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Effect of continuous low-dose γ-irradiation on rat Sertoli cell function (*)

P. KAMTCHOUING ([1]), Ghislaine PINON-LATAILLADE ([2]), V. PAPADOPOULOS ([1]), J. M. GUILLAUMIN ([3]), P. BARDOS ([3]), J. MAAS ([2]), Christine PERREAU ([4]), M. A. DROSDOWSKY ([1]), Marie-Thérèse HOCHEREAU de REVIERS ([4]), S. CARREAU ([1]) (**)

([1]) *Laboratoire de Biochimie, UA CNRS 609, CHU Côte de Nâcre, 14033 Caen, France.*
([2]) *CEA, Service de Pathologie Expérimentale, 92265 Fontenay-aux-Roses.*
([3]) *Laboratoire d'Immunologie, Faculté de Médecine, CHU, 37000 Tours.*
([4]) *Physiologie de la Reproduction, I.N.R.A., Nouzilly, 37380 Monnare.*

Summary. Continuous low-dose γ-irradiation of mature rats induced a progressive degeneration of the germ cells. Blood FSH increased by 127, 176 and 214 %, respectively, after 55, 70 and 85 days of treatment when compared to FSH levels in control rats (8.50 ± 0.60 ng/ml) ; conversely, serum LH and testosterone levels were unchanged. The Sertoli cell function was affected by the treatment from 70 days on, as attested by androgen binding protein (ABP) and transferrin secretions which diminished 35-40 %. Serum ABP levels were not altered, whatever the duration of irradiation, even though epididymal ABP contents (as well as concentrations) diminished 34-60 % when compared to those of the controls. Moreover, in purified Leydig cells, LH-stimulated intracellular cAMP levels, which were decreased by seminiferous tubule medium (STM) from control rats, were enhanced in presence of STM from treated animals. Testosterone output was stimulated 9-fold in presence of oLH and further increased (46-76 %) from stages XIV-V by STM prepared from control and irradiated rats, respectively. After 85 days the STM effects on both cAMP and testosterone syntheses were zero. These results demonstrate a probable alteration of Sertoli cell function after irradiation, but also a role of the germ cells in the regulation of the synthesis of ABP, transferrin and Sertoli cell paracrine factors.

Introduction.

Numerous studies related to seminiferous tubule damage of rat testis have shown changes in gonadotrophins and testosterone levels as well as modification of Leydig cell function. After acute or chronic irradiation, serum FSH level

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(**) All correspondance should be addressed to S. Carreau.

increased while LH and testosterone levels were less or unaffected (Verjans and Eik-Nes, 1976 ; Main *et al.*, 1978 ; Wang *et al.*, 1983 ; Pinon-Lataillade *et al.*, 1985). In addition, any alteration in the seminiferous tubule induced by cryptorchidism, vitamin A deficiency (Risbridger *et al.*, 1981 ; Bergh *et al.*, 1984) or acute irradiation of immature rats provoked hypertrophy of the Sertoli cells (Aoki and Fawcett, 1978) as well as modification of Sertoli cell secreted factors involved in the paracrine control of Leydig cell function (Papadopoulos *et al.*, 1987). The secretion of some Sertoli cell proteins (androgen binding protein : ABP, transferrin, cyclic protein 2) vary markedly at different stages of the seminiferous epithelium cycle (Parvinen, 1982 ; Mather *et al.*, 1983). The purpose of the present study was to examine changes in ABP and transferrin secretions as well as in the factors involved in paracrine control of Leydig cell function, following continuous low-dose γ-irradiation of mature rats.

Material and methods.

Animals. — Male Sprague-Dawley rats (IFFA CREDO) 3 months old were housed under standard conditions. The animals had been continuously whole-body irradiated with ^{60}Co γ-ray at a dose rate of 7 cGy per day for 40, 55, 70 and 85 days. The groups of control (n = 4) and irradiated (n = 4) rats were sacrificed by decapitation in the morning ; the blood was collected and allowed to clot at room temperature, and the serum was then stored at − 20 °C until assay. The testes and epididymides were removed, cleared of adherent fat and weighed.

