EFFECT OF CALCIUM ANTAGONISTS ON RAT'S TESTIS; SERUM PROLACTIN AND TESTOSTERONE

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INTRODUCTION

Calcium is involved in several vital cellular processes including contractile, secretory and neural activities; in most of them, calcium may be considered as a final intracellular messenger (Godfraind et al., 1986). In addition, calcium is proved to be an activator of several key enzymes of the cell.

Calcium antagonist is defined as a drug that alters the cellular function of calcium by inhibiting its entry and or its release, by interfering with one of its intracellular actions (Godfraind, 1981). Although the clinical use of calcium antagonists is at the present time mainly directed towards cardiovascular problems, yet understanding of the actions of these compounds in non vascular ailments, might increase awareness of their possible side-

effects and new therapeutic applications.

The aim of the present work is to study the effect of chronic administration of verapamil, one of the phenylalkylamine derivatives, on the rat's serum testosterone and prolactin and histopathological changes on the testis in a trial to explore the effect of this drug on fertility. Since the effects of calcium antagonists are variable, verapamil is compared with dihydropyridine derivatives (nifedipine & isradipin).

MATERIAL AND METHODS

A total of 64 mature male albino rats weighing 260-300 gms each were used in this study. They were randomly divided into 4 equal groups each of 16.

The first group, received water and served as control group.

The second group, received verapamil (Isoptin Arab Drug Co.) 20 mg/kg orally (Ludeman & John, 1985).

The third group, received nifedipine (Epilat Epico) 1/mg/kg orally (Pulse, 1979).

The fourth group, received isradipin (Lomir, Sandoze) 0.3mg/kg orally (Paget & Barnes, 1964). The drugs were dissolved in water and given orally through a stomach tube without anaesthesia. After 6 weeks, half of each group was sacrificed and the other left without treatment for another 6 weeks. Blood samples were withdrawn from the heart before sacrifice and serum was stored at - 20°C. Radioimmunoassay method was used for estimation of serum testosterone (Ismail & Astley, 1986) and prolactin levels (beth 1979 & Pepperell, 1981).

The testes were dissected and weighed. Biopsies were taken for the central portion and fixed in Bouin's solution for 18 hours. The specimens were dehydrated in ascending grades of alcohol, cleared in xylol, embedded in paraffin, cut at 6 microns and

stained with Haematoxylin and Eosin. Sections were examined under light microscopy for changes in seminiferous tubules (number, low power x60 thickness of basement membrane, density of spermatogenic cell lining and spermes in the lumen). Intertubular tissue was also examined for Leydig cell & blood vessels.

RESULTS

Serum prolactin was significantly increased after verapamil, while serum testosterone was significantly decreased after either verapamil or nifedipine. Both hormone levels were not affected by Isradipin (Table 1).

The histopathologic study demonstrated that :

- (A) The testis of the control group showed normal sized seminiferous tubules with their basement membrane. The germinal lining consisted of spermatogonia, most of them showed mitotic figure & primary spermatocytes. The tubules were separated by interstitial tissue (Fig.1).
- (B) Following verapamil administration, a significant decrease in testicular weight was observed (Table1). Seminiferous tubules

were reduced in size and their basement membrane was thicker than normal. The spermatogonia were few and most of them showed absence of mitotic figures. Nearly all tubules showed arrested maturation up to primary spermatocytes, neither spermatids nor sperms were found in the lumen. In foci, spermatogenic cells lining tubules were more reduced in density and appeared degenerated (early atrophic changes). While, the interstitial tissue separating tubules appeared more or less normal with prominent Leydig cells (Fig. 2).

Six weeks after stoppage of verapamil administration, some recovery in the pathologic changes of the testis was observed. The foci of seminiferous tubules with degenerated spermatogenic cell lining were not seen. Few tubules, on the otherhand, showed relatively more dense spermatogenic cells compared to the treated group with maturation of spermatogonia and few sperms in the lumen (hypospermatogenic tubules). Interstitial tissue was less oedematous and less cellular compared to the treated group (Fig. 3).

