Potentiation of the Crop-Sac Stimulating Action of Prolactin by ATP[1] (34670)

Y. N. SINHA[2] AND G. H. SCHMIDT
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Department of Animal Science, Cornell University, Ithaca, New York 14850

Prolactin is a metabolic hormone that produces a variety of responses (1). One of its earliest known actions is its ability to cause proliferation of the crop-sac mucosa of pigeons. This property forms the basis for a commonly used assay method for prolactin. We have observed that addition of ATP enhances the crop-sac response of minimal dose levels of prolactin. This report presents data on the effect of ATP and other compounds on the crop-sac response of prolactin.

Materials and Methods. One percent levels of ATP (Na salt) or other compounds were added to several dose levels (0.001, 0.01, 0.1, 0.5, 1, 2, 5, and 10 μg) of prolactin (NIH-P-S_8)[3]. In a second experiment, varying amounts of ATP ranging from 0.4 to 40 mg were added to 1.0 μg of prolactin. Prolactin solutions were prepared fresh in 0.85% NaCl such that 0.1 ml contained one fourth of the total dose to be injected. ATP was added to the prolactin solution on the first day of injection and the solutions were stored at 5–10° for the duration of the injections. Comparisons were made using paired assay procedure (2). The test and control solutions were injected intradermally over the right and the left crop-sacs, respectively, of adult pigeons (Homer breed). This procedure of injecting the test and the control solutions in the same bird provided a direct comparison that eliminates the variation among birds. At least seven pigeons were used in each trial and a 0.1-ml volume of the solution was injected daily for 4 days. On the fifth day crop-sacs were removed and the proliferation was rated visually in terms of Reece-Turner (R-T) units. The data were analyzed by the paired t test.

Results. Prolactin at the total dose levels of 0.001 and 0.01 μg, with or without 1% ATP, did not produce any visible proliferation of the crop-sac (Table I). At the 0.1-μg level, only one crop-sac receiving prolactin and two receiving prolactin plus ATP showed proliferation. At the 0.5-μg level, 6 of 8 crop-sacs receiving prolactin and 7 of 8 receiving prolactin plus ATP responded. The addition of 1% ATP resulted in a 81% increase ($p < 0.05$) in the response in comparison to prolactin alone. At the 1.0-μg level, ATP consistently enhanced prolactin response in the six separate trials, the percentage increase averaging 98, 77, 71, 44, 40, and 16. The pooled data showed a 57% ($p < 0.01$) increase over the controls. At 2.0-, 5.0-, and 10.0-μg levels, no significant differences ($p > 0.05$) were observed.

The addition of 1% ATP to the prolactin solution reduced the pH to 2.95. To determine if alteration in pH enhanced the action of prolactin, the pH of the control prolactin solution was adjusted to 2.95. The 1% ATP again increased the response of prolactin by 66% ($p < 0.01$), ruling out the effect of pH (Table I).

ADP (trilithium salt), bovine serum albumin, or gamma globulin in the concentration of 1% did not increase the response of 0.1 μg of prolactin, whereas 3′, 5′-cyclic AMP appeared to suppress the response slightly ($p < 0.05$). Dibutyryl cyclic AMP had no significant ($p > 0.05$) effect, but GTP, like ATP, significantly ($p < 0.05$) enhanced prolactin response. ATP, ADP, 3′, 5′-cyclic AMP, dibutyryl cyclic AMP, or

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[2] Present address: Division of Endocrinology, Scripps Clinic and Research Foundation, La Jolla, California 92037.

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TABLE I. Crop-Sac Response to Prolactin After the Addition of ATP or Other Substances.

Treatment[a]	No. of pigeons	Crop-sac response (R-T units)	Difference (%)
0.1 μg P	8	0.09 ± 0.00	
+ 1% ATP	8	0.06 ± 0.04	−33
0.5 μg P	8	0.31 ± 0.11	
+ 1% ATP	8	0.56 ± 0.17[c]	+81
1.0 μg P	47	0.65 ± 0.06	
+ 1% ATP	47	1.02 ± 0.08[d]	+57
2.0 μg P	7	1.92 ± 0.24	
+ 1% ATP	7	1.75 ± 0.12	−9
5.0 μg P	8	2.59 ± 0.20	
+ 1% ATP	8	2.84 ± 0.21	+10
10.0 μg P	8	4.09 ± 0.29	
+ 1% ATP	8	3.84 ± 0.23	−6
1.0 μg P (pH 2.95)[b]	7	0.82 ± 0.22	
+ 1% ATP	7	1.36 ± 0.29[d]	+66
1.0 μg P	8	0.25 ± 0.14	
+ 1% GTP	8	0.63 ± 0.21[c]	+150
1.0 μg P	8	1.87 ± 0.24	
+ 1% ADP	8	1.62 ± 0.25	−13
1.0 μg P	8	1.56 ± 0.23[c]	
+ 1% 3′, 5′-cyclic AMP	8	1.09 ± 0.20	−30
1.0 μg P	9	0.50 ± 0.18	
+ 1% dibutyryl cyclic AMP	9	0.64 ± 0.17	+28
1.0 μg P	8	0.47 ± 0.11	
+ 1% bovine serum albumin	8	0.47 ± 0.18	0
1.0 μg P	8	0.47 ± 0.11	
+ 1% gamma globulin	8	0.41 ± 0.08	−13