Preparation of seminiferous tubule medium (STM) for culture. — Seminiferous tubules (ST) were prepared using the transillumination technique (Parvinen and Vanha-Pertulla, 1972) and classified into the different stages (VI-VIII, IX-XIII, XIV-V) ; the purity of the seminiferous tubule preparations was checked as described by Parvinen *et al.* (1984). Increasing lengths of ST (50, 100, 200 mm) were incubated for 20 h at 32 °C in 1 ml of Ham F12-DME medium alone (transferrin assay) or supplemented with oFSH (5 µg/ml) and testosterone (200 ng/ml). The STM was collected and charcoal-treated prior to addition to the Leydig cell incubation medium.

Purification and incubation of Leydig cells. — Leydig cells from control rats were purified (> 80 %) using a discontinuous Percoll gradient (Papadopoulos *et al.*, 1985). Cells (10^5) were incubated for 5 h at 32 °C under O_2/CO_2 (95 : 5, v/v) with a saturating amount of oLH (25 ng/ml) and with or without the various STM (40 %, v/v).

Miscellaneous. — Testosterone and intracellular cAMP were determined by radioimmunoassay (RIA) as reported elsewhere (Papadopoulos *et al.*, 1987). STM ABP content and epididymal and serum ABP levels were measured by RIA (Gunsalus *et al.*, 1978) using rabbit anti-rat ABP. Antibody-bound and free ABP

were separated after incubation with sheep antirabbit γ-globulin (Papadopoulos *et al.*, 1987). Transferrin secretion was assayed in STM by RIA (Khalfoun *et al.*, 1986). Serum gonadotrophin levels were determined by RIA using specific kits provided by NIADDK (NIH, Bethesda). Total seminiferous tubule length and total number of Sertoli cells per testis were determined in previous experiments (Pinon-Lataillade, 1986) as described by Attal and Courot (1963). The number of Sertoli cells per mm of ST were then calculated and compared to transferrin and ABP secretions.

Statistical analysis. — The data were expressed as means $\pm$ SEM and Student's t-test was used to compare mean values.

Results.

Testicular and epididymal weights. — Body weights were not affected by the duration of irradiation (40, 55, 70 and 85 days) ; conversely, testicular weights decreased by 23, 45, 54 and 58 %, respectively, when compared to those of the control rats (2.13 $\pm$ 0.05 g). Epididymal weights decreased significantly (20-25 % ; $P < 0.001$) in irradiated rats, starting at 55 days, when compared to control animals (0.39 $\pm$ 0.01 g).

Serum gonadotrophin and testosterone levels. — No significant changes in serum testosterone (1.90 $\pm$ 0.02 ng/ml) and LH (0.52 $\pm$ 0.02 ng/ml) were noted in any experimental group (fig. 1) ; conversely, FSH levels increased 127, 176 and 214 %, respectively, at 55, 70 and 85 days of irradiation when compared to the controls (8.50 $\pm$ 0.60 ng/ml).

ABP levels in serum, epididymis and STM. — Serum ABP levels were unchanged in relation to the duration of irradiation ; in contrast, epididymal ABP contents (as well as ABP concentrations) were significantly diminished by 34, 42, 56 and 59 % as compared to the control (table 1). In control STM preparations (fig. 2) ABP levels increased according to ST length regardless of age ($r > 0.90$; $P < 0.05$) and were maximal at stages VI-VIII (60 $\pm$ 2 fmol/200 mm ST length/ml, which corresponded to a secretion of 1.76 fmol/10^4 Sertoli cells/ml) and did not differ from those in the STM of irradiated rats until the 55th day of irradiation. After 70 and 85 days, the ABP levels were measured separately in STM prepared from either « empty » or « dark » (with remaining germ cells, mostly elongated spermatids) tubules. As shown on figure 2, a sharp and significant decrease of ABP levels was seen in rats irradiated for 85 days (22 and 56 fmols, *i.e.* 0.42 and 1.16 fmol/10^4 Sertoli cells/ml, respectively, in empty and dark tubules). It is noteworthy that these STM ABP levels were 55 and 30 % lower than those in control STM.

