

COMPARATIVE STUDY OF EFFECTS OF NIFEDIPINE AND ISOSORBIDE DINITRATE AND THEIR INTERACTION ON ISOLATED RABBIT AORTIC STRIPS

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SUMMARY

Experiments were performed on isolated rabbit aorta in order to study the action of nifedipine and isosorbide dinitrate on noradrenaline-evoked contractions. Contractions of aorta were obtained with increasing concentrations of noradrenaline. The tissue was kept in Krebs physiological salt solution. The effects were recorded with the help of Narco Mark IV Physiograph (USA). Both nifedipine and isosorbide dinitrate caused a decrease in amplitude of contractions of the aorta. However, none caused 100 percent inhibition of the evoked contractions. Percentage decrease in the amplitude of evoked-contractions of the aorta caused by nifedipine was compared with the percentage decrease caused by isosorbide dinitrate. The effects of the combination of nifedipine + isosorbide dinitrate on the aorta were compared with the effects of the individual test drugs.

INTRODUCTION

It has been demonstrated that contractions of vascular muscle are accompanied by an increase in the concentration of free intracellular calcium (Ca^{2+}). Calcium responsible for contraction of vascular smooth muscle may arise from mobilization of intracellular stores, or by influx from the extracellular environment through voltage-operated Ca^{2+} channels or receptor-operated channels.¹

It has been demonstrated that potassium chloride-induced contractions of the rabbit aorta depend almost entirely upon the presence of extracellular calcium, whereas both extracellular and intracellular calcium pools are utilized during noradrenaline-induced contractions of the tissue.² However the relative importance of different sources of calcium for contractions varies considerably in blood vessels from different anatomical positions even under similar experi-

mental conditions.³ In general, the contribution of cell-derived calcium is greater in arteries than in veins.⁴

Nifedipine, a calcium channel blocker, synthesized in 1971, was used initially as an anti-anginal drug.⁵ It is the most commonly used calcium channel blocker alongwith verapamil in cases of hypertension and ischaemic heart diseases. It exerts its actions at nanomolar concentrations on the blood vessels in such a manner that the drug may actually plug the Ca^{2+} channels. Nifedipine and verapamil relax potassium chloride induced contractions more effectively than contractions evoked by noradrenaline in rabbit aorta. It is because calcium channel blockers inhibit the entry of Ca^{2+} through voltage-operated Ca^{2+} channels release.⁶ However, studies with isolated arteries and veins generally confirm that the relaxing effects of nifedipine differ from those of organic nitrates like nitroglycerine and isosorbide dinitrate.⁷ The organic ni-

trates are being used in different types of angina for a long time either as prophylaxis or treatment. Isosorbide dinitrate is used alone and in combination with other anti-ischaemic drugs.⁸ The present study deals with comparing vasodilator property of isosorbide dinitrate with that of nifedipine and also to see whether their interaction can be of benefit or not.

MATERIALS AND METHODS

Nifedipine, isosorbide dinitrate and noradrenaline were obtained from Sigma USA in powdered form. Nifedipine was dissolved in alcohol, isosorbide dinitrate in chloroform and noradrenaline in 1/10 N hydrochloric acid. Fresh stock solutions of all the drugs were prepared daily and kept in refrigerator. Dilutions were made in Krebs solution. The solvents used in the maximum concentrations had no significant effect on noradrenaline induced contractions of aortic strips of rabbit in our pilot study.

