

## INTERACTION OF CALCIUM CHANNEL BLOCKERS (CCBs) WITH HISTAMINE AND 5-HYDROXYTRYPTAMINE IN AORTA FROM NORMAL AND DISEASED RATS

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**Abstract :** The present study attempts to investigate the interaction of calcium channel blockers (CCBs) with histamine (H) and 5-hydroxytryptamine (5-HT) in rat isolated aortic strip preparations. In preparations obtained from rats chronically treated with various CCBs the contractile responses to H were completely blocked suggesting that this may be due to inhibition of the voltage-dependent channels and inositol 1,4,5-triphosphate induced release of calcium from intracellular stores. The decreased contractions of the aortic strip preparations with 5-HT obtained from rats chronically treated with various CCBs implies a decrease in 5-HT receptor density. DOCA-saline hypertensive rats chronically treated with various CCBs showed variable responses to H and 5-HT suggesting that these changes may be due to different isoforms of L-type calcium channels. In L-thyroxine-treated preparations or those simultaneously treated with L-thyroxine and CCBs the responses to H were abolished and those to 5-HT were partially blocked with decrease in maxima which could be secondary to the primary effect on the heart and to generalised reduced sensitivity of the rat aorta.

**Key words :** aorta  
histamine

5-hydroxytryptamine  
calcium channel blockers

### INTRODUCTION

The contractile process in vascular smooth muscle is dependent upon the presence of free calcium ion at sufficient intracellular concentration (1-3). A generally accepted scheme to explain the responses of blood vessels to agonists such as noradrenaline, angiotensin and 5-hydroxytryptamine (5-HT) is as follows (4) :

i) Breakdown of phosphatidyl inositol (PI) to yield triphosphoinositol ( $IP_3$ ) which releases calcium from intracellular stores.

ii) Opening of receptor-operated calcium channels (ROC) which allows calcium to enter the cell and produce depolarization.

iii) Depolarization causes potential-operated channels (POC) to open, which allows calcium to enter the cell.

Calcium channel blockers are established as one of the first line drugs in the therapy of essential hypertension (5). Increases in the  $K_D$  and  $B_{max}$  values for [ $^3H$ ] nitrendipine binding sites have been observed in heart membranes from 24-weeks old spontaneously hypertensive rats (SHR) (6). Clinically hyperthyroidism may be associated with systolic hypertension (7). Chronic treatment of cultured chick ventricular cell with thyroid hormone is reported to produce an increase in calcium channel density which correlated well with 1,4-dihydropyridine

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sensitive calcium channel uptake (8). In contrast, heart membranes from rats made hyperthyroid by 5-day treatment with thyroxine showed a decrease in the number of VDCCs (9).

The present study attempts to investigate the interaction of calcium channel blockers with histamine (H), and 5-hydroxytryptamine (5-HT) in aorta from normal rats, DOCA-saline hypertensive rats and hyperthyroid rats.

## METHODS

### I. Preparation of rat isolated aortic strip for recording of contractions:

Male albino rats of Wistar strain weighing 250-350 gm were used for the present experiments. Chronic treatment of the rats was started from day one and at the time of sacrifice the rats weighed between 200 to 350 gm depending on the chronic treatment with different drugs. The animals were sacrificed by a sharp blow on the head and cutting the neck blood vessels.

The thoracic aorta was rapidly removed and placed into a petridish containing oxygenated McEwen physiological salt solution (pH-7.3) of the following composition (mM) : NaCl-113; KCl-5.6; CaCl<sub>2</sub>-2.2; NaHCO<sub>3</sub>-25.0; NaH<sub>2</sub>PO<sub>4</sub>-1.21; glucose-11.1; and sucrose-13.1. The thoracic aorta was cleaned of connective tissue and adherent fat. The isolated artery was cut helically into strips as described by Furchgott and Bhadrakom (10) for the rabbit aortic strip. From each thoracic aorta strips were prepared and the same portion of the strip was used in all sets of experiments for a particular agonist response.

The aortic strip was suspended into 30 ml organ bath containing McEwen physiological salt solution (pH-7.3) maintained at 30°C ± 0.5°C. The bath medium was bubbled with oxygen (11). The aortic strip was arranged for isometric tension recording by tying the lower end to the glass tissue holder and upper end to the force displacement transducer. The tissue was allowed to equilibrate under 1 g resting tension for 60 min, during which the bathing

solution was routinely changed every 15 min. After equilibration the strips were exposed to cumulative doses of histamine (H) and 5-HT. The doses were added in a cumulative fashion at 0.3 log unit intervals. The next higher dose was added after the first dose had caused maximal contraction.

### II. Chronic drug treatment schedules:

Group of 5-10 normal or 3-4 hypertensive rats received similar chronic treatments with drugs.

