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MODULATION OF TESTICULAR FUNCTIONS BY TESTICULAR OPIOID PEPTIDES

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The possible physiological role of testicular opioid peptides in the control of testicular functions has been studied. In neonatal rats intratesticular administration of opiate receptor antagonists (naloxone, nalmefene) stimulates Sertoli cell proliferation and secretion. Both in adult and neonatal rats local injection of the testis with opiate receptor antagonists or with β-endorphin antiserum results in a decrease in steroidogenesis in long-term studies. Treatment of neonatal testis with an enkephalin analogue induces a short-term suppression of testosterone secretion. Further studies were carried out to investigate whether the above described local effects of opiate agonist or antagonist on testicular function are under the regulatory control of testicular nerves. Partial denervation of the testis was performed by testicular injection of 6-hydroxydopamine (a neurotoxin degenerating sympathetic neural structures) or by vasectomy (cutting the inferior spermatic nerve). If testicular administration of opioid agonist or antagonist was combined with partial denervation of the testis, the effects of pharmacological agents influencing testicular opioid level were not evident. The data indicate that opioid peptides synthesized in the testis are components of the intratesticular regulatory system and that local opioid actions are modulated by testicular nerves.

Key words: *opiates*; *testis*; *rat*

INTRODUCTION

There is accumulating evidence that opioid peptides derived from each of the opioid peptide genes have a widespread distribution and may act as neuromodulators and hormones. However, recent studies suggest that part of their effects can be attributed to local actions. This latter view is in line with the findings that opioid peptides are synthesized in the gonad of both sexes. Immunocytochemical studies have demonstrated that the proopiomelanocortin (POMC)-derived peptide β-endorphin together with other derivatives of this precursor protein are present in Leydig cells of five species

including the rat (1). POMC mRNA has also been localized in Leydig cells by the technique of in situ hybridization (2, 3). Prodynorphin mRNA is also expressed in the testis and immunocytochemical studies have revealed that prodynorphin-derived peptides are present in Leydig cells (4). Proenkephalin-like mRNA and its peptide products, however, have been reported to be localized in spermatogenic and Sertoli cells (5, 6).

Several data indicate that expression of POMC-derivated peptides is under the direct control of gonadotropins. In neonatal animals the percentage of Leydig cells displaying immunostainable β-endorphin increases significantly following 5 days of hCG treatment (7). The concentration of β-endorphin in the testicular interstitial fluid declines after hypophysectomy and is increased by hCG treatment (8). Acute stimulation of foetal Leydig cells kept in culture for 5 days with hCG increases β-endorphin output 12—12-fold (9). Further studies have demonstrated that hypophysectomy decreases the total amount of POMC-like mRNA of the testis. Treatment of hypophysectomized animals with hCG for 8 days results in an increase in testicular POMC mRNA (10). These studies indicate the expression of opioid peptides in the testis and suggest that POMC gene and its opioid product β-endorphin is regulated by LH/hCG. In addition, the data also suggest that testicular opioids may play a role in local regulatory processes.

The present review will summarize recent developments in the study of the local testicular actions of opioid peptides and potential physiological factors modifying local opioid effects.

RESULTS

POSSIBLE PHYSIOLOGICAL ROLE OF TESTICULAR OPIOID PEPTIDES

Effect of intratesticular administration of opioid antagonists on Sertoli and Leydig cell function

Our early studies indicated that in neonal rats local injection of 1 μg/testis of the opiate receptor antagonist naloxone stimulates testicular growth of the treated gland and enhances the extent of compensatory testicular hypertrophy that follows hemicastration (11).

Further studies using another opiate receptor antagonist nalmefene have also indicated that in neonatal (12), but not in adult rats (13), intratesticularly administered nalmefene facilitates compensatory testicular growth and increases the serum concentration of androgen-binding protein (ABP), a Sertoli cell product secreted into the vascular compartment before puberty. The effect of opiate antagonists was evident 5 days post-treatment. These obser-

vations suggested that testicular opioid peptides inhibit Sertoli cell proliferation and secretion in the early postnatal period.

