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MAGNESIUM AND RESERPINE INFLUENCE NOREPINEPHRINE SENSITIVITY OF VAS DEFERENS IN RATS

D. N. GANDHI AND H. VENKATAKRISHNA-BHATT*

Department of Neurobehavioural Toxicology,
National Institute of Occupational Health (ICMR),
Meghani Nagar, Ahmedabad - 380 016

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Abstract : Reserpine induced supersensitivity to norepinephrine (NE) in rat vas deferens was sought by alteration of Mg^{++} and Ca^{++} concentration in incubation medium in the absence and presence of EDTA. Vas deferens incubated in Mg^{++} free and Mg^{++} excess media showed supersensitivity and subsensivity to NE respectively. Alterations in the sensitivity to NE produced by varying the concentrations of Mg^{++} were comparatively less. In the presence of EDTA, vas deferens obtained from reserpinized animals showed subsensitivity in normal and Mg^{++} excess media and supersensitivity in Mg^{++} free medium. In the presence of EDTA, reserpinized preparations showed slight supersensitivity in normal Mg^{++} medium, marked supersensitivity in Mg^{++} free and lesser subsensitivity in Mg^{++} excess medium. Probably EDTA by more effectively removing Mg^{++} from the membrane binding sites by chelation makes the membrane permeable to Ca^{++} leading to supersensitivity to NE (observed only in the presence of EDTA). These results suggest that the failure of reserpine to induce supersensitivity to NE in rat vas deferens may be due to an enhanced antagonism of Mg^{++} on Ca++ movements in this preparations due to the poor capacity of rat tissue to retain Ca^{++}.

Key words : vas deferens, supersensitivity, reserpine, magnesium, norepinephrine

INTRODUCTION

Increasing evidence suggests that vascular smooth muscle from an animal pretreated with reserpine becomes non-specifically supersensitive to norepinephrine (NE), potassium (K^+) and acetylcholine (1, 2), Ba^{++} (3) and Ca^{++} (4). This type of non-specific supersensitivity has been interpreted as a postsynaptic change occurring beyond the receptor of the muscle cell, rather than a quantitative change in the drug receptors or any alteration in the inactivation of the agonists (1, 3). This change may be due to increased membrane supersensitivity to catecholamines may be due to a reduction in the overall 'uptake' of catecholamines by adrenergic neurones leaving more free agoinst to react with receptors (6).

Reserpine fails to induce supersensitivity to even nor-epinehrine (NE) in rat aorta (7-9) and in rat seminal vesicle (10) to 5-hydroxytrptamine. This failure is in contrast to the results obtained with rabbit aorta and may be due to poor capacity of the rat aortic smooth muscle to retain Ca^{++} and the relative absence of releasable firmly bound Ca^{++} movements and the sensitivity of vascular smooth muscle (2). The present investigation was undertaken to study the relationship between the modulation of rat vas deferens sensitivity by magnesium to norepinerphrine with the supersensitivity induced by reserpine and also demonstrate the ability of EDTA to alter the sensitivity of vas deferens in the presence of different concentrations of magnesium.

METHODS

Male Wistar albino rats weighing 250 to 350 g were sacrificed by a blow on the head. Vas deferens were rapidly dissected, freed from surrounding tissues and placed in petridish containing carbogenated Krebs-

*Corresponding Author

Henseliet solution of the following composition (mM): NaCl 117.6; KCl 5.2 ; CaCl $2H_2O$ 2.16 ; $MgSO_4$, $7H_2O$ 1.2 ; $NaHCO_3$ 2.5 ; NaH_2PO_4, $2H_2O$ 0.8 ; glucose 11.1 in deionized distilled water.

Each vas deferens was suspended in a 30 ml organ bath containing Krebs Henseleit solution (pH 7.4) at 37°C + 0.5°C and continuously bubbled with 95% oxygen and 5% carbon dioxide. Vas deferens was equilibrated in the bath for 30 min. Contractions were recorded on a smoked drum with an iostonic frontal writing lever which was under 500 mg tension and gave ten fold magnification.

In all preparations, the completion of the full concentration-response curve was first achieved by the administration of increasing conentrations of norepinephaine (NE : 1.6×10^{-6}M to 1.44×10^{-5}M) till no further increase in contraction was obtained. The completion of the full concentration response curve was usually accomplished within 30 min and consistent concentration response curve of NE (1.6×10^{-6}M to 1.44×10^{-5}M) in control krebs Henseleit medium, the preparation was exposed to either Ca^{++} free, Ca^{++} excess (4.2 mM) ; Mg^{++} free or Mg^{++} excess (3.6mM), Krebs solution for 30 min and the concentration response curves were redertermined.

