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INTERACTION OF CALCIUM CHANNEL BLOCKERS WITH DIFFERENT AGONISTS IN AORTA FROM NORMAL AND DISEASED RATS

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Abstract : The interactions of calcium channel blockers (CCBs) with noradrenaline (NA), phenylephrine (PE), dopamine (DA) and KCl have been investigated in rat isolated aortic strip.

In preparations from control and hypertensive (DOCA-saline) rats chronically treated with verapamil, nifedipine and diltiazem, there was partial inhibition of contractions to NA, PE and DA. However, with nimodipine, there was potentiation of responses. This could be related to the occurence of different isoforms of L-type calcium channels. In preparations obtained from hyperthyroid rats the concentration-response curves of NA, PE and KCl were shifted to the right with depressed maximal response which could be secondary to the primary effect exerted on the heart. In preparations from L-thyroxine + nimodipine/ nifedipine treated rats the concentration-response curves of NA, PE and KCl were shifted to the right and the maxima was depressed suggesting that this may be due to decreased alpha receptor density (NA and PE) and down-regulation of voltage operated channels (KCl).

Key words : rat aorta calcium channel blockers noradrenaline phenylephrine dopamine potassium chloride

INTRODUCTION

Vascular smooth muscle contraction occurs following an influx of extracellular calcium into the cell, raising intracellular concentrations of calcium approximately 100-fold (from 0.1 to 10 μmol/L) (1-4). The calcium then initiates a cascade reaction which results in increased contractile activity in an experimental preparation or an increased vascular tone in an intact circulatory system (5). Thus the contractile process in vascular smooth muscle is dependent upon the presence of free calcium ion at sufficient intracellular concentrations (6-8).

Receptor-operated channels are likely to be the most responsible in the initiation of vascular smooth muscle contraction. They may be opened by the interaction of a noradrenaline (NA) molecule with an alpha-adrenoceptor on the cell membrane or by the binding of other agonist agents such as histamine (HA), 5-hydroxytryptamine (5-HT) and prostaglandins etc. to other membrane receptors (9).

Potential-dependent channels, which open in response to a depolarising stimulus such as electrical impulse or a relatively high concentration of potassium (9, 10), are present in myogenically active blood vessels such as

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precapillary arterioles and in the portal mesenteric vasculature but usually not in other veins or larger arteries (11). In some vascular tissues, potassium depolarisation may cause release of endogenous NA, thus indirectly activating receptor-operated channels (10).

In the present study with the rat aortic strip preparations, changes in the receptor-operated channels and potential-dependent channels were studied by comparing the responses to different agonists such as NA, Phenylephrine (PE), Dopamine (DA) and KCl.

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 gms 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 of the following composition (mM) : NaCl-113; KCl-5.6; $CaCl_2$-2.2; $NaHCO_3$-25.0, NaH_2PO_4-1.21; 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 (12) 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 maintained at 30°C ± 0.5°C. The bath medium was bubbled with oxygen. The aortic strip was arranged for isometric tension recording by tying the lower end of the strip to the glass oxygenating tube 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 NA, phenylephrine, dopamine and KCl. The doses were added in a cumulative fashion. The next higher dose was added after the first dose had caused maximal contraction.

II. Chronic drug treatment schedules:

Groups 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 (PEG 400-969 g, glycerine-60 g, water-100 g) were administered via a Ryles tube once daily for 28 days.

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

Another group of rats received simultaneously the same dose of L-thyroxine sodium sc 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.

In order to check as to whether hypertension had been produced by the DOCA-saline treatment schedule, blood pressure of rats randomly selected from each group of ten rats was recorded (13).

Following confirmation of the 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 (14).