(C) Following nifedipine administration : the seminiferous tubules were reduced in size and their basement membrane was normal. The spermatogenic cells lining the tubules were reduced in number forming 3-4 layers in cross sections with appearance of mitotic activity in few of them. The majority of tubules showed arrested maturation of spermatogonia up to primary spermatocytes with neither spermatides nor sperms in the lumen. The interstitial tissue separating tubules was normal (Fig. 4). Withdrawal of treatment for 6 weeks showed no evidence of recovery.

(D) Following isradipin, most of the tubules were normal while few exhibited thinner spermatogenic cell lining with few sperms in the lumen (hypospermatogenic tubules); (Fig. 5). No further changes were observed, 6 weeks after stoppage of drug administration.

DISCUSSION

Spermatogenesis is a complex process which depends on pituitary follicular stimulating hormone (FSH) and intratesticular steroids (Ritzen et al., 1981 and Dekretser et al., 1983).

Testosterone is the principal testicular steroid (Lipsett, 1974) which is produced by leydig cells (Hall, 1974). It acts upon sertoli cells and peritubular cells of seminiferous tubules to derive spermatogenesis. Pituitary leuteinizing hormone (LH) exerts both primary acute regulatory effect on leydig cell spermatogenesis and trophic (slow effect) on leydig cell differentiation and development. Also, FSH and prolactin modify LH stimulated leydig cell steroidogenesis (Ewing, 1983).

Gonadotropin-releasing hormone (Gn-RH) is another small peptide produced by the hypothalamus and transported to pituitary gland where it stimulates the synthesis and release of LH & FSH (Mc-Cann and Moss, 1975). The secretion of GnRH is modulated by hypothalamic biogenic amines. While norepinephrine augments secretion of GnRH (Barraclough & Wise 1982) dopamine have inhibitory effect on GnRH & prolactin secretion (Vijayan & Mc. Cann 1978).

The present study demonstrated that after 6 weeks of verapamil administration there was marked decrease in testicular weight associated by histopathologic changes in the form of arrested maturation of spermatogenesis,

with absence of sperms an focal early atrophic changes. This was followed by some recovery 6 weeks after drug withdrawal. This finding is in agreement with (Junej et al., 1990 who reported similar histopathologic changes in guinea pig's testis.

Testicular pathologic changes demonstrated in this work were accompanied by highly significant increase in serum prolactin and significant decrease in serum testosterone levels. Similarly, Verga et al., (1991) demonstrated that Verapamil administration caused significant rise in serum prolactin in normal subjects. The decrease in serum testosterone and the testicular pathologic changes following verapamil could be explained by many investigators :

* Verapamil depresses the gonadotropin response to GnRH (Barbarina & Marinis, 1980 and Barbarina et al., 1983).

* It inhibits testosterone production by leydig cells (Lin et al., 1979)

* The rise in serum prolactin observed in this study may play a factor in the pathologic changes observed in the testis and the decrease in

serum testosterone. Since many published data proved that lactational, experimental or pathological hyperprolactinemia is associated with a decrease in plasma and pituitary gonadotropin with or without a decrease in plasma testosterone and reduced gonadal functions of many vertebrates (MC-Neilly et al., 1978; MC-Neilly 1980; Carter et al., 1983, Tresguerres et al., 1985). Similarly, reduction of both LH & FSH are associated with increased level of prolactin during seasonal infertility in male and female ungulates (MC-Neilly 1987). Also, reduction in plasma LH and testicular weight was observed in ring doves following intracranial injection of prolactin (Buntin et al., 1988). Hyperprolactinemia acts centrally both at pituitary gland to reduce spontaneous gonadotropin release (LH & FSH) and responsiveness to GnRH and at the hypothalamus to reduce the output of GnRH (Mc-Neilly et al., 1978, Brae et al., 1985 and Buntin et al., 1988).

