

STUDIES ON THE EFFECT OF INTRATESTICULAR ADMINISTRATION OF OPIOID PEPTIDES, NALOXONE OR N-ACETYL β -ENDORPHIN ANTISERUM ON SOME TESTICULAR PARAMETERS IN RATS

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Abstract : Opioid peptides have been localized in a variety of peripheral tissues like placenta, thyroid, pancreas, gastrointestinal tract, in the reproductive tract of male and female and in the testes of rats. Immunoassayable material was detected in extracts of gonads, reproductive tract and accessory reproductive organs. Studies with naloxone have suggested that β -endorphin may have an important role in steroidogenesis and may have a role in regulating transport of luminal material. In our studies met-enkephalin, β -endorphin, naloxone or N-acetyl β -endorphin antiserum were microinjected intra testicularly once on alternate days for one week and autopsied 24 h after the last injection. Intratesticular administration of 25, 50 and 100 μ g doses of naloxone induced significant decrease in *in vitro* secretion of testosterone *per se*, which was significantly greater with 50 μ g dose than with those of the other two doses. A 25 μ g dose had no effect on hyaluronidase or acid phosphatase activity while 50 μ g dose significantly decreased the enzyme activity. One hundred μ g dose also significantly decreased hyaluronidase activity. Intratesticular injection of 10 μ g met-enkephalin or 1 μ g β -endorphin significantly decreased hyaluronidase activity whereas 20 μ l N-acetyl β -endorphin antiserum increased the specific activity of hyaluronidase. There was no change in the weight of the testes on treatment with the above agents.

Key words : testis

opioids

naloxone

INTRODUCTION

Endogenous opioid peptides play an important role in reproduction through the regulation of pituitary gonadotrophin secretion in both male and female. They have been known to perform a regulatory role within the reproductive system itself. Transcripts of the genes have been shown

to be present in mammalian testis and ovary (1). The expression of all the peptides from POMC (pro-opiomelanocortin) precursor in the reproductive system suggested that opioid peptides act as local regulators of reproductive function (2). β -endorphin has been found in testis and epididymis in the hypophysectomized rat and other species (3). It has been shown that treatment with

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human chorionic gonadotrophin (hCG) increases POMC like m-RNA concentration in Leydig cells (4) and immunostaining for β -endorphin in Leydig cells (5). Role of opioid peptides in regulating testicular function is not known. Studies of Chandrashekhar and Bartke (6) suggested that β -endorphin inhibited testosterone secretion. Results of Ellerkmann et al (7) have attributed functional property for N-acetyl β -endorphin. So, this study was undertaken to see the effects of intratesticular injections of opioid peptides met-enkephalin, β -endorphin, N-acetyl β -endorphin anti-serum and antagonist naloxone on some of the parameters of testes.

METHODS

Methionine-Enkephalin (met-Enkephalin) was purchased from Vega Biotechnologies Inc., Tucson, Arizona. N-acetyl- β -Endorphin Antiserum (Rabbit) was purchased from Peninsula Laboratories and ^3H -Uridine specific activity 6,000 mCi/mmol was bought from Bhabha Atomic Research Centre, Bombay. Naloxone, β -endorphin were purchased from Sigma Chemical Co. Dulbecco's Modified Eagle medium was purchased from Hi Media Laboratories Pvt. Ltd., Bombay.

Adult male Wister rats, 65 days of age, were maintained in the Small Animal House facility under a 14 h light and 10 h dark regimen. They were fed on standard rat pellets purchased locally and with drinking water *ad libitum*.

Rats were microinjected Met-enkephalin, β -endorphin, naloxone or N-acetyl

β -endorphin antiserum once on alternate days for one week and autopsied 24 h later. Control rats received equal volume of saline and were autopsied at respective time intervals. Testes and accessory reproductive glands were dissected out and weighed to the nearest 2.0 mg on a torsion balance. The following parameters of testicular function were evaluated:

1. The weight of testes and accessory reproductive glands.
2. *In vitro* incorporation of ^3H -uridine into RNA in the testes.
3. Testicular hyaluronidase and acid phosphatase activity.
4. Testosterone levels in the medium of the *in vitro* incubated testis was measured.

Procedures

The estimation of enzyme activity, testosterone and incorporation assays were carried out as described (8).

