FEEDBACK REGULATION BY OESTRADIOL OF OVARIAN FUNCTION IN JAPANESE QUAIL

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Summary. Feedback regulation of gonadotrophin release from the adenohypophysis by 17β -oestradiol was examined in breeding female Japanese quail. Implants of the steroid in the preoptic–supraoptic (nuclear) regions, in ventral regions of the infundibular nuclear complex or in the adenohypophysis resulted in a ‘persistent oestrous’-like syndrome. Ovulation was prevented, but the weight and follicular size of the ovary and the weight of the oviduct did not differ from control (cholesterol implants or oestradiol implants outside of the hypothalamus) values. Since implants of cholesterol in identical regions of the hypothalamus had no effect on ovarian function, and since ovulation recurred in 10 or 16 days, depending on the site of implantation, the effects observed are attributed directly to the hormone contained in the implant and not to a physical obstruction in the hypothalamus. It is suggested that oestradiol is involved in the regulation of release of LH from the adenohypophysis.

INTRODUCTION

Considerable attention has been given to steroid regulation of ovarian function (particularly ovulation) in mammals (for review see Flerkó, 1966, 1967; Harris & Campbell, 1966; Davidson, 1969). Two varieties of feedback phenomena have been described, one stimulating the release of gonadotrophins from the adenohypophysis and the second inhibiting the release of these hormones (see Barraclough & Haller, 1970; Davidson, Weick, Smith & Dominguez, 1970; Weick & Davidson, 1970; Arimura & Schally, 1971).

In males of several avian species, intrahypothalamic implants of testosterone prevent photoperiodically induced testicular growth (Gogan & Kordon, 1964; Kordon & Gogan, 1964, 1970; Gogan, 1967, 1968; Wilson, 1970) and result in regression of fully mature testes in breeding birds (Stetson, 1971, 1972). It appears that testosterone feedback is inhibitory in nature; pituitary gonadotrophic potency is reduced and, depending on the location of the implant, the testes appear histologically to be deficient either in LH (or LH and FSH) or in FSH (Gogan, 1968; Stetson, 1971, 1972).

Relatively few investigations of the oestrogenic control of ovarian function in avian species have been reported (see Parkes & Emmens, 1944; Lorenz, 1954;

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Kobayashi, 1954; Fraps, 1954, 1955; Heald, Rookledge, Furnival & Watts, 1968; Stetson, 1971 and unpublished). Because of the paucity of data available, it was decided to test the presence of a feedback of 17β -oestradiol on the hypothalamo-hypophysio-ovarian axis of breeding Japanese quail.

MATERIALS AND METHODS

Laying female Japanese quail were housed with a male in a small wire compartment ($15.5 \times 20 \times 24$ cm), ten of which constituted one tier in a bank of breeding cages. Experimental treatment of both members of a breeding pair was identical, except that males received testosterone or cholesterol while females received 17β -oestradiol or cholesterol; analysis of the results in males has been dealt with previously (Stetson, 1972). Experimental birds were continually within sight and sound of normal breeding pairs. Environmental conditions were kept constant at 16 hr light per day, and 22 to 28° C. Food and water were continually available.

Stereotaxy (Stetson, 1968) under ether anaesthesia (Stetson, 1969a) was used to implant either fine glass capillaries (0.05 to 0.1 mm i.d.) or 22-gauge stainless steel tubing (0.4 mm i.d.) in the brains of experimental animals. The capillaries were filled with cholesterol or 17β -oestradiol, as described by Gogan (1968). After implantation, the capillaries were cut off at the surface of the brain in which they remained firmly implanted (Pl. 1, Figs 1, 5 and 6) and were removed only after the brains had been fixed in Bouin's fluid. In other experiments, a pellet of oestradiol:charcoal (1:1 by weight) or cholesterol:charcoal (1:1) was implanted through a stainless steel tube. A wire plunger was withdrawn 0.5 mm, and the steroid:charcoal mixture was tapped into the tube. After cleaning the tube externally with ethanol, it was inserted into the brain to a depth of approximately 0.5 mm dorsal to the target. The plunger was then depressed, forcing the pellet into the brain. The tube was then withdrawn. The cylindrical pellets, approximately 0.4×0.5 mm, weighed on the average ($\pm$ S.E.) 36.5 ± 1.6 μ g (cholesterol:charcoal) and 29.6 ± 1.7 μ g (oestradiol:charcoal).

