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Effect of Methallibure (ICI, 33, 828) on Pituitary Pars Distalis and Ovary of the Frog, *Rana cyanophlyctis* (Schn.)

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Methallibure administration on alternate days (for 10, 20 and 30 days) to female *R. cyanophlyctis* resulted in the hypertrophy, degranulation and vacuolization of B2 cells of the pituitary pars distalis. The staining was weak in B2 cells due to the lack of secretory granules. Gonadosomatic index (GSI) and the mean diameter of the largest oocytes decreased significantly following 20 and 30 days' treatment. The previtellogenic oocytes were normal but all the vitellogenic oocytes were atretic in the methallibure treated frogs. In controls, healthy, vitellogenic as well as mature oocytes were abundant even ten days after the cessation of treatment there was no withdrawal recovery of B2 cells, GSI value and the mean diameter of the largest oocyte. The present studies confirm the antigonadal effects of methallibure and suggest that the B2 cells are the probable source of gonadotrophins in the pars distalis of the frog.

Key Words: Pars distalis, Ovary, Methallibure, *Rana cyanophlyctis*

Introduction

The antigonadotrophic effect of methallibure, a nonsteroidal compound (ICI : 33,828), is well known in several mammalian species (Paget et al. 1961, Parkes 1963). However, its effect on the testis and the secondary sexual characters, especially the pituitary gland and ovary of non-mammalian vertebrates, is scantly known. (Mohanty & Naik 1979). Among amphibians, there is only one study dealing with the effect of methallibure on the pituitary gland (Rastogi et al. 1972) and none on the ovary. Therefore, the present work was undertaken to study the effect of methallibure on the pituitary pars distalis and the ovary of the frog *Rana cyanophlyctis.*

Materials and Methods

Adult females of *R. cyanophlyctis,*

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(wt. 25–30 g), collected locally in March/April were acclimated to laboratory conditions for 5 days and divided into two groups of 60 frogs each: (i) 0.2 ml amphibian ringer : (control), and (ii) 1 mg methallibure in 0.2 ml amphibian ringer. The two groups were given injection (ip) on alternate day. Group I received 0.2 ml of amphibian ringer (control) while Group II was given 0.2 ml of amphibian ringer containing 1 mg of methallibure. The frogs were fed with thigh muscles of other frogs on alternate days. Five animals were autopsied on 11th, 21st and 31st day after treatment and rest of the frogs were left untreated to study the withdrawal effect of methallibure and autopsied 10 days after each autopsy. During autopsy the body and ovary weights were recorded to determine the gonadosomatic index (GSI). A piece of ovary from each frog was fixed in Bouin's fluid, embedded in paraffin wax and sectioned at 6 μm thickness and stained with hematoxylin-eosin. The pituitary glands, fixed in Bouin Hollande sublimate for 24 hr were sectioned at 4 μm thickness and stained with (*i*) Herlant's tetrachrome, (*ii*) Cleveland and Wolfe's trichrome, (*iii*) Halmi's aldehyde fuchsin, (*iv*) Herlant's AB-PAS-OG, (*v*) Gabe's aldehyde fuchsin, and (*vi*) Lead hematoxylin.

Measurements

The maximum cell length and nuclear diameter of 75 cells, from each category of chromophils (A1, A2, B1, B2 and B3) and the cell area, nuclear area and cytoplasmic area of B2 cells (50 each) for each group were measured as described earlier (Masood-Hussain & Saidapur 1983). Five pituitaries were used in each group.

The diameter of the largest oocyte was determined by measuring the smallest and the largest diameters of 25 oocytes from sections of each ovary. The ovaries of five frogs were used for determining the mean diameter of largest oocytes for each group.

The data were analysed using Student's *t* test and differences were judged as significant if $P < 0.05$.

