A gonadotropin releasing hormone analog induces spermiation in intact and hypophysectomized frogs, *Rana esculenta*[1]

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Summary. The sperm-releasing activity of a gonadotropin releasing hormone (GnRH) agonist, Buserelin (GnRH*) and hypophysis homogenate (PD) preparations was studied in intact and hypophysectomized (PDX) frogs, *Rana esculenta*. In addition, human chorion gonadotropin (hCG) was tested in PDX animals, and GnRH antagonist (GnRHA) treatments were carried out in intact and PDX animals, in combination with the hormonal injections. GnRH* or PD treatments were able to elicit spermiation in intact and PDX animals. While GnRH*, injected 24 h later, was again effective in inducing spermiation in intact animals, this was not the case in PDX frogs. GnRHA counteracted GnRH* effects in intact frogs. Moreover, in PDX animals GnRHA injections counteracted the sperm-releasing activity induced by hCG or GnRH*, but failed to inhibit sperm-releasing activity induced by PD homogenate.

Key words. Testis; spermiation; gonadotropins; GnRH agonist; GnRH antagonist; Amphibia.

Spermiation in Amphibia is under hypophysial control. Gonadotropins appear to play a central role, but it has been suggested that substances other than gonadotropins may also effect spermiation[2]. In particular, a peptide called sperm-releasing substance (SPRS), which is different from LH or FSH, seems to act as an active principle[3]. Recently, a direct effect exerted on gonads by gonadotropin-releasing hormone (GnRH)-like substances has been shown in frogs. As in the rat[4], this GnRH-like material interferes with steroid production[5-11], and apparently supports spermatogenesis[7,8]. Mc Creery et al.[12] also suggested that GnRH may affect spermiation in *Rana catesbeiana*, since they found that a GnRH antagonist was able to block spermiation in frogs characterized by high gonadotropin levels in blood. The work described here was designed in order to investigate whether or not treatment with a GnRH agonist (GnRH*) provokes spermiation in the hypophysectomized frog, *Rana esculenta*.

Materials and methods

One hundred male frogs (*Rana esculenta*) were collected in the vicinity of Naples. Fifty animals were hypophysectomized (PDX) within a week from capture under anesthesia with MS 222 (Sigma). Intact and PDX frogs were divided into four groups receiving either: vehicle (Krebs Ringer bicarbonate buffer, 100 µl), vehicle plus 1 µg GnRH agonist (GnRH*; Buserelin, supplied by Hoechst), crude hypophysis homogenate (PD) (1/3 hypophysis equivalent), or GnRH* plus 10 µg GnRH antagonist (GnRHA; D-pGlu[1], D-Phe[2], D-Trp[3,6]-LHRH from Peninsula Laboratories Ltd.). Intact and PDX animals (n = 12) which responded to the GnRH* treatment received a further dose (1 µg) of the agonist 24 h later. In a second experiment male frogs (n = 28) were hypophysectomized and divided into five experimental groups receiving vehicle alone, or vehicle containing 20 µg hCG or hCG plus GnRHA (10 µg). PD homogenate (1/3 hypophysis equivalent), or PD plus GnRHA, respectively. Injections were made into the craniodorsal lymph sac. Samples were taken with a Pasteur pipette from the cloacae of the frogs at 90 and 150 min after the injections, and examined for presence of spermatozoa under a microscope (magnification ×100). All frogs were examined before the injections to eliminate false positive results. In addition, at the end of the treatment, frogs were decapitated after anesthesia with MS 222 (Sigma) and the testes were excised and placed in Bouin's fluid for histological examination. Data were evaluated statistically by Fisher's method.