EXPLANATION OF PLATE 1

Frontal sections through the hypothalamus of Japanese quail from Groups 1 to 5 showing location of the implant.

FIG. 1: Group 1. Arrow indicates location of the tip of a glass capillary that contained cholesterol. The growth of glial elements into the tube (arrow) indicates that the brain was in continual contact with the steroid within the tube. Paraldehyde thionin, $\times 34$.

FIGS 2 and 3. Group 2. Implants of oestradiol:charcoal in the ventral infundibular nuclear region of a bird from the short-term experiment (Fig. 2) and one of the long-term experiments (Fig. 3). The bird in Fig. 2 did not ovulate during the 14 days of the experiment. Postovulatory delay to the resumption of ovulation in the bird in Fig. 3 was 16 days. Paraldehyde thionin, $\times 34$ (Fig. 2), $\times 28$ (Fig. 3).

FIG. 4. Group 3. Implant of oestradiol:charcoal that had no effect on ovarian function. Paraldehyde thionin, $\times 34$.

FIG. 5. Group 4. Section through the preoptic region of the hypothalamus showing the location (arrow) of a glass capillary containing oestradiol that suppressed ovulation for 6 days. Paraldehyde thionin, $\times 34$.

FIG. 6. Group 5. Section through the preoptic region of the hypothalamus showing location of a glass capillary containing oestradiol that did not affect ovarian function. Contact between steroid and nervous elements is attested by growth (arrow) of tissue into the capillary. Paraldehyde thionin, $\times 34$.

(Facing p. 206)

Three experiments were designed to assess the long-term (33 to 35 days) and short-term (13 to 14 days) effect of oestradiol on the hypothalamo-hypophysio-ovarian axis. At the end of each experiment, the birds were decapitated and the adenohypophysis was immediately removed and desiccated in acetone (see below). The ovary and oviduct were removed and weighed to the nearest milligram on a Mettler analytical balance. The brain, ovary and oviduct were fixed in Bouin's fluid for 4 days. The brain was examined histologically (see Stetson, 1969a, 1971, for details) to verify placement of the implant.

TABLE 1

OVARIAN-OVIDUCAL WEIGHT AND FOLLICULAR DIAMETER IN CONTROL AND EXPERIMENTAL GROUPS OF JAPANESE QUAIL

Group (n)	Location of implant*	Ovary-oviduct weight (g)	Follicle diameter (mm)			
			f_1	f_2	f_3	f_4
1 (19)	Control implants	9.060 $\pm$ 0.597	15.59 $\pm$ 0.66	11.84 $\pm$ 0.69	8.02 $\pm$ 0.62	4.89 $\pm$ 0.38
2 (7)	V-INC, ME, PD	8.345 $\pm$ 0.807	15.33 $\pm$ 0.74	11.10 $\pm$ 0.81	7.34 $\pm$ 0.80	4.41 $\pm$ 0.30
3 (20)	INC	9.340 $\pm$ 0.438	15.68 $\pm$ 0.57	11.52 $\pm$ 0.57	7.57 $\pm$ 0.54	4.65 $\pm$ 0.59
4 (7)	POR, SOR	10.311 $\pm$ 1.124	15.81 $\pm$ 0.78	12.24 $\pm$ 1.40	9.31 $\pm$ 1.40	5.81 $\pm$ 1.10
5 (13)	POR, SOR	9.715 $\pm$ 0.681	16.14 $\pm$ 0.49	12.38 $\pm$ 0.65	8.67 $\pm$ 0.72	5.30 $\pm$ 0.59

Results expressed as Mean $\pm$ S.E.

* INC=infundibular nuclear complex; ME=median eminence; PD=pars distalis; POR=preoptic region of the hypothalamus; SOR=supraoptic (nuclear) region of the hypothalamus; V-INC=ventral region of the infundibular nuclear complex (includes the nucleus tubers).