Transferrin levels (table 2). — The mean secretion of transferrin increased with ST length ($r = 0.67$, $P < 0.05$) ; it varied slightly with the seminiferous epithelium cycle and was minimal in stage VI-VIII. In control rats the transferrin

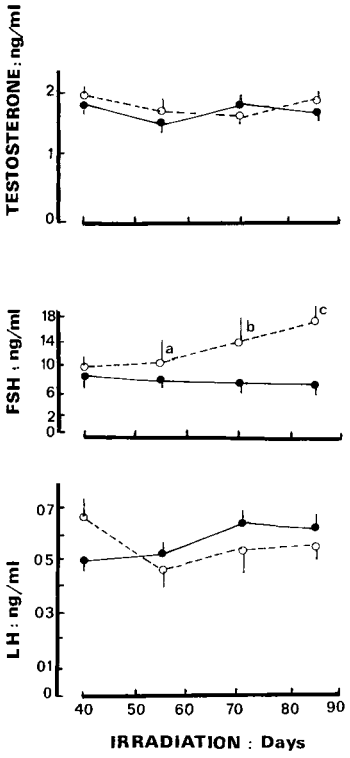

FIG. 1. — *Serum gonadotropins and testosterone levels* (results are means ± SE, n = 4).
●———● : control rat ; ○- - - - -○ : irradiated rat.
a : $p < 0.05$; b : $p < 0.01$; c : < 0.001, compared to control rat.

TABLE 1

Age-related effects of chronic irradiation on epididymal and serum ABP levels.

AGE (Day)	Epididymal ABP		Serum ABP (fmole/ml)
	Content (fmole/organ)	Concentration (fmole/g)	
90 + 40 C	15 199 ± 839	38 319 ± 1 910	898 ± 37
90 + 40 IR	10 097 ± 848**	25 291 ± 1 996**	965 ± 48
90 + 55 C	15 391 ± 1 031	34 089 ± 1 287	1 054 ± 48
90 + 55 IR	8 964 ± 367**	26 938 ± 290**	969 ± 17
90 + 70 C	21 126 ± 2 054	51 481 ± 3 414	807 ± 26
90 + 70 IR	9 297 ± 243***	29 894 ± 1 048***	900 ± 18
90 + 85 C	17 732 ± 323	45 454 ± 434	1 175 ± 56
90 + 85 IR	7 286 ± 157***	24 317 ± 888***	1 210 ± 79

Results are means + SEM (n = 4). C : control ; IR : irradiated rats.
** : $p < 0.01$; *** : $p < 0.001$ compared to control animals.

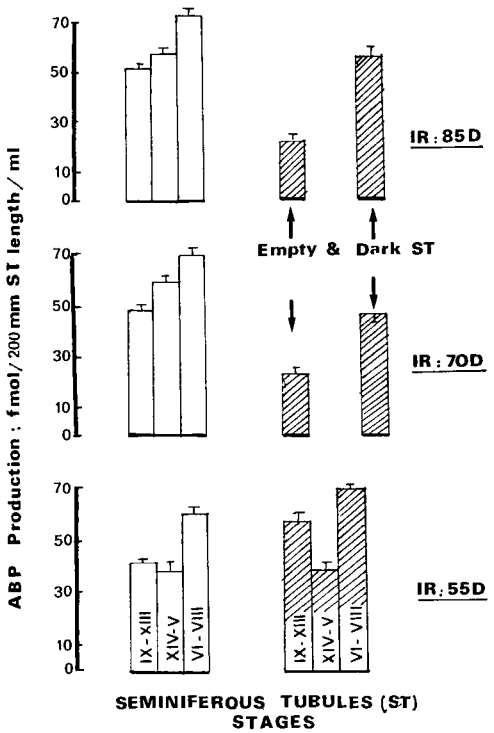

FIG. 2. — *ABP levels in seminiferous tubule* (200 mm length) *medium after 55, 70 and 85 days of irradiation* (IR). Empty & Dark ST : seminiferous tubules without (empty) or with (dark) remaining germ cells.
□ : control rat ; ▨ : irradiated rat.