Verapamil (30 mg/kg) and diltiazem (20 mg/kg) dissolved in triple glass distilled water and nifedipine (10 mg/kg) and nimodipine (20 mg/kg) dissolved in the solvent (PEG400-969 g, glycerine-60 g, water-100 g) were administered orally via a Ryles tube once daily for 28 days (12).

Drug treatment schedules for thyroxine-treated rats were as follows :

A group of rats received simultaneously the same dose of L-thyroxine sodium and nifedipine (10 mg/kg) or nimodipine (20 mg/kg) orally by Ryles tube.

### III. Diseased state models:

1. *Hypertension* : Male albino rats weighing about 100 g were kept on a diet high in sodium chloride and drinking water was replaced by 2% sodium chloride solution *ad lib*. When they attained a weight of about 250 g, they were also given deoxycorticosterone acetate (DOCA) dissolved in sesame seed oil in a dose of 10 mg/kg, sc twice weekly for 42 days (13).

In order to check as to whether hypertension had been produced by the DOCA-saline treatment schedule, blood pressure of rats was recorded (14).

Following confirmation of induction of hypertension groups of 3-4 rats received chronic treatment with drugs as under II.

2. *Hyperthyroidism* : Hyperthyroidism was induced by subcutaneous injection of 0.75 mg/

kg L-thyroxine sodium in alkaline saline solution (0.001N NaOH in 0.9% NaCl) daily for 7 days (15).

Rectal temperature and heart weights of L-thyroxine treated animals were recorded before the start of the treatment and before sacrifice. Serum was prepared from blood samples collected from the common carotid artery of rat exsanguinated on the day of the experiment and stored at  $-20^{\circ}\text{C}$ . Total serum thyroxine ( $T_4$ ), Tri-iodothyronine ( $T_3$ ) and thyroxine stimulating hormone (TSH) levels were determined by enzyme immunoassay (EIA) with a commercially available *in vitro* 'diagnostic kit' (Bio Me'rieux, France) on semi-autoanalyser (SEAC CH-100; ames marketed by Miles India, Baroda).

#### IV. Drugs:

Histamine (H), 5-hydroxytryptamine creatine sulfate complex (5-HT) was obtained from Sigma Chemical Co., Pentobarbitone sodium was obtained from National Chemicals, Baroda. The following drugs were received as free gifts : diltiazem (Sun Pharmaceutical Industries, Baroda); verapamil and nifedipine (Torrent Pharmaceutical Ltd., Ahmedabad); L-thyroxine sodium (Glaxo Laboratores (India) Ltd., Bombay); nimodipine (U.S. Vitamins, Bombay), deoxycorticosterone acetate (Infar (Inda) Ltd., Calcutta).

Polyethylene glycol 400 (E. Merck (India) Ltd., Bombay); glycerine I.P. (Metro Pharmaceutical Industry, Wadhwan City); sesame seed oil (Ahmed Mills, Bombay) were obtained and used as solvents of the drugs.

The *in vitro* diagnostic kits used for the estimation of TSH,  $T_3$  and  $T_4$  by enzyme immunoassay were of Bio Me'rieux, France obtained from "Cadila Diagnostic Division", Ahmedabad.

#### V. Statistical analysis:

Only one agonist was used for getting concentration-response curve in a given preparation. Contractile force was expressed as

mg of tension developed.  $EC_{50}$  values were determined by the method of Van Rossum (16) from the concentration-response curve against percentage of its own maximum produced by a given agonist. The  $pD_2$  values were determined by the method of Ariens (17). The results are expressed as mean  $\pm$  S.E.M. and analysed by the Student's "t" test for obtaining the level of significance (18).

## RESULTS

H ( $0.59 \times 10^{-7}\text{M}$ - $6.16 \times 10^{-5}\text{M}$ ) and 5-HT ( $0.74 \times 10^{-7}\text{M}$ - $9.70 \times 10^{-6}\text{M}$ ) produced concentration-dependent contractions of the aortic strip. The concentration-response curves of H and 5-HT were not significantly ( $P > 0.05$ ) affected in preparations obtained from rats treated with solvent (PEG 400-969 g; glycerine-60 g, water 100 g), saline or sesame seed oil.

#### Effect of calcium channel blockers on the contractile responses to H and 5-HT:

*Control rats* : In preparations obtained from rats chronically treated with verapamil, nifedipine, nimodipine or diltiazem the maximal contractions generated by aortic strips in response to 5-HT and the  $pD_2$  values of 5-HT compared to controls were significantly reduced. There was complete inhibition of responses to H ( $0.59 \times 10^{-7}\text{M}$  -  $4.72 \times 10^{-3}\text{M}$ ) with all the calcium channel blockers except verapamil which caused a significant reduction in the contractile force and  $pD_2$  value of H as compared to control (Table I).