Collection of medium for testosterone hormone assay

Treated and control set of animals were decapitated, testis were dissected out, decapsulated and the testicular tissue was incubated in 3 ml of Dulbecco's Modified Eagle Medium for 2 h at 37°C in a GFL 1083 shaking water bath (West Germany) at the speed of 65 cycles per min. After 2 h, flasks containing the tissue were placed on ice, tissue was removed, medium was collected and centrifuged at low speed (1000 rpm for 5 min) to remove the cell debris, if present, and supernatant was collected and

stored at -20°C for later radioimmunoassay of testosterone.

Statistics

Group means were compared by analysis of variance followed by Duncan's new multiple range test with $P < 0.05$ required for significance.

RESULTS

Intratesticular administration of 25, 50 and 100 μg doses of naloxone induced significant decrease in *in vitro* secretion of testosterone which was significantly greater with 50 μg dose ($P < 0.001$) (Table I). A 25 μg dose had no effect on either of the enzyme activity. Treatment with 100 μg dose decreased hyaluronidase activity but had no effect on acid phosphatase activity.

Specific activity of RNA decreased significantly after treatment with 25 and 50 μg doses of naloxone whereas higher dose of 100 μg significantly stimulated it (Table I).

Intratesticular injection of high dose of opioid peptide 10 μg met-enkephalin or 1 μg β -endorphin significantly decreased testicular hyaluronidase activity whereas treatment with 20 μl N-acetyl β -endorphin antiserum significantly ($P < 0.01$) increased specific activity of hyaluronidase. There was no significant change in the specific activity of acid phosphatase and RNA after intratesticular injection of met-enkephalin, β -endorphin and N-acetyl β -endorphin antiserum (Table II). There was no significant change on *in vitro* secretion of testosterone (not given).

TABLE I : Effect of intratesticular injection of naloxone on *in vitro* secretion of testosterone, testicular hyaluronidase and acid phosphatase activity and *in vitro* incorporation of ^3H -uridine in testes of sexually mature rats.

Treatment	Dose	Testosterone	Hyaluronidase	Acid phosphatase	Specific activity of RNA (DPM $\times 10^{-3}$) / mg RNA
Control	Saline (6)	162.00 \pm 9.00	1.20 \pm 0.17	5.95 \pm 0.15	47.4 \pm 3.6
	25 μg (6)	140.00 \pm 10.80	0.91 \pm 0.11	6.23 \pm 0.55	22.6 \pm 2.3**
Naloxone	50 μg (6)	23.45 \pm 0.90***	0.61 \pm 0.10**	4.85 \pm 0.20	30.0 \pm 2.5**
	100 μg (6)	67.50 \pm 4.50**	0.49 \pm 0.07**	6.40 \pm 0.40	63.0 \pm 8.5**

Activity of enzymes expressed as

(a) μmole NAGA formed/mg protein/h

(b) μmole PNP released/mg protein/h

(c) Secretion of testosterone expressed as ng/testes

Specific activity of uridine-6000 mCi/mmol

Control set of rats received equal volume of saline, intratesticular injections were performed on alternate days for 1 week and were autopsied 24 h after the last injection.

Values are mean \pm SEM. Values *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$) are significantly different from their respective control. Number in parenthesis represents number of animals in each group.

TABLE II : Effect of intratesticular injection of Met-enkephalin, β -endorphin and N-acetyl β -endorphin antiserum on testicular hyaluronidase and acid phosphatase activity and *in vitro* incorporation of ^3H -uridine in testes of sexually mature rats.

Treatment	Dose	Hyaluronidase	Acid phosphatase	Specific activity of RNA (DPM $\times 10^{-3}$) / mg RNA
Control	Saline	1.30 \pm 0.08	6.23 \pm 0.24	90.1 \pm 7.0
Met-enkephalin	1 μg (6)	1.42 \pm 0.10	6.00 \pm 0.29	85.4 \pm 5.0
	10 μg (6)	0.99 \pm 0.08*	6.07 \pm 0.30	80.3 \pm 8.0
β -endorphin	0.01 μg (6)	1.28 \pm 0.11	6.08 \pm 0.15	95.4 \pm 4.8
	1.0 μg (6)	0.82 \pm 0.06**	7.00 \pm 0.41	105.3 \pm 8.5**
Endo-AS	10 μg (6)	1.43 \pm 0.09	6.00 \pm 0.35	98.8 \pm 6.3
	20 μg (6)	1.70 \pm 0.15**	5.95 \pm 0.21	96.2 \pm 7.2**

Activity of enzymes expressed as

(a) μmole NAGA formed/mg protein/h

(b) μmole PNP released/mg protein/h

Control received equal volume of Saline. Values are mean \pm SEM. Values *($P < 0.05$), **($P < 0.01$), are significantly different from their respective control. Number in animals are given in parenthesis.