Reserpine was administered to rats in a dose of 7.5mg/kg (s.c) and they were sacrificed 24 hr later.

The results have been expressed as mean ±S.E. and analysed by the Student's 't' test (paired) for significance.

Drugs used were Norepinephrine - HCl (NE; Sigma); reserpine (Serpasil, Ciba); ethylenediamine tetraacetic acid disodium salt (EDTA, Pfizer); tyramine monohydrochloride (Nutrition Biochemical's Corporation); phentolamine methane sulphonate (Ciba) and methysergide (Sandoz).

RESULTS

Non-reserpinized rats :

Effects of phentolamines and methysergide on response to NE : NE (1.6×10^{-6}M to 1.44×10^{-5}M) induced concentration related contractions of the vasdeferens. Phentolamine (1.0×10^{-6}M) reduced the response to NE to 15.2±3.2% of the control but methysergide (2.0×10^{-7}M) had no effect on those to NE (n = 5).

Effects of different concentrations of Ca^{++} : Vas deferens preparations from rats incubated in Ca^{++} free as well as excess Ca^{++} medium for 30 min showed decrease in sensitivity (indicated by rightward shift of the concentration response curve) and maximal responses (Figs. 1 & 2).

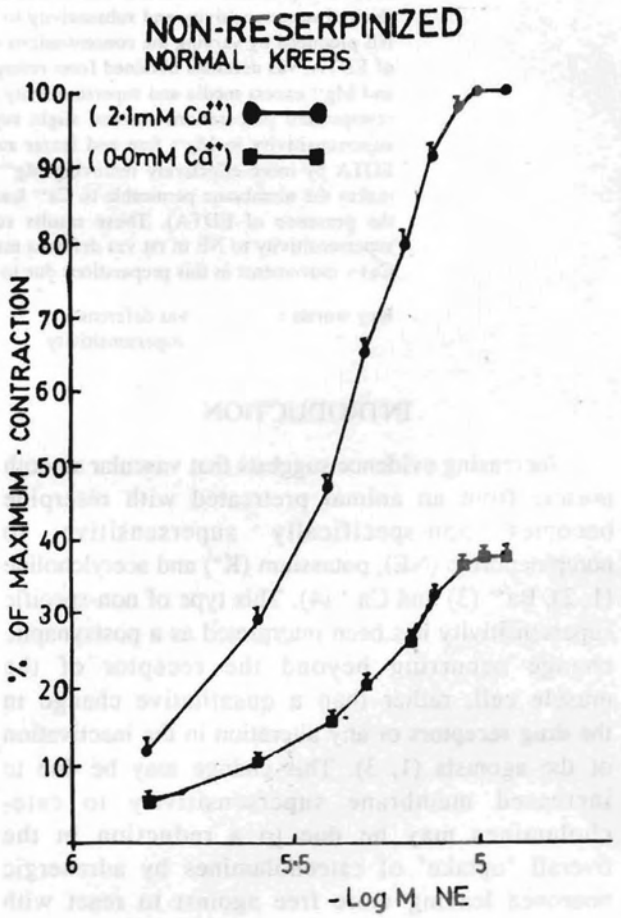

Fig. 1 : Effects of alteration of the Ca^{++} concentration in Kreb's Henseleit solution (Zero Ca^{++}) on the concentration response curve of NE in rat vas deferens. Each point of the curve represents the mean of 5 experiments and the vertical bars, the SEM.

