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EFFECTS OF INTERLEUKIN-1β ON STEROIDOGENESIS IN LEYDIG CELLS OF THE RAT TESTIS: *IN VIVO* AND *IN VITRO* STUDIES

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ABSTRACT

The effects of interleukin-1β (IL-1β) on the secretory activity of Leydig cells were investigated in hypophysectomized/human chorionic gonadotropin (hCG)-treated rats. IL-1β dose-dependently increased basal and hCG-stimulated plasma testosterone concentration (PTC) and testosterone secretion by isolated Leydig cells. Basal PTC returned to the control level 12 h after the injection of a maximal effective dose of IL-1β (10 nmol/rat), but after 24 and 36 h it was markedly lower than in control animals; after 48 h PTC again attained the control value. IL-1β did not affect basal PTC in rats injected 24 h before with 10 nmol of IL-1β (IL-1β-pretreated rats), but it dose-dependently decreased hCG-stimulated PTC. However, IL-1β dose-dependently enhanced both basal and hCG-stimulated testosterone production by dispersed Leydig cells obtained from IL-1β-pretreated rats. Corticotropin-releasing hormone (CRH) concentration was very low in the testes of control rats, but it did display a striking rise 24 h after the injection of IL-1β. As expected, CRH (100 pmol/rat) and IL-1β (10 nmol/rat) elicited a marked decrease in hCG-enhanced PTC in IL-1β-pretreated rats. (α-Helical)-CRH, a competitive inhibitor of CRH, did not alter hCG-stimulated PTC in control IL-1β-pretreated rats, but it did completely annul the inhibitory effect of both CRH and IL-1β. In light of the present findings, it seems reasonable to suggest that IL-1β exerts a two-fold modulatory action on rat testis steroidogenesis: IL-1β (i) acutely stimulates testosterone secretion by acting directly on Leydig cells, and (ii) induces the intratesticular production of CRH, which in turn inhibits Leydig-cell secretory activity. This last effect, however, requires 24 h to become manifest, since it probably involves the *de novo* expression of CRH gene.

Interleukin (IL)-1β is a polypeptide monokine released from activated macrophages and monocytes during the acute phase of the inflammatory response (for review, see 6). The bulk of evidence indicates that ILs inhibit the hypothalamo-pituitary-gonadal axis by acting both at the level of the hypothalamus and on the steroidogenic gonadal cells (a finding which parenthetically helps to explain the impairment of the reproductive parameters occurring during infections or inflammations) (for review, see 15, 17). However, different results have been reported in the male rat, where IL-1β seems to raise both basal and gonadotropin-stimulated testosterone production by cultured or isolated Leydig cells (37, 39).

It, therefore, seemed worthwhile to investigate the effects of IL-1β on rat Leydig-cell steroidogenesis *in vivo* and *in vitro*. In *in-vivo* studies we employed hypophysectomized rats treated with maintenance doses of human chorionic gonadotropin (hCG) (22). In fact, IL-1β suppresses the hypothalamo-pituitary release of gonadotropins (8, 16, 25), as well as strongly stimulates the secretion of corticotropin-releasing hormone (CRH) (27, 28, 33, 34), which is known to depress hCG-stimulated secretory activity and growth of rat Leydig cells

(22, 35).

METERIALS AND METHODS

Male Wistar rats (300 ± 30 g in body weight) were purchased from Charles-River (Como, Italy). They were hypophysectomized by the parapharyngeal approach, and the completeness of the operation was checked at the time of autopsy as previously described (2, 22). On the third day after hypophysectomy, the animals were subcutaneously infused for 7 days (Alzet osmotic pumps Mod. 2001; Alza, Palo Alto, U.S.A.) with hCG (Serono, Rome, Italy) dissolved in 0.9% NaCl vehicle (1 IU hCG/kg·h), in order to maintain the growth and secretory activity of their testes (22).