Results

Pars Distalis

In control, all the cell types (A1, A2, B1, B2 and B3) of the pars distalis were normal and their cytoplasm contained secretory granules (figure 1). In 10-days' methallibure-treated frogs there was marked hypertrophy and degranulation of B2 cells (figure 2). The cell length, the cell and the cytoplasmic areas increased significantly ($P < 0.05$) whereas the nuclear diameter and nuclear area remained unaffected (table 1). The staining intensity of B2 cells decreased (figure 2). In 20 and 30 days' treated frogs the cell length, the cell and the cytoplasmic areas of B2 cells increased further (table 1). In addition, the nuclear diameter and the nuclear area of these cells increased significantly ($P < 0.001$) following 20 and 30 days' treatment (table 1). The staining intensity of B2 cells was drastically reduced due to the absence of granules (figure 3). In 30 days' treated frogs, a few B2 cells showed distinct vacuolization of their cytoplasm (figure 5), and the cell boundaries were difficult to delimit. The other cells of the pars distalis remained normal (tables 2 & 3).

There was no withdrawal recovery of the B2 cells in terms of size or granulation, 10 days following the cessation of treatment (figures 4, 6 & table 1).

Table 1 *Cytological changes in the B2 cells of the pituitary pars distalis of 10, 20 and 30 days methallibure-treated* R. cyanophlyctis

Group*	Nuclear+		Cell+		*Mean Cytoplasmic area (μm^2)
	diameter (μm)	area (μm^2)	length (μm)	area (μm^2)	
		10 Day			
Control	6.33±0.13	32.5±1.2	16.68±0.21	95.9±4.3	63.1±4.1
Methallibure (1 mg) -treated	6.71±0.15 NS	33.4±1.3 NS	17.71±0.23 P < 0.01	111.3±3.5 P < 0.05	77.9±3.6 P < 0.05
10 days after withdrawal	6.76±0.19 NS	33.0±1.1 NS	17.64±0.26 P < 0.01	111.0±3.3 P < 0.05	77.4±2.9 P < 0.05
		20 Day			
Control	6.24±0.12	33.6±1.3	16.99±0.14	100.0±4.7	66.4±4.5
Methallibure (1 mg) -treated	7.10±0.17 P < 0.01	39.3±1.4 P < 0.05	21.54±0.24 P < 0.001	132.1±4.4 P < 0.001	92.9±4.2 P < 0.01
10 days after withdrawal	7.06±0.16 P < 0.01	38.9±1.3 P < 0.05	19.51±0.28 P < 0.001	126.9±4.3 P < 0.01	89.9±4.3 P < 0.01
		30 Day			
Control	6.24±0.12	33.7±1.6	17.34±0.20	101.8±4.4	68.1±4.1
Methallibure (1 mg) -treated	7.35±0.22 P < 0.01	41.3±1.5 P < 0.001	21.75±0.25 P < 0.001	160.4±5.4 P < 0.001	118.8±5.1 P < 0.001
10 days after withdrawal	7.16±0.21 P < 0.01	37.8±1.5 P < 0.05	19.22±0.22 P < 0.001	156.4±4.6 P < 0.001	116.7±4.3 P < 0.001

