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ECT OF CALCITONIN ON PLASMA LEVELS OF TESTOSTERONE, AND LH IN ORCHIDECTOMIZED RATS.

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MARY

Present study was performed to investigate hysiological role of calcitonin (CT) on physical testicular axis . A total number of 80 . male albino rats were divided into 4 equal ps, the first served as control, and the second p was injected with salmon calcitonin (sCT), third group was orchidectomized and the th was orchidectomized and injected with . Blood samples were collected after two ks . Plasma were separated for analysis of tesrone , FSH and LH. Results indicated a sigcant decrease in testosterone level in intact anls which were injected with sCT and a nificant increase in FSH and LH levels of orlectomized rats injected with calcitonin.

TRODUCTION

citonin (CT) is a peptide hormone produced inly by parafollicular cells (C cell) of thyroid gland in mammals, and its effect have been focused on calcium homeostasis (Azria,1989). Its receptors have been reported in brain (Hilton et al.,1995), pituitary gland (Goltzman and Mitchell,1985) and testicular leydig cells (Nakhla et al., 1989) and ovarian cells (George et al.,1997) which proved the existence of endocrine role of CT on pituitary gonadal axis. CT was also able to relive pain as it increases plasma B-endorphin level by acting through the hypothalamus and/or pituitary gland (Franceschini et al .,1993). There is a direct evidence for the critical dependence of pain behavior on calcitonin gene related receptors (Han et al.,2005). In addition, it is extremely potent suppressor of slow wave sleep when injected between the lateral part of the hypothalamic paraventericular area (PVA) and the fornix, as it is involved in sleep regulation (Slisli and Beaurepaire,1999). Calcitonin affects skin temperature castrated male rats (Yuzurihara et al.,2003), On the other hand (Kamada et al .,2005) reported an anti-inflammatory effect of calcitonin and Davey and Morris,(2005) proved the effect of single

ment with salmon calcitonin (sCT)on calcium
ostasis and bone turnover.

tion of LH and FSH is known to be con-
l by releasing factors from hypothalamic
which expressed CT receptors (Nicosia et al
). On the other hand, CT infusion in phar-
logical doses reduced the serum concentra-
of LH and FSH through a mechanism in-
g and increase in cAMP production in rats
or pituitary cells (Tsai et al.,1999)

t evidence has shown that calcitonin gene
l peptide (CGRP) is considered a key medi-
f stress ifinduced suppression of the gona-
in releasing hormone (GnRH). A little is
t about the neural pathway involved (Kin-
al., 2005). Calcitonin receptors in CNS
involved in the regulation of food intake,
genesis and behavior (Sawada, et al., 2006
aylor et al., (2006).

was found to increase the intramyocytic
m in adult cells and decrease it in neonates
et al.,2005). The hypercalcemia caused by
hydroxyvitamine D3 disrupts the endocrin-
al homeostasis that in turns temporarily dis-
the female reproductive system (Horri et
92).

ment of man suffering from infertility with
erum testosterone level by tamoxifen re-
in increased plasma level of LH,FSH and
sterone.Testostosterone has a direct influence
T secretion (Schopman et al.,1987). Also (Lu
., 2000)indicated that orchidectomy decreases
l CT release from thyroid gland.

So the present study was performed to investigate the role of CT on hypophysial gonadal axis.

MATERIALS AND METHODS

A total number of 80 adult male albino rats weighing 200 ± 50 gm, were obtained from laboratory farm animals, faculty of Veterinary Medicine, Banha university.The animals were fed on standard commercial diet, milk and water and kept in steel wire cages throughout the study

Experimental design

The animals were divided into 4 equal groups as following:

- 1st group(G1) : served as control injected with normal saline intramuscularly (I/M) daily for two weeks (Control intact) .
- 2nd group (G2): injected (I /M)for two weeks with salmon calcitonin (sCT) obtained from Novartis pharma.Sat.Cairo.in a dose of 10 MRC U/kg body weight/day (Mahruous and Nakhla,1988).
- 3rd group (G3): orchidectomized animals injected I/M with normal saline for two weeks.
- 4th group (G4): orchidectomized animals injected I/M with 10 MRC U/kg body weight /day for two weeks.

Male rats were orchidectomized 2 weeks before treatment (Chen et al., 1994) .
Orchidectomy was performed according to (Byron, 1978)

Blood sampling and hormonal assay:

Blood samples were collected on heparinized tube after scarification of the animals.Plasma were separated by centrifugation and used for hormonal

assay by radioassay system laboratories. Testosterone and FSH were determined after Abraham (1981) and LH according to Ho Yuen et al.,(1983) Obtained data were analysed using ANOVA testing according to (Noursis, 1997).

