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Effect of Calcium Channel Blockers on Increased Plasma Testosterone Level Induced by Arecoline in Male Albino Rats

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Abstract

Background: Arecoline is one of the major components of betel nuts, which have been consumed as chewing gum in Southeast Asia. Arecoline might affect the endocrine system but the mechanism is still unclear.Some studies suggest that calcium channels may play a role in this effect.

Aim: The present study was carried out to investigate the effect of calcium channel blockers on increased plasma testosterone level induced by Arecoline of betel nuts in rats.

Methods: 80 male albino rats were divided into 8 equal groups as follows: control group; arecoline group; Human chorionic ganadotropin (HCG) group; arecoline + HCG group; nifedipine group; nifedipine + arecoline group; tetrandrine group and tetrandrine + arecoline group. After 2 hours of injection, rats were sacrificed and blood samples were collected and plasma was separated for determination of testosterone, androstendione and leutinizing hormone (LH).

Results: Arecoline with and without HCG resulted in significant increase of plasma testosterone and androsteindione, and only significant increase after HCG injection of LH levels. Injection of nifedipine or tetrandrine alone caused non-significant change in plasma testosterone and androsteindione compared with the control group. Injection of nifedipine or tetrandrine with arecoline resulted in significant reduction in plasma testosterone and androsteindione levels compared with arecoline treated group.

Conclusion: Arecoline caused significant increase in testosterone secretion in male albino rats that may be due to calcium channel activation in Leydig cells.

Keywords: Arecoline, Calcium channel blockers, Testosterone, Rats.

Introduction

Arecoline is an alkaloid extracted from betel nuts (Areca catechol leaves). Four major alkaloids are found in betel nuts: Arecoline (7.5 mg/gm weight), arecaidine (1.5 mg/gm weight), guvacoline (2 mg/gm weight) and guvacine (2.9 mg/gm weight) [1]. Epidemiological studies showed a strong correlation between oral cancer and betel nut chewing habit [2]. Arecoline and its alkaloids have been shown to be carcinogenic [3], immunotoxic [4], genotoxic [5] and teratogenic in animal models [6]. The increased frequency of micronucleated cells, chromosomal aberrations and sister chromatid exchanges in exfoliated cells of buccal mucosa was observed in arecanut consumers [7,8]. In addition, it was shown that arecoline enhances the frequently of chromosomal aberrations and micronuclei in mouse bone marrow cells in vivo [9].

Arecoline mimics the actions of acetylcholine and exerts its effects at both muscarinic and nicotinic receptors [10]. Studies showed that arecoline readily penetrates the blood brain-barrier [11] and exerts its excitatory action by binding to M2 muscarinic receptors on the cell membrane of neurons of the locus coeruleus [12]. Also it was proved that cholinergic agonist arecoline stimulates the hypothalamo-pituitary-adrenal axis in rats [13], and this effect is mediated mainly by release of endogenous corticotropin-releasing hormone. It has been confirmed by the dose-dependent ability of arecoline to cause hypothalamic corticotropin releasing hormone secretion in vitro [12]. Arecoline significantly elevated

the number of micronucleated cells in a dose dependent manner [13]. Moreover, morphological abnormality and unscheduled DNA synthesis were observed in arecoline treated germ cells of the mouse [14]. Intravenous arecoline administration was followed by the increase in plasma epinephrine and ACTH [15]. It was proved that low dose of betel leaf extract was found to increase the tri-iodothyronine and decrease T_4 [16], but high dose of betel leaf extract increased $T_4$and decreased T_3 [16]. Moreover, in Alzheimer patients, elevated plasma ACTH and cortisol were observed in high dose arecoline administration [17]. These studies indicated that arecoline might affect the endocrine system but the mechanism is still unclear. Some studies suggest that calcium channels may play a role in this effect [18].

Nifedipine and tetrandrine are calcium channel blockers used as antihypertensive and in treatment of angina. Calcium is vital in many biological processes including hormonal secretion, mitosis, reproduction, fertility, and regulation of gene expression [18]. Reports exist which suggest that calcium-channel blockers may have anti-reproductive effects in males on long-term treatment. Problems with sexual function have been a long-standing concern in the treatment of hypertension and may influence the choice of treatment regimens and decisions to discontinue drugs [19]. The aim of this work was to investigate the effect of calcium channel blockers on increased plasma testosterone level induced by arecoline of betel nuts in male albino rats.

Materials and Methods

The present study was carried out on 80 male albino rats weighing 200-250 grams. All the experiments were conducted according to the National Research Council's guidelines. Animal handling was followed according to Helsinki declaration of animal ethics. The rats were housed singly and were fed milk bread and had free access to water and food.