IV. Drugs:

Noradrenaline (± Arterenol; NA) was obtained from Sigma Chemical Co. Potassium chloride was obtained from Qualigen Fine Chemicals, Bombay. Pentobarbitone sodium was obtained from National Chemicals, Baroda. The following drugs were received as free gifts, dopamine hydrochloride (TTK Pharma Ltd., Madras); diltiazem (Sun Pharmaceutical Industries, Baroda); verapamil and nifedipine (Torrent Pharmaceutical Ltd., Ahmedabad); L-thyroxine sodium (Glaxo Laboratories (India) Ltd., Calcutta); nimodipine (U.S. Vitamins, Bombay); phenylephrine (Dr. T.V. Subhaiah, Alembic Chemical Works Co. Ltd., Baroda); deoxycorticosterone acetate (Infar (India) Ltd., Bombay).

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.

V. Statistical methods:

Only one agonist was used for getting concentration-response curve in a given preparation. Test responses are expressed as percentage of the corresponding control maximal response to a given agonist. The results are expressed as mean ± S.E.M. and analysed by the Student's "t" test for unpaired observation for obtaining the level of significance (15).

RESULTS

Contractile responses of the aortic strip to NA, DA, PE and KCl:

Control : NA(0.39×10^{-7}M-1.20×10^{-6}M), DA(0.86×10^{-7}M-1.11×10^{-5}M), PE(0.65×10^{-7} M-2.07×10^{-6}M) and KCl (1.79×10^{-4}M-1.14×10^{-2}M) produced concentration-dependent contractions of the aortic strip.

Chronic treatment with calcium channel blockers : In preparations obtained from rats chronically treated with verapamil, diltiazem or nifedipine the concentration-response curves of NA/PE were shifted ($P < 0.01$) to the right and the maximal response was reduced ($P < 0.01$) (Fig. 1 a, b, c) and (Fig. 2 a, b). With chronic nimodipine treatment the concentration-response curve of NA was shifted to the left at the lower concentration (0.39×10^{-7}M), and to the right ($P < 0.01$) at higher concentrations (2.73×10^{-7}M - 2.45×10^{-6}M). The maximal response was reduced ($P < 0.01$) (Fig.1 d). The concentration-response curve of PE was shifted to the left at all concentrations and there was increase of maximal response (Fig. 2 c).

In preparations obtained from rats chronically treated with verapamil, there was no response to DA over a concentration range of 0.86×10^{-7}M - 1.98×10^{-4}M. Chronic treatment with diltiazem, nifedipine or nimodipine shifted ($P < 0.01$) the concentration response curve of DA to the right and reduced ($P < 0.01$) the maximal response (Fig. 3a, b, c).

In preparations obtained from rats chronically treated with verapamil, diltiazem, nifedipine or nimodipine the concentration-response curve of KCl was shifted to the right ($P < 0.01$) and the maximal response was reduced ($P < 0.01$) (Fig. 4a, b, c, d).

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

Contractile responses of the aortic strip to NA, PE and KCl in hypertensive rats:

Control preparations : In preparations obtained from rats chronically treated with