In-Vivo *Experiments*

(I) A group of rats received a bolus intraperitoneal (ip) injection of 100 IU/kg hCG dissolved in 0.2 ml of 0.9% NaCl, 60 min before the sacrifice. Another group of animals was injected with the vehicle. A number of rats (n=6) in each of the two groups had intraperitoneally received, together with hCG or the vehicle, increasing doses (from 3×10^{-14} to 3×10^{-7} mol/kg) of IL-1β (Calbiochem, La Jolla, U.S.A.). (II) Other rats were given a bolus ip injection of IL-1β (3×10^{-8} mol/kg), and then sacrificed, in groups of 6 each, 1, 6, 12, 24, 36 or 48 h later. (III) Other rats were given a bolus ip injection of IL-1β (3×10^{-8} mol/kg) 24 h before the sacrifice, and then were treated as in Experiment I. (IV) Other rats were given a bolus ip injection of IL-1β (3×10^{-8} mol/kg) 24 h before the sacrifice and a bolus ip injection of 100 IU/kg hCG 60 min before the sacrifice. They were divided into 3 groups (n=16). One group served as a control, and the other two groups received together with hCG, CRH (3×10^{-10} mol/kg) or IL-1β (3×10^{-8} mol/kg). Half of the animals in each group had intraperitoneally received with the second injection (α-helical)-CRH_{9-41} (α-CRH; Peninsula, Merseyside, U.K.), a CRH antagonist (26), at a dose (3×10^{-8} mol/kg) which was previously found to completely annul *in-vivo* CRH effects on ACTH release and adrenal steroidogenesis (24).

The rats were decapitated between 10 00 and 11 00, and their trunk blood was collected. Testosterone was extracted from heparinized plasma, and extracts were chromatographed on Amprep™ minicolumns RNP 1910 (Amersham, U.K.). Plasma testosterone concentration (PTC) was measured by radioimmunoassay (RIA), using a commercial kit (Testosterone/dihydrotestosterone-|^{3}H| Assay Kit; Amersham). Sensitivity: 10 fmol/ml. Cross-reactivity: testosterone, 100%; 5α-dihydrotestosterone, 45%; 5α-androstane-3β,17β- diol, 7.1%; 5α-androstane-3α,17β-diol, 4.5%; androst-4-ene-3,17-dione, 2.1%; 3β-dihydrotestosterone, 1%; epitestosterone, 0.9%; other steroids, less than 0.05%. Intra-assay and inter-assay variations: 7.1% and 9.2%, respectively.

The testes of rats in experiment II were removed, decapsulated and frozen at −70°C. CRH was extracted from the frozen tissue, according to Naito *et al.* (23). Briefly, tissue from each rat was boiled for 5 min in a 20-fold excess (v/w) of 1.0 N acetic acid and 0.1 N HCl, cooled on ice, homogenized and centrifuged at 1,000 *g* for 30 min at 4°C. The supernatants were re-centrifuged at 12,000 *g* for 30 min at 4°C, then defatted with 3 vol of petroleum ether; aqueous extracts were evaporated to dryness. The residues were then dissolved in 1 ml of phosphate-buffered saline (PBS), and CRH-like immunoreactivity (CRH-ir) was determined by RIA, using a commercial kit (CRH |human-rat|-RIA Kit; Peninsula). Sensitivity: 3 fmol/ml. Cross-reactivity: CRH (human-rat), 100%; CRH (ovine), 0.1%; sauvagine and GH-RH (rat), less than 0.001%. Intra-assay and inter-assay variations: 7.5% and 9.3%, respectively.

In-Vitro *Experiments*

Testes were obtained from (I) control rats (n=24), and (II) animals intraperitoneally injected 24 h before with IL-1β (3×10^{-8} mol/kg) (n=24). The testes of each of the 24 rats of each group were decapsulated, pooled 4 by 4 to obtain 6 samples and then chopped in small fragments. Leydig cells were isolated from each sample according to Van Damme *et al.* (36), as described earlier (1).

Dispersed Leydig cells obtained from each sample were incubated (3×10^5 cells/ml) with increasing concentrations of IL-1β (from 10^{-12} to 10^{-5} M), in the presence or absence of hCG (5 IU/ml). The incubation was carried out in a shaking bath at 37°C for 90 min, in an atmosphere of 95% O_2 and 5% CO_2. At the end of the experiment, the incubation tubes were centrifuged at 4°C, and testosterone concentration in the supernatants was measured by RIA as described above. Intra-assay and inter-assay variations: 6.8% and 7.5%, respectively.