TABLE 2

PREOPERATIVE AND POSTOPERATIVE OVULATORY FREQUENCIES, POSTOPERATIVE DELAY TO RESUMPTION OF OVIPOSITION, AND PITUITARY GONADOTROPIC POTENCY IN CONTROL AND EXPERIMENTAL GROUPS OF JAPANESE QUAIL

Group	Preoperative frequency (eggs/day)	Postoperative frequency (eggs/day)	Delay (days)	Pituitary GTH*
1	0.8 $\pm$ 0.03	0.7 $\pm$ 0.05	3.4 $\pm$ 0.6	2.7 $\pm$ 0.1
2	0.7 $\pm$ 0.03	0.2 $\pm$ 0.05†	16.6 $\pm$ 3.0†	2.9 $\pm$ 0.05
3	0.7 $\pm$ 0.03	0.6 $\pm$ 0.02	3.8 $\pm$ 0.6	2.6 $\pm$ 0.2
4	0.7 $\pm$ 0.04	0.3 $\pm$ 0.05†	10.4 $\pm$ 1.8†	2.8 $\pm$ 0.1
5	0.7 $\pm$ 0.04	0.7 $\pm$ 0.05	3.2 $\pm$ 0.7	2.6 $\pm$ 0.1

Results expressed as Mean $\pm$ S.E.

* Pituitary gonadotrophic potency in μ g-equivalents of NIH-LH-s13 or s15 per gland.

† Significantly different from controls ($P < 0.001$).

In addition to ovarian and oviducal weight, several other criteria were used to monitor the effects of implants on ovarian function. Oviposition was recorded during, and for several weeks before, each experiment; ovulatory frequencies were expressed as the number of eggs laid per day, e.g. 0.75=three eggs laid in 4 days. Daily records indicated the postoperative delay to the resumption of oviposition. Finally, the four largest follicles from every ovary were measured with a pair of vernier callipers.

After each experiment, the adenohypophysis of each bird was pooled with

glands from other birds in which implants in similar locations had a similar effect on ovarian function. The gonadotrophic potency (in μg -equivalents of NIH-LH-s12 or s15) of the pooled glands was determined by the method of

TEXT-FIG. 1. Diagrammatic representation of the location of the implants on a mid-sagittal plane of the ventral hypothalamus of the Japanese quail. $\square$, Control implants of cholesterol; $\blacksquare$, control implants of oestradiol; $\circ$, effective implants that resulted in a significant postoperative delay to the resumption of ovulation; $\bullet$, one pellet that broke within the third ventricles, leaving fragments in the locations indicated, the main body of the pellet being located just dorsal to the median eminence; Δ , ineffective implants of oestradiol; g, glass capillary, located just lateral to mid-line; l, pellet of oestradiol : charcoal located laterally in the region of the hypothalamus indicated; p, pellet of oestradiol : charcoal located in the mid-line. Unmarked triangles indicate implants in mid-line that are excluded from the 'sensitive zone'. All symbols represent either the approximate centre of a pellet or the tip of a glass capillary. Insert: A, anterior division of the median eminence; AC, anterior commissure; INC, infundibular nuclear complex; nt, nucleus tuberis (included in this report in the ventral INC); OC, optic chiasma; P, posterior division of the median eminence; PN, pars nervosa; POR, preoptic region; SOD, supraoptic (nuclear) region; 3, third ventricle.

Breneman, Zeller & Creek (1962) as modified by Follett & Farner (1966). The assays were of the '2+2' design and were analysed statistically as described by Bliss (1952). All other statistical analyses were determined with Student's *t* test.

RESULTS

The control group, Group 1, contained nineteen birds, ten of which received implants of cholesterol, while nine others received oestradiol implants that were placed incorrectly in the forebrain of the optic chiasma or optic tracts (Pl. 1, Fig. 1; Text-fig. 1). Regardless of the composition of the implant, the postoperative delay to the onset of oviposition, the ovarian and oviducal weights and the follicular dimensions (Tables 1 and 2) were identical to those of control groups in investigations of the effects of hypothalamic lesions (ovarian-oviducal weight = 10.269 ± 0.299 g; delay = 3.2 ± 0.7 days) or transections (8.775 ± 0.718 g; 3.41 ± 1.3 days) on ovarian function (Stetson, 1971).

