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# EFFECT OF CALCIUM ION CHANNEL ANTAGONISTS ON CHORIONIC GONADOTROPIN SECRETION

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## SUMMARY

By using calcium ion channel antagonists such as Verapamil and Nifedipine, we demonstrated that calcium ion channels are involved in human chorionic gonadotropin (hCG) secretion stimulated by gonadotropin releasing hormone (GnRH). Addition of 1 μM Verapamil (Class II inhibitor) resulted in an inhibition of GnRH-stimulated hCG secretion by placenta without affecting basal release. However, while addition of 10 μM Nifedipine (Class I inhibitor) resulted only in partial inhibition, significant inhibition was observed at 100 μM concentration and above. These results suggest that calcium ion channels in placenta appear to be similar although not identical with the channels reported in the pituitary.

Key words: Calcium ion channel, Placenta, Verapamil, Nifedipine, hCG secretion.

## INTRODUCTION

Human chorionic gonadotropin is a glycoprotein hormone produced by the placenta. It has two dissimilar subunits alpha and beta held together by non-covalent interactions (1). It shares both structural and functional homology with pituitary LH which is subjected to positive modulation by gonadotropin releasing hormone (2,3). Studies from our own laboratory as well as from others have shown that hCG synthesis and secretion by placenta is also regulated by GnRH (4,5). Earlier studies have established that extracellular calcium is required for the normal secretion of hCG by placenta. In addition it has been observed that the presence of calcium is obligatory for the increase in hCG secretion following addition of GnRH (6,7). Electrophysiological studies on pituitary gonadotropes revealed that in the depolarization of the

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Abbreviations used:
hCG, human chorionic gonadotropin; LH, luteinizing hormone; GnRH, gonadotropin releasing hormone; BSS, balanced salt solution; FTHP, first trimester human placenta; PRIA, plastic tube radioimmunoassay; DMSO, dimethyl sulfoxide; PPO, diphenyl oxazole; POPOP, 1,4-bis[5-phenyl-2-oxozolyl]-benzene; EGTA, ethylene glycol-bis (β–aminoethyl ether) N,N,N',N'-tetraacetic acid.

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plasma membrane, voltage fluctuations were not involved in GnRH stimulated FSH/LH secretion (8). However, stimulation of gonadotropin secretion by GnRH was calcium-dependent and this could be inhibited by calcium antagonists; hence it was suggested that the stimulatory effect of GnRH was mediated by conductance changes associated with the activation of calcium ion channels. Use of Verapamil and Methoxyverapamil which are specific inhibitors for calcium ion channels in the pituitary were found to inhibit the GnRH-stimulated LH release in a dose dependent manner (9). In view of the fact that results of our studies using human placental minces also revealed that GnRH stimulated hCG secretion is mediated by mobilization of extracellular calcium, it was felt that calcium ion channels may be involved in the secretory phenomenon as in the case of pituitary. In this study an attempt has been made to characterize calcium ion channels that mediate hCG secretion in human placenta by using Nifedipine and Verapamil which are class I and class II specific inhibitors of calcium ion channels respectively (10).

## MATERIALS AND METHODS

All the chemicals used were of analytical grade and were obtained from Sarabhai Chemicals (Bombay, India). Verapamil, Nifedipine, EGTA, PPO, POPOP and DMSO were obtained from Sigma Chemical Co (St Louis, MO, USA). GnRH was a gift from Ayerest and Wyeth laboratories (USA). $^{45}CaCl_2$ (1.9 Ci/mole) was obtained from Bhaba Atomic Research center (Bombay, India). Normal BSS at pH 7.2 was prepared with the following composition: 1.3 mM calcium chloride, 5.4 mM potassium chloride, 0.824 mM magnesium chloride, 116.4 mM sodium chloride, 26.2 mM sodium bicarbonate, 0.7 mM sodium dihydrogen phosphate, and 5.6 mM glucose. Calcium-deficient BSS is the same as BSS except that calcium chloride was not added. Calcium free medium is the same as calcium deficient BSS except that 1 mM EGTA was added to chelate all the calcium, if present as impurity in other chemicals used for making BSS. Stock solutions of Verapamil and Nifedipine were always freshly prepared just before use in DMSO and dilutions and incubations were carried out in the dark to avoid inactivation of the drug by light.