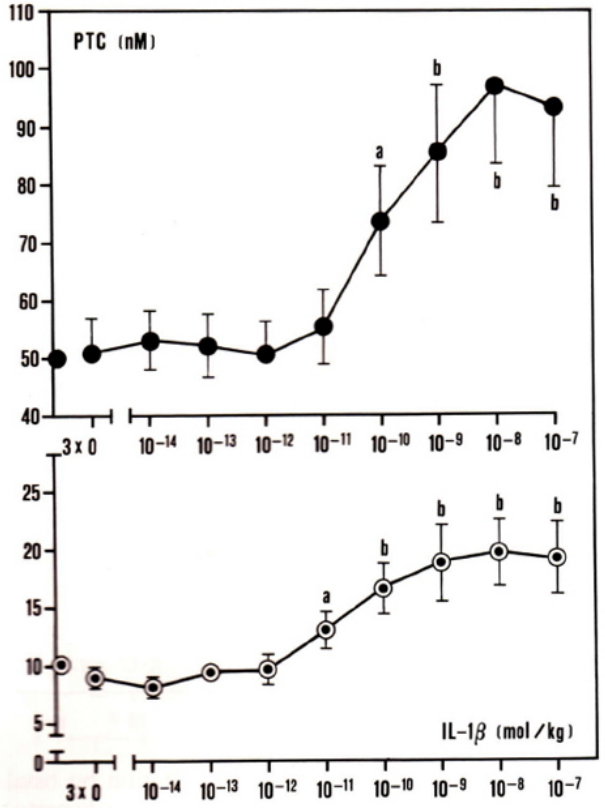

Fig. 1 Acute effect of IL-1β administration on basal (◉) and hCG-stimulated (●) PTC in hypophysectomized/hCG-infused rats. Symbols on the ordinate represent basal and hCG-stimulated PTC in sham-hypophysectomized rats. Results are means ± SE (n=6). a, $P<0.05$ and b, $P<0.01$ from control (not IL-1β-administered) groups

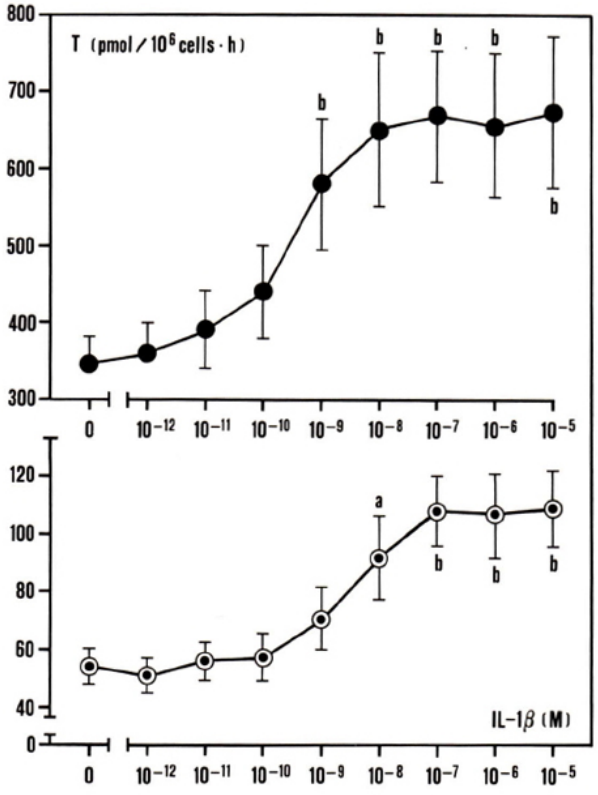

Fig. 2 Acute effect of IL-1β exposure on basal (◉) and hCG-stimulated (●) testosterone (T) production by isolated rat Leydig cells. Results are means ± SE (n=6). a, $P<0.05$ and b, $P<0.01$ from control (not IL-1β-exposed) groups

Statistics

The data were averaged per experimental group, and SE was calculated. The statistical comparison of the results was performed by ANOVA, followed by the Multiple Range test of Duncan.