Results and discussion

Spermiation occurs in intact frogs (table 1) treated either with GnRH or with PD homogenate, which confirms that gonadotropin has a central role in sperm-releasing activity[2]. The GnRH antagonist inhibits the GnRH*-induced effect, thus indicating that the GnRH action is mediated via specific receptors located at the pituitary or testicular level[8,12]. Histological sections of the testes

Table 1. Sperm-releasing activity in intact frogs treated with vehicle (I), hypophysis homogenate (PD), buserelin (GnRH*) or GnRH* plus an antagonist (GnRHA)

I			I + GnRH*			I + PD			I + GnRH* + GnRHA		
	n			n			n			n	
+		−	+		−	+		−	+		−
0		10	14		1	14		1	1		10
	a			b			b			a	

a vs b, $p < 0.001$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

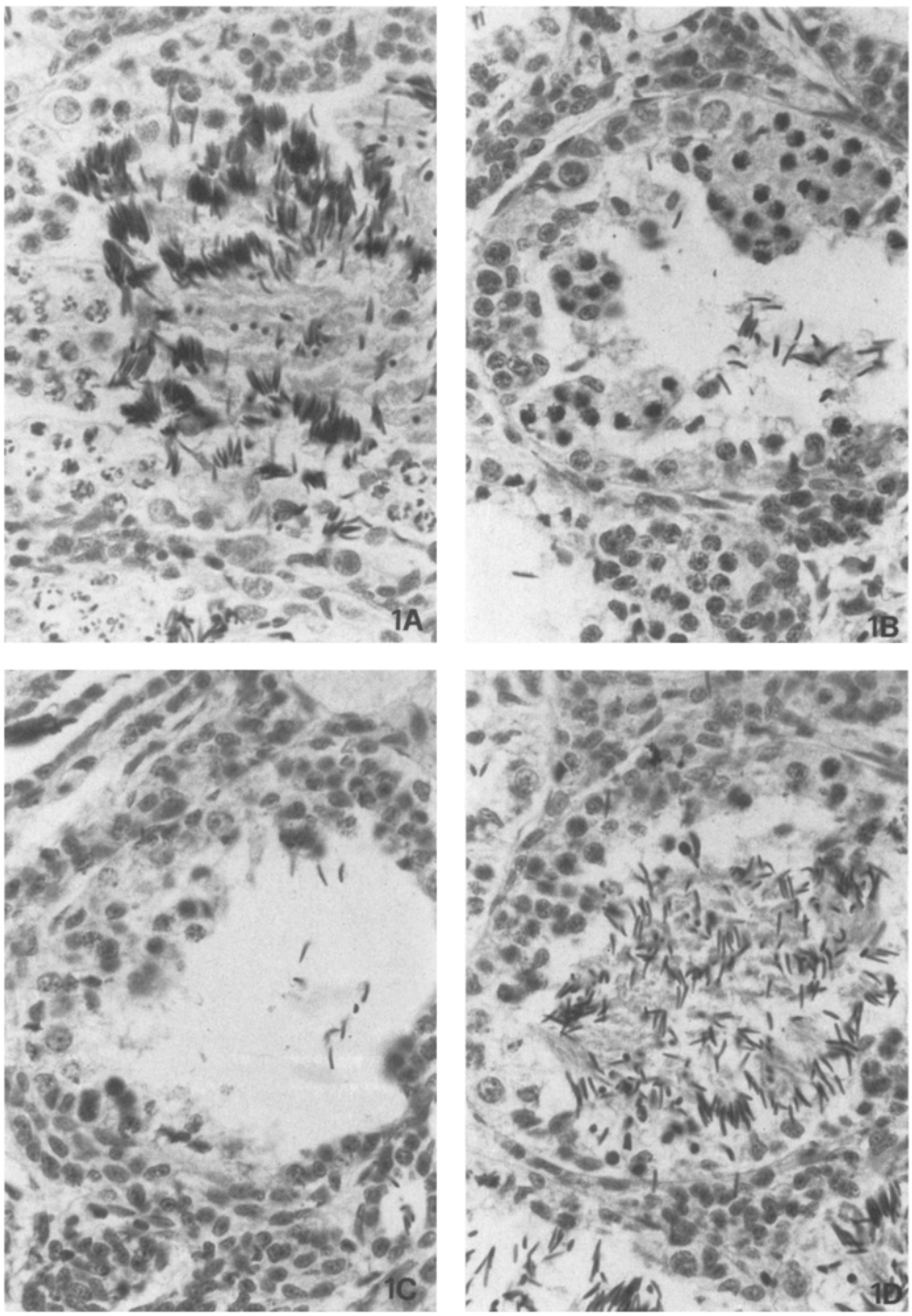

Figure 1. Seminiferous tubule full of spermatozoa from intact frogs treated with vehicle (*A*) or with GnRH* plus GnRHA (*D*) in combination, and practically empty seminiferous tubule from frogs treated with pituitary homogenate (*B*) or GnRH* (*C*), ×350.