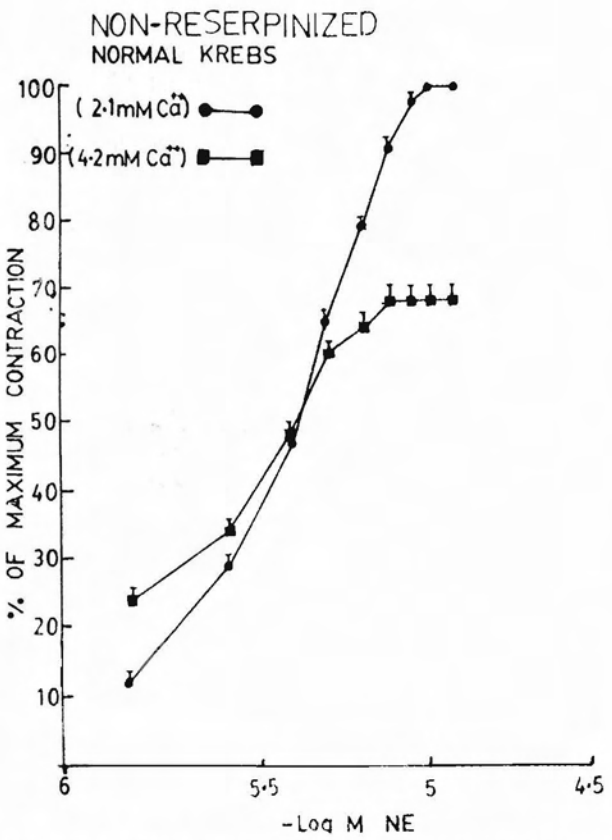

Fig. 2 : Effects of alteration of the Ca^{++} concentration in Kreb's Henseleit solution (high Ca^{++}) on the concentration-response curve of NE by the rat vas deferens. Each point of the curves represent the mean of 5 experiments and the vertical bars, the SEM.

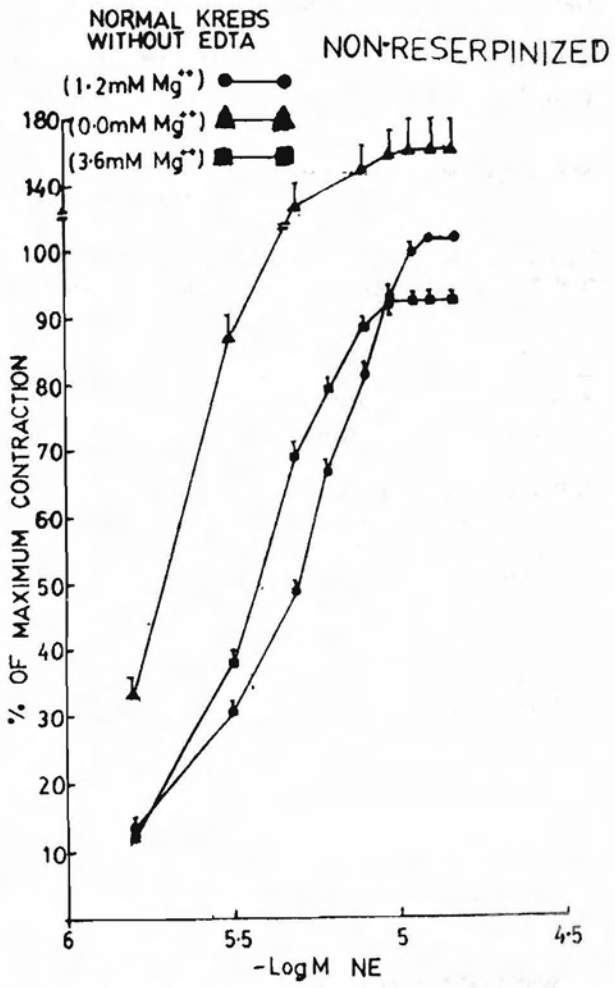

Fig. 3 : Effects of alteration of the Mg^{++} concentration in Kreb's Henseleit solution (without EDTA) on the concentration response curves of NE in rat vas deferens obtained from non-reserpinized animals. Each point of the curve represents the mean of 5 experiements and the vertical bars, the SEM.

Effects of different concentrations of Mg^{++} in Krebs medium without EDTA : Vas deferens preparations from non-reserpinized rats incubated in Mg^{++} free medium for 30 min, showed increase in sensitivity to NE (indicated by leftward shift of the concentrations-response curve) and maximal responses. The foot of the concentration response curve was raised. Incubation in medium containing Mg^{++} excess depressed the sensitivity, the maximal responses and the foot of the concentration response curve (Fig. 3; Table I).

Effects of different concentrations of Mg^{++} in Krebs medium with EDTA (3.0×10^{-5} M) : Preparations incubated in Mg^{++} free medium with EDTA for 30 min, showed increase in sensitivity and maximal responses to NE; however, this was less than that obtained in the absence of EDTA. Incubation in medium containing Mg^{++} excess increased the sensitivity as well as the maximal responses (Fig. 4; Table I), the increase were much less compared with that obtained in the absence of EDTA. Moreover, the foot of the

TABLE I: Effects on maximum response to norepinephrine in non-reserpinized and reserpinized rat vas deferens preparations exposed to Mg^{++} (1.2, "O" and 3.6 mM) in Kreb's Henseleit media without and with EDTA ($3x10^{-5}M$).