They were divided into 8 equal groups as follows:

Group (1): The control group, received 1 ml saline intraperitoneally.

Group (2): The arecoline treated group, received arecoline (Sigma) in a dose of 1 microgram/kg intraperitoneally [20].

Group (3): The human chorionic gonadotropin (HCG) treated group, received HCG (Sigma) in a dose of 5 IU/kg intraperitoneally [20].

Group (4): The arecoline and HCG treated group, received 1microgram/kg arecoline and 5 IU/kg HCG intraperitoneally [20].

Group (5): Nifedipine treat group, received 25 mg/kg nifedipine (Sigma) intraperitoneally [21].

Group (6): Tetrandrine treated group, received 25 mg/kg tetrandrine (Sigma) intraperitoneally [21].

Group (7): Nifedipine and arecoline treated group, received 1 microgram/kg arecoline and nifedipine 25 mg/kg intraperitoneally [21].

Group (8): Tetrandrine and arecoline treated group, received 1 microgram/kg arecoline and 25 mg/kg tetrandrine intraperitoneally [21].

After 2 hours of injection, all rats were sacrificed and blood samples were collected and plasma was separated for determination of plasma testosterone hormone according to the method of Cox et al. [22], plasma leutinizing hormone (LH) and plasma androsteindione according to the method of Kulllin and Santiner [23].

Statistical analysis

Data were presented as mean ± standard error of mean (SEM). Student's t-test was used for evaluating the statistical significance of differences in means. P value of less than 0.05 was considered to be statistically significant [24].

Results

The results are shown in Figures 1-3. They showed that administration of 1microgram/kg arecoline intraperitoneally resulted in significant increase in plasma testosterone and androstenedione levels ($P<0.05$) and non-significant change in plasma LH level compared to control group. The results also showed significant increase in plasma levels of testosterone, androstenedione and LH after injection of HCG alone and injection of HCG and arecoline ($P<0.05$) compared to the control group.

The results showed non-significant change in plasma testosterone, androstenedione and LH levels ($P>0.05$) after injection of nifedipine or tetrandrine compared with the control group. There were significant reduction in plasma levels of testosterone and androstenedione after injection of both arecoline and nifedipine or arecoline and tetrandrine ($P<0.05$) and non-significant change in plasma LH levels compared with the arecoline treated group.

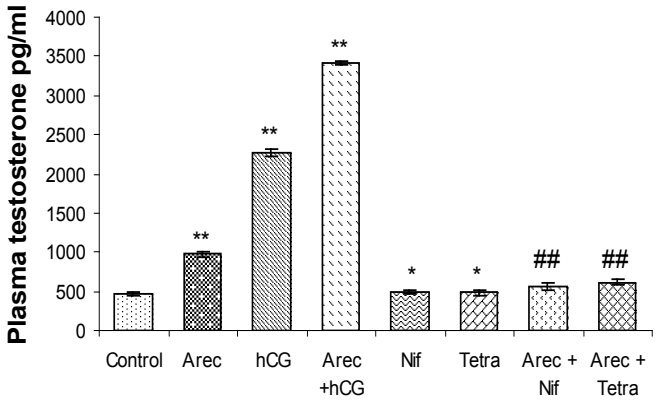

Figure 1: Effect of arecoline, HCG and calcium channel blockers on plasma testosterone in male albino rats (Mean ± SEM)

*Non significant compared to the control group ($P>0.05$)

** Significant compared to the control group ($P<0.05$)

#Non significant compared to arecoline group ($P>0.05$)

Significant compared to arecoline group ($P<0.05$)

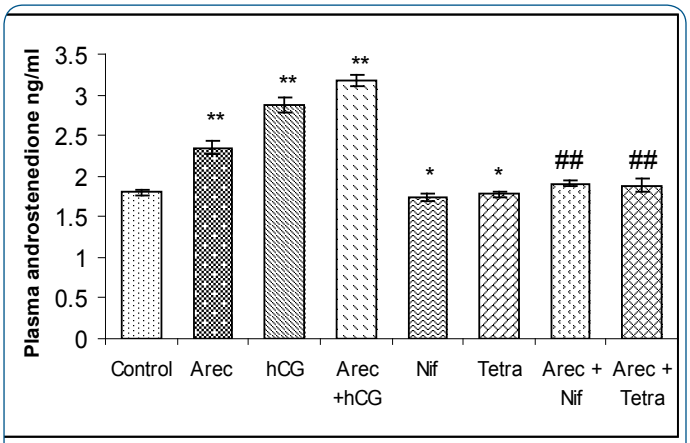

Figure 2: Effect of arecoline, HCG and calcium channel blockers on plasma androstenedione in male albino rats (Mean ± SEM)

*Non significant compared to the control group (P>0.05)