Rabbits (1-3 Kg) of either sex were used for the experiments. They were sacrificed by cutting their throats and their chests opened. The aortic strips (20-30 mm long and 2-4 mm wide) were prepared from the thoracic aorta as described by Carron et al., 1991.⁹ The strips were tied to the transducer (F-60 Myographs, Narco Biosystem USA) with the help of a nylon thread.¹⁰ Physiograph (Narco Biosystem Mark IV Recorder USA) was used as a recording system. The preparations were allowed to settle and equilibrate with the Krebs solution for 1 hr.¹¹ Contractions of the aortic strips were induced with increasing concentrations of noradrenaline till maximum contractions were obtained. Contractions of the tissue were recorded after each dose of noradrenaline for 5 minutes.¹² The last maximum contraction induced by noradrenaline was considered as control.¹³ After recording the control response with noradrenaline the tissues were washed and exposed to test drugs in various concentrations for 30 minutes each.¹⁴ After this time

the tissue response to the control dose of noradrenaline was elicited. The doses of the test drugs were increased in a multiple of 10 till maximum inhibition was recorded. The resultant decrease in the amplitudes of contractions was expressed as percentage of control.¹⁵

CALCULATION OF POTENCIES

Since none of the test drugs (nifedipine and isosorbide dinitrate) produced complete inhibition of noradrenaline-induced contractions of the aortic strips, their potencies were expressed by calculating their IC 25 doses.¹⁶ It is defined as the mean concentration of the test drugs which inhibits 25% of the maximum amplitude of noradrenaline-induced control contractions of rabbit aortic strips.¹⁷ The IC 25 was calculated by plotting a graph. The point representing percentage changes were marked on the graph paper. The points were joined together. The log doses crossing the linear time-line at points producing 25% changes were noted (Fig.2). The relative potencies between the drugs on the tissues were calculated on the basis of the differences in their IC25.¹⁸ Comparison was made between the effects of single doses (IC 25) of nifedipine and isosorbide dinitrate. Also the action of their combined administration on the tissues was compared to their individual effects.

RESULTS

1. EFFECTS OF NORADRENALINE

Concentrations of noradrenaline of 10nM and above produced biphasic contractions of the aortic strips after the tissue had been equilibrated with Krebs solution for one hour. The biphasic responses consisted of an initial fast and short lived phasic component which reached a maximum in about 15-30 seconds followed by slower sustained tonic contraction. The tonic contractions rose slowly in five minutes to maximum tension and were maintained for

a prolonged period of time, till the tissue was washed (Fig.1). Noradrenaline in IuM doses produced maximal contractile response and was used as a standard agonist dose for eliciting the response of aortic strips in all the experiments. The mean amplitude of contraction was 18.62 ± 1.88 mm (mean \pm standard error, n=17). The mean percentage of the initial fast component was 61.71 ± 2.53 (mean \pm SE, n = 17) (Table-I).

2. EFFECTS OF NIFEDIPINE

Nifedipine produced a concentration dependent reduction in the amplitudes of contractions of rabbit aortic strips. However, it could not inhibit the contractions com-

pletely even in maximum doses used in the present study. The doses of nifedipine were 10^{-9} M to 10^{-6} M (Table-II). An example of the effects of various concentrations of nifedipine is shown in Fig.1. Fig.2 shows the concentration inhibition curve obtained with nifedipine. The overall effect of nifedipine on the aortic strip relaxation was statistically significant ($P < 0.001$).

3. EFFECT OF ISOSORBIDE DINITRATE

Isosorbide dinitrate produced a concentration dependent reduction in the amplitudes of contraction of rabbit aortic strips. However, it could not inhibit the contrac-

TABLE - I
EFFECT OF IUM NORADRENALINE ON ISOLATED AROTIC STRIPS OF RABBIT.

S. No.	AMPLITUDE OF CONTRACTIONS			
	Total (mm)	Initial Fast Phase (mm)	Second Tonic Phase (mm)	Percentage of Initial Fast Component
1.	12.50	7.2	5.30	57.63
2.	6.50	3.50	3.00	53.84
3.	19.00	13.50	5.50	71.05
4.	26.50	20.00	6.50	75.47
5.	8.00	5.00	3.00	62.50
6.	26.00	18.00	8.00	69.23
7.	31.00	24.00	7.00	77.42
8.	18.00	13.00	5.00	72.22
9.	8.50	5.50	3.00	64.71
10.	9.50	6.50	3.00	68.42
11.	18.00	10.00	8.00	55.55
12.	29.50	16.00	13.50	54.24
13.	26.50	17.50	9.0	66.04
14.	17.50	10.00	7.50	57.14
15.	16.50	6.00	10.50	36.36
16.	20.00	11.00	9.00	55.00
17.	23.00	12.00	11.00	52.17
Mean	18.62	11.69	6.95	61.71
SE	\pm 1.88	\pm 1.47	\pm 0.66	\pm 2.53

