The Early Effect of Gonadotropins on ^{32}P Uptake by the Immature Chicken Testes[1]

FRANK J. ZELLER
Department of Zoology
Indiana University, Bloomington 47401

Abstract

Cockerels were injected with ^{32}P and gonadotropins and then killed at time intervals of 20, 40, 60, 80, and 100 minutes. The radioactivity of testes from treated birds was compared with that of birds receiving ^{32}P alone. Dosage levels of 2.5 μg luteinizing hormone (LH) resulted in a significant ^{32}P increase over controls within 20 minutes while 100 μg of follicle stimulating hormone (FSH) was needed to produce the same effect at this time interval. Both FSH and LH treatment resulted in the same general response, an increased ^{32}P uptake reaching a peak around 40-60 minutes which then began to decrease. Pregnant mare serum gonadotropin (PMS) and human chorionic gonadotropin (HCG) produced a more gradual rise in ^{32}P uptake which when reaching its peak was maintained for several hours. HCG was the least potent of the gonadotropins as far as ^{32}P uptake was concerned in that a significant increase in the isotope uptake was not noted until 8 hours after HCG injection. Chicken pituitary gonadotropin extracts also significantly stimulated ^{32}P uptake within 20-40 minutes.

Introduction

The stimulation of testis ^{32}P uptake has been used as an assay method for total anterior pituitary gonadotropins for several years (1, 2). Several questions arise in considering this ^{32}P stimulation, the major one perhaps being what biochemical system(s) in the testis is being stimulated by the gonadotropin to result in increased ^{32}P uptake. This assay method is sensitive to either follicle stimulating hormone (FSH) or luteinizing hormone (LH) alone, therefore, another question is whether these two gonadotropins act in similar fashion in stimulating ^{32}P uptake. As a preliminary step in answering these questions, studies have been performed to note the initial time of the stimulatory response. FSH, LH, pregnant mare's serum gonadotropin (PMS), human chorionic gonadotropin (HCG), as well as chicken anterior pituitary gland gonadotropin extracts were tested. The purpose of this paper is to report the initial time of effect of a variety of gonadotropins on ^{32}P uptake by the testes of the immature chicken.

Materials and Methods

Newly-hatched single comb White Leghorn cockerels were obtained from the Farm Bureau Co-op, Indianapolis, Indiana, and used exclusively in these experiments. The birds used were between 5-9 days of age. The procedure was as follows. Birds were injected subcutaneously at time "0" with 4.0 or 5.0 μc ^{32}P and the gonadotropin and then killed at various time intervals, usually 20, 40, 60, 80, and

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100 minutes. The effects of PMS and HCG treatments were observed over a 24-hour period. Control animals (^{32}P but no gonadotropins) were also killed at each time interval. The animals were killed by cervical dislocation and the testes were quickly removed, weighed, and placed on planchets. There was one pair of testes per planchet. The testes were air dried overnight and then counted on a Nuclear Chicago gas flow counter. The results were expressed as counts per minute per milligram of wet-weight of testes (cpm/mg). The ^{32}P was purchased from New England Nuclear as carrier free H_3PO_4 in 0.02 N HCl. Ovine FSH and LH was generously provided by the Endocrine Study Section of NIH. Dr. J. B. Jewell of the Ayerst Laboratories graciously supplied the PMS and HCG. Water extracts of anterior pituitary material from older chickens were also used as a source of gonadotropin.

Results

Luteinizing Hormone

Table 1 presents the data on ^{32}P uptake from a series of LH dosages as a function of time. Dosages of 2.5, 5.0, 10.0, and 20.0 μg LH per bird all caused significant increases in ^{32}P uptake over the controls at 20 minutes, and in one group of 20 μg LH treated birds a significant increase was noted as early as 15 minutes.