TABLE III : Effect of intratesticular injection of Naloxone, Met-enkephalin, β -endorphin or N-acetyl β -endorphin antiserum on the weight of testes and accessory reproductive glands in sexually mature male rats.

Treatment	Dose	Testes (mg)	Seminal vesicle (mg)	Ventral prostate (mg)
Control	Saline (6)	1290 \pm 82	162 \pm 10	165 \pm 8
	25 μg (6)	1185 \pm 20	179 \pm 20	187 \pm 9
Naloxone	50 μg (6)	1216 \pm 40	180 \pm 30	175 \pm 16
	100 μg (6)	1285 \pm 80	188 \pm 16	180 \pm 14
Met-enkephalin	1 μg (6)	1215 \pm 60	183 \pm 15	170 \pm 10
	10 μg (6)	1190 \pm 57	170 \pm 10	182 \pm 17
β -endorphin	0.1 μg (6)	1310 \pm 110	180 \pm 18	180 \pm 10
	1 μg (6)	1285 \pm 45	182 \pm 9	185 \pm 12
Endo-AS	10 μg (6)	1310 \pm 52	188 \pm 15	188 \pm 19
	20 μg (6)	1267 \pm 43	188 \pm 12	189 \pm 15

Control set of rats received equal volume of Saline. Number in parenthesis represents the number of animals in each group. Values are mean \pm SEM.

Values are expressed in mg/total body wt.

Intratesticular administration of any of these agents did not produce any change in the weight of Testes, Seminal Vesicle (SV) and Ventral Prostate (VP) (Table III).

DISCUSSION

Intratesticular injection of met-enkephalin or β -endorphin induced significant reduction in hyaluronidase activity. It is known that pro-enkephalin gene is expressed in germ cells and somatic cells of testes (2, 9). Products derived from the transcripts of the gene may be having inhibitory effect on the function of germ cells or may be inhibitory via affecting the function of somatic cells. Data from several laboratories suggest that spermatogenic cells can alter the function of neighbouring somatic cells (10, 11, 12). It is suggested that germ cell associated opioid peptides may mediate such interactions (2). This study suggests that met-enkephalin containing peptides or endorphin may be regulating the activity of testicular hyaluronidase as there was no change in the rest of the testicular parameters. N-acetyl β -endorphin present in spermatogonia and primary spermatocytes (13) is having some regulatory role in testicular function. Intratesticular injection of N-acetyl endorphin antiserum stimulated hyaluronidase activity suggesting endogenously present endorphins have some regulatory role on hyaluronidase activity or in development of spermatogonia and spermatocytes.

Intratesticular injection of naloxone

decreased testosterone secretion from the testicular tissue incubated *in vitro*. This is in accordance with the studies of Gerendai and Bardin (14) who showed that intratesticular administration of naloxone in hemicastrated rats caused decrease in the basal secretion of testosterone *in vitro*.

The presence of opioid peptide receptors were shown on Sertoli cells but were unable to detect on Leydig cells and it was also indicated that β -endorphin produced within the Leydig cells may behave as a paracrine inhibitor of Sertoli cell function (15, 16). Basal secretion of testosterone *in vitro* had decreased on treatment with opioid peptide antagonist *in vivo* which suggests that blockade of opioid receptors might have disturbed the communication network between Sertoli, Leydig and germ cells producing an inhibitory effect on steroidogenesis and on hyaluronidase activity. Inhibitory effect on the activity of this enzyme may be secondary to the effect on testosterone. There may be direct inhibitory effect of opioid peptides on germ cell functions or may be via altering the function or secretion of Sertoli cells.-

Till now no studies have been undertaken with opioid peptides which gives an insight in the regulation of the reproductive functions at the gonadal level. Our study suggests that opioid peptides as well as N-acetyl β -endorphin have some role in regulating reproductive functions at the gonadal level, but still further studies are required to get a clear picture of their role.

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