The possible role of endogenous opioid peptides in the control of steroidogenesis has also been studied. Intratesticular injection of naloxone or nalmefene was accompanied by a significant decrease in the serum testosterone level and basal testosterone secretion in vitro. The suppressive effect of opiate receptor antagonists on steroidogenesis could be observed both in neonatal and adult rats (12, 13). The effective dose of nalmefene was 0.01 μg/testis and 100 μg/testis in neonatal and adult rats, respectively.

Effect of intratesticular injection of anti-β-endorphin antiserum on Leydig cell function

Five-day-old hemicastrated rats were treated intratesticularly with 1 μl of anti-β-endorphin antiserum (dilution: 1 : 100; 1 : 10; undiluted). Control animals were treated intratesticularly with the same amount of normal rabbit serum (NRS). Similarly to the effect of opiate receptor antagonists, the antiserum significantly suppressed basal testosterone secretion *in vitro* 5 days posttreatment *Fig. 1.*

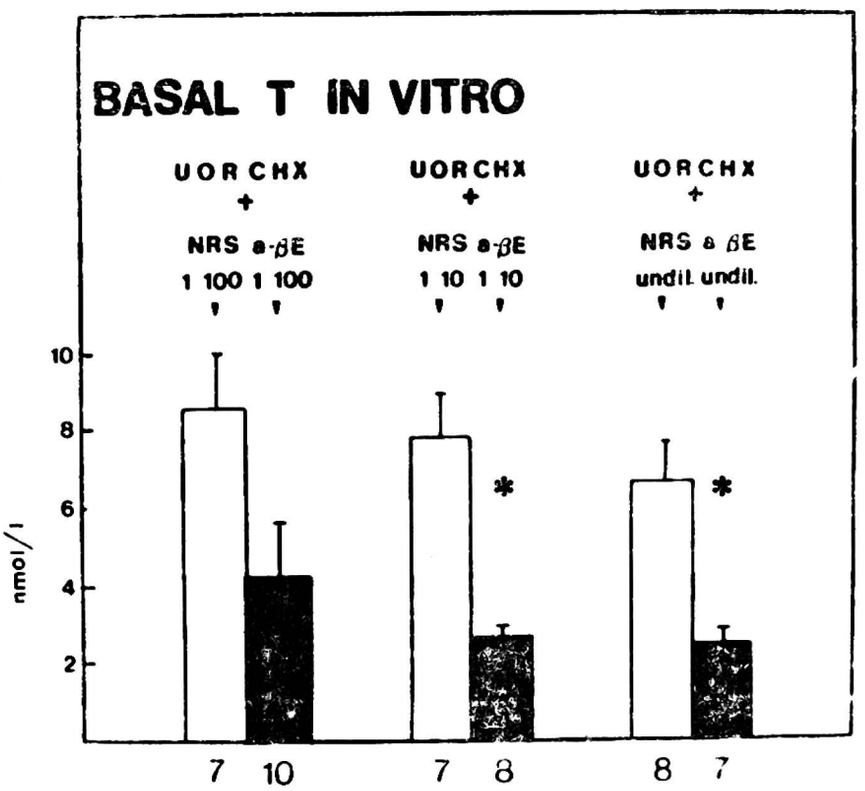

Fig. 1. Effect of β-endorphin antiserum treatment of the remaining testis on basal testosterone (T) secretion in vitro in hemicastrated rats. Treatment and hemiorchidectomy were performed on day 5 of age and the animals were killed 5 days after treatment and hemicastration. Values are mean±SE. Figures indicate the number of animals. UORCHX: unilateral orchidectomy; NRS: normal rabbit serum; a-βE: anti-β-endorphin antiserum. An asterisk indicates significant difference from the corresponding control value.

In adult hemicastrated rats after 5 days administration of 200 µl/testis of anti-β-endorphin antiserum (dilution: 1 : 10) resulted in a significant decrease in basal testosterone secretion in vitro.

Taken together, the results obtained from opiate antagonist (naloxone, nalmefene) treated rats and anti-β-endorphin treated testes indicate that testicular opioid peptides, namely the POMC-derived β-endorphin, appears to facilitate steroidogenesis.

These studies suggest that testicular β-endorphin might play a regulatory role in the steroidogenesis of Leydig cells.