RESULTS

The results of the present study were indicated a significant decrease in plasma levels of testosterone in all rat groups in comparison to intact control rats. while FSH and LH levels were significantly elevated in all orchidectomized rats incomparison with the intact rats

DISCUSSION

There are many reports which indicated that the inhibitory effect of sCT on basal PRL release was reversed by treatment with either the Ca2+ ionophore A23187. (Shah et al., 1990 and 1996), thyrotropin hormone (Mitsuma et al.,1984),growth hormone (Guisting et al., 1990), gonadotropin (Chen et al.,1994), testosterone (Wang et al.. 1994),estradiol (Mahrous and Nakhla, 1988) and the progesterone secretion (Tsai et al., 1999). The present study was indicated that I/M injection of sCT for two weeks resulted a significant reduction at $p<0.001$ in plasma levels of testosterone and these results come in agreement with Mulder (1993) whom proved that the mechanism by which sCT reduced plasma testosterone level may

Plasma levels of testosterone, FSH and LH in intact and orchidectomized rats injected with sCT/ 2 weeks

Groups / Hormones	(G4)	(G3)	(G2)	G (1)
Testosterone ng/ml	0.743 ±0.035	0.580[a] ±0.063	0.462[ab] ±0.011	0.456[a,c] ±0.021
LH IU/ml	0.069 ±0.014	0.052[a] ±0.016	0.197[a,b] ±0.024	0.130[a,c] ±0.011
FSH IU/ml	0.089 ±0.050	0.090 ±0.067	0.210[a,b] ±0.015	0.160[a,c] ±0.037

a: significant differences between G (1) of the same line
b: significant difference between G (1) and G3
c: significant difference between G (1) and G4

be due to direct effect on the testis and/ or indirect effect on hypothalamo-hypophysial testicular axis. On the other hand (Yuzurihara et al., 2003)proved that calcitonin gene related peptide (CGRP)is the most potent peptide in the family that elevates the skin temperature in male rats. The elevation of such temperature was more affected by testosterone deficiency resulting from castration, which suggest that CGRP is involved in mechanisms underlying hat flases in man.

The reduction of plasma testosterone level was accompanied by a significant decrease in both LH, FSH at $p<0.001$, which indicate that the inhibitory effects of sCT on testosterone secretion might be affected pituitary gland and these results were proved by (wang et al., 1994) as they concluded that sCT caused a direct inhibition on gonadotropins hormone release from anterior pituitary through mechanisms involving an increase in cAMP through the action of protein kinase A (PKA) which block Ras pathway and then inhibits release of LH (Marx, 1993). Recently (Kinsey, et al., 2005) were reported that CGRP is considered a key mediator of stress induced suppression of gonadotropin releasing hormone (GnRH) although little is known about the neural pathway involved. Sawada et al (2006) suggest that calcitonin receptor stimulating peptide-1(CRSP-1) can function as a ligand for the CT-R and may act as a catabolic signaling molecule in the CNS.

On the other hand the present study showed that the level of both FSH and LH were significantly increased at $p<0.001$ in orchidectomized animals, while it decreased significantly after administration of sCT which may be due to direct inhibitory effect on the pituitary gland or indirect through decreasing secretion of gonadotropin releasing hormone from the hypothalamus (Nicosia et al., 1986) or through inhibition of basal release of LH (Tsai et al., 1999).

The general effect of CT could be attributed to a change in intracellular calcium of the secreting cell (Isaac et al., 1980) and pituitary cells; furthermore, the widespread recognition of CT-like immunoreactivity in adenohypophysis and in portion of the central nervous system suggests that CT may be a neurotransmitter or paracrine regulator. (Ceda et al.,1982). The calcium signal plays an important role in the control of the secretory processor some adenohypophyseal hormones, which responds to the administration of calcitropic substances by a marked change (Zofkova et al., 1989)

The effects of CT on gonads may be indirectly via high centers-as illustrated above- or directly on gonads, as the findings of (Nakhla et al., 1989) who reported that the possibility that the action of calcitonin on steroid receptors might be mediated by another messenger such as calcium (ca2+) was therefore considered. Progressively lowering the concentration of Ca2+ in the culture medium of the cells from 1.5mM to less than 0.01 mM decreased the concentration of both androgen and estrogen receptors. Returning the Ca2+ concentration to normal levels (1.5mM) restored steroid receptors levels. Receptor levels were also decreased when the extracellular Ca2+ concentration was lowered to 0.5 mM, and treatment with the Ca2+ ionophore, A23187

(1microM), restored receptor levels to normal. The calcium channel blocker, verapamil, decreased the androgen receptor concentration but unexpectedly increased the concentration of estrogen receptors. It was concluded that calcitonin stimulates cAMP formation and testosterone secretion, and increase the concentration of sex steroid receptors. These observations provide evidence that the previously demonstrated calcitonin receptors on Leydig cells may be coupled to several biologic responses in this cell type.