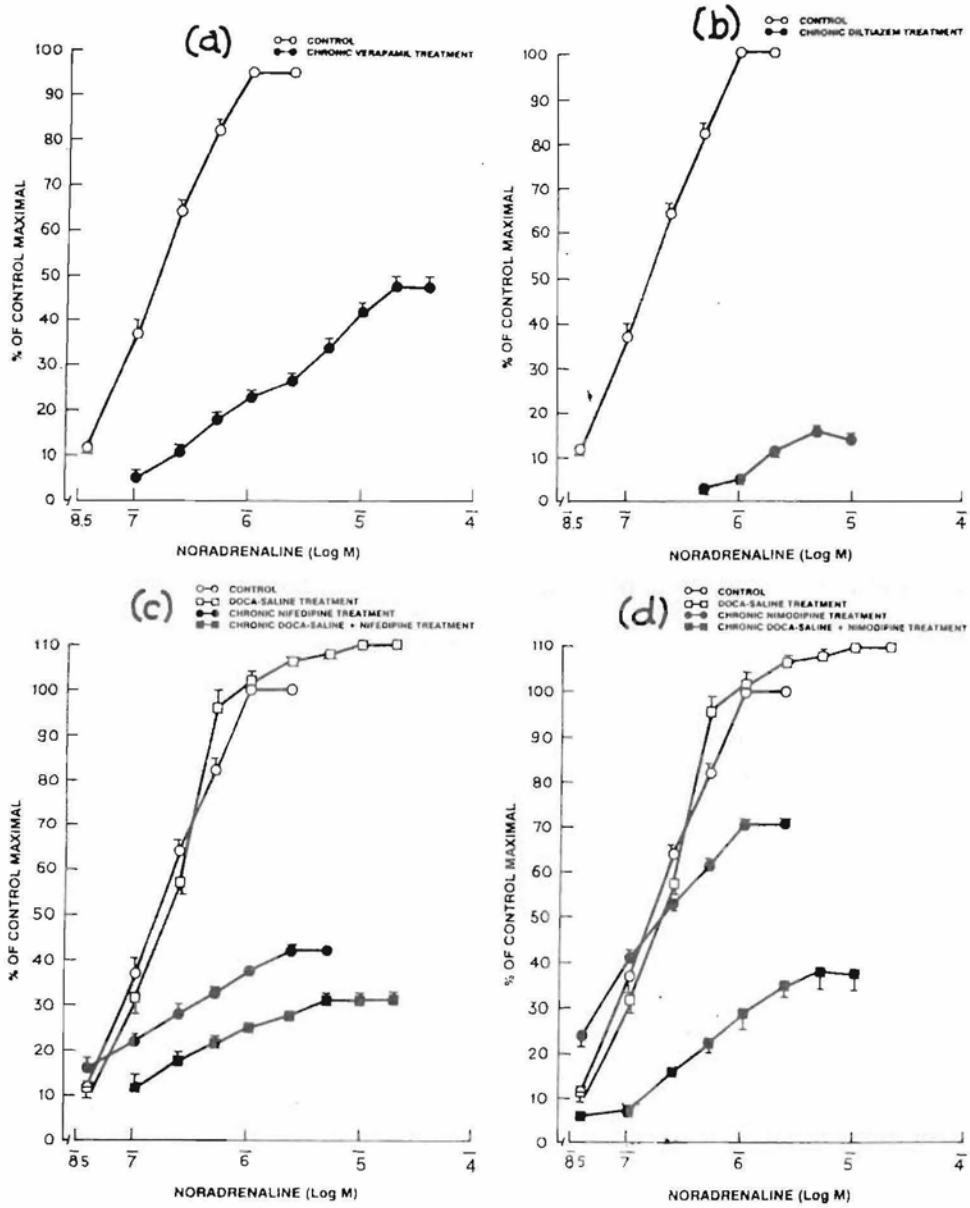

Fig. 1 : Effect of calcium channel blockers on the contractile responses of rat aortic strip to NA. Responses are calculated as percentage of the control maximal response and plotted against the logarithm of the molar concentration of NA. Each value is the mean of 5-6 experiments of control and chronic calcium channel blocker treatment and 3 experiments each for chronic DOCA-saline treatment and chronic DOCA-saline and nifedipine or nimodipine treatment. Vertical lines indicate SEM.

