EFFECT OF CA^{++} CHANNEL BLOCKERS AND SARALASIN ON ANGIOTENSIN II INDUCED CONTRACTION IN RABBIT AORTIC STRIP

K. KHETERPAL, S. B. LALL, S. RAJDEV AND Y. K. GUPTA

Department of Pharmacology,
All-India Institute of Medical Sciences, New Delhi - 110 029

(Received on November 19, 1985)

Summary To investigate the role of calcium in angiotensin II (A II) induced contractions in rabbit aortic strip, the action of verapamil, nifedipine, cinnarizine and saralasin was studied. The cumulative dose response curves obtained with A II shifted to right with increasing concentrations of all these four agents. The antagonism was noncompetitive. The pD'_2 value of saralasin was 8.49 of nifedipine, 8.15 and or verapamil 7.92. Cinnarizine which mainly acts at intracellular site had pD'_2 value 5.54. The results indicate that A II induced contractions critically depend on entry of calcium through channels which appear to be closely associated with angiotensin receptors.

Key words : angiotensin II, cinnarizine, verapamil, nifedipine, saralasin, rabbit aortic strip

INTRODUCTION

The entry of calcium ions (Ca^{++}) into the cell interior is of prime importance for the contraction of the vascular smooth muscle cells (4). Vascular smooth muscle agonists, such as angiotensin II (A II) and noradvrnaline (NA) are capable of inducing contractions of smooth muscles by this mechanism (1,11). Controversial reports are available on the roles of intracellular or extracellular Ca^{++} in A II induced contractions. Antonio *et al.* (3) demonstreted 40% reduction in the maximal response to A II in Ca^{++} free medium thereby stressing the importance of extracellular Ca^{++}. In contrast Freer (9) showed insignificant blockade by verapamil of A II induced contractions in rabbit aorta and suggested a major role of intracellular Ca^{++}. Present study was conducted to further investigate the role of extracellular Ca^{++} in A II induced contractions.

MATERIAL AND METHODS

Rabbits of either sex (2-2.5 *kg*) were used. Descending thoracic aorta was removed immediately after stunning and exsanguinating the animal. Oxygenated modified Kreb's bicarbonate solution (NaCl, 6.9 : KCl, 0.35; $CaCl_2$, 0.28; $NaHCO_3$, 0.21; KH_2PO_4, 0.16; $MgSO_4$. $7H_2O$, 0.26; glucose 1 *g/lit.*) was used. Contractions of isolated helical aortic strip were recorded isotonically under 4 *g* of tension (7). Cumulative dose responses (3) to A II were taken in the presence and absence of various doses of calcium channel blockers and saralasin. Similar studies were carried out using verapamil and phenoxybenzamine against NA for comparison. pD'_2 and slope of regression line (5) for all the antagonists were calculated.

RESULTS

The response to A II was reduced in the presence of various Ca^{++} channel blockers and saralasin (Fig. 1). No tachyphylaxis occured following the continous use

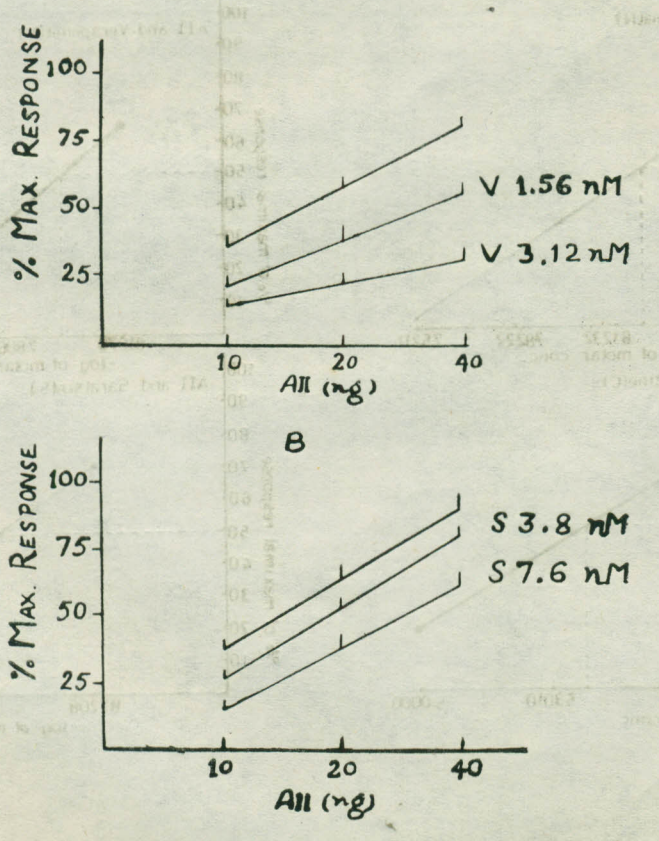

Fig. 1 : Effect of Verapamii and saralasin on the cumulative dose response for angiotensin II on rabbit aortic strip. A : shift of dose response curve to right in the presence of Verapamil (V). B : shift of dose response curve to right in the presence of saralasin(S). Note the decrease in maxima with boto the antagonists used.

of A II as complete recovery of cumulative dose response was obtained at the end of each experiment. The results are comparable to that of Khairallah (8) and Altura (2) who have also demonstrated lack of tachyphylaxis for A II in rabbit aorta. The dose response curve to A II showed a nonparallel shift to right in presence of Ca^{++} channel blockers with clear depression of maxima although the shift was parralel in the presence of smaller doses of saralasin. Linear relationship between percentage of maximal response and negative log of molar concentration of various antagonists was observed (Fig. 2). The pD'_2 value and slope of regression line of these antagonists against A II and NA are shown in Table I.