Short-term effect of intratesticular injection of enkephalinamide on Leydig cell function

In 5-day-old hemicastrated rats intratesticular administration of 0.1 µg/testis of (D-Met^2-Pro^5) enkephalinamide a superactive enkephalin analogue (14), significantly decreased basal testosterone secretion in vitro 2 hrs post-treatment. The suppressive effect of enkephalinamide was dose-dependent. Similar studies performed on 10-day-old rats (effective doses: 1.0 and 3.0 µg/testis) resulted in a reduced serum testosterone level and basal testosterone production *in vitro*. Intratesticular treatment with naloxone prior to enkephalinamide administration prevented the opioid-induced decrease in steroidogenesis (15). These results suggest that enkephalins might be among the intratesticular factors regulating Leydig cell function.

It is of note that in control experiments systemic administration of either naloxone, nalmefene or enkephalinamide was ineffective in altering the testicular parameters studied.

FACTORS MODULATING INTRATESTICULAR OPIOID ACTIONS

Local naloxone action following systemic testosterone treatment

We have studied whether or not the well-established local effect of the opiate receptor antagonist naloxone on Sertoli and Leydig cell function could be modified if gonadotropin and testosterone secretion are suppressed by daily systemic administration of testosterone.

Five-day-old rats were treated intratesticularly on one side with 1 µg/testis of naloxone. Immediately after treatment the contralateral testis was removed. Starting from the day of testicular intervention the rats were treated daily with 20 µg/g body weight/day of testosterone propionate subcutaneously. The animals were killed at 10 days of age. Control rats were treated intratesticularly with physiological saline and systemically with sesame oil.

Five days after hemicastration the remaining testis had increased in weight as compared with age-matched controls with two testes. As in our previous

studies (11, 12), intratesticular administration of naloxone resulted in a further increase in compensatory testicular growth and serum ABP concentration. Daily testosterone treatment of hemicastrated animals not only prevented the development of compensatory gonadal hypertrophy but resulted in a testicular weight significantly lower than that of intact control rats with two testes. If testosterone treatment was combined with testicular administration of the opiate antagonist the testicular weight was similar to that observed in hemicastrated, testosterone treated rats, i. e. naloxone did not counteract