(fig. 1) show empty tubules in spermiating PD or GnRH* treated animals, whereas in GnRH* plus GnRHA injected frogs the testes are full of spermatozoa. Interestingly, spermiation occurs in GnRH*-treated PDX animals and this effect is prevented by GnRHA injection which suggests a direct action of GnRH* on frog testes (table 2). Once again, empty tubules are observed in spermiating PD or GnRH treated frogs, whereas spermatozoa appear in GnRH* plus GnRHA injected animals (fig. 2). PD is also able to induce sperm release 24 h later, while GnRH* does not (table 3), which strongly suggests that PD and GnRH* act via different mechanisms. In this respect,

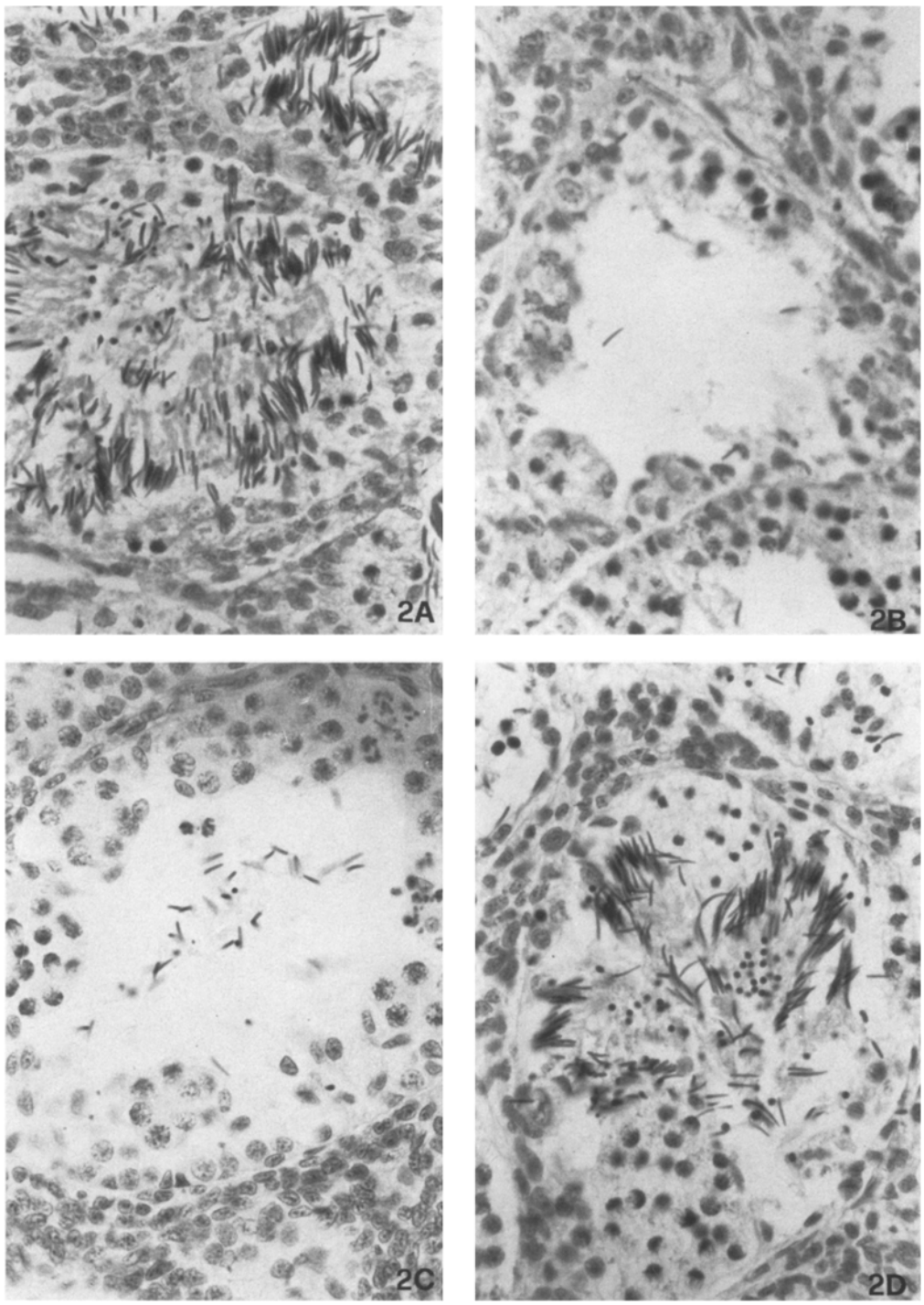

Figure 2. Seminiferous tubule full of spermatozoa from hypophysectomized frogs treated with vehicle (*A*) or with GnRH* plus GnRHA (*D*) in combination, and practically empty seminiferous tubule from frogs treated with pituitary homogenate (*B*) or GnRH* (*C*), ×350.

it is noteworthy to mention that the antagonist treatment does not prevent the sperm-releasing activity induced by PD injections (table 4).

On the other hand, GnRHA inhibits the hCG effect. At present we have no explanation for this phenomenon. We can only speculate on it, and remember that hCG treatment stimulates an intratesticular GnRH activity in rats[13].

The effect of GnRH* and GnRHA on spermiation, described here for the first time in a vertebrate, further supports the hypothesis that there is an endogenous GnRH-like material which regulates gonadal activity in

Table 2. Sperm-releasing activity in hypophysectomized frogs treated with vehicle (PDX); hypophysis homogenate (PD), Buserelin (GnRH*) or GnRH* plus an antagonist (GnRHA)

PDX		PDX + GnRH*		PDX + PD		PDX + GnRH* + GnRHA	
n		n		n		n	
+	−	+	−	+	−	+	−
0	10	15	4	10	1	1	9
a		b		b		a	

a vs b, $p < 0.001$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

Table 3. Sperm-releasing activity in intact (I) and hypophysectomized (PDX) frogs treated with GnRH* twice (at 0 h and 24 h later)

