THE IN VITRO RELEASE BY THE TOAD BLADDER OF AN INHIBITOR OF OXYTOCIN*

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Oxytocin and the other naturally occurring neurohypophyseal hormones, as well as some synthetic analogs, have the effect of increasing the permeability to water of the amphibian skin and bladder. This effect has been studied extensively in the urinary bladder of the toad, Bufo marinus (Bentley, 1958; Rasmussen, et al., 1960; Leaf and Hays, 1961). Recently, it was found that an ammonium sulfate extract of the bladder of Bufo marinus is capable of inactivating oxytocin and its hormone analogs (Karlin, 1962). This extract appears to contain an aminopeptidase, differing in some respects from the oxytocinase found in pregnancy serum and characterized by Tuppy and co-workers (Tuppy, 1961). An attempt has been made to determine whether this oxytocin-inactivating enzyme present in the toad bladder appreciably influences the action of oxytocin on the intact bladder.

The method employed was similar to that of Bentley (1958). Half-bladders were excised, washed, and tied, bag-like, to tubes, with the serosal side out and the mucosal side in. Each half-bladder was then suspended in 25 ml. aerated Ringer's solution bathing the serosal side (110 mM NaCl,

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1 mM KCl, 1 mM $NaHCO_3$, 1 mM K_2HPO_4, 0.2 mM KH_2PO_4, 2 mM $CaCl_2$, 5 mM glucose, 5 mM Na-pyruvate, pH 7.7). Within each half-bladder, bathing its mucosal side, was 5 ml. distilled water. Water thus moved down its activity gradient through the bladder from mucosal to serosal solution. The rate of loss of water from the mucosal solution was determined by periodically weighing the tube and attached half-bladder enclosing the mucosal solution. Following each weighing, the amount of water lost during the previous period was added back to the mucosal solution, so that the volume of this solution was maintained fairly constant. The decrease in osmotic gradient due to dilution of the serosal solution was not appreciable during the time of an experiment.

When the time course of the response of the bladder to various concentrations of oxytocin was determined, it was found that from 10 to 20 min. after the addition of oxytocin to the serosal solution the water permeability (mg. water lost per min.) reached a maximum; thereafter the permeability declined, reaching about 50% of the maximum at 100 min. (Figure 1). What is the cause of this decline?

Slowly reversible or irreversible changes in the bladders may be ruled out. Half-bladders were exposed to 2×10^{-8} M oxytocin for several weighing intervals, and their response (mg. water lost during the first hour after addition of hormone) was determined. When the half-bladders were then placed in fresh Ringer's solution their permeability fell quickly to resting values. When these half-bladders were then exposed a second time to the same concentration of oxytocin in fresh Ringer's solution, they gave the same response the second time as the first ($P > 0.2$) (Figure 2A). Decline due to depletion of exogenous substrates may similarly be ruled out, for omission of glucose and pyruvate from the serosal solution during the second test period did not alter the response to the same concentration of oxytocin.

Also, the pH of the serosal solution did not alter during the time of an experiment.

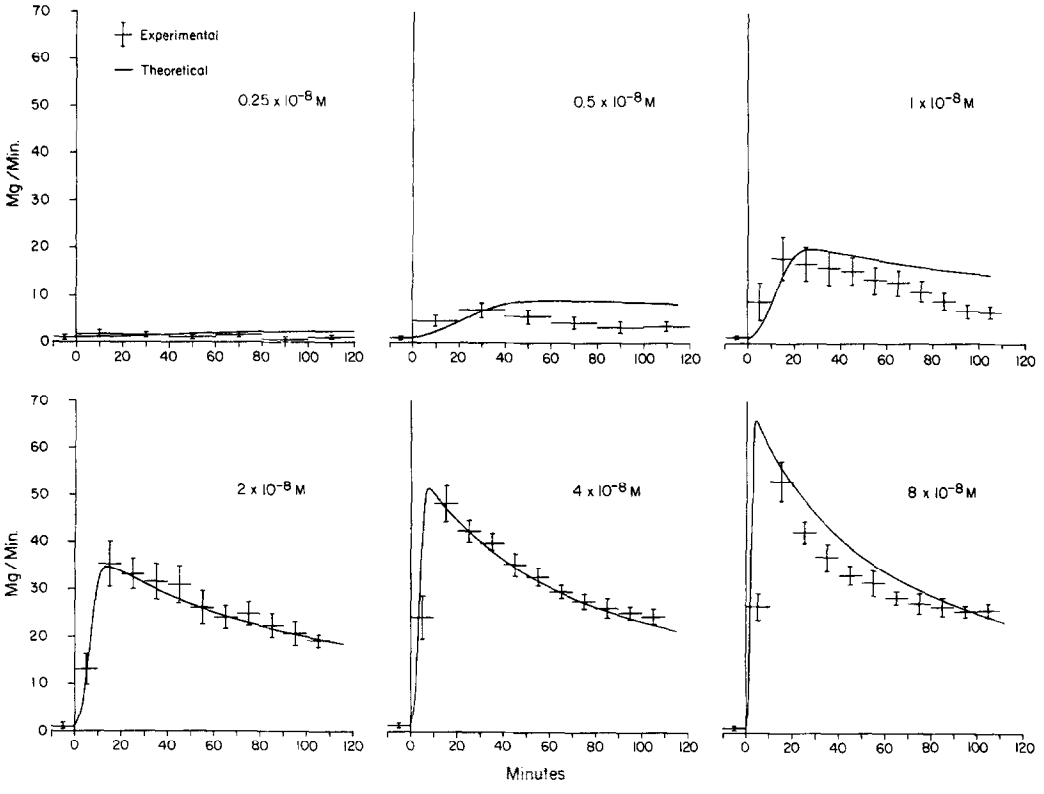

Figure 1. The time course of the effect of various concentrations of oxytocin on the water permeability of the toad bladder.