** Significant compared to the control group (P<0.05)

#Non significant compared to arecoline group (P>0.05)

Significant compared to arecoline group (P<0.05)

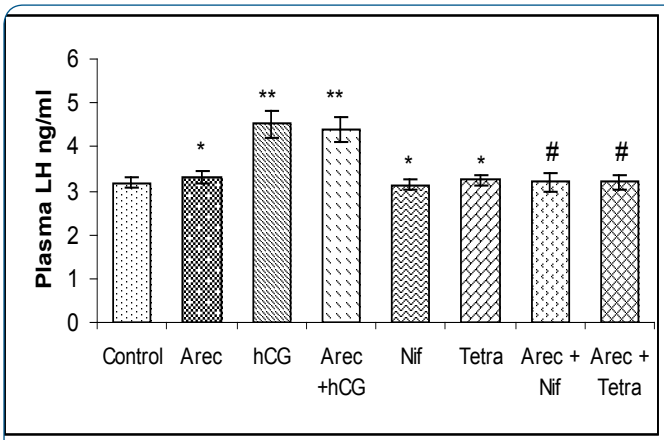

Figure 3: Effect of arecoline, HCG and calcium channel blockers on plasma LH in male albino rats (Mean ± SEM)

*Non significant compared to the control group (P>0.05)

** Significant compared to the control group (P<0.05)

#Non significant compared to arecoline group (P>0.05)

Significant compared to arecoline group (P<0.05)

Discussion

Betel is a masticatory substance and betel chewing is a popular oral habit in southern Taiwan, although the significant correlation of betel chewing and the incidence or oral cancer and oral submucosal fibrosis [3]. Arecoline is one of the major ingredients in betel muts in addition to the other three ingredients; arecaidine, guvacoline and isoguvacine [1]. Each betel quid contains 7.5 mg/gm of Arecoline [25]. Although a number of studies showed that betel chewing is strongly related to oral cancer, betel has been used for the treatment of diarrhea, edema, throat inflammation and tapeworm infection [26].

The results of the present work showed that arecoline with or without HCG, significantly stimulated testosterone secretion. The increased secretion of testosterone may be explained by the ability of arecoline to stimulate steroidogenesis and also HCG-induced plasma testosterone and androstenedione was enhanced by arecoline injection. The effect of arecoline on testosterone was observed by Chiao et al. [27] who noted that significant increase in plasma testosterone in rats after 60 min from arecoline after the LH secretion from the anterior pituitary gland alone or after HCG treatment, in spite of the increase in plasma testosterone which suggested that the action of arecoline on testosterone is not LH dependent [28]. It was observed that, arecoline stimulated testosterone secretion from isolated Leydig cells which may be through nicotinic or muscarinic receptors in the tissues [29]. Testosterone is synthesized in Leydig cells by several metabolic steps known as steroidogenesis. The results of the present work showed significant increase in androstenedione by arecoline injection which can explained the significant increase in testosterone secretion [30], because androstenedione, testosterone precursor, which can be converted to testosterone by 17 beta hydroxylase enzyme [31].

The mechanism of testosterone hormone secretion can be explained by that, stimulation of LH on the steroidogenesis in the cells is through binding to specific receptors, activation of adenylate cyclase, formation of cAMP and protein kinase A activation, phospholipids turnover, inositol triphosphate formation and the increase of intracellular calcium [31]. The increase of cytoplasmic Ca^{+2}, either released from endoplasmic reticulum or transported from calcium channels in the cell membrane, is involved in enhancement of testosterone production by rat Leydig cells [32]. The results of the present study showed that arecoline-induced testosterone secretion was reduced by administration of calcium channel blockers. These results can be explained by that, HCG induced a slow onset and sustained monophasic intracellular Ca^{+2}concentration that was dependent on extracellular Ca^{+2}.

Rossato et al. [32] reported that, the transient but not sustained rise in Ca^{+2} was observed in Leydig cells treated with thapsigargin and cyclopiazonic acid, which are two sarcoplasmic reticulum calcium adenosine triphosphatase inhibitors, in Ca^{+2} free medium. This rise in Ca^{+2} indicated that thapsigargin and cyclopiazonic acid are able to empty the intracellular Ca^{+2} influx [32]. This could be explained by that, the influx of external Ca^{+2}induced by the emptying of internal stores in Leydig cells, that could occur through Na^{+} dependent depolarization of the plasma membrane [33], not through voltage activated Ca^{+2}channels [34], which significantly reduced by administration of either T and L calcium channel blockers, such as nifedipine, with arecoline.

Conclusion

Arecoline stimulates testosterone production in rats via activation of calcium channels in Leydig cells that can be blocked significantly by calcium channel blockers.

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