TABLE – II
EFFECT OF VARIOUS CONCENTRATIONS OF NIFEDIPINE ON THE AMPLITUDES
OF NORADRENALINE-INDUCED CONTRACTIONS OF THE RABBIT AORTIC
STRIP PREPARATIONS.

Amplitude of Contractions (Control)	14.25 ±2.73	13.67 ±2.63	14.61 ±2.68	15.04 ±2.65	7.41 ±3.50
Dose of Nifedipine (Moles/Litre)	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵
Amplitude of Contraction (After the Dose)	11.92 ±2.38	10.73 ±2.14	10.91 ±2.07	10.29 ±2.05	3.46 ±1.29
Percentage Decrease	16.56 ±1.88	22.24 ±1.60	26.40 ±2.31	34.19 ±2.11	52.36 ±5.15
Number of Experiments	14	15	14	13	6

Amplitudes of contractions are in millimeters.
Each value represents the mean ± standard error.

tions completely even in maximum doses used in the present study. The doses of isosorbide dinitrate were 10⁻⁹ M to 10⁻⁵ M (Table-III). An example of the effects of isosorbide dinitrate is shown in Fig.1. Fig.2

shows the concentration inhibition curve obtained with isosorbide dinitrate. The overall effects of isosorbide dinitrate on the aortic strips relaxation was statistically significant (P < 0.05).

TABLE – III
EFFECT OF VARIOUS CONCENTRATIONS OF ISOSORBIDE ON THE AMPLITUDES
OF NORADRENALINE-INDUCED CONTRACTIONS OF THE RABBIT AORTIC
STRIP PREPARATIONS.

Amplitude of Contractions (Control)	8.62 ±1.25	8.22 ±1.17	10.43 ±2.20	10.29 ±2.00	13.25 ±3.51
Dose of Isosorbide Dinitrate (Moles/Litre)	10 ⁻⁹	10 ⁻⁸	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵
Amplitude of Contraction (After the Dose)	7.45 ±1.50	7.02 ±1.06	8.28 ±1.62	8.02 ±1.62	8.00 ±2.43
Percentage Decrease	11.86 ±1.14	15.33 ±1.95	21.26 ±1.60	23.86 ±2.12	44.37 ±5.24
Number of Experiments	8	9	8	12	6

Amplitudes of contractions are in millimeters.
Each value represents the mean ± standard error.