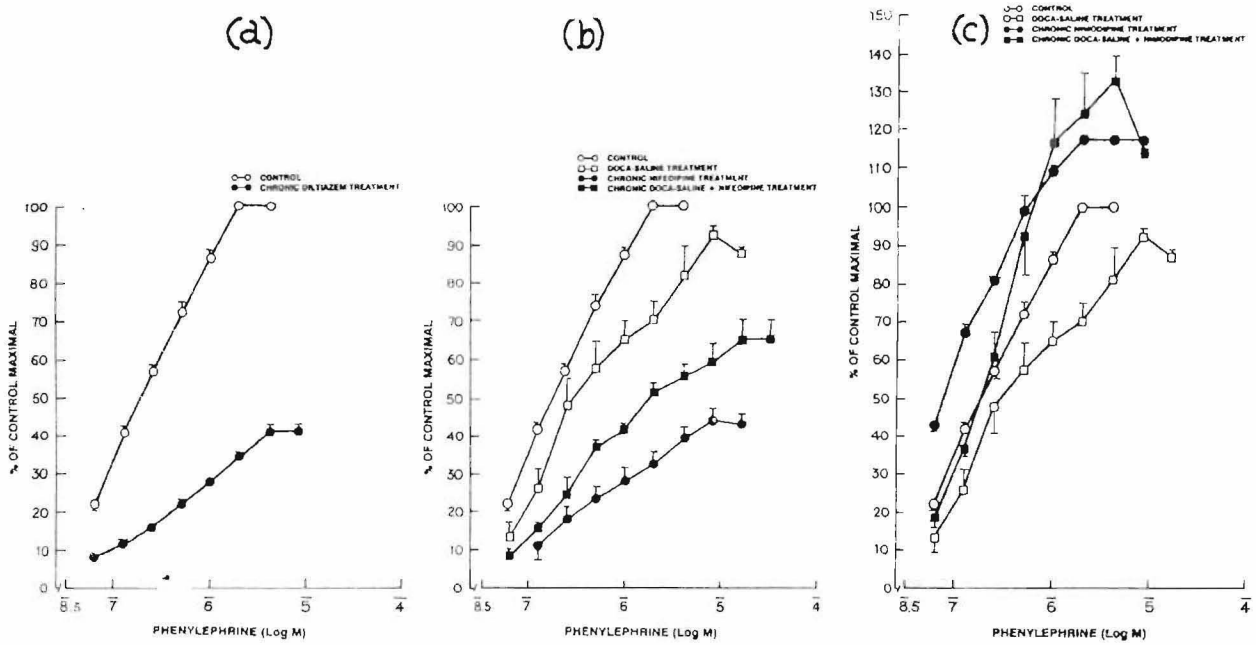

Fig. 2 : Effect of calcium channel blockers on the contractile responses of rat aortic strip to PE. Responses are calculated as percentage of the control maximal response and plotted against the logarithm of the molar concentration of PE. Each value is the mean of 5-6 experiments for control and chronic calcium channel blocker treatment and 3 experiments each for chronic DOCA-saline treatment and chronic DOCA-saline and nifedipine or nimodipine treatment. Vertical lines indicate SEM.

