Bovine Pituitary LH and Prolactin Release During Superfusion *in Vitro* (36424)

JOSEF ZOLMAN AND EDWARD M. CONVEY
(Introduced by H. A. Tucker)

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48823

In vitro techniques, used successfully to study hormone synthesis and release by anterior pituitary tissue (1), permit evaluation of discrete variables on hormone release. But the commonly used procedure for pituitary culture (2) is a static system which does not allow evaluation of rate changes in hormone release. In addition, this system does not allow for constant precursor repletion, product removal or control of treatment exposure time. Preliminary evidence (3, 4) based on periodic exchange of medium in a static system indicates that the hormone releasing or release inhibiting action of hypothalamic principles varies with exposure time.

Continuous superfusion used to evaluate adrenal steroid synthesis *in vitro* (5, 6), eliminates most of the restrictions imposed by static *in vitro* cultures; it permits continuous exchange of medium, timed treatment exposure and convenient postincubation effluent collection. Recently, Serra and Midgley (7) described experiments utilizing a continuous superfusion system to evaluate the response of whole rat anterior pituitary glands to crude hypothalamic extracts.

The purpose of this investigation was to develop an *in vitro* superfusion system for bovine anterior pituitary and to evaluate several incubation variables on prolactin and luteinizing hormone release.

Materials and Methods. Incubation procedure. The incubation apparatus is illustrated in Fig. 1. Pituitary explants were retained in glass chambers (A) of approximately 0.5 ml volume, by perforated polyethylene disks (B). A Dekastaltic pump continuously moved incubation medium through Tygon tubing (1.52 mm i.d., Technicon Instruments, Corp., Chauncey, NY) from the reservoir (C), through the efferent tube (D), through the culture chamber (A) and out the efferent tube (E). The effluent was fraction-collected as it left the outlet tubing. Incubation chambers and reservoirs containing incubation medium were submerged in a thermoregulated bath at 37°. Incubation media were kept under continuous gassing (300 ml/min) with humidified 95% O_2:5% CO_2. Rate of flow of incubation media was 0.5 ml/min and 2 ml samples were collected at hourly intervals.

Experimental procedure. Variables studied were: (a) anterior pituitary source (steers or cows), (b) bicarbonate concentration in incubation medium (0.035 or 0.224%), (c) area of anterior pituitary (peripheral or central), (d) number of pieces (1 or 5 pieces of equiv wt), and (e) incubation period or interval. The pituitary was removed from each of four cows and four steers, the posterior pituitary was discarded and the anterior pituitary was cut midsagitally. Each anterior pituitary half was further divided into central and peripheral anterior pituitary regions. Tissue from each region was cut into 8 ribbons of approximately equal size (1 × 1 × 10 mm). To determine whether explant surface area influenced hormone release, four ribbons from each region were incubated without further division (1 × 1 × 10 mm) and four were cut into a total of 20 pieces of approximately equal size (1 × 1 × 2). Two ribbons or 10 pieces were incubated in each incubation

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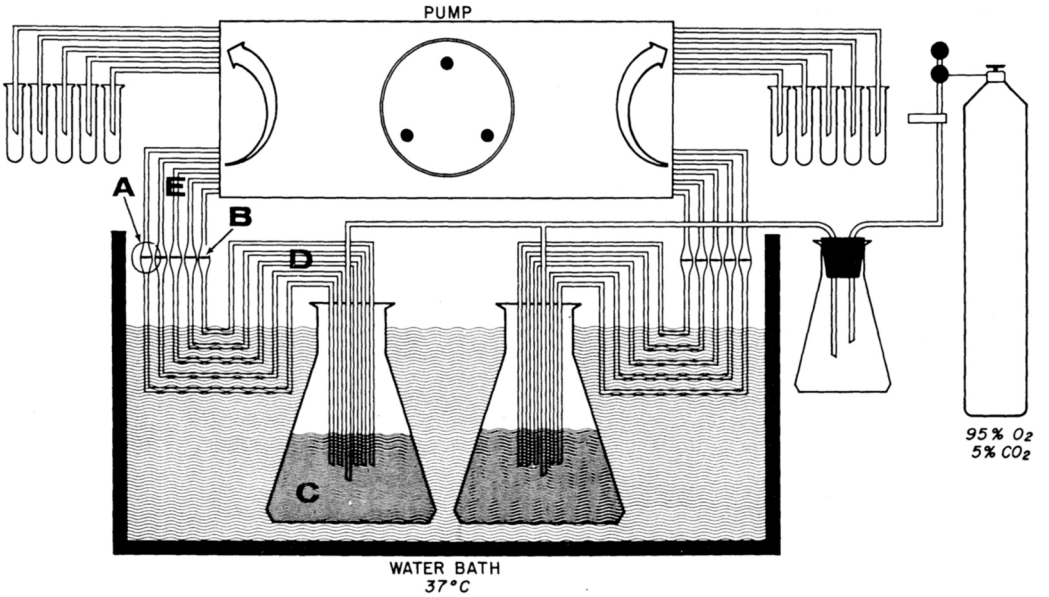

FIG. 1. Schematic representation of incubation apparatus used for *in vitro* superfusion of bovine pituitary pieces.

chamber. Incubation usually was initiated within 30 min after the anterior pituitary was removed from the donor.