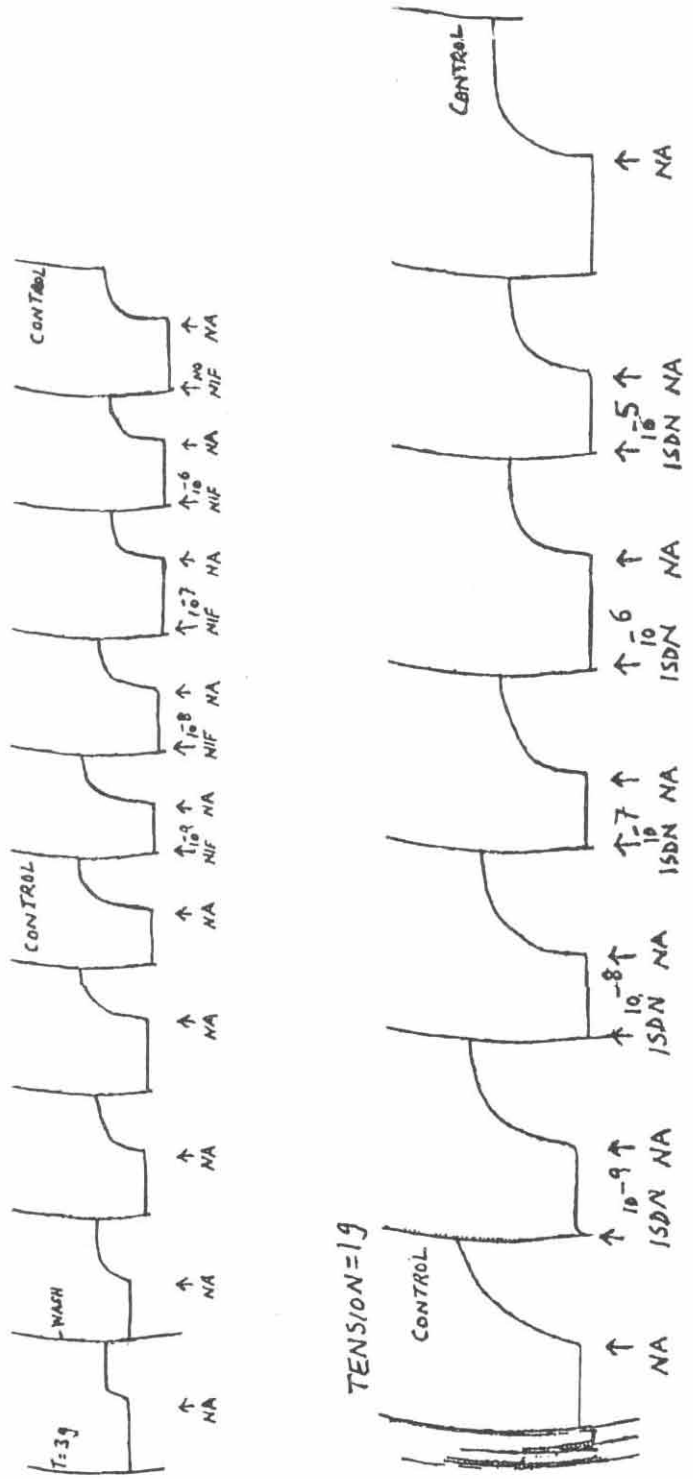


Fig. 1. Graph showing the effects of various concentrations of nifedipine (NIF) and isosorbide dinitrate (ISDN) on the amplitude of concentrations of rabbit aortic strips induced with 1uM noradrenaline (NA).