+The values represent mean ±SE

**In each no. of animals = 5; *P* values were calculated using Student's *t* test; NS = Nonsignificant

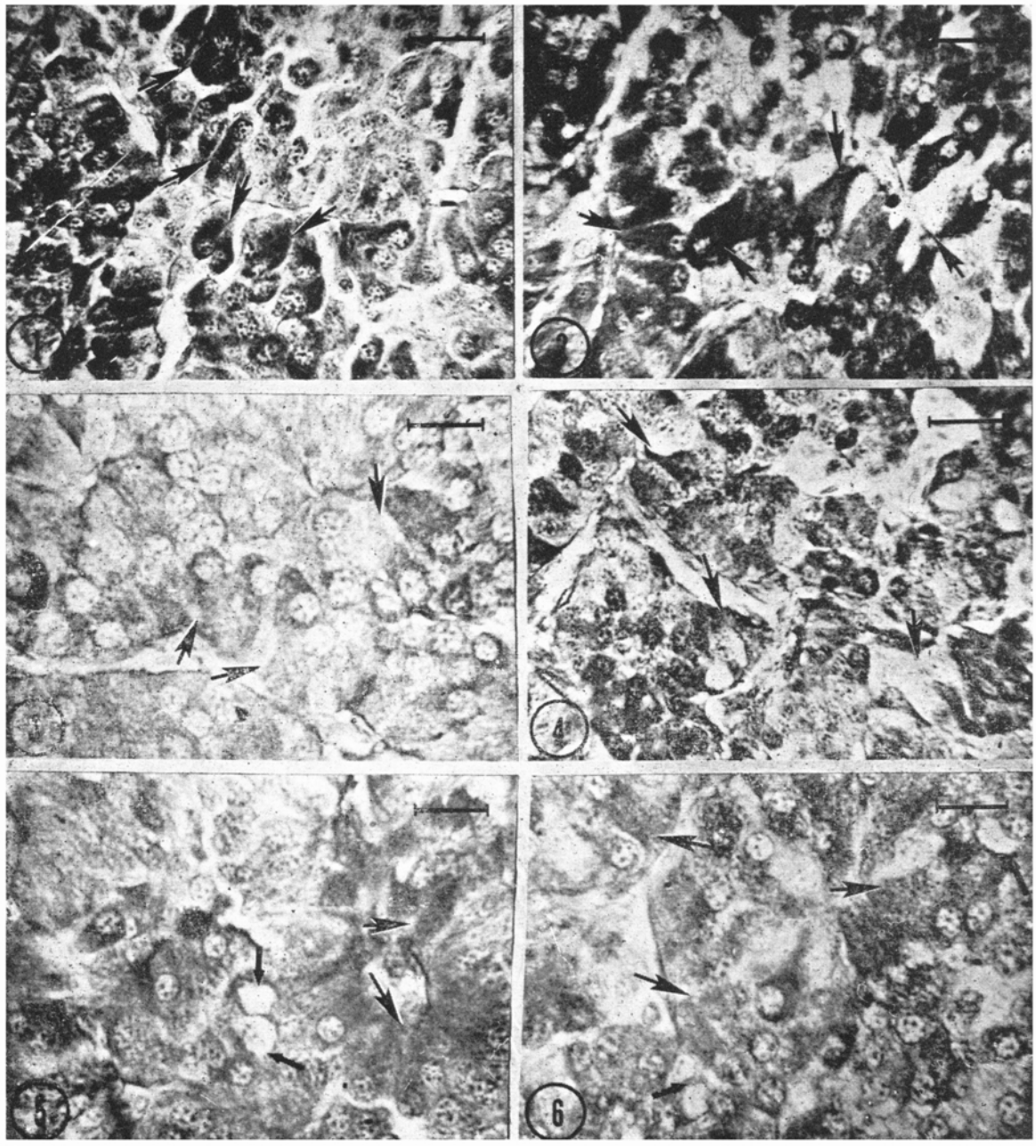
*Mean cytoplasmic area were obtained by substracting the nuclear area from the cell area of corresponding cell