[a] The dose of prolactin shown is the total dose injected over a period of 4 days. The addition of 1% ATP or other substances resulted in the injection of 4 mg of the substance.

[b] pH adjusted to 2.95.

[c] $p < 0.05$.

[d] $p < 0.01$.

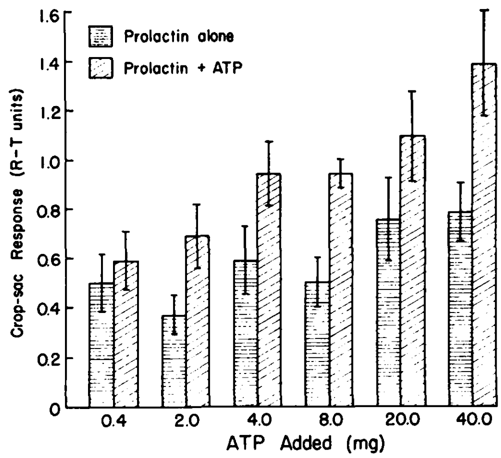

FIG. 1. Effect of varying levels of ATP on the crop-sac response to 1.0 μg of prolactin. The bars represent mean response of 7 to 8 pigeons ± the standard error of the mean.

GTP when injected alone in 1% concentrations did not cause any visible proliferation of the crop-sac (Table I).

Figure 1 shows the effect of adding varying amounts of ATP to a constant dose of prolactin (1.0 μg). The addition of 0.1% ATP (*i.e.*, an injection of 0.4 mg of ATP) did not produce any significant increase. But 0.5, 1, 2, 5, and 10% ATP caused further increases in the proliferation of the crop-sac. A slight concomitant increase in the control side of the 5 and 10% ATP groups, however, prevented a linear response. Each pigeon received 20 or 40 mg of ATP, respectively, in these two groups and it is possible that this large amount of ATP may have affected the contralateral side by way of systemic circulation.

Discussion. The potentiation of a hormone action by ATP has been shown previously. ATP has been found to enhance steroidogenesis *in vitro* from calf adrenals either with or without ACTH stimulation (4). In the present experiments, ATP increased the response of prolactin but did not show any visible response when given alone. Because only ATP and GTP exhibited this property, it appears that the phenomenon may be related to the energy supply mechanisms. ATP is intimately involved in many cellular synthetic and energy requiring processes. Since prolactin stimulates protein synthesis in the crop-sac (3), it is conceivable that ATP may aid prolactin by providing energy for the many chemical reactions, such as activation of amino acids and their increased transport into the cells. The fact that ATP potentiated the action of only low doses (0.5 and 1.0 μg) of prolactin suggests that high level of prolactin is able to mobilize enough endogenous ATP so that any additional ATP does not contribute further.

Recently 3′,5′-cyclic AMP has been found to be a mediator in many hormone actions (5). The possibility that the crop-sac may convert the additional ATP into cyclic AMP in the presence of prolactin and thus produce greater response could not be substantiated in the limited trials of this experiment. Neither 3′,5′-cyclic AMP nor dibutyryl cyclic AMP showed any significant advantage when added to prolactin nor did they produce any response by themselves. In fact, 3′,5′-cyclic AMP suppressed the prolactin response by 30%, probably due to minimizing the absorption of prolactin. However, their role in prolactin action at subcellular level can not be ruled out on the basis of these data, which represent only gross measurements.

Summary. The influence of various chemicals on the proliferation of pigeon crop-sac in response to prolactin was studied. A 1% concentration of ATP or GTP significantly enhanced the crop-sac response of low levels of prolactin. ADP, 3′,5′-cyclic AMP, dibutyryl cyclic AMP, bovine serum albumin and gamma globulin had no beneficial effect. ATP added in increasing concentrations to a constant dose of prolactin (1.0 μg) caused further increases in the crop-sac response.

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