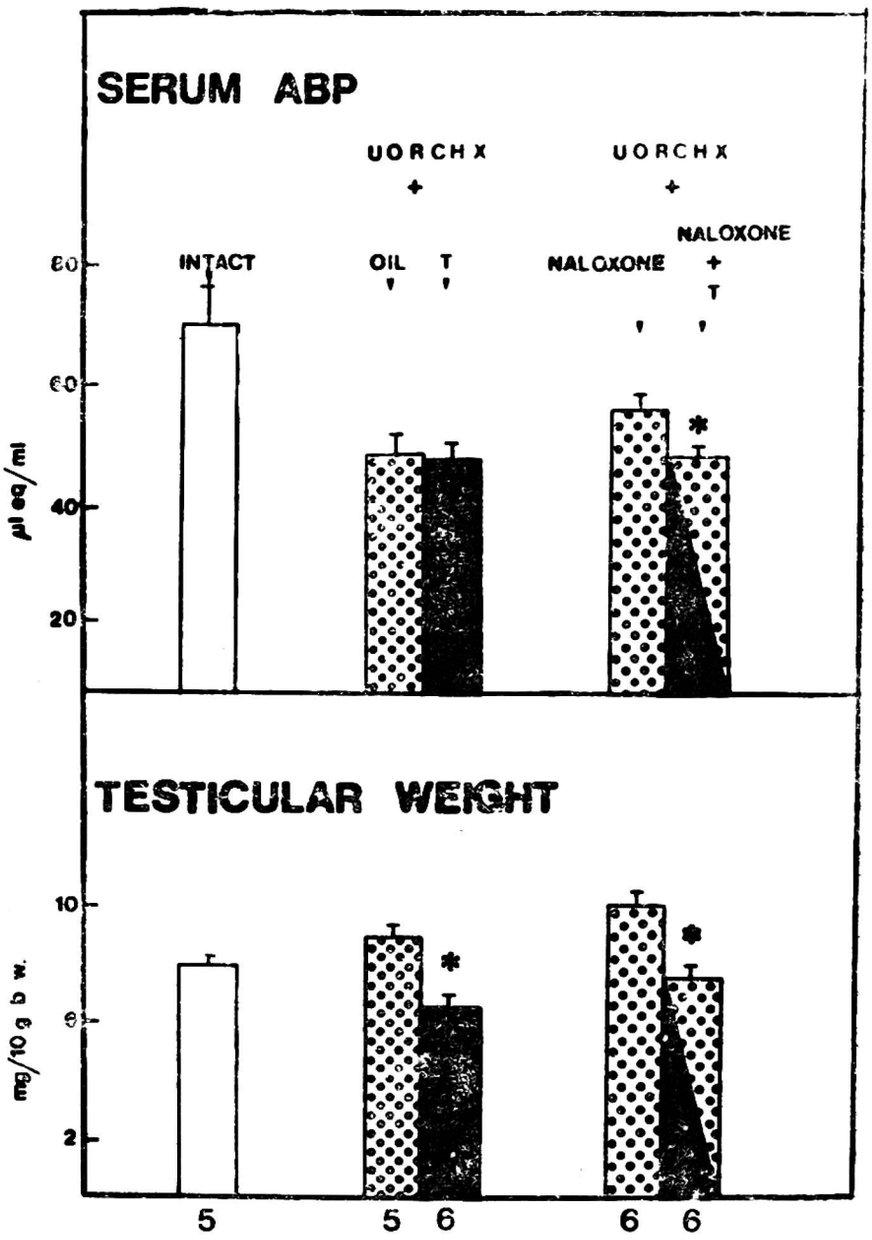

Fig. 2. Effect of intratesticular injection of 1 µg of naloxone on testicular weight (lower panel) and serum androgen-binding (ABP) level (upper panel) in hemicastrated rats treated daily with 20 µg/g body weight of testosterone propionate subcutaneously. Testicular injection and hemiorchidectomy were performed on day 5 of age, testosterone treatment being started on the day of testicular intervention. The animals were killed on day 10 of age. Values are: mean±SE. Figures indicate the number of animals. UORCHX: unilateral orchidectomy; T: testosterone. An asterisk indicates a significant difference from the corresponding control value.

the suppressive effect of testosterone on testicular weight *Fig. 2, lower panel.* Testosterone administration did not modify serum ABP concentration but prevented the naloxone-induced rise in the serum protein level *Fig. 2, upper panel.* These observations suggest that the effects of naloxone on Sertoli cell proliferation and secretion are not present if the serum testosterone concentration is continuously high.

Basal testosterone secretion in vitro of animals treated daily with testosterone or subjected to a single intratesticular administration of naloxone is markedly reduced. Combined use of the two suppressive agents did not cause an additive effect, since there was no further decrease in steroidogenesis.