Ovary

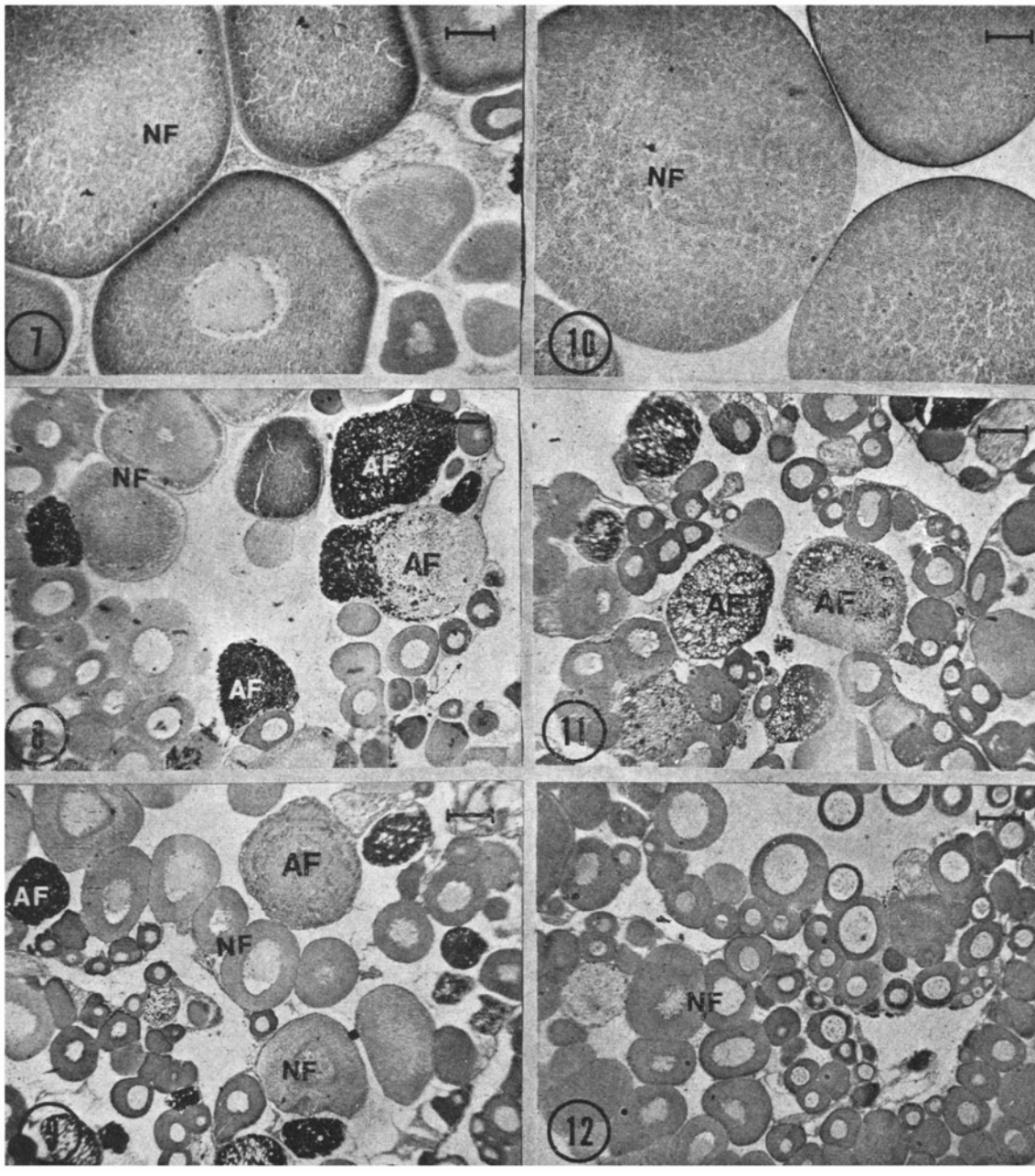
The ovaries of 10 days' control frogs contained previtellogenic and vitellogenic oocytes. In 20 and 30 days' control frogs most of the oocytes were mature and fully ladden with yolk (figures 7 & 10). The previtellogenic oocytes were fewer in number. A few atretic follicles were present in all the ovaries.

In the 10 day's methallibure treated frogs there was no significant effect on the ovary. Following 20 or 30 days' treatment GSI values decreased significantly (table 4). All vitellogenic and large previtellogenic oocytes started degeneration. Normal previtellogenic oocytes were present in fewer number whereas healthy vitellogenic oocytes were completely absent (figures 8 & 11). The mean diameter of the largest oocytes was reduced significantly (table 4).