### Effect of addition of calcium ion channel inhibitors on GnRH-stimulated hCG secretion by human placenta

Collection of first trimester human placenta and incubations were performed as described earlier (11). In brief, 80-100 mg of placental minces was incubated with or without GnRH in 0.5 ml normal BSS or calcium free BSS or different concentrations of Verapamil or Nifedipine at $37^0$ C in Dubnoff metabolic shaker for 4 h under 95 % oxygen and 5 % carbon dioxide. Following the incubation, the medium was separated by centrifugation at 800 x g, tissue was washed extensively with BSS and homogenized in a known volume of BSS. An aliquot of the tissue homogenate and medium was used for quantitation of hCG by PRIA (12). Another aliquot was used for protein estimation by Lowry's method using BSA as standard (13).

**Effect of addition of Verapamil on GnRH-stimulated $^{45}Ca$ uptake by first trimester human placental minces**

Placental minces were incubated in calcium deficient BSS for 30 min at $37^0$ C under 95 % oxygen and 5 % carbon dioxide. Following the incubation, the tissue was extensively washed with calcium-deficient BSS. One hundred mg of tissue minces was incubated in tubes containing 2 μCi of $^{45}CaCl_2$ in normal BSS with or without GnRH (1 μM) or Verapamil (1-10 μM) or GnRH + Verapamil for 5 min. At stipulated time points the incubated placental samples were diluted with two ml BSS and filtered under vaccum using GFC filters in a Millipore filter assembly. Filters were washed with BSS and the tissue on the filter was digested by boiling in 2 ml of 2 M NaOH. An aliquot of the digest was used to determine the radioactivity using 5 ml scintillation fluid (10% naphthalene, 7.5 % PPO and 0.3 % POPOP in dioxane).

## RESULTS

**Effect of addition of Verapamil and Nifedipine on GnRH-stimulated hCG secretion**

As expected, addition of GnRH to FTHP minces resulted in 2 fold increase in the hCG secreted into the medium over the control (Fig 1). Verapamil at concentrations of 1-50 μM was found to inhibit only GnRH-stimulated hCG secretion and had no effect on basal secretion at all the concentrations tested (Fig 1). Similarly, addition of Nifedipine had no effect on basal hCG secretion at the concentrations tested, while at 10 mM concentration, only partial inhibition (35.5 %) of GnRH-stimulated hCG secretion was observed (Fig 2).

**Effect of Verapamil on GnRH-stimulated $^{45}Ca$ uptake**

Addition of verapamil alone (1-10 μM) was found to have no effect on $^{45}Ca$ up take, while it inhibited GnRH-stimulated $^{45}Ca$ uptake at both the concentrations tested with 10 μM being more effective (Fig 3).

## DISCUSSION

Our earlier studies revealed that GnRH-stimulated hCG release in human placenta depends on the presence of extracellular calcium in the medium (6,7). It was also demonstrated that there was a mobilization of calcium within minutes, following addition of GnRH to the placenta (14). In the present study the use of calcium ion channel inhibitors revealed that GnRH-stimulated extracellular calcium movement into the cytosol takes place through voltage-sensitive calcium ion channels. Verapamil addition resulted in an inhibition of GnRH stimulated hCG secretion in placenta without affecting basal secretion. An inhibitory effect was observed at low