RESULTS AND DISCUSSION

IL-1β acutely and dose-dependently enhanced both basal and hCG-stimulated PTC and testosterone secretion by isolated Leydig cells (Figs. 1 and 2). The *in-vivo* effect was already apparent at a dose of $10^{-11}/10^{-10}$ mol/rat (about 40%) and reached its maximum (2-fold) at a dose of 10^{-8} mol/rat (Fig. 1). The *in-vitro* effect started to appear at an IL-1β concentration 10^{-9}-10^{-8} M (about 70%) and became maximum at a concentration 10^{-7}-10^{-6} M (2-fold) (Fig. 2). We have not actually assayed the concentrations reached by IL-1β in the plasma of treated rats. However, by assuming a 70% rate of peritoneal absorption of the monokine and about 12 ml of total blood volume (2), we can roughly estimate that *in-vivo* minimal significant and maximal effects were obtained with plasma levels of IL-1β of about 5×10^{-9} M and 5×10^{-7} M, respectively. Thus, a strict correspondence occurs between the *in-vivo* and *in-vitro* effective concentrations of IL-1β.

According to previous studies (37, 39), IL-1β exerts a strong acute secretagogue action of rat Leydig cells. Moreover, our *in-vitro* data suggest that IL-1β acts directly on Leydig cells, and are in keeping with the demonstration of the presence of IL-1 receptors in the testis interstitial tissue and Leydig cells (19, 30).

Basal PTC remained 2-fold raised 6 h after the

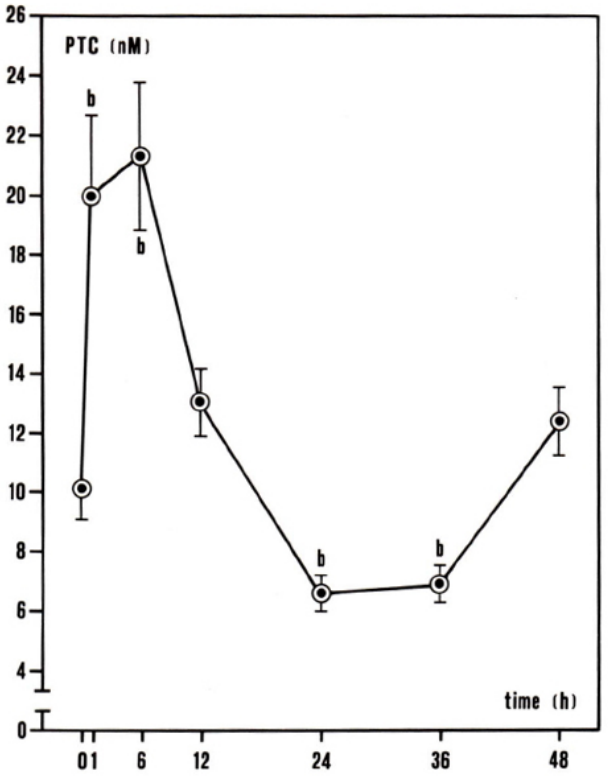

Fig. 3 Basal PTC in hypophysectomized/hCG-infused rats as a function of the time elapsed from a bolus ip injection of IL-1β (3×10^{-8} mol/kg). Results are means ± SE (n=6). b, $P<0.01$ from control (0-time) group