Ten days following the cessation of treatment, there was no recovery in the mean diameter of the largest oocytes and the GSI value (table 4). Histologically, ovaries of these frogs resembled that of the respective treatment groups (figures 9 & 12).



Figures 1–6, Sagittal Sections of the pituitary pars distalis (centrorostral region) of R. cyanophlyctis **1**, Presence of secretory granules and intense staining in B2 cells (arrows) in controls; **2**, Hypertrophy and slight degranulation of B2 cells (arrows) following 10 days methallibure treatment; **3-4** Hypertrophy, degranulation and vacuolization of B2 cells (arrows): (**3**) following 20 days methallibure treatment and (**4**) 10 days following cessation of the treatment; **5-6** the hypertrophy, degranulation and vacuolization of B2 cells (arrows). (**5**) following 30 day methallibure treatment, and (**6**) 10 days following cessation of the treatment. Herlant's AB-PAS-OG, Scale lines = 20 μm.



Figures 7–12, *Cross sections of ovary of R. cyanopylyctis.* **7,** A portion of ovary of the control frog showing healthy oocytes in various stages of development; **8–9** Showing small previtellogenic oocytes and several atretic follicles (AF) following 20-day methallibure treatment (figure 8), and also 10 days following cessation of the treatment (figure 9); **10,** A portion of the ovary of 30 days control frog showing yolky oocytes; **11–12,** Showing previtellogenic oocytes following 30 days' methallibure treatment (figure 11) and also 10 days following cessation of the treatment (figure 12).

NF, normal follicle; **AF,** atretic follicle. **Scale lines** = 100 μm

Table 2 *Changes in the cell length* of B1, B3, A1 and A2 cells of the pituitary pars distalis of 10, 20 and 30 days methallibure-treated* R. cyanophlyctis

Group+	Cell length (μm)			
	B1	B3	A1	A2
		10 Day		
Control	10.38±0.18	13.06±0.18	12.29±0.13	12.63±0.17
Methallibure treated (1 mg)	10.61±0.25†	13.31±0.21†	12.56±0.21†	12.46±0.25†
10 days after withdrawal	10.39±0.26†	13.09±0.23†	12.40±0.23†	12.29±0.26†
		20 Day		
Control	10.37±0.14	13.15±0.14	12.37±0.25	12.34±0.17
Methallibure treated (1 mg)	10.51±0.16†	13.29±0.21†	12.51±0.21†	12.23±0.19†
10 days after withdrawal	10.46±0.19†	13.23±0.26†	12.49±0.22†	12.31±0.23†
		30 Day		
Control	10.22±0.13	13.29±0.17	12.29±0.29	12.38±0.25
Methallibure treated (1 mg)	10.47±0.19†	13.51±0.22†	12.33±0.17†	12.26±0.19†
10 days after withdrawal	10.41±0.18†	13.43±0.23†	12.27±0.19†	12.23±0.19†

* Values are mean ± S.E.; + No. of animals in each group = 5; †Nonsignificant

Discussion

The studies show that methallibure induced specific changes in the B2 cells of the pituitary pars distalis and degeneration of vitellogenic and mature oocytes in the ovary of *R. cyanophlyctis*. The degranulation of gonadotrophs following methallibure-treatment as observed in *R. cyanophlyctis* is also reported in *Cymatogaster aggregata* (Leatherland 1969), *Plectroplitus ambigus* (Mackay 1971), *Tilapia mossambica* (Dadzie 1975), *Rana esculenta* (Rastogi et al. 1972), *Calotes versicolor* (Mohanty & Naik 1979) and black tailed deer *Odocoileus hemionus columbianus* (West & Nordan 1976). Interestingly, in *Brachydanio rerio* methallibure treatment resulted in the increase of cytoplasmic granules and the size of gonadotrophs (van Ree 1976).