Treatment		N*	% Maximum response±SEM (Non-reserpinized)	% Maximum response±SEM (Reserpinized)
Norepinephrine				
	Mg^{++} (1.2mM)	5	100 ± 0.0	97.88 ± 3.5
	('0'mM)	5	162 ± 13.84**	160 ± 6.7**
Without EDTA	(3.6mM)	5	91 ± 2**	89.94 ± 0**
With EDTA ($3x10^{-5}M$)	Mg^{++} (1.2mM)	5	100 ± 0.0	110.49 ± 0
	('0'mM)	5	133 ± 7.2**	180.58 ± 3.4**
	(3.6mM)	5	119 ± 8.6**	109.3 ± 2.3**

N*— Number of experiments
**— P<0.05 from corresponding 1.2 mM Mg^{++}

TABLE II: Effect on neg log molar ED_{50} values of the does response curves of NE in vas deferens obtained from rats treated with reserpine (7.5 mg/kg/sc/24 hr) and exposed to Mg^{++} (1.2, '0' and 3.6 mM) to Krebs medium, without and with EDTA ($3.0x10^{-5}M$).

Treatment		*N**	*Mean neglogmolar ED_{50} value ± SE*	*P value*
Norepinephrine				
Without EDTA				
1.2 mM Mg^{++}	Control	5	3.70 ± 0.12	0.05
	Reserpine	5	5.00 ± 0.09	
'0' mM Mg^{++}	Control	5	3.30 ± 0.16	NS
	Reserpine	5	3.50 ± 0.13	
3.6 mM Mg^{++}	Control	5	3.60 ± 0.08	0.05
	Reserpine	5	4.90 ± 0.04	
With EDTA, $3.0 \times 10^{-5}M$				
1.2 mM Mg^{++}	Control	5	3.70 ± 0.13	NS
	Reserpine	5	3.75 ± 0.15	
'0' mM Mg^{++}	Control	5	3.25 ± 0.12	0.01
	Reserpine	5	4.12 ± 0.14	
3.6 mM Mg^{++}	Control	5	3.30 ± 0.11	NS
	Reserpine	5	3.40 ± 0.13	

N*—Number of experiments
NS—Not significant

concentration response curve with Mg^{++} excess medium in the presence of EDTA was higher than that in the absence of EDTA (Compare Figs. 3 & 4).

Reserpinized rats :

Effects of different concentrations of Mg^{++} in Krebs medium without EDTA : Reserpinized preparations incubated for 30 min in normal Mg^{++} medium without EDTA exhibited a slight subsensitivity and reduction of maximum responses to NE while those incubated in Mg^{++} free medium showed increase in sensitivity and maximum responses. However, the

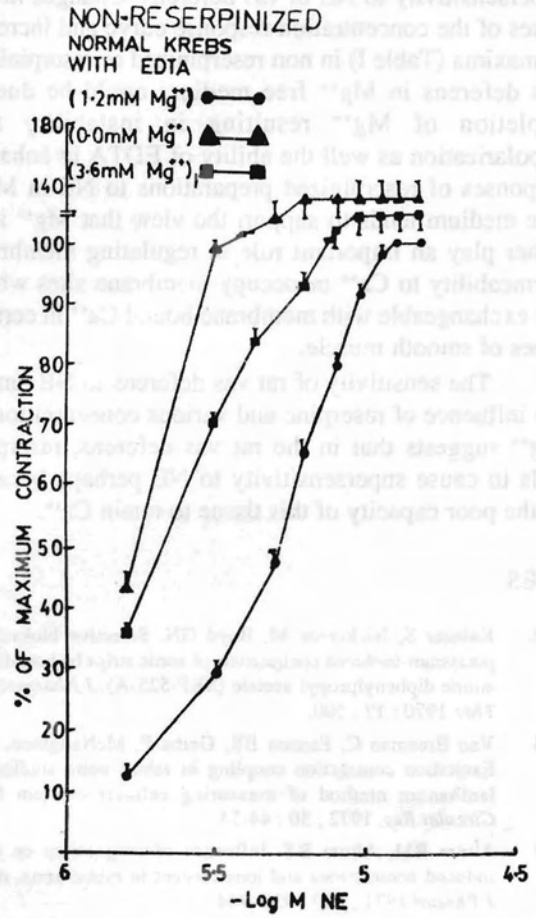

Fig. 4 : Effects of alteration of the Mg^{++} concentration in Kreb's Henseleit solution containing $3x10^{-3}$M EDTA on the concentration-response curves of NE in rat vas deferens obtained from non-reserpinized animals. Each point of the curve represents the mean of 5 experiments and the vertical bars, the SEM.

increases were not different from those observed in non-reserpinized tissues (Table I & II).