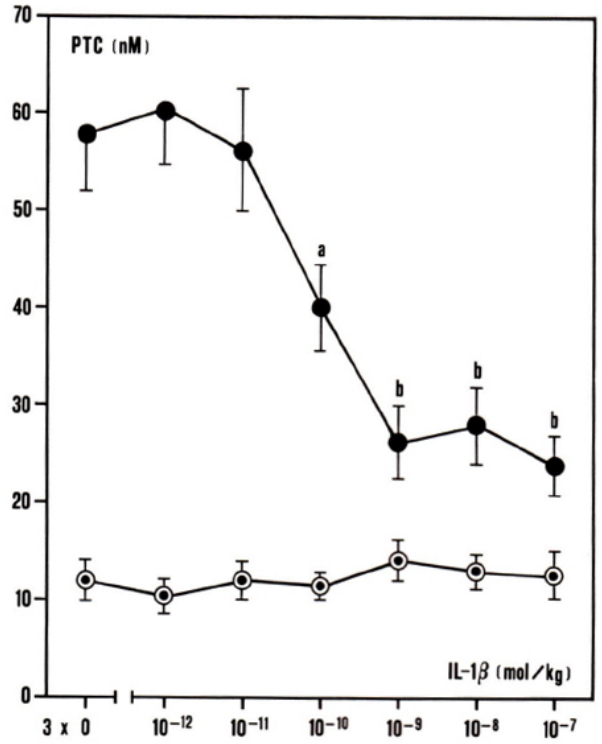

Fig. 4 Acute effect of IL-1β administration on basal (◉) and hCG-stimulated (●) PTC in hypophysectomized/hCG-infused rats, given 24 h before a bolus ip injection of IL-1β (3×10^{-8} mol/kg). Results are means ± SE (n=6). a, $P<0.05$ and b, $P<0.01$ from control (not IL-1β-administered) groups

bolus ip injection of the maximal effective dose of IL-1β (10 nmol/rat), and returned to the control level after 12 h. After 24 and 36 h from IL-1β administration, PTC was significantly below the control level (about −34%), then after 48 h it again attained the control value (Fig. 3). This last rather unexpected finding would suggest that IL-1β is able to trigger in the rat testis some events, requiring about 24 h to become manifest and resulting in the depression of the secretory activity of Leydig cells.

To elucidate this point, we studied the *in-vivo* and *in-vitro* acute secretory responses to IL-1β of Leydig cells of rats given 24 h before the sacrifice a bolus ip injection of 10 nmol of IL-1β. Basal PTC did not display any significant change following the acute IL-1β administration. Conversely, IL-1β dose-dependently decreased hCG-stimulated PTC (Fig. 4). The inhibitory effect was apparent at a monokine dose of 10^{-10} mol/rat (−31%) and reached its maximum at a dose of 10^{-9}-10^{-8} mol/rat (about −57%). However, dispersed Leydig cells obtained from IL-1β-pretreated rats responded to IL-1β acute exposure as those of animals not pretreated with the monokine. In fact, IL-1β dose-dependently enhanced both basal and hCG-stimulated testosterone output (Fig. 5). The effect was significant at a monokine concentration of 10^{-8} M (basal output, 37%; hCG-stimulated output, 73%) and attained its maximum at a concentration of 10^{-7}-10^{-6} M (basal output, 83%; hCG-stimulated output, 2-fold).

Our *in-vivo* findings appear to accord well with those reported previously, showing that a prolonged exposure (from 24 to 72 h) to ILs depresses testosterone secretion (mainly that stimulated by hCG) from cultured Leydig cells (4, 10, 12, 19, 21). Evidence indicates that the prolonged (until 6 days) continuous exposure to IL-1 markedly inhibits hCG- or cyclic-AMP-stimulated P-450_{scc} and P-$450_{17\alpha}$ gene expression in rat and mouse Leydig-cell cultures (13, 20). However, our *in-vitro* data clearly show that the mechanism underlying the *in-vivo*