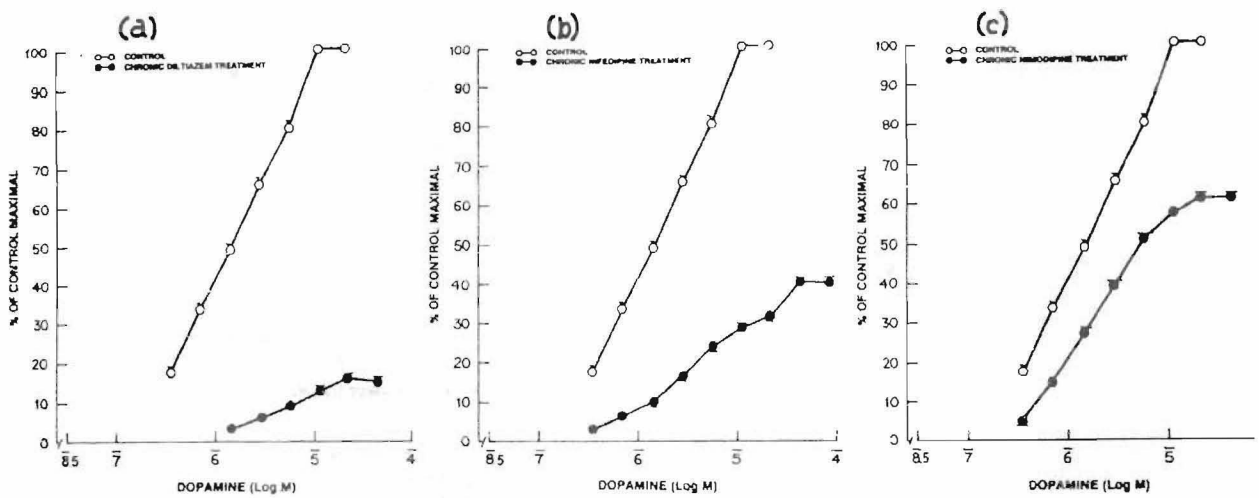

Fig. 3 : Effect of calcium channel blockers on the contractile responses of rat aortic strip to DA. Responses are calculated as percentage of the control maximal response and plotted against the logarithm of the molar concentration of DA. Each value is the mean of 5-6 experiments of control and chronic calcium channel blocker treatment. Vertical lines indicate SEM.

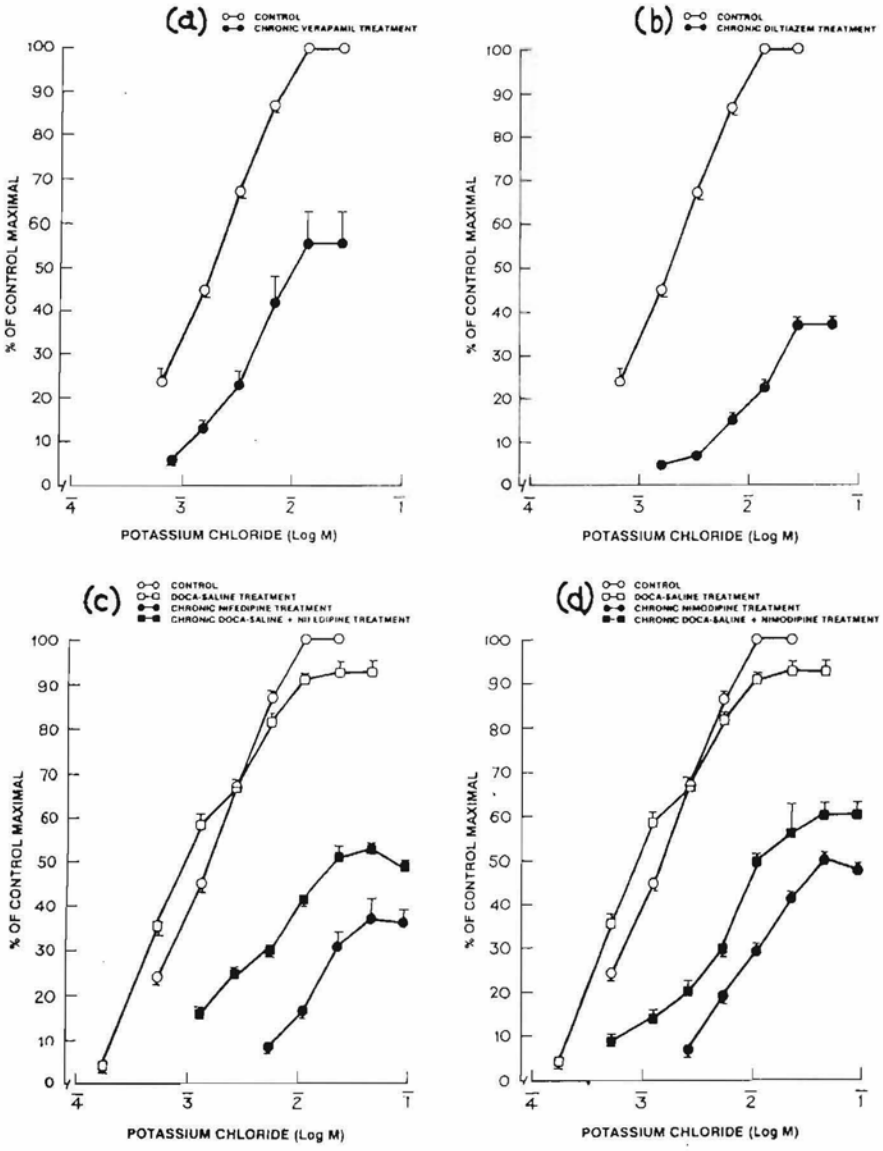

Fig. 4 : Effect of calcium channel blockers on the contractile responses of rat aortic strip to KCl. Responses are calculated as percentage of the control maximal response and plotted against the logarithm of the molar concentration of KCl. Each value is the mean of 5-6 experiments for control and chronic calcium channel blocker treatment and 3 experiments each for chronic DOCA-saline treatment and chronic DOCA-saline and nifedipine or nimodipine treatment. Vertical lines indicate SEM.

