0014-4754/93/010065-03$1.50 + 0.20

Dopamine regulation of testicular activity in intact and hypophysectomized frogs, *Rana esculenta*[1]

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Received 9 December 1991; accepted 31 August 1992

Abstract. In intact frogs, both GnRHA and L-dopa were able to increase testicular and plasma androgen levels and to induce spermiation. The dopamine antagonist pimozide inhibited both the effects of L-dopa but not those of GnRHa. Hypophysectomy reduced androgen levels, but spermiation was still induced by both GnRHa and L-dopa, suggesting that these agents can directly influence the testis through a route not involving the pars distalis. Again, pimozide antagonised spermiation induced by L-dopa but not that induced by GnRHa.
Key words. Testis; spermiation; L-dopa; pimozide; Amphibia.

In amphibians, spermiation is primarily under gonadotrophin control, but other substances also may affect the sperm-releasing process[2]. In particular, a peptide called sperm-releasing factor seems to act on the frog testis[3]. Recently, we have demonstrated that a GnRH agonist is able to induce spermiation both in intact and hypophysectomized (HPX) frogs, *Rana esculenta*[4], and this effect is counteracted by a GnRH antagonist. GnRH can also be involved in the reproductive behaviour in amphibians by altering catecholaminergic activity, as shown in rats[5]. Wilson et al.[6,7] observed that, in conscious adult cane toads (*Bufo marinus*), treatment with a GnRH analog caused a dose-dependent increase in plasma catecholamines, while a specific GnRH antagonist blocked the agonist-induced rise in plasma noradrenaline concentration in bullfrog and salmon[8]. High levels of catecholamines have been shown to induce mating behaviour and sperm release in other amphibian species besides bullfrogs[9,10]. In a previous study, Stutinsky et al.[10] demonstrated that electrical stimulation of two indpendent diencephalic centers is able to induce spermiation in *Rana esculenta*. The stimulation of one of these areas caused gonadotrophin discharge from the pituitary, while the other activated the adrenal gland, inducing the release of adrenaline.

Since GnRH and catecholamines seem to play a role in the spermiation process, the present work investigates whether dopamine affects testicular activity in intact and hypophysectomized frogs, *Rana esculenta*, and whether the GnRH-stimulated sperm release is exerted via catecholaminergic activity.

Materials and methods

100 male frogs, *Rana esculenta*, were collected in the vicinity of Naples during February 1991. 50 frogs were hypophysectomized (HPX) within a week of capture, under anesthesia with MS 222 (Sigma). After a further 4 days, intact and HPX frogs were divided into 5 experimental groups (n = 10), receiving respectively: vehicle (Krebs Ringer bicarbonate buffer, 100 µl/frog), vehicle plus 1 µg GnRH agonist (GnRHa, Buserelin, Hoechst) per frog, vehicle plus L-dopa (Simes) 20 µg/frog, vehicle plus 20 µg L-dopa plus 200 µg L-dopa antagonist (pimozide, Le Petit) per frog, vehicle plus 1 µg GnRHa plus 200 µg pimozide per frog. The sample size of HPX frogs was reduced (n = 5) due to mortality after operation.

Injections were made into the dorsal sac. A curious observation is the presence of a black pigment, probably dopacrome, in the water of the box containing animals treated with L-dopa alone. Samples were taken with Pasteur pipettes from the cloacae of the frogs 90 and 150 min after the injection and were examined for the presence of spermatozoa by light microscopy (magnification × 100). All frogs were examined before the injection to eliminate false positive results.

After the injections (2.5 h), frogs were anesthesized with MS 222 (Sigma) and blood and testes were collected. Plasma and left testes were stored at −80 °C until androgen measurement by RIA as previously described[11]. Since the antiserum (G. F. Bolelli, Bologna, Italy) crossreacts strongly with testosterone and 5a-dihydrotestosterone, data are expressed as 'androgens'. The remaining testes were placed in Bouin's fluid for histological examination. Data were evaluated statistically by Fisher's method and ANOVA when appropriate.

Results and discussion

Spermiation occurs in intact frogs (table 1) treated either with GnRHa or with L-dopa. The effect observed after injecting GnRHa confirms the role played by gonadotrophins in sperm-releasing activity (Weiner et al.[12], for review). Pimozide inhibits the L-dopa-induced effect, thus indicating that the action of L-dopa is mediated via specific receptors. Pimozide does not block

Table 1. Sperm-releasing activity in intact frogs treated with vehicle, buserelin (GnRHa), dopamine (L-dopa) or L-dopa plus an antagonist (Pim) 150 min after the injection

Treatment	Response Spermiated	Non-spermiated	Significance
Vehicle	0	10	a
GnRHa	10	0	b
GnRHa + Pim	10	0	b
L-dopa	10	0	b
L-dopa + Pim	0	10	a

a vs b $p < 0.1$; a vs a NS.; b vs b NS.