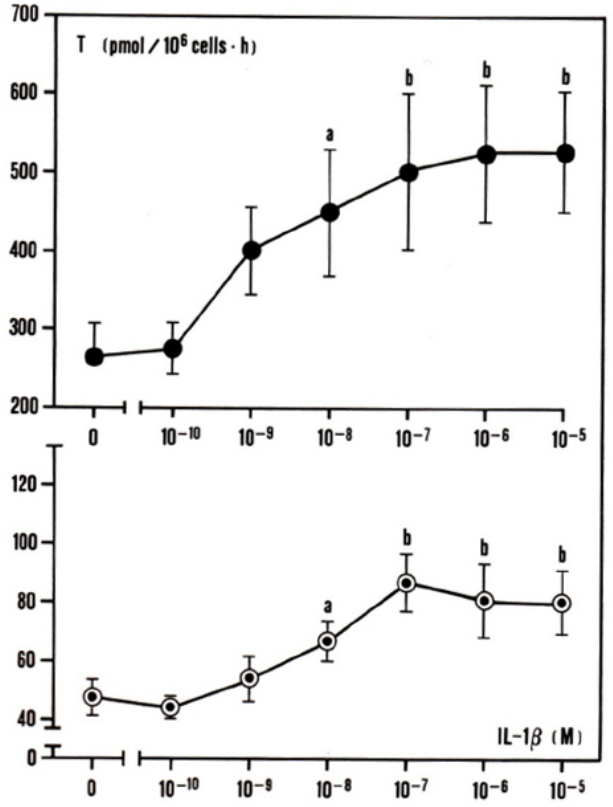

Fig. 5 Acute effect of IL-1β exposure on basal (◉) and hCG-stimulated (●) testosterone (T) production by isolated Leydig cells obtained from hypophysectomized/hCG-infused rats, given 24 before a bolus ip injection of IL-1β (3×10^{-8} mol/kg). Results are means ± SE (n=6). a, $P<0.05$ and b, $P<0.01$ from control (not IL-1β-exposed) groups

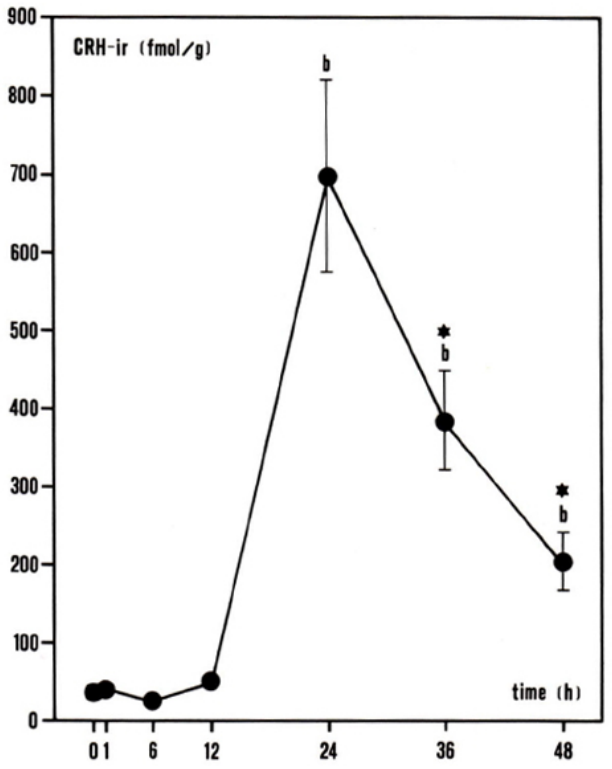

Fig. 6 CRH-ir concentration in the rat testis as a function of the time elapsed from a bolus ip injection of IL-1β (3×10^{-8} mol/kg). Results are means ± SE (n=6). b, $P<0.01$ from control (0-time) group; *, $P<0.01$ from 24-h group

antisecretagogue effect of IL-1β pretreatment does not involve any modification of the steroidogenic machinery of Leydig cells, inasmuch as they are able to normally respond to both hCG and IL-1β. The apparent discrepancy between *in-vivo* and *in-vitro* results may be explained by assuming that the acute inhibitory *in-vivo* effect of IL-1β on Leydig cells of IL-1β-pretreated rats is indirect, i.e. it is mediated by the intra-testicular release of some factor(s), whose synthesis had been induced by IL-1β pretreatment and which in turn is (are) able to inhibit, in a paracrine manner, Leydig-cell secretory activity.

Among the various possible factors, one of the main candidates appears to be CRH. The *in-vivo* response of Leydig cells from IL-1β-pretreated rats to the acute administration of the monokine is similar to that of 'normal' Leydig cells to CRH (22): in fact, CRH, when acutely administered, notably decreases hCG-stimulated (but not basal) PTC, probably by inhibiting adenyl-cyclase/hCG-receptor coupling (35). Evidence is available that CRH may exert an autocrine/paracrine modulatory action on the functions of the testis: CRH and CRH-mRNA are present in the testis interstitial tissue (9, 31, 41), serotonin stimulates the intratesticular release of CRH (32), and rat Leydig cells are provided with CRH binding sites (5, 35). IL-1β is well known not only to stimulate the hypothalamic CRH synthesis and release (see introductory remarks), but also the local production of CRH in the rat adrenal zona medullaris (2, 3).

The hypothesis that the stimulation of a local production and release of CRH may mediate the acute antisecretagogue effect of IL-1β on Leydig cells of IL-1β-pretreated rats is indirectly confirmed by the following experiments.