DOCA-saline there was no significant ($P > 0.05$) shift of the concentration-response curve of NA. However, there was a significant ($P < 0.01$) increase in the maximal response (Fig. 1c, d). The concentration-response curve of PE was shifted to the right ($P < 0.01$) and the maximal response was reduced ($P < 0.01$) (Fig. 2b, c).

In preparations obtained from rats chronically treated with DOCA-saline the concentration-response curve of KCl was shifted ($P < 0.01$) to the left at lower concentrations (1.79×10^{-4}M-1.25×10^{-3}M); while at higher concentrations (1.14×10^{-2}M-2.30×10^{-2}M) the shift was rightward ($P < 0.01$). The maximal response was reduced ($P < 0.01$) (Fig. 4c, d).

Chronic treatment with calcium channel blockers : In hypertensive rats chronically treated with nifedipine there was a rightward shift ($P < 0.01$) of the concentration-response curves of NA and PE and reduction in the maximal response ($P < 0.01$) (Fig. 1c) and (Fig. 2b). Nimodipine acted like nifedipine on the concentration-response curve of NA but the concentration-response curve of PE was shifted to the left and the maximal responses were increased (Fig. 1d) and (Fig. 2c).

With both nifedipine and nimodipine there was a rightward shift ($P < 0.01$) of the concentration-response curve of KCl and reduction of the maximal response ($P < 0.01$) (Fig. 4c, d).

Contractile response of the aortic strip to NA, P_1 and KCl in hyperthyroid rats:

Control preparations : In preparations obtained from rats chronically treated with L-thyroxine, there was a significant ($P < 0.01$) shift of the concentration-response curve of NA, PE and KCl to the right and reduction ($P < 0.01$) in the maximal response (Fig. 5a, b, c, d, e, f).

Chronic treatment with calcium channel blockers : Chronic treatment of rats with L-thyroxine and nifedipine produced no significant ($P > 0.05$) change in the concentration-response curve of NA; whereas chronic treatment with L-thyroxine and nimodipine produced a leftward shift ($P < 0.05$) of the concentration-response curve of NA and increased ($P < 0.01$) the maximal response (Fig. 5a, b).

Chronic treatment of rats with L-thyroxine and nifedipine or nimodipine shifted ($P < 0.01$) the concentration-response curve of PE and KCl to the right and reduced ($P < 0.01$) the maximal response (Fig. 5c, d, e, f).

DISCUSSION

Postjunctional alpha$_1$ and alpha$_2$ adrenoceptors both mediate contraction of vascular smooth muscle (16, 17). Pressor responses mediated by each subtype exhibit differential sensitivity to calcium channel blockers. It has been suggested that alpha$_1$-adrenoceptors are coupled to both the release of cellular bound calcium and the opening of calcium channels, while alpha$_2$-adrenoceptors are linked on to the latter (18). The sustained contraction of rat aorta following alpha$_1$-adrenoceptor activation is partially inhibited by Ca^{2+} channel blocker (19-22).

In low concentrations DA activates postsynaptic DA-1 receptors mediating vasodilatation and presynaptic DA-2 receptors inhibiting NA release. In higher concentration DA activates beta$_1$-adrenoceptors and at very high concentrations it can activate postsynaptic alpha$_1$ and alpha$_2$-adrenoceptors mediating vasoconstriction as well as presynaptic alpha$_2$-adrenoceptors inhibiting NA release (23).