The experimental values are the mean of 10 determinations. The height of the vertical bars is twice the standard deviation of the mean. The width of the horizontal bars is the length of the weighing interval. The curves were calculated from a model of hormone action (Karlin, 1962).

On the other hand, when half-bladders were exposed to 2 x 10^{-8} M oxytocin, then placed in fresh Ringer's solution to recover resting permeability, and finally transferred for a second test period back into the first Ringer's solution containing the original oxytocin, the second response was considerably less than the first ($P < 0.001$) (Figure 2B). By comparing the responses with a standard dose-response curve, the ratio of the hormone activities present in the serosal solution at the beginnings of the two test

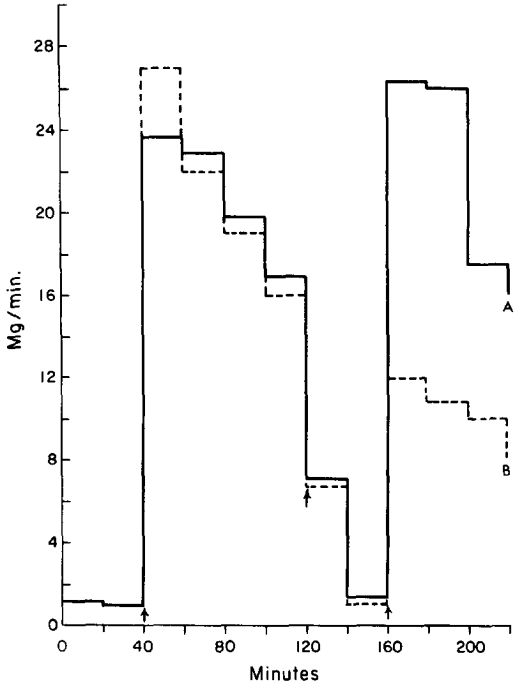

Figure 2. The mean response of six half-bladders.

A. After an initial resting period (0-40 min.), the half-bladders were transferred to Ringer's solution containing 2 x 10^{-8} M oxytocin for the first test period (40 - 120 min.). At 120 min. the half-bladders were transferred to fresh Ringer's solution for a recovery period (120-160 min.). At 160 min. the half-bladders were transferred to fresh Ringer's solution containing 2 x 10^{-8} M oxytocin for a second test period (160-220 min.).

B. The procedure was the same as in A. except that at 160 min. the half-bladders were transferred for a second test period (160-220 min.) into the same Ringer's solution that they were in during the first test period (40-120 min.).

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periods could be estimated. The activity during the second test period was 45% of that during the first. When, however, just before the second test period, the amount of oxytocin estimated to have been lost by inactivation during the first test period was added to the original Ringer's solution, the second response was still significantly less than the first ($P < 0.01$). In this case, the activity during the second test period in the serosal solution

containing the original dose plus the additional dose was only 60% of the activity during the first test period. It was found that only by adding an additional dose of oxytocin equal to the first did the response during the second test period equal that during the first ($P > 0.4$). Even in this case, by extending the first test period to 100 min., and by decreasing the initial concentration of oxytocin to 10^{-8} M, the second response became less than the first ($P < 0.05$). Thus it became clear that hormone inactivation alone could not account for the decline in response, that in fact it appeared that a substance was accumulating in the Ringer's solution bathing the serosal side of the bladders which prevented a given concentration of oxytocin from exerting its expected effect.

It was thus possible to prepare an inhibitory Ringer's solution by suspending half-bladders in the usual way in Ringer's solution for 60 min. The response to 2×10^{-8} M oxytocin which was then added to the inhibitory Ringer's solution was considerably less ($P < 0.01$) than the response to the same concentration of oxytocin in fresh Ringer's solution. The loss of hormone activity in the inhibitory Ringer's solution was 50%. When oxytocin was added to the inhibitory Ringer's solution, together with the half-bladders, at the beginning of the test period, the loss in activity was not significantly different ($P > 0.3$) than when the same concentration of oxytocin was pre-incubated with the inhibitory Ringer's solution for 100 min. prior to the beginning of the test period. Hence the inhibition does not appear to be due to the accumulation of an inactivating enzyme in the serosal solution.

Inhibitor Ringer's solution was prepared by adding 8 half-bladders, freshly excised and washed, to 100 ml. Ringer's solution with slow stirring for 2 hours. This solution was dialyzed overnight against fresh Ringer's solution at 4° C. The dialyzed solution was finally diluted to 150 ml. with

fresh Ringer's solution. The response of half-bladders to 2×10^{-8} M oxytocin in this solution was considerably less than the response to the same concentration of oxytocin in fresh Ringer's solution ($P < 0.001$), the inhibition being 43%. Hence the inhibitory factor was not readily dialyzed.

Work is in progress to characterize this inhibitor.

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