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Tissue and Cell 38 (2006) 171–176

Tissue&Cell

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Partial development of the steroidogenic ultrastructural features in degenerative corpora lutea after a single injection of pituitary extract in the Western painted turtle (*Chrysemys picta*)

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Received 25 November 2005; received in revised form 31 January 2006; accepted 7 February 2006
Available online 20 March 2006

Abstract

Pituitary glands were removed from sexually mature female turtles (*Chrysemys picta*) and they were injected intraperitoneally (i.p.) into other mature females of the same species (experimental). In addition mature females of the same species received saline injection only (controls). Initially all the turtles used in this study were steroidogenically inactive with corpora lutea already undergoing luteolysis (degeneration) as these turtles had ovioposited their eggs approximately 2 weeks earlier. Forty-eight hour post injection the corpora lutea were removed from the control and experimental turtles. In the experimental turtles, the lutein granulosa cells developed ultrastructural features such as tubular and cisternal smooth endoplasmic reticulum (SER) and mitochondria with tubular cristae associated with lipid droplets. However, the controls maintained degenerative corpora lutea without steroidogenic ultrastructural features. The circulating progesterone (Pro) levels in the experimental turtles were significantly higher than the controls ($P < 0.049$). Although the 48 h development of steroidogenic ultrastructural features in the lutein granulosa cells was only partial in development, the effect of the pituitary taken from the inactive donor triggered an activating process within a short period, clear evidence of gonadotropic effect on the inactive corpora lutea. The present data offer interesting information on the short-term effect of gonadotropins during the non-reproductive period. This information may have useful implication under natural conditions particularly during the onset of a new reproductive cycle where the ovary is still inactive.

Keywords: Corpora lutea; Lutein granulose; Western painted turtle

1. Introduction

It has been documented that the chelonian ovary is a steroidogenic organ (Licht and Crews, 1976; Cyrus et al., 1978; Klicka and Mahmoud, 1972, 1973; Mahmoud et al., 1980; Callard et al., 1976; Mahmoud and Licht, 1997). The reproductive cycle of the Western painted turtle was thoroughly described by Moll (1979). It commences in early spring and ends in late fall.

The Western painted turtle is a polyclutch species which lays about 2–3 clutches in Wisconsin. However, in the southern sectors of its range, it may lay higher number of clutches because of the warmer climate. The ovarian cycle beings in late May with full growing preovulatory follicles ready to ovulate.

Ovulation occurs in late May and the eggs are maintained in the reproductive tract for almost 2 weeks before oviposition. After ovulation the granulosa cells of the corpus luteum undergo hypertrophy (luteinization) to become granulosa lutein cells, similar to those of other reptiles and mammals (Cyrus et al., 1978; Mahmoud and Licht, 1997). The corpora lutea in fresh water turtles are sterodogenically active for almost 3 weeks but remain in the ovarian stroma for a span of 5–6 weeks (Cyrus et al., 1978; Mahmoud et al., 1980). While the eggs are still in the oviducts, luteolysis (degeneration) begins and continues for approximately 2–3 weeks after oviposition before the corpora lutea are

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doi:10.1016/j.tice.2006.02.001

completely involuted from the ovarian stroma (Cyrus et al., 1978).

In the freshwater turtles including the Western painted turtles, involution of the corpora lutea is marked by a gradual decrease in the diameter of the corpora lutea. In addition the corpora lutea remain active for most of the time while in the oviduct (Mahmoud et al., 1980). In the snapping turtle, corpora lutea of approximately 7 mm diameter are considered active (Mahmoud et al., 1980). As involution commences in corpora lutea there is a gradual regression in size, beginning shortly after oviposition. In the snapping turtles, as well as other freshwater turtles, corpora lutea measuring less than 4.0 mm were considered inactive (Mahmoud et al., 1980; Cyrus et al., 1978).

Vitellogenesis commences in August in which the follicles attained preovulatory size prior to hibernation and will be ready for the following spring.