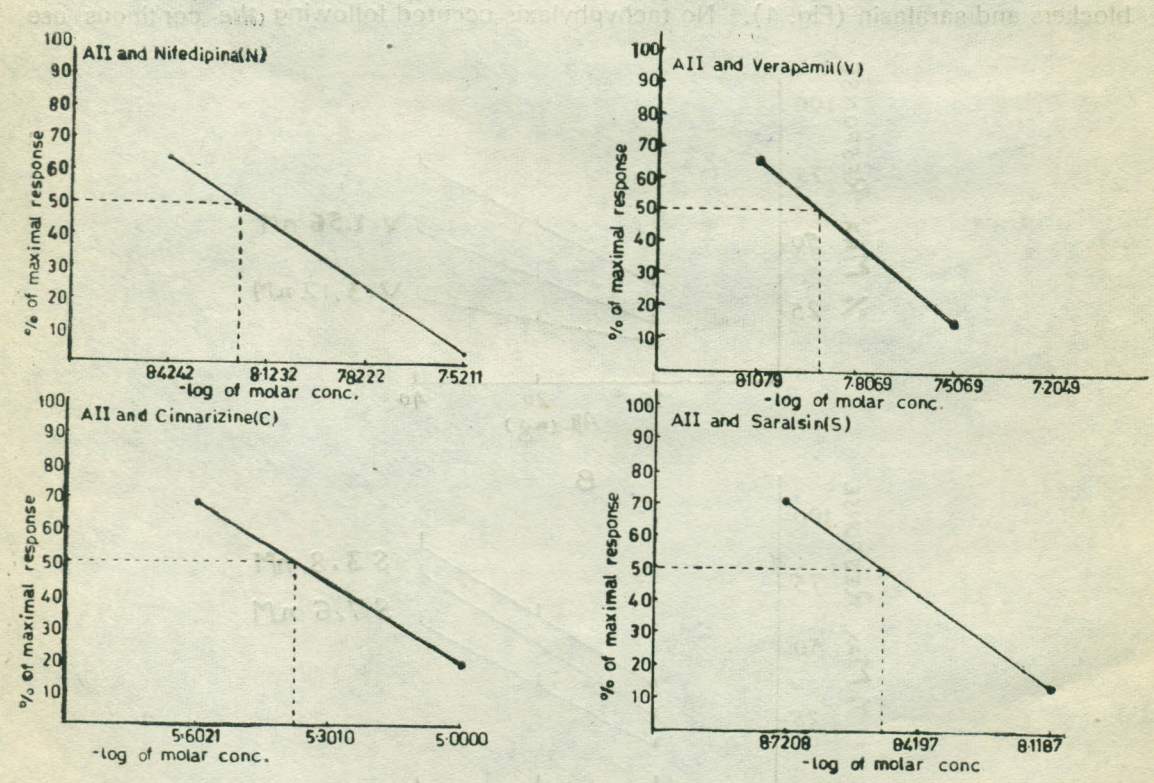

Fig. 2 : Percentage of maximal response to angiotensin II in the presence of different concentrations of calcium channel blockers and saralasin.

TABLE I : Slope of repression line and pD'_2 volues of various antagonists against angiotensin II and noradrenaline.

Antagonists	*Angiotensin II*		*Noradrenaline*	
	pD'_2	*Slope*	*pD'_2*	*Slope*
Verapamil	7.9291	1.000	6.409	0.38
Nifedipine	8.0408	0.80	—	—
Cinnarizine	5.4060	0.80	—	—
Saralasin	8.4999	0.90	—	—
Phenoxybenzamine	—	—	9.03	0.96

DISCUSSION

In the present study inhibition of contractile responses to A II by verapamil and nifedipine in a dose related manner suggests a dependence of A II induced contractions on influx of extracellular Ca^{++} into the cells. Tachyphylaxis was ruled out by complete recovery of cumulative dose response at the end of each experiment. Role of extracellular Ca^{++} in A II induced contractions has also been suggested by Antonio *et al.* (3) who showed 40% reduction of contractions of rabbit aortic strip in Ca^{++} free medium. However, Freer (9) observed insignificant blockade of A II induced contractions in rabbit aorta by verapamil. The fact that there was markedly less degree of inhibition by cinnarizine, claimed to be a weak Ca^{++} channel blocker but having a significant effect at intracellular site (10), further supports the importance of extracellular Ca^{++} in A II induced contractions.

Existence of receptor operated Ca^{++} channels in vascular smooth muscle is still uncertain. Saralasin, A II receptor blocker significantly inhibited the A II induced contractions of rabbit aorta. The closeness of pD'_2 value of saralasin to nifedipine and verapamil supports the existence of receptor operated Ca^{++} channels in rabbit aorta. Douglas *et al.* (6) have also suggested the close proximity of the A II receptors and divalent cation sites on the plasma membrane of the smooth muscle cells.

From the result it appears that A II induced contractions mainly involve extracellular Ca^{++} influx through receptor operated channels, however intracellular Ca^{++} mobilization may also contribute to the effect.

ACKNOWLEDGEMENTS

Authors are thankful to Bayer AG, Germany; Janssen Pharmaceuticals, Belgium; Norwich Eator Pharmaceuticals, Inc., New York and Boehringer-Knoll Ltd., Bombay for providing gift samples of nifedipine, cinnarizine, saralasin and verapamil.

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