*Hypertensive rats* : The mean blood pressure of control rats given sesame seed oil sc for 42 days was  $103 \pm 3.3$  mm Hg ( $n=3$ ); the mean blood pressure of rats treated with DOCA - saline for 42 days was  $132.5 \pm 2.5$  mm Hg ( $n=4$ ). The rise in blood pressure was significantly ( $P < 0.01$ ) higher.

In hypertensive preparations the contractile force generated by the aortic strip in response to H and 5-HT was reduced significantly in relation to control preparation. However, the  $pD_2$  values of the agonists were not significantly

TABLE I: Effect of CCBs on maximal contractile force and  $pD_2$  generated in rat isolated aorta by 5-HT and histamine in control, DOCA-saline hypertensive and hyperthyroid rats.

Treatment group	5-HT			Histamine		
	Maximum contractile force (mg)	(n)	$pD_2$ value	Maximum contractile force (mg)	(n)	$pD_2$ value
Control	630 ± 25	(10)	5.99 ± 0.04	774 ± 22	(8)	5.27 ± 0.05
Chronic Verapamil	225 ± 19*	(6)	5.04 ± 0.03*	372 ± 09*	(6)	4.83 ± 0.04*
Chronic Nifedipine	214 ± 14*	(5)	5.01 ± 0.06*	No response	(5)	—
Chronic Nimodipine	504 ± 15*	(5)	5.62 ± 0.03**	No response	(5)	—
Chronic Diltiazem	184 ± 08*	(5)	4.42 ± 0.05*	No response	(5)	—
DOCA - saline	306 ± 10*	(3)	6.08 ± 0.07 <sup>NS</sup>	252 ± 18*	(3)	5.27 ± 0.01 <sup>NS</sup>
DOCA - saline+Chronic Nifedipine	306 ± 58 <sup>NS</sup>	(3)	5.77 ± 0.01**	198 ± 10*	(3)	5.54 ± 0.14 <sup>NS</sup>
DOCA - saline+Chronic Nimodipine	271 ± 10 <sup>NS</sup>	(3)	6.07 ± 0.01 <sup>NS</sup>	220 ± 22 <sup>NS</sup>	(3)	5.60 ± 0.10 <sup>NS</sup>
L-Thyroxine	282 ± 06*	(3)	6.18 ± 0.06 <sup>NS</sup>	228 ± 26*	(3)	5.60 ± 0.07*
L-Thyroxine+Chronic Nifedipine	255 ± 00**	(3)	6.27 ± 0.04 <sup>NS</sup>	No response	(3)	—
L-Thyroxine+Chronic Nimodipine	210 ± 06*	(3)	6.27 ± 0.04 <sup>NS</sup>	No response	(3)	—

Values are cited as means ± S.E.M.

\* $P < 0.01$  and \*\* $P < 0.05$  indicate significant differences.

Comparisons were made between (i) chronic calcium channel blocker/DOCA-saline/L-thyroxine and control; (ii) chronic DOCA-saline and chronic DOCA-saline + calcium channel blocker, (iii) chronic L-thyroxine and L-thyroxine + calcium channel blocker.

affected. Chronic treatment with nifedipine or nimodipine did not produce any significant changes in the contractile force in response to 5-HT as compared to that in DOCA - saline control preparations. However, the  $pD_2$  value of 5-HT was significantly reduced by nifedipine, but was unaffected by nimodipine. In case of H, the maximal contractile force was significantly reduced by chronic nifedipine treatment without any change in the  $pD_2$  value of H. Chronic nimodipine neither significantly affected the maximal contractile force nor the  $pD_2$  value (Table I).

**Hyperthyroid rats :** Various somatic parameters were measured following induction of hyperthyroid state and treatment with calcium channel blockers. This was done to monitor the efficacy of various treatment protocols. In hyperthyroid state the heart weight was increased ( $P < 0.01$ ), the rectal temperature was enhanced ( $P < 0.01$ ), the  $T_3$  and  $T_4$  levels was raised ( $P < 0.01$ ) and the TSH level was reduced ( $P < 0.01$ ). Chronic nimodipine and nifedipine treatments reduced ( $P < 0.01$ ) the heart weight and rectal temperature, had no effect ( $P > 0.05$ ) on  $T_3$  and  $T_4$  levels and enhanced ( $P < 0.01$ ) the TSH levels (Table II).

