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# Failure of Oxytocin and Vasopressin to Combine with an Anti-insulin Antibody

by

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The similarity of the 6-11 ring of insulin with oxytocin and vasopressin (6, 7, 8) provides *a priori* a reason for suggesting that this part of the molecule may be intimately concerned with its biological activity and therefore, perhaps, its antigenicity.

The chemical studies, reported by CECIL and MCPHEE (1) appear to leave this possibility open. Any study on oxytocin and vasopressin in relation to an insulin-like action would be of great interest.

Although the relationship between molecular structure and biological action of insulin, on the one hand, and its antigenic effect in combining with the specific antibody on the other, has not yet shown, the present paper deals with an attempt to consider whether synthetic oxytocin and vasopressin exert an insulin-like action in combining with an anti-insulin serum.

## Material and Methods

The possible effect of oxytocin and vasopressin in combining with the insulin antibody, was studied by using HALES and RANDLE'S (2) immunological method; this method is very convenient for studies of this sort because of its great sensitivity and relative simplicity.

The bovine insulin used for the secondary standard in the immunological test was obtained from Eli Lilly & Co., Indianapolis, USA. The anti-insulin guinea pig serum for crystallized ox insulin was provided by the Radiochemical Centre and Wellcome Brand. The anti-(guinea pig-γ-globulin) serum of rabbits came from Wellcome Brand and the $^{125}$I-labeled insulin used for the immunological assay was supplied by the Radiochemical Centre, Amersham, England. Synthetic oxytocin and vasopressin were obtained from the Sigma Chemical Co.

## Results

Unlabeled insulin acts as a competitive inhibitor in the formation of the antigenic-antibody complex between labeled insulin and anti-insulin serum. Samples containing insulin, oxytocin and vasopressin are mixed with a fixed amount of $^{125}$I-labeled

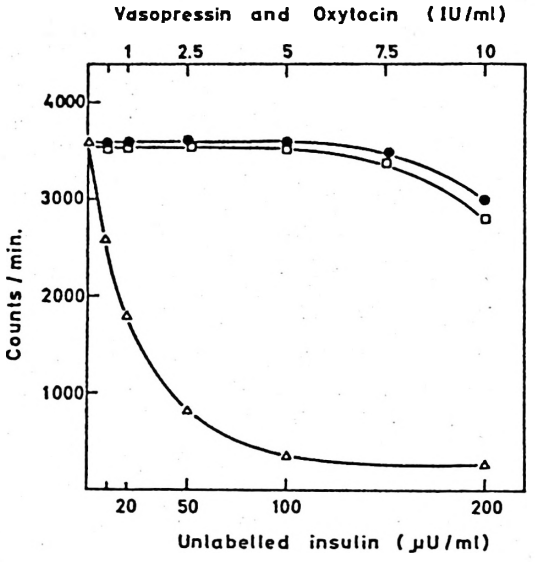


FIG. 1. Effect of additions of bovine insulin and synthetic oxytocin on the recovery of radioactivity in insulin-antibody precipitate.
△ insulin; □ oxytocin and ● vasopressin.
Dilution of anti-insulin serum 1:8.000.
Dilution of precipitable anti-serum 1:16. Concentration of labeled insulin 250 μμg/0,1 ml.

insulin and a limited amount of anti-insulin serum prepared in guinea pigs is added to the mixture. The hormone that has become bound to antibody is then quantitatively precipitated by the addition of a second anti-serum (made in rabbits against guinea pig serum). Test samples of insulin, oxytocin and vasopressin were assayed in triplicate samples by method A or C.

The effect of increasing concentration of bovine insulin and oxytocin on the recovery of $^{125}$I-labeled ox insulin bound by guinea pig anti-ox insulin, is shown in figure 1.

As expected, the greater the concentration of insulin in the sample, the lower the radioactivity of the precipitate, which reveals the ability of the unlabeled insulin to inhibit competitively the binding of $^{125}$I-labeled insulin to insulin antibodies. This effect clearly shows the well-known antibody action which occurs when the antigenic potential of insulin is neutralized.

On the other hand, under the same experimental conditions oxytocin produces no change in the radioactivity recovered in the precipitate. It was necessary to increase the concentration of the synthetic hormone to 10 units to get almost the same reading as that obtained with 10 microunits of insulin. Increasing the concentrations of oxytocin to more than 10 units/ml does not change the results.

Experiments were made simultaneously with synthetic vasopressin. The results were very similar to those obtained with oxytocin (Fig. 1).

## Discussion

Comparing the effects of oxytocin and insulin on the oxidation of glucose-1-C$^{14}$, MIRSKY and PERISUTTI (5) reported that insulin is approximately 300 times more potent than oxytocin in stimulating the oxidation of glucose and lipogenesis by adipose tissue. Accordingly, assuming the potency of insulin to be approximately 25 units per mg, and of oxytocin about 500 units per mg, the potency of 20 μg of oxytocin would be equivalent to less than 0.4 μmg of insulin, which means that insulin would combine with an anti-insulin anti-body about 50.000 times more potent than oxytocin.

The experiment by WILSON, DIXON and WARDLAW (9) supplies the most convincing evidence to date, that antigenicity of insulin is controlled by the A chain. Since the only known characteristic shared in common by insulin, oxytocin and vasopressin is the presence of a disulfide bridge, it became pertinent to find out whether the disulfide bridge *per se* might play a role in the antigen-antibody reaction of insulin. To as shown here oxytocin and vasopressin do not exert an insulin-like

action as regards combination with an anti-insulin antibody, this suggests the possibility that these particular portions of the molecule (disulfide bridge) *per se* might not play a role in the antigen-antibody reaction. On the other hand since the biological activity is the same in insulin from different species and in other compounds with structural differences showing insulin-like activity, one may speculate that the biologically active part is likely to be found in those portions of these molecules that are common to all of them. Similarly, it is reasonable to suggest that the antigenic potential of insulin is determined by a structural feature which is not only the disulfide bridge (3, 4).

## Summary

Synthetic oxytocin and vasopressin do not exert an insulinlike action in combining with an ox anti-insulin serum. Since the only characteristic of both insulin and these peptides is the presence of a disulfide bridge, it became pertinent to show that these particular portions of the molecule *per se*, do not play a role in the antigen-antibody reaction.

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