Seven birds received implants of oestradiol in ventral regions of the infundibular nuclear complex and median eminence. These birds (Group 2, Table 1, Text-fig. 1; Pl. 1, Figs 2 and 3), had an average postoperative delay to oviposition of 15 to 17 days (Table 2). Four of these, from one of the long-term experiments (35 days' duration), had postoperative delays that were greater than the duration of the short-term experiments (13 to 14 days); the lengthiest delay was 33 days. From the short-term experiment, three birds which complete Group 2, failed to ovulate after receiving implants of oestradiol. Five of the seven implants were in postero-ventral regions of the infundibular nuclear complex and/or posterior division of the median eminence, one was dorsal to the anterior division of the median eminence, and one was in the cephalic lobe of the pars distalis (Text-fig. 1, Table 1). All implants were located in, or immediately lateral to, the mid-line. At autopsy, the ovaries of all seven birds, including the three in which resumption of ovulation had not yet occurred, were similar in weight and follicular size to those of the controls of Group 1 (Table 1), and pituitary gonadotrophic potency in the two groups was nearly equivalent (Table 1). Both the postoperative delay to the resumption of oviposition and the postoperative frequency of ovulation in Group 2 were significantly different from control values (Table 2).

Oestradiol implanted into the infundibular nuclear complex of twenty other birds (Group 3) failed to affect the ovarian control system (Tables 1 and 2). The majority of implants were located dorsal to those in Group 2 (see Text-fig. 1), but seven were located in nearly identical locations. Six of these (one implant resided in the caudal lobe of the pars distalis) were glass capillaries of small diameter; the seventh was in pellet form and was located to the right of mid-line in the infundibular nuclear complex, just dorsal to the 'effective area' of Group 2 (Text-fig. 1). Histological examination of the implantation sites revealed that the oestradiol within the glass capillaries was in contact with neural tissue since there had been growth of glial elements into the tubes as the oestradiol was dissolved. Three other implants located in the ventral infundibular nuclear region were displaced laterally. The hypothalamo-hypophysis-ovarian axis in the birds in Group 3 was, in all respects, similar to that of the birds in Group 1 (Tables 1 and 2). Group 3 can, therefore, be taken as an experimental control group for Group 2.

In the seven birds of Group 4, oestradiol implants in the preoptic and supra-optic regions of the hypothalamus (see Text-fig. 1) resulted in a significant delay

to the resumption of oviposition (Table 2). Six of the seven implants were medial (Pl. 1, Fig. 5) in location; the seventh was located dextrorlaterally in the preoptic region and, in addition, had severed the ipsilateral *tractus septomesencephalicus*. The significantly greater postoperative delay to the onset of oviposition in Group 4 was coincident with the statistically smaller postoperative ovulatory frequency which was similar to that of Group 2 (Table 2). In addition, the average pituitary gonadotrophic potency of Group 4 was also similar to that of Group 2 (Table 2). The ovarian and oviducal weight and follicular diameter of the birds in Group 4 did not differ statistically from those in Group 1 (Table 1).

An additional thirteen females (Group 5) also received implants of oestradiol in the preoptic and/or supraoptic regions of the hypothalamus (Table 2). The majority of implants were dorsal and/or lateral to those in Group 4 (Pl. 1, Fig. 6), but four were similarly placed; two of these were glass capillaries containing oestradiol, which, from the growth of glial elements into the capillary (see Text-fig. 1; Pl. 1, Fig. 6), were presumably in continual contact with neural tissue. The two other implants were in pellet form, and were located in the preoptic recess and adjacent tissue and in the preoptic region, just to the right of the mid-line. The hypothalamo-hypophysio-ovarian axis in the birds of Group 5 was normal in every category investigated (Tables 1 and 2); Group 5 can, therefore, be regarded as an experimental control group for Group 4.