Meanwhile, nifedipine administration for 6 weeks induced less damage to the testis than verapamil, and no recovery was observed. These pathologic changes were accompanied by

insignificant increase in plasma prolactin and highly significant decrease in testosterone level. It was reported that nifedipine, neither, affects human plasma prolactin (Verga et al., 1990), nor inhibits the *in vivo* release of pituitary hormone (Struthers et al., 1983). While, the stimulatory effect of GnRH on leydig cells is calcium dependent and can be blocked by nifedipine (Lin, 1984 and Sullivan & Cooke, 1984). Furthermore, nifedipine blocked testosterone production evoked by phorbol ester (Lin 1985).

On the other hand, isradipin neither induced significant change in serum prolactin and testosterone nor histopathologic changes in the testis. While, Sundsteadt et al., (1989) reported that no haematologic or biochemical changes were attributable to isradipin.

Calcium antagonists elicit their effect on the so-called slow or L- type calcium channels of which there are probably subtypes.

Generally, the more potent compounds have a high affinity. This is typical for compounds of the dihydropyridine type e. g. nifedipine. Verapamil, on the other hand, appears to be less sensitive with respect to different

tissues and therefore, its pattern of cardiovascular activity is different from that of dihydropyridines. Isradipin is a new calcium antagonist with a very high affinity for Ltype calcium channels. As a result, it shows a marked selectivity on specific tissues as sinus nod, coronary, cerebral and skeletal muscle vascular bed (Hof et al., 1984a&b).

CONCLUSION

In the light of these findings, it could be suggested that the adverse effects on fertility are less with dihydropyridine derivatives (nifedipine & isradipin) than phenylalkylamine derivatives (e.g. verapamil). Further, isradipin produces no testicular damage and insignificant hormonal changes. Accordingly, it is more safe for long term therapy specially in young males.

SUMMARY

The effect of chronic administration of phenylalkylamine derivatives (verapamil) on the gonadal structure and function of adult male rats were investigated and compared with dihydropyridine derivatives (nifedipine & isradipin). 64 male albino rats were in-

cluded in this study, they were divided into four equal groups. The first group received water and served as control. Verapamil 20 mg/kg/day, nifedipine 1 mg/Kg/day and isradipine 0.3 mg/Kg/day were given orally through a stomach tube without anaesthesia for 6 weeks to group II, III & IV respectively. Serum testosterone and prolactin were estimated by radioimmunoassay method. Furthermore, histopathological study of testis was performed. Verapamil induced marked alteration in histopathology of testis, which was followed by some recovery after drug withdrawal. Serum prolactin was significantly increased while serum testosterone was significantly decreased. The histopathologic changes in testis following nifedipine were less marked but irreversible. However, no significant changes in serum prolactin and highly significant decrease in serum testosterone levels were observed. Isradipine, neither, induced histopathological changes in testis, nor changes in serum prolactin & testosterone. Thus, this study shows that the effects of calcium antagonists are variable on fertility, though isradipine is more safe for long term therapy.

Fig. 1 : Testis of control rats showing normal seminiferous tubules with all spermatogenic cell lining and numerous sperms in the lumen. (Hx & E. stain x 160).

Fig. 3 : Testis of rats after stoppage of verapamil, showing hypospermatogenic tubules with thin spermatogenic cell lining and few sperms in the lumen (Hx & E. x 160).

Fig. 2 : Testis of rats treated with verapamil showing atrophic changes. Seminiferous tubules are small sized with thick basement membranes, lined by few degenerated cells without sperm formation. Interstitial tissue is oedematous with mild hyperplasia of Leyding cells (Hx & E. 160).

Fig. 4 : Testis of rats treated with nifedipine showing tubules with arrest of spermatogenesis and no sperms in the lumen (Hx & E. x 160).