TABLE 2

Effect of chronic α-irradiation (7 cGy/day) *on seminiferous tubule* (ST) *parameters and transferrin* (Tf) *output in ST culture media.*

Treatment	ST length per testis (m) (1)	Sertoli cell number/10 mmST × 10^4 (1,2)	Tf levels ng/10 mmST/ml	Tf output/10^4 Sertoli cell/ml (3)
Control (4)	19.9	1.7	21.8 ± 5.0 (36*)	12.8
90 + 55 IR (2)	17	2	23.6 ± 4.6 (15)	11.8
90 + 70 IR (1)	14	2.4	38.5 ± 12.6 (3)	16
90 + 85 IR (1)	14	2.4	12.9 ± 6.1 (4)	5.4

Number of animals in parentheses ; * : number of STM tested.
1) From Pinon-Lataillade (1986). 2) The mean total number of Sertoli cells/testis observed was 65 × 10^6 (Pinon-Lataillade, 1986). After correction for their nuclear size, the true number was 34 × 10^6. 3) Number of Sertoli cells/10 mm and Tf secretion were obtained in 2 independent series of animals.

output was 21.8 ng/10 mm ST length, which corresponded to a secretion of 12.8 ng/10^4 Sertoli cells/ml. In irradiated animals no significant variation of the mean secretion of transferrin was observed ; however when compared to the number of Sertoli cells per 10 mm of ST, the secretion of transferrin dropped at 85 days of irradiation.

Leydig cell cAMP and testosterone levels : effects of STM. — In purified rat Leydig cells, LH-stimulated intracellular cAMP levels (2.57 pmol/10^5 Leydig cells) were decreased slightly (non-significant) by STM from those of the 40-day old control rats, whatever the ST stage ; however as already reported (Papadopoulos *et al.*, 1986), with a 100 mm length of ST this effect was less than with a 200 mm length. The STM from irradiated rats induced an increase of cAMP at least during the first 70 days of irradiation, although there was no effect of STM in rats treated for 85 days (table 3). Testosterone output was enhanced 9-fold by LH (13.115 ng/10^5 Leydig cells) and the addition of control STM provoked a further increase of testosterone production. This effect was noted for STM collected from stages XIV-V and VI-VIII and was more marked with ST from stages XIV-V. The addition of STM from irradiated rats also induced an augmentation of testosterone production until the 55th day of irradiation. After 70 days, the STM effect had decreased and was negligible on day 85. Increasing the length of ST from 25 to 100 mm enhanced the effect of STM on Leydig cell testosterone output, even though an opposite effect was noted for a 200 mm length (data not shown).

TABLE 3

Effects of STM (40 %, v/v) *on LH-stimulated Leydig cell function.*

Age (Day)	Intracellular cAMP (% of control : 2.57 pmol)			Testosterone (% of control : 13.11 ng)		
	a	b	c	a	b	c
90 + 40 C	− 10	− 10	− 18	− 7	+ 47	+ 52
90 + 40 IR	− 3	+ 25	− 7	− 3	+ 41	+ 62
90 + 55 C	+ 6	− 19	+ 18	0	+ 46	+ 33
90 + 55 IR	+ 60	+ 33	+ 75	+ 81	+ 76	+ 79
90 + 70 C	0	0	0	0	+ 44	+ 23
90 + 70 IR	Empty	Tubules	+ 110	Empty	Tubules	+ 20
90 + 85 C	0	+ 9	+ 11	+ 5	+ 54	+ 32
90 + 85 IR	Empty	Tubules	+ 5	Empty	Tubules	+ 5

Control : Leydig cells + LH.
Results expressed per 10^5 Leydig cells/5 h.
a : Seminiferous tubule stage IX-XIII (100 mm length).
b : Seminiferous tubule stage XIV-V (100 mm length).
c : Seminiferous tubule stage VI-VIII (100 mm length).

Discussion.