The progressive decline of GSI values, arrest of vitellogenesis, degeneration of large follicles in the frog ovary following methallibure treatment are analogus to that observed after hypophysectomy (Saidapur & Nadkarni 1973). Similar results were obtained after the administration of methallibure in female teleosts (Hoar et al. 1967, Dadzie 1975, van Ree 1976).

Different interpretations may emerge with regard to the effect of methallibure on the pituitary alone and when considered

Table 3 *Chances in the nuclear diameter* of B1, B3, A1 and A2 cells of the pituitary pars distalis 10, 20 and 30 days methallibure-treated* R. cyanophlyctis

Group+	Nuclear diameter (μm)			
	B1	B3	A1	A2
		10 Day		
Control	5.95±0.13	5.95±0.14	6.15±0.13	6.13±0.17
Methallibure (1 mg) treated	6.23±0.26 NS	5.73±0.11 NS	6.09±0.14 NS	5.83±0.16 NS
10 days after withdrawal	6.11±0.21 NS	5.77±0.16 NS	6.06±0.15 NS	5.89±0.21 NS
		20 Day		
Control	6.28±0.11	5.85±0.14	6.07±0.12	6.24±0.31
Methallibure (1 mg) treated	6.64±0.21 NS	5.81±0.13 NS	6.19±0.13 NS	6.16±0.11 NS
10 days after withdrawal	6.73±0.19 NS	5.83±0.15 NS	6.12±0.15 NS	6.12±0.15 NS
		30 Day		
Control	5.58±0.12	5.78±0.09	6.13±0.11	6.19±0.12
Methallibure (1 mg) treated	5.72±0.16 NS	5.93±0.14 NS	6.23±0.13 NS	6.13±0.15 NS
10 days after withdrawal	5.95±0.19 NS	5.93±0.15 NS	6.21±0.13 NS	6.17±0.16 NS

* Values are mean ± SE; + No. of animals in each group = 5; NS †Nonsignificant

Table 4 *Changes in the ovary of 10, 20 and 30 days methallibure-treated* R. cyanophlyctis

Group+	Wt of ovary*/g 100 g body wt	Mean diameter of largest oocyte (μm)
	10 Day	
Control	3.26±0.39	882.44±20.04
Methallibure treated (1 mg)	3.09±0.29†	822.78±15.76†
10 days after withdrawal	2.91±0.33†	809.63±16.91†
	20 Day	
Control	3.31±0.23	982.21±21.64
Methallibure treated (1 mg)	1.93±0.21 $P < 0.001$	367.20±9.36 $P < 0.001$
10 days after withdrawal	1.22±0.14 $P < 0.001$	354.00±9.24 $P < 0.001$
	30 Day	
Control	3.61±0.15	1036.20±24.60
Methallibure treated (1 mg)	1.33±0.10 $P < 0.001$	306.20±19.32 $P < 0.001$
10 days after withdrawal	0.99±0.13 $P < 0.001$	303.80±10.14 $P < 0.001$

* Values are mean ± SE; + No. of animalsin each group = 5; †Nonsignificant

along with its effects on the gonads. The hypertrophy and degranulation of B2 cells in the present work is indicative of hyperactivity of these cells. However, methallibure is known to be antigonadotrophic and therefore the degranulation and hypertrophy of B2 cells may not, in fact, reflect the increased synthesis and release of gonadotrophins. Recently, it has been shown that methallibure treatment causes a reduction in the gonadotrophin content of the pituitary in *Heteropneustes fossilis* (Raizada & Singh 1977). The gonadal regression following methallibure treatment may be due to reduced gonadotrophin output by the pituitary gland. The hypertrophy of gonadotrophs in the frog is probably due to the depression of the secretion of ovarian steriods as a result of the action of the drug at the hypothalamo-hypophyseal level/or ovarian level. However, it remains to be clarified whether methallibure acts directly on both pituitary and ovary and if so on which one first.

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