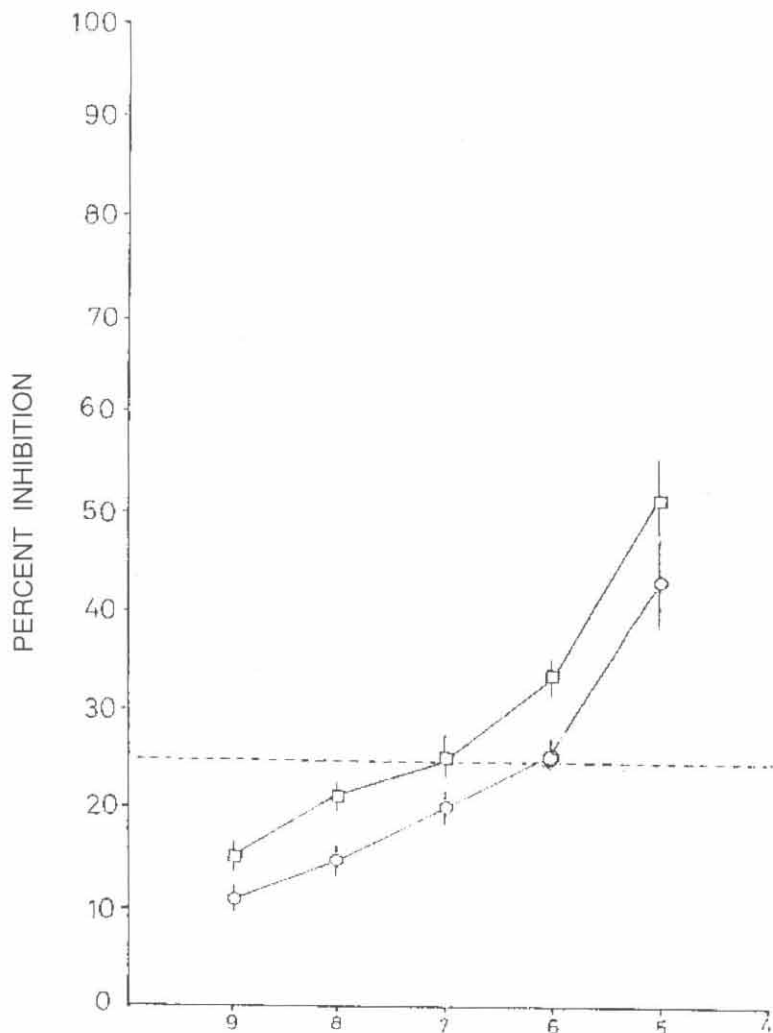


Fig. 2. Comparison of the effects and calculation of IC25s of nifedipine (Squares n=6-15) and isosorbide dinitrate (Circles n=6-12) on the amplitude of contractions of rabbit aortic strips induced by $1\mu\text{M}$ noradrenaline. The ordinate scale shows the percentage inhibition of the control response and the abscissa scale the concentration (M) of the agent used. Symbols represent the arithmetic mean of the number (n) of experiments and vertical bars the standard error mean. The dotted line crosses the IC25s of the test drugs.

IC25 of nifedipine = $8.5 \times 10^{-8}\text{M}$

IC25 of isosorbide dinitrate = $8 \times 10^{-7}\text{M}$

4. EFFECTS OF IC 25 DOSES OF NIFEDIPINE AND ISOSORBIDE DINITRATE

In a series of experiments, the mean values of the amplitudes of contractions were 25.14 ± 6.78 mm before and 16.75 ± 5.12 mm after administration of IC 25 doses of nifedipine (8.5×10^{-8} M). In these experiments the mean percentage decrease in the amplitude of contractions was 32.72 ± 2.24 (Fig. 3 & 4) {Table-IV}. The difference in these values was statistically significant (P 0.01).

In a series of 15 experiments, the mean values of amplitudes of contractions were 18.22 ± 3.85 mm before and 14.63 ± 3.37 mm after the administration of IC 25 dose of isosorbide dinitrate dose. The mean percentage decrease was 22.25 ± 1.77 (Fig.3 & 4) {Table-IV}. The difference in these values was statistically significant (P 0.05).

5. EFFECTS OF COMBINATION OF NIFEDIPINE AND ISOSORBIDE DINITRATE

In a series of experiments, the mean values of the amplitudes of contractions were 25.14 ± 6.78 mm before and 12.54 ± 3.61 mm after the administration of both nifedipine and isosorbide dinitrate using their IC 25 doses. In these experiments the mean percentage decrease in the amplitudes of contractions was 52.39 ± 2.83 (Fig.3 & 4) {Table-IV}. The difference in these values was statistically significant (P < 0.001).