In the present study chronic treatment with verapamil, nifedipine or diltiazem produced a partial inhibition of contraction with NA and PE. With chronic nimodipine treatment there was potentiation of responses to lower concentration of NA and all concentrations of PE. These results are opposite to those with other calcium channel blockers and could be related to the occurence of different isoforms of L-type calcium channels (24).

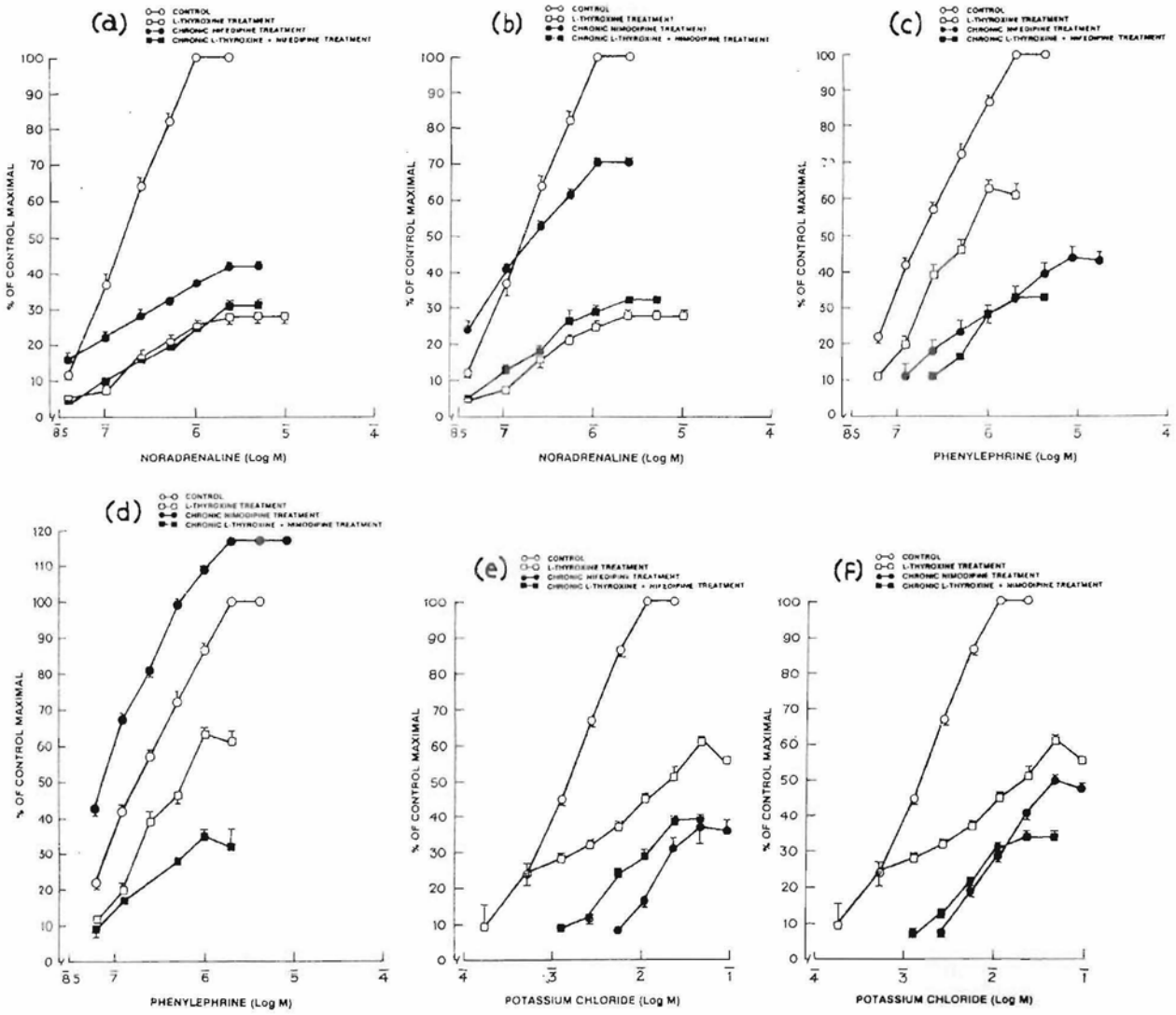

Fig. 5 : Effect of calcium channel blockers on the contractile responses of rat aortic strip to NA. (a, b), PE (c, d) and KCl (e, f). Responses are calculated as percentage of the control maximal response and plotted against the logarithm of the molar concentration of agonists respectively. Each value is the mean of 5-6 experiments for control and chronic calcium channel blocker treatment and 3 experiments each for chronic L-thyroxine treatment and chronic L-thyroxine and calcium channel blocker treatment. Vertical lines indicate SEM.

The existence of a component in the contraction which is resistant to Ca^{2+} blockers suggests two possibilities (1) this component does not require the enhanced Ca^{2+} entry, or (2) Ca^{2+} entry through 'receptor-operated' Ca^{2+} channels which are insensitive to organic Ca^{2+} channel blockers (25, 26) contributes to this contraction. Alternatively there is the possibility of down-regulation of the alpha-adrenoceptors on chronic treatment with nifedipine, verapamil, diltiazem, or nimodipine.

Finally the inhibitory effect on the contractile response with DA on chronic verapamil treatment may be related to blockade of voltage-operated L-calcium channels as already proposed by Morel and Godfraind (27) for the NA-evoked contraction of rat aorta.

KCl depolarizes the vascular smooth muscle membrane to allow Ca^{2+} influx into the cell through voltage-operated channels; NA (either exogenous or endogenous) admits Ca^{2+} by way of receptor-operated channels. Nifedipine has demonstrated activity consistent with its classification as inhibitor of voltage-operated channels at higher concentrations (28-30). Nifedipine has also been shown to inhibit both spontaneous and drug-induced (PGF 2alpha, oxytocin, vasopressin and potassium) contractile activity in isolated human pregnant myometrium at midterm and in the immediate post-partum period and uterine activity in menstruating, pregnant, post-partum and dysmenorrhagic females (31-39). Nifedipine