11) exert the same effect as γ-type endorphins, which suggests that these peptides may also interact with DA auto-receptors in the nucleus accumbens. Some evidence for an influence on dopamine transmission is available, since Glowinski et al.[11] have shown that SP and SP-(4-11) stimulate the release of DA from nerve terminals in the caudate nucleus of the rat, and SP inhibits apomorphine induced hypolocomotion in rats (Van Ree et al., personal communication).

The biotransformation of β-endorphin or vasopressin and oxytocin has been shown to generate powerful neuropeptides with different, opposite, selective and more potent effects[12]. It is possible that SP requires processing by enzymatic cleavage to active moieties which elicit the various behavioral effects that have been found in the present experiments and those reported by Stewart et al.[6].

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Effect of cadmium chloride on steroidogenic enzymes in the Bidder's organ of the toad *(Bufo melanostictus)*

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Summary. Injection of cadmium chloride in a toad increases both the Δ^5-3β-hydroxy-steroid dehydrogenase and 17β-hydroxysteroid dehydrogenase activities in Bidder's organ.

Although Bidder's organ in the male toad has been considered as a rudimentary ovary containing corpus luteum[1] and follicle[2], little is known about the occurrence of steroid-forming enzymes in this organ in *Bufo melanostictus.* In vitro studies have shown that the Bidder's organ of *Bufo vulgaris* can synthesize steroids like the ovary of the same species[3]. Previous studies also indicate that the removal of both testes stimulates of Bidder's organ to change into a functional ovary[4] while administration of testosterone results in atrophy of the same organ[2].

Cadmium chloride is known to inhibit spermatogenesis[5] and testicular 17β-hydroxysteroid dehydrogenase activity in toads[6]. The present experiments were undertaken to demonstrate the activities of Δ^5-3β-hydroxysteroid dehydrogenase (Δ^5-3β-HSD) and 17β-hydroxysteroid dehydrogenase(17β-HSD) in the Bidder's organ of normal toads and those treated with cadmium chloride.

Materials and methods. For the present investigation, 330 male toads *(B. melanostictus)* of average weights 50-60 g were collected from their natural environment during the breeding season (August). The animals were divided equally into 2 groups. A single s.c. injection of 0.5 mg of cadmium chloride, dissolved in 0.2 ml amphibian saline, was given to one group of animals while the remaining group received vehicle only. All the animals were provided with food (ant eggs) ad libitum every other day and were sacrificed after 7 days. 320 animals were used for biochemical estimation of Δ^5-3β-HSD and 17β-HSD activity, and 10 animals were used for histochemical demonstration. For estimation of Δ^5-3β-HSD and 17β-HSD, both control and treated groups were divided equally and the Bidder's organs of 10 animals were pooled in both control and treated animals for estimation of each enzyme activity.

For assay of Δ^5-3β-HSD activity, the Bidder's organs were removed and dropped into ice cold homogenizing medium consisting of equal parts of 0.65% sodium chloride and 0.1 M sodium phosphate buffer, pH 7.4, to give a tissue concentration of 5 mg/ml. The enzyme was assayed by spectrophotometric measurement of the production of Δ^4-androstenedione from dehydroepiandrosterone (DHEA)[7].

The activity of 17β-HSD was measured by the method of Jarabak et al.[8]. Pooled Bidder's organs were homogenized in 20% spectroscopic grade glycerol, 5 mM potassium phosphate and 1 mM EDTA and centrifuged at 10,000 × g for 30 min. 1 ml of the supernatant was mixed with 440 µmoles sodium pyrophosphate buffer, pH 10.2; 25 mg crystalline bovine serum albumin and 0.3 µmoles testosterone. The enzyme activity after addition of 1.1 µmoles NADP was measured spectrophotometrically at 340 nm against a blank (without NADP). One unit of enzyme

Effect of cadmium chloride on Δ^5-3β-HSD and 17β-HSD activities in Bidder's organ of toad

Treatment	Δ^5-3β-HSD activity nmoles/mg tissue/h	17β-HSD activity units/mg tissue/h
Control	3.9 ± 0.44	16.84 ± 1.64
Cadmium	6.62 ± 0.88	26.68 ± 1.98

Each value represents mean ± SD. $p < 0.01$ (Wilcoxon test) control vs cadmium, where N = 8.