DISCUSSION

I. NORADRENALINE

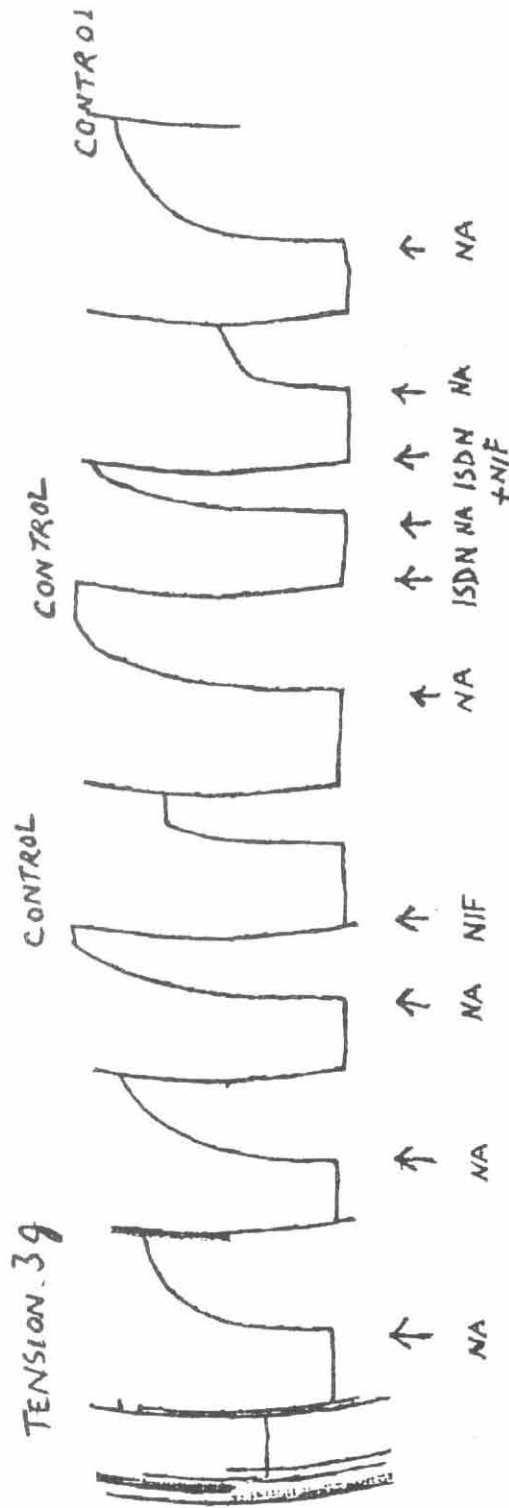
Aortic strips had to be induced to contract with noradrenaline before observing the effects of nifedipine and isosorbide dinitrate. Noradrenaline and potassium chloride are the two most widely used experimental means of in vitro activation of vascular smooth muscle.¹⁹

Bolton¹ demonstrated that contractile responses in vascular smooth muscle depend upon an elevation of intracellular calcium via (i) release of cellular bound or sequestered calcium stores, (ii) entry of calcium via voltage-operated calcium channels and (iii) entry of calcium via receptor-operated calcium channels. His views were later on collaborated by Godfraind⁶, Marriot³ and Daly et al.,⁴. Contractions of rabbit aortic strips caused by potassium chloride depend exclusively on entry of extracellular calcium through the voltage-operated calcium channels.^{1,20} Such contractions are abolished in the calcium free medium²¹ or in the presence of calcium channels blockers.²² However, contractions of the tissue evoked by noradrenaline are due to influx of calcium from extracellular environment and release from the intracellular stores.²³ Noradrenaline activates the alpha adrenoceptors distributed on the myoplasmic membrane and also releases the calcium from the tissues sequestered stores.²⁴

Our results show that the noradrenaline-induced contractions can be resolved into two components, an initial fast component and a second sustained component. These observations are in accordance with the findings of Bolton¹. Heaslip and Rahwan² demonstrated in the rabbit aortic strips that the release of intracellular calcium mediated the fast component of the biphasic contractile responses and that the tonic component was associated with an increase in calcium influx through the receptor-operated channels. These researchers noticed that the strips contracted in response to noradrenaline but not to potassium chloride in calcium free Krebs's solutions, thus indicating that such contractions were due to release of calcium from the intracellular stores.

II. NIFEDIPINE

In the present study, nifedipine produced concentration dependent reduction of noradrenaline-induced contractions of the



EFFECT OF COMBINATION OF ISDN + NIF
ON NA-INDUCED CONTRACTIONS OF
RABBIT AORTIC STRIPS

Fig. 3. Graph showing the effects of nifedipine (NIF) and isosorbide dinitrate (ISDN) used individually and in combination on the amplitude of contraction of rabbit aortic strips induced with 1uM noradrenaline (NA). The doses of nifedipine and isosorbide dinitrate were their IC25, i.e. $8.5 \times 10^{-8}M$ and $8 \times 10^{-7}M$ respectively.