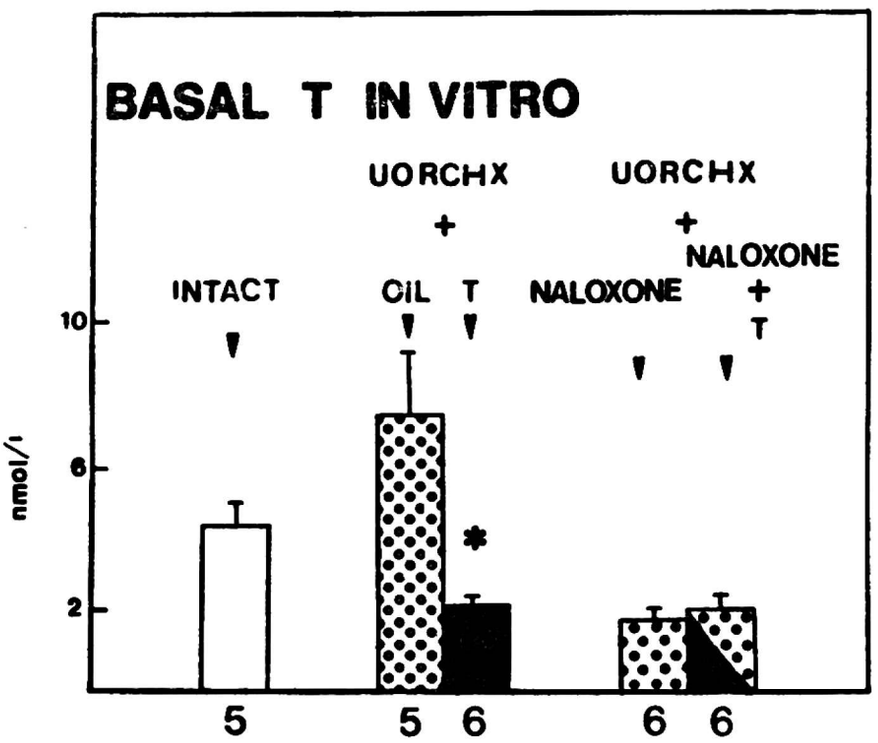

Fig. 3. Basal testosterone secretion in vitro of hemicastrated animals subjected to a single intratesticular administration of naloxone (1 μg/testis) and daily systemic injection of testosterone propionate (20 μg/g body weight). Testicular injection and hemiorchidectomy were performed on day 5 of age, testosterone treatment being started on the day of testicular intervention. The animals were killed on day 10 of age. Values are: mean±SE. Figures indicate the number of animals. UORCHX: unilateral orchidectomy. An asterisk indicates a significant difference from the corresponding control value.

Taken together, these results indicate that if gonadotropin hormone secretion and steroidogenesis is significantly reduced, testicular opioids cannot exert their effects on Sertoli and Leydig cell functions. However, the mechanism of action of this interaction is not clear. These findings are consistent with the view that synthesis of testicular opioid peptides is under the regulatory control of gonadotropic hormones.

Local naloxone action following pharmacological sympathectomy of the testis

Several lines of evidence support the view that testicular innervation and peripheral catecholamines are involved in the control of male gonadal function, particularly before puberty (16, 17, 18).

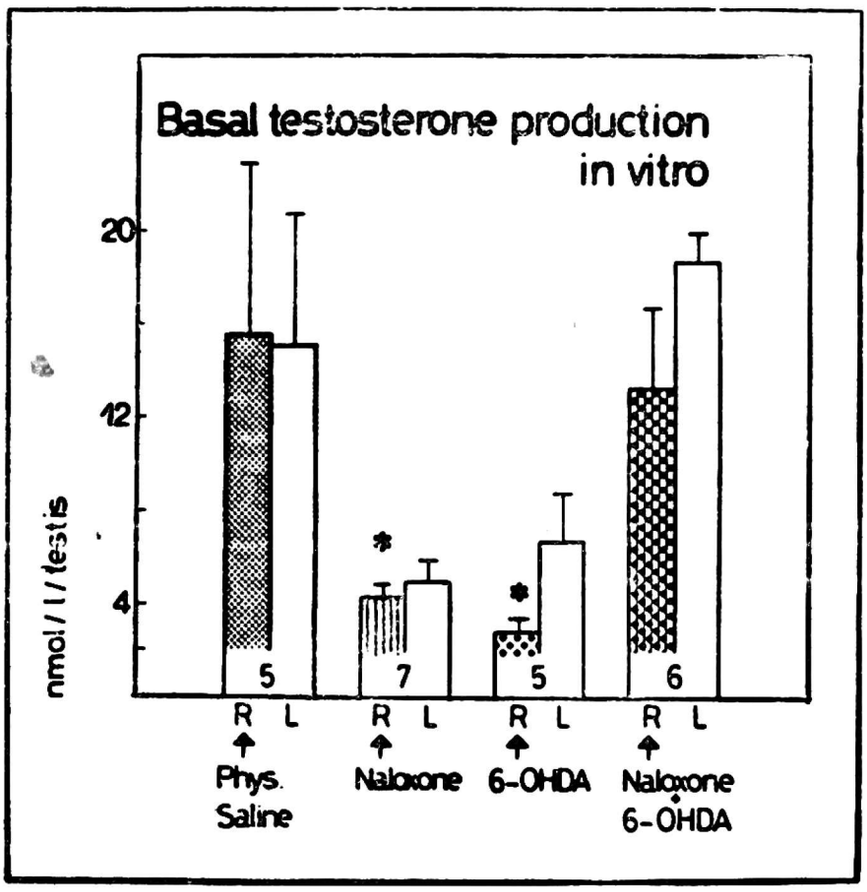

Fig. 4. Effect of intratesticular administration of 1 μg of naloxone and/or 330 μg of 6-hydroxydopamine of the right testis on basal testosterone secretion in vitro. Testicular treatment was performed on day 5 of age and the animals were killed 5 days post-treatment. Values are: mean ± SE. Figures indicate the number of animals. R: right testis; L: left testis; 6-OHDA: 6-hydroxydopamine. An asterisk indicates a significant difference from the physiological saline-treated control value.