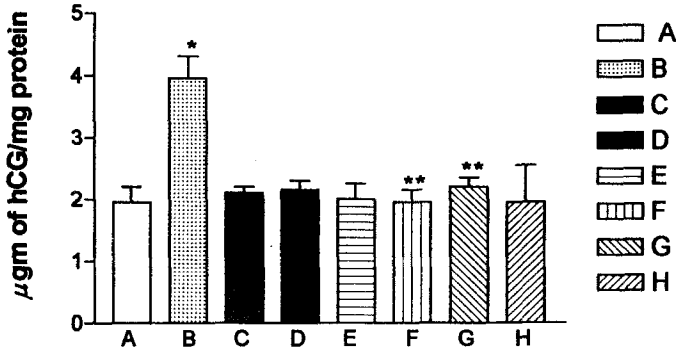


Fig 1. Effect of addition of Verapamil on GnRH-stimulated hCG secretion by FTHP. Each value is the mean of three observations ± standard error. Minced FTHP was incubated with no addition (A), with 1 μM GnRH (B), 1 μM Verapamil ( C ), 10 μM Verapamil (D), 50 μM Verapamil (E), 1 μM GnRH + 1 μM Verapamil (F),1 μM GnRH + 10 μM Verapamil (G) and 1 μM GnRH + 50 μM Verapamil (H) for 4 h at 37 $^0$C under 95 % oxygen and 5 % carbon dioxide. hCG was quantitated in the medium by PRIA. *p<0.01 compared with control, **p<0.01 compared with the group treated with GnRH.


Fig 2. Effect of addition of Nifedipine on GnRH-stimulated hCG secretion by FTHP. Each value is the mean of three observations ± standard error. Minced FTHP was incubated with no addition (A), with 1 μM GnRH (B), 10 μM Nifedipine ( C ), 100 μM Nifedipine (D), 500 μM Nifedipine (E), 1000 μM Nifedipine (F), 1 μM GnRH + 10 μM Nifedipine (G), 1 μM GnRH + 100 μM Nifedipine (H), 1 μM GnRH + 500 μM Nifedipine (I) and 1 μM GnRH + 1000 μM Nifedipine (J) for 4 h at 37$^0$ C under 95 % oxygen and 5 % carbon dioxide. hCG was quantitated in the medium by PRIA. *p<0.01 compared with control, **p<0.01 compared with the group treated with GnRH.

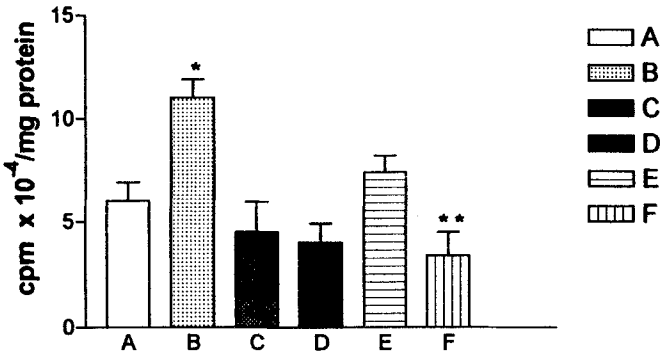


Fig 3. Effect of addition of Verapamil on GnRH-stimulated $^{45}Ca$ uptake by FTHP minces. Each value is the mean of three observations ± standard error. Minced FTHP was incubated in BSS containing no addition (A), with 1 μM GnRH (B), 1 μM Verapamil ( C ), 10 μM Verapamil (D), 1 μM GnRH + 1 μM Verapamil (E) and 1mM GnRH + 10 μM Verapamil (F) for 5 min under 95 % oxygen and 5 % carbon dioxide. The tissue was processed for monitoring the radioactive calcium.*$p<0.01$ compared with control, **$p<0.01$ compared with the group treated with GnRH.

concentrations of 1 μM. However, while Nifedipine showed only a partial inhibition of GnRH-stimulated hCG secretion at 10 μM, a significant inhibition was observed at 100 μM and above. Interestingly, it was reported earlier that Verapamil showed greater inhibition of GnRH-stimulated LH secretion in the pituitary and Nifedipine virtually had no effect (15). It should be noted that in smooth muscle, the class II inhibitors are less potent and class I inhibitors are the most powerful antagonists of calcium ion channel (10). Although a good deal of information is available on the action of these inhibitors at calcium ion channels the precise loci of action within the channel remains unclear. The present study suggests that GnRH-stimulated hCG secretion in placenta is through calcium ion channels which appears to be chemically similar although not identical with the channels reported in pituitary gonadotropes, as Nifedipine (class I inhibitor) also inhibited the hCG release. Our studies also revealed that verapamil (1-10 μM) inhibited GnRH-stimulated $^{45}Ca$ uptake which supports the view that calcium ion channels are involved in GnRH action. Further studies are required to characterize how calcium channels of the placenta differ from calcium channels present in the pituitary and in smooth muscle.

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