TABLE 1. *The effect of various dosages of luteinizing hormone (LH) on ^{32}P uptake by the testes as a function of time. The LH and ^{32}P (4.0 or 5.0 μc) were both given at time "O" and there were 10 or 11 birds in each series.*

Control cpm/mg ± SE	2.5 μg LH cpm/mg ± SE	Time minutes	Control cpm/mg ± SE	5.0 μg LH cpm/mg ± SE
39 ± 2	45 ± 2*	20	80 ± 3	94 ± 5***
32 ± 1	51 ± 3***	40	63 ± 2	97 ± 5***
35 ± 2	52 ± 3***	60	57 ± 3	102 ± 7***
36 ± 3	53 ± 4***	80	58 ± 3	105 ± 4***
42 ± 3	57 ± 3**	100	62 ± 4	102 ± 3**
Control	10.0 μg LH	Time	Control	20.0 μg LH
61 ± 3	80 ± 3***	20	57 ± 4	78 ± 3***
50 ± 2	89 ± 4***	40	48 ± 2	96 ± 5***
51 ± 2	93 ± 5***	60	52 ± 3	108 ± 8***
55 ± 6	88 ± 4***	80	49 ± 3	104 ± 5***
55 ± 3	92 ± 4***	100	55 ± 3	97 ± 4***
Control	20.0 μg LH	Time		
70 ± 1	90 ± 4***	15		
55 ± 2	91 ± 3***	30		
45 ± 1	91 ± 3***	50		
45 ± 4	92 ± 4***	70		
44 ± 2	86 ± 3***	90		

Control vs LH significance levels: * = 5% ; ** = 1%, *** = 0.1%.

Follicle Stimulating Hormone

The results of 3 dosage levels of FSH are presented in Table 2. The 40 μg and 50 μg levels produced significant increases in ^{32}P

uptake at 40 minutes, while the higher level, 100 μg, brought about a significant increase at 20 minutes. These results again demonstrate that on a microgram basis this assay method is more sensitive to LH than it is to FSH. A possible event of interest was the decrease in FSH stimulating activity with the two lower doses at 80 minutes, followed by a second rise in activity.

TABLE 2. *The effect of various dosages of follicle stimulating hormone (FSH) on ^{32}P uptake by the testes as a function of time. The FSH and ^{32}P (4.0 or 5.0 μc) were both given at time "O". Each series had 10 or 11 birds.*

Controls cpm/mg ± SE	40.0 μg FSH cpm/mg ± SE	Time minutes	Controls cpm/mg ± SE	50.0 μg FSH cpm/mg ± SE
35 ± 2	37 ± 2	20	37 ± 2	40 ± 2
30 ± 2	45 ± 3**	40	31 ± 1	43 ± 3**
31 ± 2	44 ± 5*	60	32 ± 3	56 ± 6**
38 ± 8	46 ± 3	80	36 ± 2	53 ± 5*
30 ± 3	55 ± 4***	100	33 ± 2	61 ± 4***
Controls	100.0 μg FSH	Time		
45 ± 2	60 ± 4**	20		
46 ± 2	74 ± 6***	40		
39 ± 2	79 ± 4***	60		
45 ± 3	82 ± 4***	80		
46 ± 2	78 ± 6***	100		

Controls vs FSH significance levels: * = 5% ; ** = 1% ; *** = 0.1%.

Pregnant Mare Serum Gonadotropin

The administration of 25 units of PMS did not bring about a significant uptake in ^{32}P until 100 minutes after injections as shown in Table 3. Although not shown in the Table, the uptake remained fairly constant for 4 hours with a second peak in uptake seen at 6 and 12 hours and a still higher level at 24 hours. A dose of 30 units of PMS, however, brought about a significant increase in ^{32}P uptake at 40 minutes. Again as with the 25 unit treatment, once the peak was established it was maintained for a considerable period of time. This pattern of response was in contrast with the early peak and subsequent drop characterized by the FSH and LH treatments.