Effects of different concentrations of Mg^{++} in Krebs medium with EDTA ($3.0x10^{-3}$M) : Reserpinized preparations incubated in normal Mg^{++} medium containing EDTA, showed a slight supersensitivity and increase in maximum responses ; those incubated in Mg^{++} free medium showed a marked increased in sensitivity to NE and maximal responses. Preparations incubated in medium containing Mg^{++} excess showed decrease in sensitivity and maximal responses (Table I and II).

DISCUSSION

In the present study it has been demonstrated that contractions of rat vas deferens induced by NE were blocked by higher concentration of Ca^{++} (4.2mM) as well as Ca^{++} free (Figs. 1 & 2) followed by depression of maxima. Since contration of smooth muscle is dependent upon Ca^{++} (11) and different contractile agents are known to utilize different sources of Ca^{++} for the development of contractile response. Since NE induced contractile response is initially dependent upon the intracellular Ca^{++} through the release from bound sites (8, 12, 13), reduction of response in Ca^{++} free medium observed in the present study is easily understandable. The reduction of response to NE observed in a medium containing Ca^{++} excess could be due to stabilization of the cell membrane (11). Accordingly, Mg^{++} may compete with the permeability and availability of Ca^{++} to the contractile proteins depending upon the extracellular concentration of these divalent ions.

In the present study, increased sensitivity of rat vas deferens to NE in Mg^{++} free medium whereas in higher Mg^{++} concentration (3.6mM) decreased the sensitivity followed by the depression of maxima. The results of the present study as well as those of the previous studies (14–16) indicating the existence of competion between Ca^{++} and Mg^{++} for the same binding sites at the membrane and intracellular sequestering sites like sarcoplasmic reticulum and mitocondria (17, 18).

Disodium EDTA is known to have greater affinity for trace metals and Ca^{++} than for Mg^{++} at neutral pH (19), it has been reported that EDTA chelates both Ca^{++} and Mg^{++} to a similar degree (20). In the presence of disodium EDTA, withdrawal of Mg^{++} produced lesser leftward shift of the concentration response curve and lesser increase in maximal response ; furthermore in Mg^{++} excess medium the block of responses to NE was much less in the presence of EDTA than in its absence (compare Figs. 3 & 4). This was to be expected that EDTA effectively removes Mg^{++} from membrane binding sites by chelation thereby making the membrane more unstable and permeable to Ca^{++}.

Reserpine failed to cause supersensitivity of the vas deferens to NE incubated under any Mg^{++}

concentration (Table I) containing no EDTA could be due to the prevalence of Ca^{++} and Mg^{++} competition at the binding sites, thereby making less Ca^{++} available to contractile mechanism. However, incubation of reserpine treated vas deferens in Mg^{++} free medium with EDTA, produced leftward shift of the concentration response curve, raised the resting tension and increased the maximum by 47.58% +3.2 (Table I) Recently, Gandhi et al (10) and Krishnamurty and Gulati (21) also provided evidence that reserpine treatment (1 mg/kg/day) neither enhanced Ca^{++} uptake nor delayed $^{45}Ca^{++}$ efflux from Rabbit aorta incubated in normal Mg^{++} (1.2 mM) but withdrawal of Mg^{++} from the medium enhanced Ca^{++} up take delayed the $^{45}Ca^{++}$ efflux, perhaps, since NE induced contraction is dependent upon intracellular Ca^{++} (8, 13), the initial rise in resting tension may have marked the onset of reserpine induced supersensitivity to NE in vas deferens. Changes in the bases of the concentration response curve and increase in maxima (Table I) in non reserpinized or reserpinized vas deferens in Mg^{++} free medium could be due to depletion of Mg^{++} resulting in instability and depolarization as well the ability of EDTA to enhance responses of reserpinized preparations to NE in Mg^{++} free medium tends to support the view that Mg^{++} ions either play an important role in regulating membrane permeability to Ca^{++} or occupy membrane sites which are exchangeable with membrane bound Ca^{++} in certain types of smooth muscle.

The sensitivity of rat vas deferens to NE under the influence of reserpine and various concentration of Mg^{++} suggests that in the rat vas deferens, reserpine fails to cause supersensitivity to NE perhaps because of the poor capacity of this tissue to retain Ca^{++}.

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