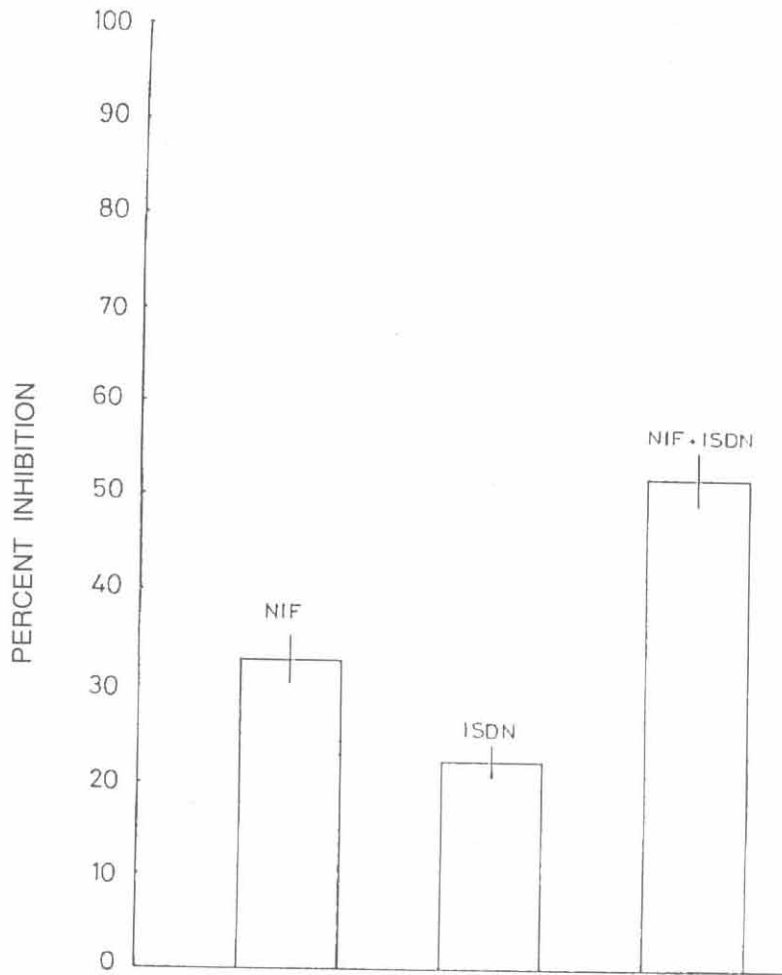


Fig. 4. Comparison of the effects of IC₂₅ of nifedipine (n=7) and isosorbide dinitrate (n=15) used individually and in combination (n=7) on the amplitude of concentrations of rabbit aortic strips induced by IuM noradrenaline. The ordinate scale shows the percentage inhibition of the control response and the abscissa the IC₂₅ doses of the agents used. The bars represent the arithmetic mean of the number (n) of experiments. Vertical lines indicate the standard of error mean.

IC₂₅ of nifedipine = $8.5 \times 10^{-8}M$

IC₂₅ of isosorbide dinitrate = $8 \times 10^{-7}M$

TABLE – IV
COMPARISON OF THE EFFECTS OF NIFEDIPINE AND ISOSORBIDE DINITRATE
USED IN DOSES OF IC₂₅ INDIVIDUALLY AND IN COMBINATION, ON THE
AMPLITUDES OF NORADRENALINE INDUCED CONTRACTIONS OF RABBIT
AORTIC STRIP PREPARATIONS.