has also been shown to inhibit the contractile responses to potassium and barium chloride in the human isolated bladder and ureter (40). In the present study with chronically-treated aortic strip preparations, there was inhibition of contractile responses to KCl in all preparations suggesting the possibility that there may be a down-regulation of the voltage operated channels.

Chronic treatment with DOCA-saline results in the development of hypertension (41). This model was successfully produced as indicated by a rise in blood pressure and an increase in the maximal responses of aorta to NA. Results from several studies have demonstrated that NA levels in a variety of vascular and non-vascular tissues are elevated in spontaneously hypertensive rats compared to their normotensive genetic control, the Wistar-Kyoto rats (42). The tissues examined include mesenteric artery (43), caudal artery (44), kidney, aorta and vas deferens (43). There is evidence to suggest that the elevated NA concentration in these tissues is due to a greater sympathetic innervation in the hypertensive strain. All calcium channel blockers except nimodipine blocked responses to NA, PE and KCl. With nimodipine treatment there was leftward shift of the concentration-response curve and increase of maximal response to PE. As suggested earlier this could be related to the occurence of different isoforms of L-type calcium channels (24), suggesting that the several agonists employed utilise different sources of calcium as discussed above.

Compared to the well reported structural cardiac changes engendered by hyperthyroid state (45), the vasculature does not seem to get affected. In fact in the hyperthyroid preparations the concentration-response curves of NA and PE were shifted to the right with depressed maximal responses. This could be secondary to the primary effect exerted on the heart. All CCBs shifted the concentration-response curves of NA and PE to the right and depressed the maximal responses. It is suggested that this may be due to the decreased alpha receptor density in hyperthyroid state reported by Gunasekera and Kariyama (46). The rightward shift and depression of maxima of concentration-response curves of KCl with chronic L-thyroxine treatment alone or simultaneously with CCB may be secondary to the primary effect exerted on the heart or due to down-regulation of the voltage operated channels.

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