CRH-ir concentration was very low in the testis of control rats and did not display significant changes 1, 6 and 12 h after the administration of

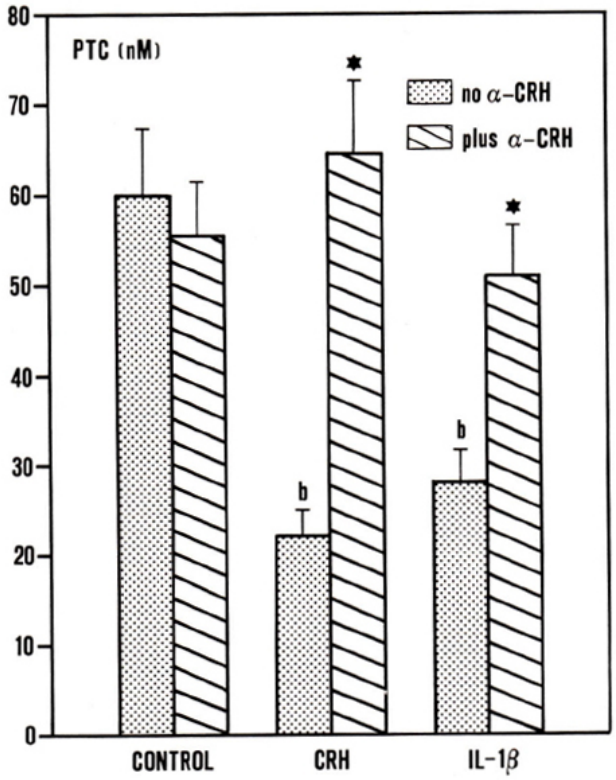

Fig. 7 Acute inhibitory effects of CRH (3×10^{-10} mol/kg)- and IL-1β (3×10^{-8} mol/kg)-administration on hCG-stimulated PTC in hypophysectomized/hCG-infused rats, given 24 h before a bolus ip injection of IL-1β (3×10^{-8} mol/kg), and their reversal by the simultaneous administration of α-CRH (3×10^{-8} mol/kg). Results are means ± SE (n=8). b, $P<0.01$ from control group; *, $P<0.01$ from the respective not α-CRH-injected group

IL-1β (10 nmol/rat). It underwent a striking increase (about 20-fold) after 24 h, then it slowly decreased as a function of the time elapsed from the monokine injection (Fig. 6). As expected, both CRH (100 pmol/rat) and IL-1β (10 nmol/rat) acutely induced a clear-cut decrease (−63% and −53%, respectively) of hCG-enhanced PTC in rats administered 24 h before with IL-1β (10 nmol/rat). α-CRH (10 nmol/rat), a competitive inhibitor of CRH (26) did not affect PTC in control animals, but it did completely annul the acute inhibitory effects not only of CRH, but also of IL-1β (Fig. 7).

In conclusion, our present findings suggest that IL-1β exerts a two-fold modulatory action on rat testis steroidogenesis: IL-1β (i) acutely stimulates testosterone secretion by acting directly on Leydig cells, and (ii) induces the intratesticular production of CRH, which in turn inhibits Leydig-cell secretory activity. The second effect of IL-1β, however, manifests itself after a delay of 24 h, since it is probably associated with the *de-novo* expression of CRH gene.

Caution, however, must be used in hypothesizing the involvement of IL-1β in the fine tuning of the testicular steroid secretion. In fact, the concentrations of IL-1β found to be effective in the present experiments (from 10^{-8} to 10^{-6} M) exceed of about 2 or 3 orders of magnitude that observed in septic patients (10^{-10}-10^{-9} M) (7). Testis interstitial tissue is very rich in macrophages (14), which are the main producers of monokines and are known to influence testosterone secretion by Leydig cells (40). Moreover, rat testis (especially Sertoli cells) produces large amounts of IL-1-like proteins (11, 18, 29), and recently IL-1 mRNA has been found to be expressed in primary cultures of rat Leydig cells (38). Thus, it seems reasonable to conceive that a local production of IL-1 may easily determine an intra-testicular concentration of the monokine of the order of those found to be effective in our present experiments.

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