Incubation media were TC 199 containing 68 μg/ml penicillin-G-phosphate and either 0.224 or 0.035% $NaHCO_3$. Following 2 hr of culture one-half of the pituitary tissue in each chamber was removed and incubation of the remaining tissue was continued for an additional 4 hr. The experimental design is illustrated in Fig. 2.

Pituitary tissue was prepared for hormone assay by sonification in phosphate (0.01 *M*) buffered (pH 7.0) sodium chloride (0.14 *M*) (PBS). Pituitary tissue and incubation media samples were stored at —20° until assayed in duplicate by double antibody radioimmunoassay for luteinizing hormone (LH) and prolactin as previously described (8, 9).

Results and Discussion. Prolactin release from steer pituitary explants averaged 20.5 ng/mg/min (Table I), 187% greater ($p \cong .03$) than the comparable average for explants from cows. Although average prolactin concentration in explants after 2 and 6 hr superfusion was 46% greater in steers than in cows, the difference was not significant ($p \cong .25$).

Explants cultured in media containing 0.224% $NaHCO_3$ released 59% more prolactin than explants cultured in similar media containing 0.035% $NaHCO_3$ ($p < .01$). Greater prolactin release by explants incubated in 0.224% bicarbonate is reflected in a 35% low-

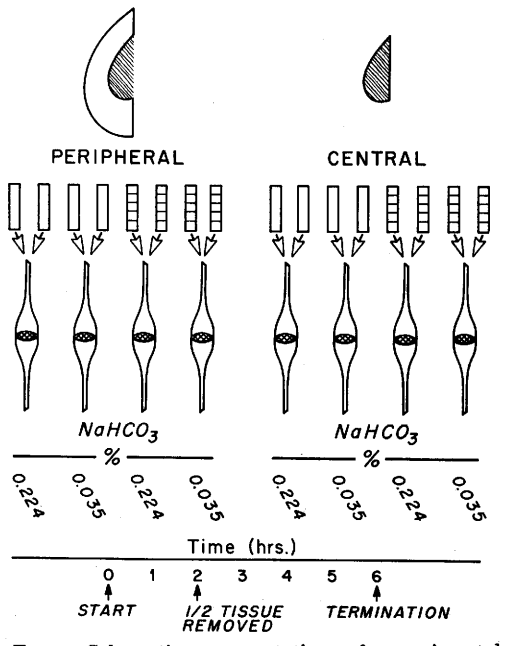

FIG. 2. Schematic representation of experimental design.

TABLE I. Bovine Pituitary Superfusion *in Vitro*—Release and Explant Concentration of Prolactin.

Variable	Prolactin in media (ng/mg/min)	p level	Prolactin in explants (μg/mg)	p level
Pituitary donor				
Steer	20.5	.03	16.3	.25
Cow	7.1		11.2	
Bicarbonate in medium				
0.034%	10.7	.001	15.8	.001
0.224%	17.0		11.6	
Pituitary region				
Peripheral	17.7	.01	18.6	.001
Central	10.0		8.9	
Explant dimensions				
One; 1 × 1 × 10 mm	12.3	.09	13.5	.63
Five; 1 × 1 × 2 mm	15.3		14.0	
Incubation interval (min)				
60	13.0	.001		0.14
120	10.5		13.3	
180	12.1			
240	13.0			
300	14.1			
360	20.2		14.2	

er average prolactin concentration in the tissue postculture ($p < .01$). These responses in superfused bovine pituitaries to composition and buffering capacity of incubation media are similar to responses in prolactin release by rat pituitaries *in vitro* (10).

Jubb and McEntee (11) demonstrated tinctoral zonation of cell types in the bovine pituitary which have been associated with secretion of different hormones. Thus, selection of explants from given regions of the pituitary may reduce within animal variation in hormone release. Explants cut from the peripheral area of the pituitary released 77% more prolactin ($p < .01$) and contained 110% more prolactin postculture ($p < .01$) than explants from the central pituitary area. These results are consistent with the greater density of prolactin producing acidophils in the peripheral area of the bovine pituitary (11).

Explant size influenced hormone production by rat pituitary explants *in vitro* (12). However, cutting our explants into five pieces increased prolactin release only about 25% ($p \cong .08$), and explant size did not influence prolactin concentration postculture ($p > .50$).