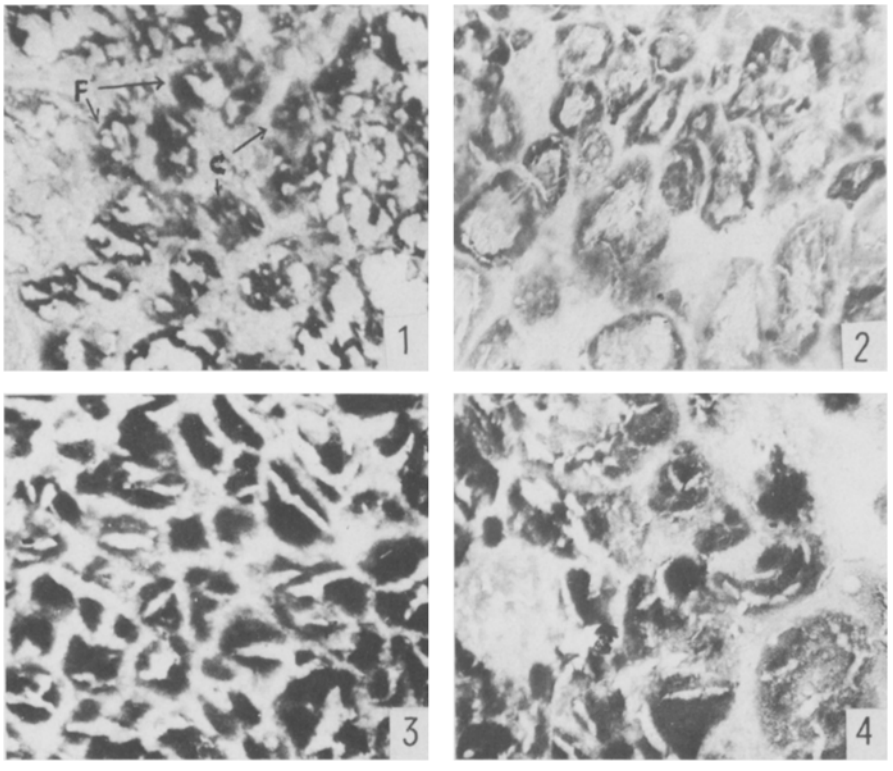

Figure 1. Δ^5-3β-Hydroxysteroid dehydrogenase in the Bidder's organ of normal toad. × 125. 'F' denotes follicle while 'C' denotes corpus luteum.

Figure 2. 17β-Hydroxysteroid dehydrogenase in the Bidder's organ of normal toad. × 125.

Figure 3. Δ^5-3β-Hydroxysteroid dehydrogenase in the Bidder's organ of cadmium-treated toad. × 125.

Figure 4. 17β-Hydroxysteroid dehydrogenase in the Bidder's organ of cadmium-treated toad. × 125.

activity is equivalent to a change in absorbancy of 0.001/min at 340 nm.

For histochemical demonstration of the enzymes, fresh frozen sections were cut at 20 µm. The sections were incubated at 37 °C in the appropriate media for demonstrating the activity of the enzyme Δ^5-3β-HSD, using DHEA as substrate[9] and of 17β-HSD, using testosterone as substrate[10]. Parallel sections incubated in substrate-free medium served as controls. After 30 min incubation the sections were fixed and mounted in glycerol-gelatin.

Results. Histochemical preparation showed Δ^5-3β-HSD (fig. 1) and 17β-HSD (fig. 2) activities in both corpora lutea and follicles in the Bidder's organ of control animals. Cadmium injections caused a rise of both the enzyme activities over that seen in controls (figs 3 and 4). Biochemically, the activities of Δ^5-3β-HSD and 17β-HSD appeared to increase significantly in the Bidder's organ as compared to controls (table).

Discussion. The present study demonstrates that the enzymes Δ^5-3β-HSD and 17β-HSD are present in both the corpus luteum and follicle in the Bidder's organ of toad. Since both the enzymes play an important role in steroid hormone synthesis, the present findings indicate that the Bidder's organ in toads is also a source of steroid hormones, like the ovary[11,12] and testis[13,14]. Chieffi and Lupo[3] showed that addition of precursors in the incubating medium enhances steroid biosynthesis in the Bidder's organ.

The mechanism of stimulation of steroid-producing enzymes in the Bidder's organ after cadmium treatment has not been elucidated. Our previous studies indicate that cadmium chloride injection in toads causes spermatogenic arrest[5] and also inhibits testicular 17β-HSD activity[6]. On the other hand, stimulation of Bidder's organ after gonadectomy has been considered to be due to the absence of any inhibitory effect of testicular hormone[4]. Ganz[15], Taleisnik and McCann[16] have observed that there is a definite increase in the luteinizing hormone content of blood after gonadectomy due to a low level of circulating testosterone. Several investigators have also reported a high LH activity of the anterior pituitary in cadmium-treated mice[17,18].

The possibility remains, therefore, that cadmium chloride stimulates steroidogenic enzymes in the Bidder's organ of toad indirectly by increasing gonadotrophins.

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