In contrast to calcitonin inhibition of TRH-induced PRL release, sCT failed to prevent the stimulation of PRL release by either IonophoreA23187, PMA, vasoactive intestinal, peptide, or forskolin (Shah et al., 1990)

Although of all previous reports, Chen et al., (1994) found that their results clearly demonstrated a plasma calcium-unrelated inhibitory effect of hCT on LH secretion.

REFERENCES

Abraham,G.E (1981): Radioassay system in clinical endocrinology. Marcal. Dekker,Inc., New York

Azria. M (1989): Endogenous calcitonin. In calcitonins: physiology and pharmacology. Basel, Switzerland ; Karger,chapter 2: 22

Bick.R.J; Poindexter.B.J; Davis. R. A and Schiess.M.C (2005):Determination of the site of action of calcitonin gene-related peptide in the alteration of intracellular calcium levels in adult and neonatal rodent myocytes. Peptides. 26(11): 2231-8

Byron (1978): Physiology, laboratory manual 4 th edition p.p. 249.Saint.Pouis.

Ceda.G.P; Valenti.G; Dall Aglio.E; Palummeri.E; Provvedini.D and Butturini.U (1982): Does calcitonin modulate anterior pituitary hormone secretion? J Endocrinol Invest.5(5):327-30.

Chen. J. J,Tsai. S. C; Lu. C. C; Ice. K. Y; chien. C. Tl; Tsai. C. L weng. P. S and chein. E. J (1994): Effect of human calcitonin on the secretion of luteinizing hormone in orchidectomized rats. Clin.J.Physiol. 37(3).161-3.

Davey. R. A and Morris M. A (2005): The effects of salmon calcitonin-induced hypocalcemia on bone metabolism in ovariectomized rats. J. Bone. Miner. Metab; 23(5): 359-65.

Franceschini. R, Cataldi . A, Cianiosi. P, Garibdi. A, Corsini. G, Barreca. T and Rolandi. E (1993): Calcitonin and beta ñ endorphin secretion. Biomed. Pharmacother. 47 (8): 305

George,S.S.E Bungay.P., J. and Naylor L.H (1997): Functional coupling of endogenous serotonin (5-HTIB) and calcitonin (Cla) receptor in CHO cells to a cyclic AMP-responsive luciferase receptor gene. J. Neurochem. 69: 1278.

Goltzman. D and Mitchell, J (1985) Interaction and calcitonin gene related peptide at receptors site in target tissue. Science.227.1343.

Guisting. A, Bodini. C, Bossoni. S, Doga. M, girelli. A, Pizzocolo. G and Wehrenberg. W. B (1995) : Effects of calcitonin on GH response to pyridostigmine in compinetion with h GHRH (1 - 29) nh 2 in normal edult tsudyects. Cbin - Endocrinof . Oxf, 33 (3) : 375.

Han. J. S, Li. W and Neugebauer. V (2005) : critical role of calcitonin gene-related peptide 1 receptors in the amygdale in synaptic plasticity and pain behavior. J. Neurosci. 1b; 25(46):10717-28.

Hilton J.M, Chai.S.Y. and Sexton. P.M.(1995): In vitro auto radiographic localization of calcitonin receptors isoforms Cla and Clb in rat brain. Neuroscience. 69

(4):1223-37

Ho Yuen, B., Moon,Y.S. Mincey, E. K. and Li, D. (1983): Adrenal and sex steroid hormone production by a virilizing adrenal adenoma and its diagnosis with computerized tomography. Am.J.Obster. Gyncol.145:164.

Horii, I. Takizawa. S; and Fujii.T(1992): Effect of 1,25-dihydroxyvitamin D3on the female reproductive system in rats. J Toxicol Sci. 17(3):91-105.

Isaac.R; Merceron.R; Caillens.G; Raymond.J.P and Ardaillou.R (1980): Effects of calcitonin on basal and thyrotropin-releasing hormone-stimulated prolactin secretion in man. J.Clin Endocrinol Metab. 50(6):1011-5.

Kamada. K, Gaskin. F. S, Yamaguchi. T, Carter. P., Yoshikawa. T, Yusof M. and Korthuis. R. J (2005): Role of calcitonin gene related peptide in the postischenic anti-inflammatory effects of antecedent ethanol ingestion. Am. J. physiology. Heart. Circ.Physiol. Sep. 2.