Average prolactin release measured at the end of each hour of incubation, remained unchanged through 5 hr but was increased ($p < .001$) after 6 hr. The interaction between sex of donor and incubation interval was significant ($p < .01$); it resulted from a linear increase ($p < .01$) in prolactin release with time from explants from steers but not cows. Similarly, the interaction between level of bicarbonate and incubation interval was significant ($p < .01$); it resulted from a linear increase ($p < .001$) in prolactin release from explants incubated in medium 199 buffered with 0.035 but not 0.224% $NaHCO_3$.

Explants from cow pituitaries released 137% more LH during culture ($p \cong .05$) and contained 49% more LH postculture ($p \cong .06$) than explants from steer pituitaries (Table II). To the authors knowledge there are no reports in the literature concerning pituitary and plasma LH concentrations in steers. A previous report from our laboratory (13) demonstrated increased serum LH and decreased pituitary LH concentration after ovariectomy in cows. Pituitary and serum LH are increased in castrate male rats relative to nonoperated controls (14). Pituitary LH activity increases in orchidectomized rats as indicated by progressive hypertrophy, hyper-

TABLE II. Bovine Pituitary Superfusion *in Vitro*—Release and Explant Concentration of LH.

Variable	LH in media (ng/mg/min)	*p* level	LH in explants (μg/mg)	*p* level
Pituitary donor				
Steer	0.22	0.05	0.87	0.06
Cow	0.51		1.30	
Bicarbonate in medium				
0.034%	0.36	0.93	1.15	0.09
0.224%	0.36		1.02	
Pituitary region				
Peripheral	0.33	0.05	0.80	0.001
Central	0.39		1.37	
Explant dimensions				
One; 1 × 1 × 10 mm	0.34	0.32	1.08	0.97
Five; 1 × 1 × 2 mm	0.38		1.08	
Incubation interval (min)				
60	0.50	0.001		0.01
120	0.29		1.01	
180	0.30			
240	0.33			
300	0.35			
360	0.42		1.16	

plasia and vacuolation of pituitary delta cells (15). In contrast, the delta cells of steers are smaller than those of bulls or cows and contain less PAS positive material (11), indicating a reduced capacity to synthesize and release LH. Our finding of less LH release and less pituitary LH in steers than in cows support this view.

Histological investigation of the bovine pituitary had previously revealed a predominance of gonadotrophs in the central area of the pituitary (11). Therefore, it is not surprising that explants from the central pituitary area released 17% more LH ($p \cong .05$) and contain 72% more LH postculture ($p < .01$) than did explants cut from the periphery of the gland. These results provide additional evidence for functional hormonal zonation in the bovine pituitary.

Neither explant dimension nor bicarbonate concentration in the media influenced LH release or postculture tissue concentration.

LH release into the media (ng/mg/min) decreased from 0.50 at 60 min to 0.29 at 120 min and increased thereafter to 0.42 ng/mg/min at 360 min ($p < .01$). LH release from explants from cows showed no consistent pattern with time while LH release from steer explants was relatively stable. Average LH concentration in tissue after 6 hr of incubation was 15% greater than at 2 hr of incubation ($p < .01$). This fact coupled with increased rate of release with increased incubation interval suggest active LH synthesis *in vitro*. We have demonstrated previously that bovine fetal pituitary tissue is capable of prolactin and LH synthesis during 72 hr static incubation *in vitro* (16).

Net hormone synthesis during the last 4 hr of culture was estimated; average postincubation pituitary hormone concentration (6 hr) plus average media hormone concentration minus hormone concentration of explants after 2 hr incubation. In this regard, explants synthesized on the average 1.98 and 0.23 μg/mg prolactin and LH, respectively, during 4 hr of culture under conditions of constant superfusion. It is also interesting to note that the ratio of prolactin to LH release (approx 40:1) observed here compared favorably with the same ratio for bovine plasma as measured in our laboratory.

The variables tested in this study accounted for 90% of the overall variability associated with prolactin release and for 75% of the variability associated with LH release.

We found no serious obstacles for use of this superfusion system to study the bovine anterior pituitary *in vitro*.

Summary. Explants were taken from central and peripheral regions of the anterior pituitary, cut into small (1 × 1 × 2 mm) or large (1 × 1 × 10 mm) pieces, and incubated by continuous superfusion in TC-199 with 0.035 or 0.224% $NaHCO_3$ for 6 hr. Explants from cows released 137% more LH than those from steers but 65% less prolactin. Average LH concentration 2 and 6 hr postculture was 49% greater in explants from cows. Prolactin release but not LH release was greater into medium containing 0.224% than 0.035% $NaHCO_3$ and postculture concentration in the pituitary tissue inversely reflected this pattern. Release and postculture tissue concentration of LH and prolactin was greatest for explants from the central and peripheral pituitary regions, respectively. Explant size did not influence release of prolactin or LH but release of both hormones was increased significantly during the incubation period.

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