Test Drug	No. of Expts.	Amplitude of Contraction (mm)		Decrease in Amplitude (mm)	Percentage Decrease in Amplitude
		Control	After Test Drug		
Nifedipine	7	25.14 ± 6.78	16.75 ± 5.12	8.34 ± 1.90	32.72 ± 2.24
Isosorbide Dinitrate	15	18.22 ± 3.85	14.63 ± 3.37	3.53 ± 0.62	22.25 ± 1.77
Isosorbide Dinitrate + Nifedipine	7	25.14 ± 6.78	12.74 ± 3.61	12.61 ± 3.84	52.39 ± 2.93
P	> 0.001				

Each value represents the mean ± standard error.

strips. However, it did not abolish the contractions completely. Its effect was mainly confined to the reduction of the second component of the biphasic contractions of the tissue. It had little effect on the initial fast phase of contractions. This phase, as discussed earlier, depends on the release of intracellular calcium. Our observations that nifedipine inhibited only the second sustained phase of contractions are due to the fact that calcium channels blockers have little inhibitory effect upon the release of intracellular calcium but suppress the influx of extracellular calcium. Nifedipine in low concentrations (i.e. 10⁻⁹ M) produced negligible effect whereas its maximum concentration used in the present study (10⁻⁵ M) reduced the amplitude of contractions by 52.36 ± 4.15 percent. Our results in the present study are similar to those of Godfraind⁶ and Kanmura et al.,²⁴

III. ISOSORBIDE DINITRATE

In the present study, isosorbide dinitrate inhibited both the fast component and the tonic component of the biphasic response of the tissues to noradrenaline 10⁻⁵ M. Isosorbide

dinitrate reduced the amplitude of contractions by 44.37 ± 5.24 percent.

In some experiments, we found a biphasic relaxation, an initial transient relaxation, followed by slow sustained relaxation which was less in magnitude than the initial relaxation. Our findings correlate with the observations of Needleman and Johnson¹⁶

Itoh et al.,²⁵ had similar observations regarding effect of organic nitrates on the aortic tissue. They demonstrated that organic nitrates suppressed the contractions caused by noradrenaline in calcium free solutions. It was thus concluded that organic nitrates suppressed the internal calcium stores rather than the calcium influx Lincoln²⁶ in his studies has observed a biphasic relaxation properties of organic nitrates. Karaki et al.,²⁷ postulated that the sustained relaxation phase caused by organic nitrates is due to activation of guanylate cyclase. It has been accepted now that all organic nitrates and nitrosocompounds exert their vasodilation actions through the activation of guanylate cyclase and that their actions on vascular

tissues are qualitatively similar.²⁸ This activation of the enzyme is the major factor in causing relaxation of the tissue.²⁹ The enzyme is stimulated by nitric oxide released from organic nitrates. Guanylate cyclase in turn stimulates guanylate triphosphate into cyclic guanylate monophosphate (cGMP). cGMP dephosphorylates myosin phosphate into myosin light chain which relaxes the smooth muscle.³⁰

IV. COMBINATION OF NIFEDIPINE AND ISOSORBIDE DINITRATE

In our studies the combined effects of nifedipine and isosorbide dinitrate using their IC 25 doses on the amplitude of contractions caused an inhibition which almost equal to the sum of their individual effects (Fig.4). This is expected as both drugs decrease availability of calcium within the tissue cells. Itoh et al.,³¹ have suggested that whereas nifedipine mainly blocks the influx of extracellular calcium in the tissues, Isosorbide dinitrate acts to suppress the release of calcium from intracellular stores. However even this combined administration of the two drugs could not completely abolish the induced contractions. The intracellular stores of calcium in thoracic aorta are particularly well developed and difficult to be totally inhibited by the combination.⁴

CONCLUSION

It is evident from our results that nifedipine can partially inhibit the noradrenaline-induced contractions of the rabbit aortic strips. Isosorbide dinitrate also could not completely inhibit the contraction. In our experiments, nifedipine was more potent inhibitor of the contractions than isosorbide dinitrate. The two drugs had added vasodilator effects when administered in combination.

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