Chronic treatment with L-thyroxine significantly ( $P < 0.01$ ) reduced the maximal contractile force generated with both 5-HT and H; however the sensitivity was reduced ( $P < 0.01$ ) only in the case of H as compared to control. Chronic treatment with L-thyroxine and nifedipine or nimodipine significantly ( $P < 0.05$ ;  $P < 0.01$ ) reduced the contractile force with 5-HT, but had no significant ( $P > 0.05$ ) effect on the  $pD_2$  values as compared to L-thyroxine control. Both nifedipine and nimodipine treatments abolished responses to H (Table I).

## DISCUSSION

H can initiate smooth muscle contraction by releasing calcium from intracellular stores or by opening either receptor-operated or voltage-operated calcium channels (19). Calcium from intracellular stores released by H-receptor stimulation has been demonstrated in endothelial cells (20), air way smooth muscle (21), rat aorta (22), although in most cases there is also an appreciable stimulation of transmembrane calcium influx (23).

In the present study with aortic strip preparations treated with verapamil there may have been some decrease in the density of H

TABLE II : Effect of chronic treatment with calcium channel blockers on various somatic parameters measured following altered thyroid state in rats.

Variable	Treatment group			
	Control	L-thyroxine	L-thyroxine + nifedipine	L-thyroxine + nimodipine
Body weight (g)	250.00 ± 0.0	216.66 ± 10.54*	316.66 ± 10.15*	225.00 ± 11.18 <sup>NS</sup>
Rectal temperature in °C	37.81 ± 0.03	39.56 ± 0.15*	37.75 ± 0.14*	37.75 ± 0.17*
Heart weight (ng 100 <sup>-1</sup> g body wt.)	362.00 ± 7.0	594.00 ± 1.8*	407.30 ± 14.8*	552.50 ± 22.0*
Serum thyroxine (T <sub>4</sub> ) (MOG%)	11.30 ± 0.3	38.26 ± 0.35*	38.26 ± 0.35 <sup>NS</sup>	36.46 ± 1.37 <sup>NS</sup>
Serum triiodothyronine (T <sub>3</sub> ) (NG/ML)	1.23 ± 0.08	8.68 ± 0.5*	8.87 ± 0.13 <sup>NS</sup>	7.00 ± 1.90 <sup>NS</sup>
Serum TSH (Mu/ML)	3.83 ± 0.03	0.24 ± 0.02*	1.60 ± 0.33*	2.10 ± 0.90 <sup>NS</sup>

Values are cited as means ± S.E.M.

\*P<0.01 and \*\*P<0.05 indicate significant differences.

Comparison were made between (i) chronic L-thyroxine treatment and control (ii) chronic L-thyroxine treatment and chronic L-thyroxine + calcium channel blocker treatment.

receptors as evidenced by decrease in the pD<sub>2</sub> value. This is similar to the finding of Morel et al (24) who have shown that the contractile response to H<sub>1</sub> receptor stimulation in the intestinal smooth muscle is largely sensitive to inhibition by dihydropyridine-based antagonists of voltage-dependent calcium channels.

The tension developed at high concentrations of H in intestinal smooth muscle is partly resistant to nifedipine indicating that a component of the contractile response may be mediated by inositol 1,4,5-triphosphate-induced release of calcium from intracellular stores (24, 25). In the present study with chronically nifedipine, diltiazem or nimodipine-treated preparations, there was complete block of contractile response to H suggesting that this may have been mediated by inhibition of inositol 1,4,5 triphosphate-induced release of calcium from intracellular stores.

5-HT is considered to contract the rat aorta by stimulating 5-HT<sub>2</sub> receptors only. Alpha-adrenoceptor are not involved as prazosin and idazoxan (selective alpha and alpha<sub>2</sub> adrenoceptor antagonists, respectively) have no effect on the 5-HT responses (26). In the present study chronic treatment with verapamil, nifedipine, diltiazem or nimodipine reduced contractile force and sensitivity of 5-HT receptor density.

Chronic nifedipine treatment of DOCA-saline hypertensive rats reduced the contractile force with H, but had no significant effect on the contractile force with 5-HT. This treatment did not affect the sensitivity to both the agonists. It is possible that these differential changes with the two calcium channel blockers may be related to the occurrence of different isoforms of L-type calcium channels (27).

In L-thyroxine treated preparations the contractile force with both 5-HT and H was reduced but the sensitivity was reduced only in the case of H. The pD<sub>2</sub> value of 5-HT was unaffected, but the maximal response was decreased by chronic treatment with thyroxine and nifedipine or nimodipine. Simultaneous treatment with nifedipine or nimodipine and L-thyroxine abolished the concentration response of H. These effects could be due to the generalised reduced sensitivity of the rat aorta and or secondary to a primary effect exerted on the heart. A significant reduction in heart weight (Table II) produced by chronic nifedipine or nimodipine administration in the thyroxine treated rats would support the latter suggestion. Further work with specific radioligands would be necessary to confirm the above suggestion.

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