Table 2. Sperm-releasing activity in hypophysectomized frogs treated with vehicle, buserelin (GnRHa), dopamine (L-dopa) or L-dopa plus an antagonist (Pim) 150 min after the injection

Treatment	Response Spermiated	Non-spermiated	Significance
Vehicle	0	5	a
GnRHa	5	0	b
GnRHa + Pim	5	0	b
L-dopa	5	0	b
L-dopa + Pim	0	5	a

a vs b $p < 0.01$; a vs a NS.; b vs b NS.

spermiation in frogs treated with GnRHa, indicating that the effect exerted by GnRHa does not occur via dopamine activation. Results obtained 90 min or 150 min post-injection are similar. Histological observations show empty tubules in spermiating animals, whereas in L-dopa plus pimozide-treated animals, testes are full of spermatozoa (not shown).

GnRHa, injected alone or in combination with pimozide and L-dopa alone both cause a significant ($p < 0.05$) increase in androgen levels in plasma and testes of intact frogs (fig. 1). The increase in androgen concentration in plasma and testes following L-dopa treatment is probably due to a dopamine-induced gonadotrophin release as suggested for mammals, where both a stimulatory and inhibitory role for dopamine in the regulation of GnRH secretion has been described[13]. However, it is well documented in teleosts that dopamine inhibits GnRH-induced gonadotrophin release, while pimozide and other dopamine antagonists potentiate the response of gonadotropes to GnRH[14,15].

L-dopa and GnRHa also provoke spermiation in HPX frogs. This effect is prevented by pimozide treatment in L-dopa-injected animal. Conversely, pimozide does not counteract GnRHa-induced spermiation. Data collected 90 min or 150 min post-injection are similar. These results confirm previous observations of a direct effect exerted by GnRHa on spermiation induction[4], and indicate that L-dopa may induce spermiation either directly through interaction with specific testicular binding sites or indirectly by enhancing GnRH release. It is clear, however, that GnRHa does not operate by stimulating dopamine release (table 2). The histological observations indicate that the testes of HPX animals are characterized by empty tubules in spermiating animals, while testes full of spermatozoa are observed in the non-spermiating frogs (fig. 2). A significant decrease in plasma androgen concentration is observed in control animals (0.95 ng/ml plasma) as compared with the intact control group (5.3 ± 1 ng/ml plasma; $p < 0.01$) due to the lack of gonadotrophins, while no significant differences are observed between experimental groups. Thus dopamine, while directly inducing sperm release, does not affect androgen production in HPX animals (shown also for serotonin in Pierantoni et al.[16]).

In conclusion, our results indicate that dopamine acts in frog testes via central and, probably, local mechanisms.

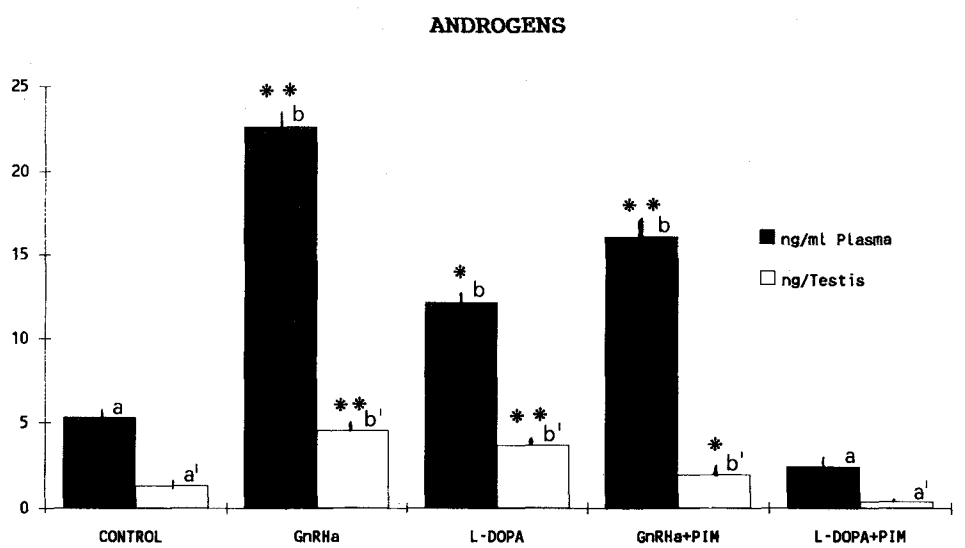

Figure 1. Androgen levels in plasma and testes of intact frogs, *Rana esculenta*. Values are expressed as means ±SD. * $p < 0.05$ (a vs b) and (a′ vs b′) ** $p < 0.01$ (a vs b) and (a′ vs b′)