Human Chorionic Gonadotropin

One dosage level (50 units) was used and its stimulatory activity was followed at intervals over a 24-hour period. This amount of HCG did not bring about an increase in ^{32}P uptake until 8 hours after injection and then the significance was only at the 5% level (Table 3). This minimal stimulatory level was then maintained for the remainder of the 24-hour period.

Anterior Pituitary Gland Extract

Table 4 presents the results of injecting water extracts of older male chicken pituitary glands into the week-old cockerels. Two dosages were used, 0.5 mg from 75-day old donors, and 1.0 mg from 55-day

old chickens. The higher level, 1.0 mg equivalent fresh pituitary per bird, caused a significant increase in ^{32}P uptake within 20 minutes while the 0.5 mg dose was not effective until 40 minutes. Interestingly, the pituitary gland treatment resulted in a "dip" in effect around 80 minutes, a situation similar to that seen with the lower dosages of FSH.

TABLE 3. *The effect of pregnant mare serum gonadotropin (PMS) or human chorionic gonadotropin (HCG) on testes ^{32}P uptake as a function of time. Gonadotropin and 5.0 μc ^{32}P were injected at the same time. Each series had 10 or 11 birds.*

Control cpm/mg ± SE	25 IU PMS cpm/mg ± SE	Time minutes	Control cpm/mg ± SE	30 IU PMS cpm/mg ± SE
44 ± 2	50 ± 3	20	45 ± 2	48 ± 3
48 ± 3	56 ± 4	40	46 ± 2	60 ± 3**
37 ± 3	44 ± 4	60	39 ± 2	57 ± 4***
40 ± 2	46 ± 2	80	48 ± 4	71 ± 3***
34 ± 2	45 ± 2**	100	46 ± 2	68 ± 4***
Control	50 units HCG	Time		
29 ± 2	28 ± 2	20		
26 ± 2	26 ± 1	40		
37 ± 3	37 ± 3	60		
40 ± 2	45 ± 4	80		
34 ± 2	40 ± 2	100		
37 ± 2	43 ± 2	4 h		
41 ± 3	52 ± 2*	8 h		
36 ± 3	47 ± 3*	12 h		
37 ± 3	47 ± 3*	16 h		
36 ± 2	46 ± 3*	24 h		

Control vs experimental significance levels: * = 5% ; ** = 1% ; *** = 0.1%.

TABLE 4. *The effect of chicken anterior pituitary gland extract on ^{32}P uptake by the testes as a function of time. The pituitary extract and 4.0 μc ^{32}P were both given at time "O" and there were 10 or 11 birds in each series. Pituitary dosage is mg equivalent wet weight.*

Time, minutes	Controls cpm/mg ± SE	Pituitary extract cpm/mg ± SE	% increase
0.5 mg anterior pituitary from 75 day old male chickens			
20	36 ± 2	36 ± 2	0
40	35 ± 2	46 ± 2***	31
60	45 ± 2	54 ± 5*	29
80	35 ± 2	35 ± 1	0
100	33 ± 2	37 ± 3	12
1.0 mg anterior pituitary from 55 day old male chickens			
20	23 ± 1	29 ± 1***	26
40	23 ± 2	38 ± 1***	65
60	26 ± 2	42 ± 3***	62
80	29 ± 3	38 ± 1**	31
100	21 ± 2	43 ± 4***	109

Significance levels controls vs treated: * = 5% ; ** = 1% ; *** = 0.1%.

Discussion

The results presented here indicate that mammalian FSH and LH can exert an effect on the chicken testis within 20 minutes as measured by ^{32}P uptake. The response resulting from either FSH or LH was similar in reaching an early peak and then tapering off. PMS and HCG exhibited a different response from that of the two anterior pituitary gonadotropins in that a more gradual increase was followed by the maintenance of this peak. Despite the similarity in LH and HCG action in mammals and also their immunological similarity, HCG was much less stimulatory than was LH at the levels used in these experiments. Studies on the distribution of ^{32}P are now in progress to determine whether there is a difference produced by the various gonadotropins in the ^{32}P metabolism of the testes.

Literature Cited

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