In 5-day-old rats pharmacological sympathectomy of the testis was performed by local injection of 330 μg/testis of the neurotoxic drug 6-hydroxydopamine (6-OHDA) (19, 20). Five days after 6-OHDA administration, basal testosterone secretion *in vitro* of the treated gonad had decreased significantly. If 6-OHDA treatment was followed by local administration of naloxone which — as previously described, also suppresses steroidogenesis, *in vitro* testosterone production of the testes was similar to that observed in vehicle-treated controls *Fig. 4*, despite the fact that either naloxone or 6-OHDA alone reduces basal testosterone secretion *in vitro* (21).

Local naloxone or enkephalinamide action in hemivasectomized rats

Partial denervation of the testis by vasectomy that also involves transection of the inferior spermatic nerve interferes with testicular functions (22, 23, 24).

In 15-day-old rats we performed hemivasectomy or administered locally 4 μg/testis of naloxone. An additional group of age-matched animals underwent hemivasectomy just prior to local injection of the opiate antagonist into the ipsilateral testis (submitted for publication). As was previously reported both vasectomy (23) and local naloxone treatment (12) alone resulted in a significant decrease in the steroidogenesis of immature testes 7 days after surgery or treatment. Unexpectedly, if the two interventions were combined, basal testosterone secretion *in vitro* did not show a further decrease but resulted in steroid production which significantly exceeded that observed in intact control gonads.

In further studies, 5-day-old hemicastrated rats were vasectomized immediately before testicular injection of 0.3 μg/testis of enkephalinamide. Two hours after hemivasectomy+enkephalinamide treatment basal testosterone secretion was similar to that in hemicastrated or opioid analogue treated rats.

These results suggest that partial denervation of the testis interferes with local effects of opiate antagonist or agonist on steroidogenesis.

DISCUSSION

The results summarized above indicate that besides their action at the hypothalamo-hypophyseal level (25, 26, 27), endogenous opioid peptides participate in the control of reproductive functions also by local gonadal mechanisms.

Data obtained from studies following local treatment with opiate receptor antagonists indicate that testicular opioid peptides inhibit testicular growth, compensatory testicular hypertrophy, ABP secretion (11, 12) and Sertoli cell proliferation (28). The fact that intratesticular administration of β-endorphin antiserum increases Sertoli cell proliferation strongly suggests that POMC--derivated β-endorphin is the endogenous opioid peptide responsible for the effects of the universal opiate antagonists on Sertoli cell function. In support of this view it has been reported that Sertoli cells have opiate receptors and that chronic treatment of Sertoli cell cultures with β-endorphin inhibits basal and FSH-stimulated ABP secretion (29).

On the basis of the available data it seems likely that β-endorphin of Leydig cell origin is a component of the regulatory communication system between Leydig and Sertoli cells.

The biological consequences of testicular opioid production on Leydig cell function have also been studied.

Both in adult and neonatal rats intratesticular injection of opiate receptor antagonists decreases steroidogenesis in long-term experiments (7 and 5 days post-treatment, respectively). These effects implied that opiates in some way influence Leydig cell testosterone secretion (11, 12, 13). The observation that β-endorphin stimulates androgen secretion by primary Leydig cell cultures (30), while local treatment of neonatal testes with β-endorphin antiserum suppresses testosterone production, further suggest that the POMC--derived opioid peptide β-endorphin may function as an ultra-short-loop or autocrine regulator of Leydig cells.

The paracrine control of Leydig cell streoidogenesis by opioid peptides is suggested by the findings that in short-term studies local injection of enkephalin analog suppresses basal testosterone secretion in vitro (15).

Taken together, these data indicate that different opioid peptides (β-endorphin, enkephalin) produced by different cell types of the testis might modulate androgen production by different mechanisms (autorcine or paracrine).

Furthermore, the studies reviewed demonstrate that testicular innervation might play a role in the modulation of local testicular action exerted by endogenous opioid peptides.

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