PDX		I + GnRH*		PDX + GnRH*	
n		n		n	
+	−	+	−	+	−
0	4	4	0	0	4
a		b		a	

a vs b, $p < 0.05$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

Table 4. Sperm-releasing activity in hypophysectomized frogs treated with vehicle (PDX), hypophysis homogenate (PD), hCG, GnRH* plus hCG or GnRH* plus PD

PDX		PDX + hCG		PDX + hCG + GnRHA		PDX + PD		PDX + PD + GnRHA	
n		n		n		n		n	
+	−	+	−	+	−	+	−	+	−
0	25	18	9	2	23	27	2	25	2
a		b		a		b		b	

a vs b, $p < 0.001$; a vs a, N.S.; n = sample size; + and − represent spermiating and non-spermiating animals.

frogs locally[5–11]. The finding of multiple sites of action is in agreement with results in male and female rats[4,14]. In particular, it is interesting to note that in the female rat GnRH* induces ovulation via plasminogen activator induction[14]. A similar activity may be responsible for the sperm release from Sertoli cells in frogs.

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Effects of estradiol on parathyroid cell activity

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Summary. Beta-estradiol-3-benzoate provoked an initial centrifugal membrane shift in rat parathyroid cells and, later, enlargement of the compartments concerned with parathyroid hormone secretion, which suggests that estradiol modulates not only transport and release of parathyroid hormone but also the capacity for its synthesis, packaging and storage.
Key words. Estradiol; parathyroid; exocytosis; membrane synthesis; rat.

Parathyroid (PT) glands are responsible for maintaining calcium homeostasis by secretion of parathyroid hormone (PTH) which acts on kidneys, intestine and bones[1]. The secretory activity is largely controlled by the serum calcium concentration via a unique feedback mechanism[2]. There are many other secretagogues such as mono-, di- and trivalent cations, catecholamines and hormones, e.g. glucagon, prostaglandins and serotonin,