Fig. 5 : Testis of rats treated with isradipin, few tubules show thinner spermatogenic cell lining with few sperms in the lumen (Hx & E. x 160).

Effect of Verapamil, Nifedipine and Isradipin on Testicular Weight, Serum Prolactin and Testosterone levels.

Drugs administered for 6 weeks	Testicular weight (gms) Mean $\pm$ SE	Serum prolactin (ng / ml.) Mean $\pm$ SE	Serum testosterone (ng / ml.) Mean $\pm$ SE
Control group : (8 rats) / 1ml water	3.44 $\pm$ 0.10	20.8 $\pm$ 0.95	2.47 $\pm$ 0.16
Verapamil 20mg / kg / day orally	1.38 $\pm$ 0.07 $p < 0.01$	34.88 $\pm$ 0.91 $p < 0.01$	1.75 $\pm$ 0.27 $p < 0.05$
Nifedipine 1 mg / kg / day Orally	2.99 $\pm$ 0.19 $p > 0.05$	22.13 $\pm$ 1.36 $p > 0.05$	0.381 $\pm$ 0.04 $p > 0.01$
Isradipin 0.3 mg / kg / day Orally	3.19 $\pm$ 0.12 $p > 0.05$	21.18 $\pm$ 1.27 $p > 0.05$	2.02 $\pm$ 0.21 $p > 0.05$

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تأثير قافلات قنوات الكالسيوم على خصية الفئران التستيريون والبرولاكتين في مصل الدم

أجرى هذا البحث لدراسة التأثير المزمّن لبعض قافلات قنوات الكالسيوم (فيراپاميل - نفيديبين - ازريدين) على التركيب النسيجي والوظيفي للخلايا الجنسية في ذكور الفئران البيضاء وكذلك دراسة تأثير هذه الأدوية على مستوى هرمون التستيريون والبرولاكتين في مصل دم الفئران البيضاء. اشتمل هذا البحث على ٦٤ فأر من الجرذان البيضاء مقسمة إلى أربعة مجموعات متساوية العدد. اتخذت المجموعة الأولى ضابطة أما المجموعات الثلاثة الأخرى فقد أعطيت على التوالي الفيراپاميل (٢٠ مجم/كجم) النفيديبين (١ مجم / كجم) ازريدين (٣ مجم/كجم) يومياً عن طريق الفم للفئران البيضاء لمدة ٦ أسابيع متتالية ولقد لوحظ أن عقار الفيراپاميل أحدث تغييرات مرضية واضحة في أنسجة الخصية مؤقتة والتي زال بعضها بعد توقف الدواء بالإضافة إلى حدوث انخفاض ذو دلالة احصائية في مستوى هرمون التستيريون وكذلك حدوث زيادة ذات دلالة احصائية في مستوى هرمون البرولاكتين في مصل الدم. وقد وجد أن - التغيرات الباثولوجية في الخصية التي أحدثها عقار النفيديبين كانت أقل من التي حدثت مع الفيراپاميل ولكنها مستديمة حيث لم تزول بعد وقف اعطاء الدواء وكذلك فقد حدث انخفاض في مستوى التستيريون ذو دلالة احصائية عالية ولكن لم يحدث أي - تغيير ذو دلالة احصائية في مستوى هرمون البرولاكتين في مصل الدم. أما عند اعطاء عقار الازريدين فقد وجد أنه لم يحدث أي تغير باثولوجي في أنسجة الخصية أو في مستوى هرمون التستيريون والبرولاكتين في مصل الدم.

ومن هذا البحث نستنتج أن استخدام قافلات قنوات الكالسيوم لفترات طويلة لها تأثير مختلف على الخصية وكذلك أظهر هذا البحث أن عقار الازريدين هو أكثر هذه الأدوية أماناً عند استعماله لفترات طويلة ولذا يفضل في علاج الأشخاص البالغين صغار السن.

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