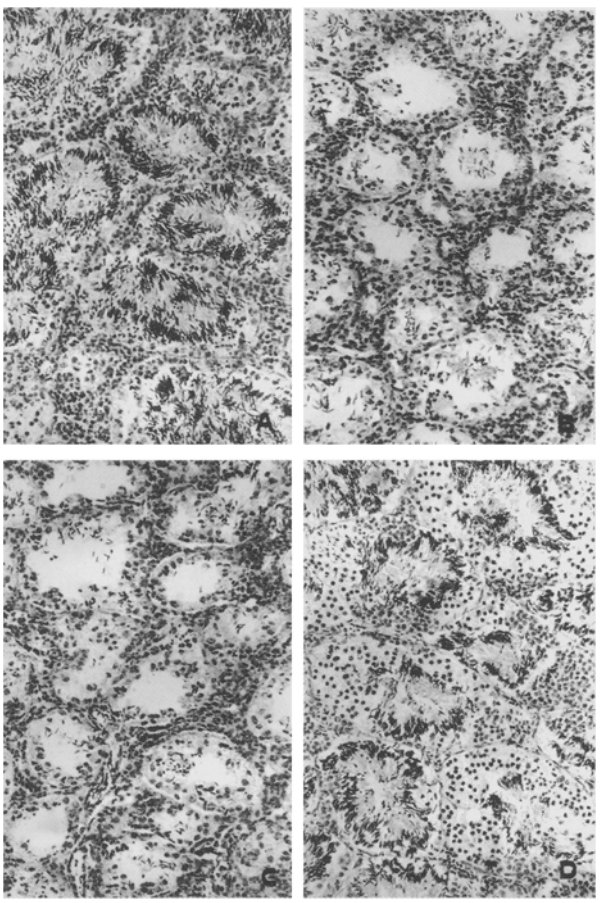

Figure 2. Seminiferous tubules full of spermatozoa from hypophysectomized frogs treated with vehicle (A) or with L-dopa + pimozide (D) in combination, and practically empty seminiferous tubules from frogs treated with L-dopa (B) or GnRHa + pimozide (C). × 190.

In particular, at the testicular level spermiation may be induced through specific binding sites.

1 This work was supported (40% and 60%) by the Italian Ministry of Education and the C.N.R. Special Project.

2 Renlund, S., and Hallin, P., Gen. comp. Endocr. *60* (1985) 333.
3 Lakaomaa, E., Acta Univ. Ups. Abstr., Uppsala Diss. Fac. Sci. *316* (1974).
4 Minucci, S., Di Matteo, L., Chieffi Baccari, G., and Pierantoni, R., Experientia *45* (1989) 1118.
5 Mora, S., and Diaz-Veliz, G., Pharmac. Biochem. Behav. *24* (1986) 433.
6 Wilson, J. X., Br. J. Pharmac. *85* (1985) 647.
7 Wilson, J. X., Van Vliet, B. N., and West, N. M., Neuroendocrinology *39* (1984) 437.
8 Wilson, J. X., Saleh, K. J., Armogan, E. D., and Jaworska, E. J., Can. J. Physiol. Pharmac. *65* (1987) 2379.
9 Easley, K. A., Culley, D. D., Horseman, N. D., and Penkala, J. E., J. Exp. Zool. *207* (1979) 407.
10 Stutinsky, F., and Befort, J. J., Gen. comp. Endocr. *4* (1964) 370.
11 Pierantoni, R., Fasano, S., Di Matteo, L., Minucci, S., Varriale, B., and Chieffi, G., Molec. Cell. Endocr. *38* (1984) 215.
12 Weiner, R. I., Findell, P. R., and Gordon, C., in: The Physiology of Reproduction, vol. 1, p. 1235. Eds E. Knobil and J. D. Neil. Raven Press, New York 1988.
13 Chieffi, G., Pierantoni, R., and Fasano, S., Int. Rev. Cytol. *127* (1991) 1.
14 Peter, R. E., Chang, J. P., Nahorniak, C. S., Omeljaniuk, R. J., Sokolowska, S. H., Shih, S. H., and Billard, R., Rec. Prog. Horm. Res. *42* (1986) 513.
15 Timmers, R. J. M., Goos, H. J. T., Lambert, J. G. D., and van Oordt, P. G. W. J., Fish Physiol. Biochem. *6* (1989) 207.
16 Pierantoni, R., Varriale, B., Minucci, S., Di Matteo, L., Fasano, S., D'Antonio, M., and Chieffi, G., Gen. Comp. Endocr. *64* (1986) 405.