DISCUSSION

The foregoing experiments appear to be the first attempt to demonstrate a potential feedback from the ovary to the hypothalamus and/or adenohypophysis in a bird, using intrahypothalamic implants of oestradiol. The results suggest that this system exists (Groups 2 and 4; Tables 1 and 2) and that it involves those regions of the hypothalamus that have been implicated previously in the LH-control system (Stetson, 1969b, 1971) in this species. The effect of the implant in preventing ovulation, while a normal-sized ovary and oviduct were maintained (see Heald *et al.*, 1968), suggests impairment of normal function of the LH-release system. Although it is not possible to state with certainty that ovarian (follicular) and oviducal size in Groups 2 and 4 were maintained at normal preovulatory levels throughout the postoperative delay to the onset of oviposition, two facts suggest that this was so: (a) the birds in Group 2 from the short-term experiment had not ovulated (examination of the ovaries revealed no recent postovulatory follicles, and no signs of ova or yolk were observed in the oviduct and abdominal cavity) at the time of autopsy (13 to 14 days after operation), yet the weights of the ovaries and oviducts and follicular diameters were indistinguishable from those of controls, and (b) bilateral electrolytic lesions in the regions of the hypothalamus in which implants resided in Groups 2 and 4 produced identical ovarian dysfunctions. In several birds, the ovary and oviduct were maintained in a preovulatory state from Day 21 (laparotomy) to Day 60 and, in three others, large ovaries and oviducts persisted from Day 21 to Day 133 when the experiment was terminated without a single egg being laid. The results presented here are similar to those reported in the lizard, *Sceloporus cyanogenys* (Callard & McConnel, 1969). In mammals, however,

regression of the ovaries and uterus occurs in rats with implants of oestradiol benzoate in the basal medial hypothalamus, indicating an alteration of the tonic release of FSH or FSH and LH. Implants in the preoptic–anterior hypothalamic region, the 'LH-cyclicity center,' are without effect (Lisk, 1960, 1965; Davidson, 1969).

Since, in the short-term experiment, the ovary and oviduct of birds with effective implants were in a state resembling that of persistent oestrus in mammals, it is unlikely that the implants affected the release of FSH from the adenohypophysis. The release of LH, however, may have been either enhanced or diminished while the release of FSH was either enhanced or remained the same. The bioassay used in this investigation is not specific for a particular pituitary gonadotrophin (Furr & Cunningham, 1970), but, contrary to the situation in male quail (Stetson, 1972), pituitary gonadotrophic potency was not altered by the implants. This suggests that oestradiol feedback is not inhibitory. The possibility exists (Gay, Midgley & Niswender, 1970) that high or increasing concentrations of circulating oestrogens may trigger the preovulatory release of LH, or LH and FSH, resulting in ovulation of the largest follicle and perhaps a concomitant, though relatively brief, decrease in plasma oestrogen levels. In this instance, a true inhibitory feedback (Stetson, 1972) would exist whereby oestradiol (testosterone in males) would exert some control over its own rate of release (see Schwartz, 1969; Schwartz & Waltz, 1970). This model is consistent with the observations of Nalbandov (1953), Breneman (1955) and Heald *et al.* (1968) who describe high levels of circulating oestrogens preceding ovulation in the domestic fowl. It could also explain the results of Fraps (1954, 1955) in the same species, who found that a single intravenous injection of oestradiol (1.0 or 2.5 mg/bird) prevented the second, but not the first ovulation in a clutch of eggs, whereas injections of smaller amounts of the hormone (0.2 to 0.5 mg/bird) had no effect.

In females as in males (Stetson, 1972), the steroid-sensitive areas in the hypothalamus appear to be situated centrally, or just lateral to the mid-line (Pl. 1, Figs 2, 3 and 5). The adenohypophysis cannot be excluded as a possible site of action of oestradiol. The implant in one bird of Group 2 was located primarily within the cephalic lobe of the adenohypophysis, and resulted in an 18-day delay in oviposition. Since there was some overlap in the location of effective and ineffective implants in both the ventral infundibular and preoptic regions of the hypothalamus, and since all but two of the ineffective implants were glass capillaries of narrow diameter (see Text-fig. 1), the criterion determining efficacy of an implant may be the volume of tissue contacting the steroid.

The effects observed in Groups 2 and 4 (Text-fig. 1, Table 1) probably represent a direct and localized action of the steroid; it is unlikely that interruption of ovulation resulted from peripheral diffusion of the steroid, unless perhaps such diffusion was ventral, toward the adenohypophysis (see Palka, Ramirez & Sawyer, 1966). It is equally unlikely that the effects observed in Groups 2 and 4 resulted from the creation of a physical lesion since (a) unilateral electrolytic lesions approximating to the size of the implants (0.4×0.5 mm) were ineffective when located in similar regions of the hypothalamus

of female quail (Stetson, 1971), (b) the effect was temporary, despite the persistence of the implant (Pl. 1, Figs 1 to 6) and (c) implants of cholesterol, of similar size and location, were without effect.

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