Continuous low-dose γ-irradiation for up to 85 days had no effect on the body weight of mature rats but caused a decrease of testicular weight as a

consequence of seminiferous tubule depletion in germ cells. As reported by Pinon-Lataillade *et al.* (1985), this treatment did not change the LH and testosterone levels in plasma, which explains why epididymal weights were not decreased very much after irradiation ; moreover, the perivascular Leydig cell profile area was not modified (Pinon-Lataillade *et al.*, 1987). Conversely, serum FSH levels increased in relation to the duration of irradiation and were maximal after 85 days of treatment when the proportion of seminiferous tubules containing germ cells (pachytene spermatocytes and round spermatids) was less than 10 % of that of the controls (Pinon-Lataillade, 1986). The augmentation in FSH levels is interpreted as decreased Sertoli cell production of inhibin (Setchell *et al.*, 1977) ; indeed, the Sertoli cell secretory products we studied (ABP, transferrin, STM factors) did show changes depending on the duration of irradiation. Nevertheless serum ABP levels were similar to those of the controls, while epididymal ABP decreased ; this was a result of a reduction in seminiferous tubule fluid excretion since ST length and diameter were reduced (Pinon-Lataillade *et al.*, 1985). Similarly, the profile area of the Sertoli cells decreased significantly in irradiated rats when compared to the controls (Pinon-Lataillade *et al.*, 1987). These observations support the hypothesis that the factors involved in the bipolar excretion of ABP are different (Carreau, 1986) and the decrease in STM ABP content, observed after 70 days of treatment, indicates an interaction between germ cells (mainly elongated spermatids, Pinon-Lataillade, 1986) and Sertoli cells. However, we cannot exclude an earlier signal provided by the pachytene spermatocytes, the first germ cells destroyed and known to stimulate Sertoli cell ABP synthesis (Galdieri *et al.*, 1984). As for ABP, the transferrin secretion expressed per Sertoli cell was maintained up to day 70 of treatment, whatever the germ cell type present ; a sharp drop was then observed at 85 days of irradiation when the elongated spermatids disappeared, especially in empty tubules which represented 40 % of the ST (Pinon-Lataillade, 1986).

Concerning the STM factors involved in paracrine control of Leydig cell function (intracellular cAMP and testosterone levels), we have previously reported that these factors produced by mature Sertoli cells were under germ cell control (Papadopoulos *et al.*, 1987). Here, we confirm this hypothesis and moreover have shown that STM from stages XIV-V is more effective on Leydig cell testosterone synthesis than STM from stages VI-VIII ; conversely, STM collected from ST stages IX-XIII is ineffective.

Moreover, it should be noted that, after 55 days of irradiation, the STM factors, involving Leydig cell synthesis of both cAMP and testosterone, were more effective when compared to STM factors from the controls. Our results confirm those of Parvinen *et al.* (1984) and Syed *et al.* (1986).

Taken together, these data show alterations of Sertoli cell function after chronic irradiation but also the different roles of the germ cells in these processes.

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Résumé. *Effets de l'irradiation continue γ à faible dose sur la fonction sertolienne du rat.*

L'irradiation continue du rat mature induit une dégénérescence progressive des cellules germinales et une augmentation de la FSH sérique (127, 176 et 214 %, respectivement après 55, 70 et 85 jours de traitement) par rapport au taux de FSH des rats contrôles (8,50 $\pm$ 0,60 ng/ml) ; les taux de LH et de testosterone sériques sont inchangés. La fonction sertolienne, explorée en dosant les sécrétions d'ABP et de transferrine qui sont diminuées de 35-40 %, est donc affectée par le traitement à partir du 70^{e} jour. Les taux d'ABP sériques ne sont pas modifiés alors que les contenus épididymaires d'ABP (ainsi que les concentrations) sont diminuées (34 à 60 %, entre 40 et 85 jours) par rapport à ceux des rats contrôles. De plus, dans les cellules de Leydig, la stimulation de la synthèse d'AMP cyclique par LH est diminuée par le STM de rat non traité et augmentée par le STM de rat irradié. En présence de oLH, la synthèse de testostérone qui est stimulée 9 fois, est accrue (46-76 %) par l'addition de STM provenant de tubes séminifères des stades XIV-V, préparés chez les rats normaux ou traités, respectivement. Après 85 jours de traitement, les effets du STM sur les synthèses d'AMP cyclique et de testostérone sont abolis. Ces résultats démontrent une altération de la fonction sertolienne après irradiation induite par la disparition progressive des cellules germinales et le rôle de celles-ci dans la régulation des synthèses d'ABP, de transferrine et des facteurs paracrines sertoliens.

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