Effect of serum thymic factor (FTS) on splenic weight, concanavalin-A responsive spleen cell proliferation and blood leukocyte number in lethally or half-lethally X-irradiated mice.

Treatment[a]	Spleen weight (mg)	^{3}H-TdR uptake[b] by spleen cells ($\times 10^{-3}$ cpm/well)	Peripheral blood leukocyte number ($\times 10^{-3}$/mm^3)
None	116 ± 5[c]	74.8 ± 2.8	4.7 ± 0.4
Saline + 567 cGy	33 ± 4	3.6 ± 2.3	0.4 ± 0.1
FTS + 567 cGy	66 ± 11*	59.0 ± 9.0**	2.7 ± 0.8*
Saline + 756 cGy	38 ± 3	ND	0.3 ± 0.04
FTS + 756 cGy	70 ± 11*	ND	1.2 ± 0.7

[a] The animals were injected daily with saline or FTS twice before and 7 times after irradiation and killed 18 h after the last injection (i.e., 7 days after irradiation). Each group consisted of 5–12 mice. [b] 4 assays from 2 studies. Radioactive thymidine uptake was negligibly small in the absence of concanavalin A (16–23 cpm/well). [c] Means ± SEM. * Significantly different from the saline groups (* $p < 0.05$, ** $p < 0.01$). ND, not done.

(table). Furthermore, spleen cells of the treated animals retained a greater proliferative activity than those of control animals, as evidenced by ^{3}H-thymidine uptake in response to concanavalin A. The results suggest that T-cells competent to respond to the proliferative stimulus had been increased in number in the spleen of FTS-treated mice. In parallel with the increased weight and proliferative activity of spleen, there was a significant increase in the number of leukocytes in the peripheral blood of these animals.

The figures in the table for the protected mice have larger variations (i.e., larger standard errors, SE) than the figures for the unprotected animals. This is a reflection of the fact that FTS increased the number of animals which had recovered from the hematologic disorder, while it left some animals as sick as the unprotected ones. The hematologic picture agreed well with the mortality pattern described above, i.e. that some animals were saved but others were killed even in the FTS-treated group. The precise mechanism of the radioprotective action of FTS is unclear at present. Increase in leukocyte number is presumably not due to a direct effect of FTS on hematopoiesis, but it must contribute greatly to the rescue of mice from radiation-induced death.

Conclusion

FTS has the following advantages over other known radioprotective substances: first, it is a natural peptide hormone; second, its toxicity is very low; third, it can be chemically synthesized; fourth, it is quite stable; fifth, it is effective even when administered after irradiation. The present study raises the possibility of using FTS as a prophylactic or therapeutic drug in cancer radiotherapy as well as after radiation accidents.

Acknowledgment. We thank E. Kuramoto, O. Mizuno, T. Sasaki, T. Ohtawa, T. Ohya, Y. Miyama, Dr H. Ishii and Dr K. Tsuneoka for their continuous cooperation.

1 Rojas, A., and Denekamp, J., Experientia *45* (1989) 41.
2 Neta, R., Oppenheim, J. J., and Douches, S. D., J. Immun. *140* (1988) 108.
3 Neta, R., and Oppenheim, J. J., Blood *72* (1988) 1093.
4 Butturini, A., De Souza, P. C., Gale, R. P., Cordiero, J. M., Lopes, D. M., Neto, C., Cunha, C. B., De Souza, C. E. P., Ho, W. G., Tabak, D. G., Sanpai, J. M., Burla, A., and the Navy Hospital Radiation Team, Lancet *ii* (1988) 471.
5 Hanks, G. E., and Ainsworth, E. J., Radiat. Res. *32* (1967) 367.
6 Bach, J.-F., Dardenne, M., Pleau, J. M., and Rosa, J., Nature *266* (1977) 55.
7 Pleau, J.-M., Gastinel, L. N., and Bach, J.-F., Meth. Enzym. *116* (1985) 269.
8 Dardenne, M., Pleau, J.-M., Nabarra, B., Lefrancier, P., Derrien, M., Choay, J., and Bach, J.-F., Proc. natl Acad. Sci. USA *79* (1982) 5370.
9 Nagai, Y., Osanai, T., and Sakakibara, K., Japan. J. exp. Med. *52* (1982) 213.
10 Kato, S., and Nakamura, H., Acta neuropath. (Berl.) *75* (1988) 337.
11 Tsuneoka, K., Takagi, Y., and Shikita, M., J. Radiat. Res. *18* (1977) 102.
12 Miyanomae, T., and Frindel, E., Exp. Hemat. *16* (1988) 801.

0014-4754/90/050484-03$1.50 + 0.20/0

The effect of guanethidine treatment of testicular blood flow and testosterone production in rats

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Received 4 April 1989; accepted 1 December 1989

Summary. Testicular blood flow was measured by means of Xenon-133 clearance in control rats and rats treated with guanethidine for 3 weeks. Plasma and testis testosterone concentrations were also measured, and the effect of hCG-treatment was examined. No difference in testicular blood flow between the control group and the guanethidine-treated group was found. However, in guanethidine-treated rats, plasma and testis testosterone concentrations after hCG-treatment were significantly decreased. The results may indicate that adrenergic nerves are involved in the regulation of Leydig cell function.
Key words. Guanethidine; testis; testicular blood flow; hCG; testosterone.

