°C (Koshland 1959; Levy et al. 1959).

Mendez-Bauer et al. (1960) reported that the specificity of the rabbit mammary strip was high and that it was unaffected by heparinized blood or plasma. We have shown that of a large number of substances including haemolyzed blood, only substances with oxytocin-like structures and acetylcholine were effective. Acetylcholine could be blocked by atropine. Atropine produced no interference with the action of oxytocin. Arginine vasopressin was shown to have 15% of the activity of oxytocin. This agrees well with the results of van Dyke et al. (1955) who found that i. v. vasopressin had 17% of the activity of oxytocin as measured by the milk ejection response in the rabbit. Smith (1961) found that arginine vasopressin was only 3.2% as effective as oxytocin upon the rat mammary strip in vitro. The disparity is probably due to a species difference.

The rabbit mammary strip may prove to be a useful method of detecting the biological activity of oxytocin. Although not as sensitive as the rat mammary strip which can detect as little as 0.002 mU oxytocin/ml (Rydén & Sjöholm 1962), it is more specific in that it distinguishes oxytocin from histamine, adrenaline, noradrenaline and serotonin.
Hawker & Robertson (1958) reported that blood extracts which had a potency of 18 to 40 mU/ml oxytocin activity when tested on the rat uterus produced no measurable change in the intra-mammary pressure of the rabbit although pressure changes were produced by 0.5 mU oxytocin.

Coutinho & Csapo (1959) reported that the Ca-deficient rabbit uterus also responds to serotonin and acetylcholine. The range of the sensitivity of this preparation was reported as 0.2 to 0.8 mU/ml and the range of the calcium-deficient rat uterus was reported as 0.1 to 0.5 mU/ml. In both cases, tension was described as a log-function of the oxytocin concentration. The rabbit mammary strip has a much greater range of response than this, responding in a near linear relationship to concentrations varying by two orders of magnitude.

REFERENCES


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