Kinsy - Jones - J. S;Li. X. F; Bowe. J. E; Brain.S. D; Lightman. S. L; and O. Byrne. K. T (2005) : Effect of calcitonin gene. Relateel peptide on gonadotrophin releasing hormone mRNA expression in GTL - 7 - cells. J. Neuroendocrinal. Sep; 17 (9) : 541 - 4.

Lu. C. C; Tsai. S. C; chien. E. J; Tsai. C. L; and Wang. P. S (2000) : Age related differences in the secretion in male rats. Metabolism. 49 (2) : 253.

Mahrous.T.S and Nakhla.A.M.(1988): Pharmalogical doses of calcitonin reduce sex hormone level in rat plasma. Acta. Endocrino.Copenh.119(4):525.

Marx. J(1993):Two major signal pathway linked. Science. 262:988.

Mitsuma.T.,Nogimori. T and Chaya. M (1984): Periferal administration of eel calcitonin inhibits thyrotropin secretion in rats. Eur.J. Pharmacol.,102;123.

Mulder.H(1993):calcitonin-testosterone-interrelationship.A classic feed back system? Neth. J. Med.42(5-6):209.

Nakhla.A.M; Bardin.C. W; Salomon.Y; Mather.J.P and Janne.O.A(1989): The actions of calcitonin on the TM3 Leydig cell line and on rat Leydig cell-enriched cultures. J Androl. 10(4):311-20.

Nicosia. S, Guidobono. F, Musantim. and Pecile. A. (1986) : Inhibitony effect of calcitonin on adenylate cyclase activity in different rat brain areas . Life. Sci., 39 : 2253.

Noursis, M. J (1997) : Statistical package for social science (spss) base - 8 - 0 for windows. Usera quide. Chichago. IL L : spss.

Sawada.H., Yamaguichi.H.,Shimbara.T., Toshinai.K., Mondal.M.S., Date,Y., Murakami,N. and Katafuchi.T., Minamino.N., Nunoi.H., Nakzato.M.(2006): Central Effects of Calcitonin Receptor-Stimulating Peptide-1 on Energy Homeostasis in Rats. Endocrinology. Jan 12:(Epub ahead)

Schopman. W, Slager. E, Hackeng. W. Lt and Mulder. H (1987) : stimulation of calcitonin secretony capacity by increased serum levels of testosterone in men treated with tamoxifen. Int. J. Androl. 10 (6) : 747.

Shah.G.V; Wang.W. Grosvenor.C.E and Crowley.W.R (1990): Calcitonin inhibits basal and thyrotropin-releasing hormone-induced release of prolactin from anterior pituitary cells: evidence for a selective action exerted proximal to secretagogue-induced increases in cytosolic Ca2+. Endocrinology. 127(2):621-8.

Shah.G.V; Pedchenko. V; Stanley. S. Li. Z and Samson. W. K (1996) : Calcitonin is a physiological inhibitor of prolactin secretion in ovariectomized female rats. Endocrinology. 137 (5) : 1814.

Slisli. Y, and De-Beaurepaire. R (1999): Interleukin-1 beta and calcitonin but not corticotrophin releasing factor, alter sleep cycles when injected into the rat hypothalamic lateral paraventricular area. Neurosci. Lett. 265(1):29.

Taylor, M.M., Baglay,S.L. and Samson, W.K. (2006):Intermedin/Adrenomedullin-2 inhibits growth hormone release from cultured, primary anterior pitui-

tary cells. Endocrinology. 147(2):859-64.

Tsai. S. C; Lu. C. C; Chen. J. J; Wang. S. W; Hawang J.J and Wang. P. S (1999) : Inhibition of salmon calcitonin on secretion of progesterone and GnRH - stimulated pituitary luteinizing hormone. Am. J. Physiol. 277 (40) : E 49.

Wang. P. S; Tsai. S. C; Hwang. G. S; Wang. S. W; lu. C. C; Chen. J. J; Liu. S. R; Lee. K. Y; Chien. E. J; Chien. C. H; Lee. H. Y; Lau. C. P and Tsai. C. L (1994) : Calcitonin inhibits testosterone and luteinizing hormone secretion through a mechanism involving an increase in cAMP production in rats. J. Bone. Mine. Res. 9 (10) : 1583.

Yuzurihara. M, I Karashi. Y, Noguchi. M, Kase. Y, Takeda. S and Aburada. M. (2003): Involvement of calcitonin gene-related peptide in elevation of skin temperature in castrated male rats. Urology, 62(5):947-51.

Zofkova.I; Scholz.G and Starka.L (1989): Effect of calcitonin and 1,25(OH)2-vitamin D3 on the FSH,LH and testosterone secretion at rest and LHRH stimulated secretion. Horm Meta Res. 21(12):682-5.