The important role of the hypothalamus and pituitary in the regulation of testicular function is well established, while little is known about the role of the nervous system. Examination of intratesticular innervation in several spe-

cies [1] revealed catecholamine-containing nerve fibers in a moderately dense plexus around the testicular artery. Norberg et al. [2] described adrenergic nerve terminals associated with blood vessels in the rat testis, but probably with no contact with Leydig cells. Several authors have also reported a decreased testicular blood flow after systemic and local administration of catecholamines [3,4]. It has been suggested that the testicular nerves mediate the compensatory testicular hypertrophy occurring after hemiorchidectomy [5]. Furthermore, systemic administration of catecholamines reduced plasma testosterone concentration in rat and man [6–8]. Beta-adrenergic receptors have also been demonstrated in Leydig cells, and catecholamines stimulate adenylate cyclase and steroidogenesis in cultured Leydig cells [9,10]. In a recent study [11], it was shown that long-term treatment with isoproteronol resulted in a marked Leydig cell hypertrophy. Thus, there are several lines of evidence suggesting that nerves play a role in testicular physiology.

The present study was designed to investigate the effect of long-term treatment of rats with the sympathetic-blocking agent guanethidine on testicular blood flow and testosterone secretion. Previous studies have shown that guanethidine is effective in blocking the adrenergic neurones supplying the reproductive organs in the male rat [12,13].

Experimental

Adult male rats of the Sprague-Dawley strain (ALAB, Sweden) were kept in a controlled environment and fed a standard rat diet. The rats were anesthetized with sodium pentobarbitone given i.p. as a single injection of a dose of 40 mg/kg. Guanethidine was dissolved in saline and injected daily i.p. for 3 weeks in a dose of 15 mg/kg body weight and control rats received i.p. injections of saline. Testicular blood flow was measured using the Xenon-133 clearance technique as previously described [14]. Plasma and testis testosterone were measured using radioimmunoassay [15].

Results and discussion

The testicular blood flow measurements revealed no significant difference between control and guanethidine-treated rats, as shown in table 1. It is well known that local administration of catecholamines to the testis induces vasoconstriction [4,16]. However, based on the present results, it appears that the testicular adrenergic innervation, at least under basal conditions, is of minor importance for the regulation of testicular vascular resistance. Basal plasma and testis testosterone concentrations were also similar in the two groups of rats, while these concentrations were decreased in guanethidine treated rats 2 h after injections of hCG (table 2). The mechanism behind the latter finding is unknown. It may be due to a direct effect on the Leydig cells, since adrenergic substances are able to stimulate adenylate cyclase and steroidogenesis in cultured Leydig cells [9,10]. In contrast, however, is the finding that catecholamines inhibited hCG-induced accumulation of cAMP in primary cultures of porcine Leydig cells [17]. In conclusion: adrenergic denervation of the testis by means of long-term guanethidine treatment does not induce any major change in testicular blood flow or testosterone production. There is, however, a small but significant reduction of plasma and testis testosterone concentration after hCG-treatment, indicating a possible role of adrenergic neurones in the fine regulation of Leydig cell function.

Table 1. Testicular blood flow in control and guanethidine-treated rats

Treatment	No.	Testicular blood flow (ml/100 g × min)
Control	20	18.4 ± 1.0
Guanethidine	20	16.9 ± 0.9

Values expressed as mean ± SEM.

1 Dayan, A. D., Experientia *26* (1970) 1359.
2 Norberg, K. A., Riesley, P. L., and Ungerstedt, U., Z. Zellforsch. *76* (1967) 278.
3 Setchell, B. P., Waites, G. M. H., and Thorburn, G. D., Circulation Res. *18* (1966) 755.
4 Free, M. J., and Jaffe, R. A., Am. J. Physiol. *223* (1972) 241.
5 Gerendai, J., Nemeskeri, A., and Csernus, V., Exp. clin. Endocr. *84* (1984) 27.
6 Lewin, J., Lloyd, J., Lobotsky, J., and Friedrich, E. H., Acta endocr. *55* (1967) 184.
7 Damber, J.-E., and Janson, P. O., Acta endocr. *88* (1978) 390.
8 Götz, F., Stahyl, F., Rhode, W., and Dorner, G., Exp. clin. Endocr. *83* (1983) 239.
9 Damber, J.-E., and Selstam, G., Acta endocr. *103* (1983) abstr. 250.
10 Cooke, B. A., Golding, M., Dix, C. J., and Hunter, M. G., Molec. cell. Endocr. *27* (1982) 221.
11 Bergh, A., Blom, H., Damber, J.-E., and Henriksson, R., Andrologia *19* (1987) 448.
12 Evans, B., Gannon, B. J., Heath, J. W., and Burnstock, G., Fert. Steril. *23* (1972) 657.
13 Gannon, B. J., Iwayama, T., and Burnstock, G., Med. J. Aust. *2* (1971) 207.
14 Damber, J.-E., and Janson, P. O., Acta physiol. scand. *101* (1977) 278.
15 Damber, J.-E., and Janson, P. O., Acta endocr. *88* (1978) 390.
16 Damber, J.-E., Lindahl, O., Selstam, G., Tenland, T., Acta physiol. scand. *115* (1982) 209.
17 Renier, G., Gaulin, J., Gibb, W., Collu, R., and Ducharme, J. R., Can. J. Physiol. Pharmac. *65* (1987) 2053.

0014-4754/90/050486-02$1.50 + 0.20/0

Table 2. The effect of guanethidine treatment on plasma and testis testosterone concentration

Treatment	Basal testosterone concentration		Testosterone concentration 2 h after hCG	
	Plasma (ng/ml)	Testis (ng/g)	Plasma (ng/ml)	Testis (ng/g)
Control	3.2 ± 0.4 (20)	111 ± 12 (20)	32.8 ± 2.7 (10)	393 ± 22 (10)
Guanethidine	3.1 ± 0.3 (18)	114 ± 15 (17)	23.8 ± 1.3 (10)	321 ± 25 (10)

Values expressed as mean ± SEM. Number of rats in parentheses. *$p < 0.05$. Significantly lower than in control rats according to Mann-Whitney's U-test.