The current study attempts to analyze the effect of gonadotropins by removing pituitary glands from sexually mature Western painted turtles (*Chrysemys picta*) and injecting them intraperitoneally (i.p.) into other sexually mature turtles of the same species. The ovaries of the donors and recipients were steroidogenically inactive. The experiments were conducted while the corpora lutea in these turtles were undergoing luteolysis. This preliminary study may offer some information on the role of gonadotropins in the ovarian development and steroidogensis.

2. Materials and methods

2.1. Animals

A total of nine mature females of Western painted turtles were collected from the field during June 25–27. At this time the turtles had already laid their first clutch, as most turtles in Wisconsin lay their first clutch in late May–early June (Callard and Lance, 1977; Klicka and Mahmoud, 1977). The turtles weight ranged between 400 and 500 g.

After the turtles were captured in the field, they were immediately palpated behind the bridge for the presence of oviductal eggs. Only those turtles without eggs were collected. At the time of collection, most of the turtles had their corpora lutea undergoing luteolysis with a diameter of less than 4.0 mm.

Within 24 h of capture, three of the turtles were sacrificed and their pituitary glands were dissected out. Each pituitary was injected intraperitoneally into a female using physiological saline as a vehicle. The other three turtles (controls) were injected with saline only. The active corpora lutea in freshwater turtles including the Western painted turtles are approximatly 7.00 mm diameter (see Section 1). The turtles used in this investigation had corpora lutea approximately 4.00 mm diameter which is an indication of degeneration (luteolysis).

2.2. Collection, storage and extraction of serum

Blood samples were taken from the caudal vessels prior to the injection from both groups and 48 h post injection when the animals were sacrificed.

Blood samples were allowed to clot, then spun at 300 g for 10 min. The serum was immediately frozen and stored at −20 °C until assayed. Extraction of progesterone (Pro) was accomplished with hexane at a ratio of 0.3 plasma to 8 hexane.

The pre and post injection values of progesterone were compared. The solvents used in extraction and chromatography were spectroquality and obtained from Fisher Scientific (Chicago, IL). Celite was obtained from the JT Baker Chemical Co. (Phillipsburg, NJ).

2.3. Transmission electron microscopy

The corpora lutea were removed from the ovaries 48 h post injection, minced and fixed in Karnovsky's fixative buffered with sodium cacodylate to a pH of 7.4 for several hours. Following the primary fixation the tissues were washed in 0.1 M cacodylate buffer and post fixed in 1% aqueous solution of osmium tetroxide for 1 h. The tissues were then dehydrated in a series of alcohol before embedding in Spur low viscosity epoxy. Semi-thin and ultra-thin sections were cut using Leica ultramicrotome R. The semi-thin sections were stained with toluidine blue and the ultra-thin sections were stained with uranyl acetate and post stained in lead citrate. The sections were examined using EM Joel-1230.

2.4. Chromatography

Random aliquots of serum extracts were purified on celite micro columns using the procedure described by Abraham et al. (1971). The purified aliquots were compared by radioimmunoassay, with extracts, which were not chromatographed). After adjusting for recovery, there was no significant difference in steroid concentration between extracted sera and chromatographed sera. Therefore, sera was extracted, but not chromatographed, prior to radioimmunoassay.

The tritiated steroid used to determine recovery during chromatography was [1,2,6,7-N-^{3}H] progesterone [sa, 96 Ci/mmol], which were purchased from New England Nuclear Corp. (Boston, MA) and purified chromatographically prior to use. All tritiated samples were counted in a Packard Minaxi Model 4430 liquid scintillation counter with a counting efficiency of 61% for tritium (Packard, Downers Grove, III).

2.5. Radioimmunoassay

Progesterone standard and samples were diluted with a 0.02 M phosphate buffer (pH 7.4) containing purified protein and assayed in duplicate following the kit protocol. Briefly, the equivalent of 75 μl original serum was assayed. Approximately 0.02 μCi of iodinated *P* was added, fol-