EFFECT OF ENDOTHELIUM ON THE REPETITIVE CONCENTRATION RESPONSE CURVES TO NOREPINEPHRINE IN RABBIT AORTA *

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SUMMARY

Endothelial cells release a relaxant factor, endothelum derived relaxing factor (EDRF), and mediate relaxation of vascular smooth muscle induced by various agonists. On the other hand, removal of endothelium potentiates vasoconstrictor effect of norepinephrine (NE). In the present study, it is shown that removal of the endothelium of isolated rabbit thoracic aortic strips, causes a significant increase in the slopes of the concentration-response curves to NE obtained repetitively after the control curve. An earlier desensitization was observed in rubbed preparations when compared with unrubbed strips. These findings suggest that removal of endothelium alters the characteristics of the concentration-response curves to NE and this may cause wrong interpretations if endothelium was not kept intact.

Key Words: Rabbit thoracic aorta; Norepinephrine; Endothelium.

INTRODUCTION

Furchgott and Zawadzki have reported that relaxation of arterial smooth muscle induced by acetylcholine is due to release of endothelim-derived relaxing factor (EDRF)(1). Relaxation of vascular smooth muscle induced by various agonists such as histamine(2) and 5-hydroxytryptamine (3) seems to be also due to the same mechanism.

On the other hand, the removal of the endothelium increases maximum tension level and/or decreases EC_{50} (effective concentration$_{50}$) values for norepinephrine (NE) in various vascular preparations[4,5], possibly as a result of lack of EDRF which counteracts the contracting effect of NE.

Isolated rabbit thoracic aorta is a commonly used preparation which is a good model to investigate the effects of various vasoactive substances as well as their relationship with various antagonists. For both purposes, repetitive concentration-response curves to the agonists are important to be evaluated. The effect of the endothelium on the repetitive concentration-response curves to NE in rabbit thoracic aortic strips was investigated in the present study.

MATERIALS AND METHODS

Helical strips of thoracic aorta were obtained from white rabbits of either sex weighing about 2 kg. The strips were mounted in an organ bath of 15 ml containing Krebs' solution of the following composition (mM): NaCl, 113.8; KCl, 4.7; $CaCl_2$, 2.4; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; and glucose, 11.4; and gassed with 95 % O_2 + 5 % CO_2 at 37C. The contractile tension of the muscle strips was recorded isometrically via a force-displacement transducer (Grass, Model PT03) on a polygraph (Beckman, Type RB). The strips were maintained under a stable tension of 1.5 g for at least 90 min, with changes of bathing fluid every 10 min, before the introduction of drugs. Concentration-response curves were obtained with stepwise cumulative additions of NE by increasing the concentration each time three fold, three times repetitively with one-hour intervals.

In a separate group of experiments, endothelial cells were removed by rubbing, before the strips were mounted in the organ bath. Acetylcholine (3.67×10^{-6}M) was added to the bath after the preparation was contracted by NE, in order to be sure of the destruction of endothelium. Unlike preparations with intact endothelium which relaxed in response to acetylcholine under these conditions, acetylcholine did not cause any relaxation of the rubbed aortic strips. Individual experimental data were obtained from at least 6 strips isolated from different animals. Results were calculated as % of the maximal response to the first application of NE and expressed as "mean ± S.E.M.". The slopes and EC_{50} values were calculated by means of Hill transformation and linear regression analysis. Differences were tested for significance by means of Student's t-test. The level of statistical significance was $P < 0.05$.

RESULTS

Isolated rabbit aorta with intact endothelium:

In isolated rabbit thoracic aorta with intact endothelum the slopes of the repetitive concentration-response curves to NE were not significantly differenet from each other, although the maximal contraction in curves related to the second and third cumu-

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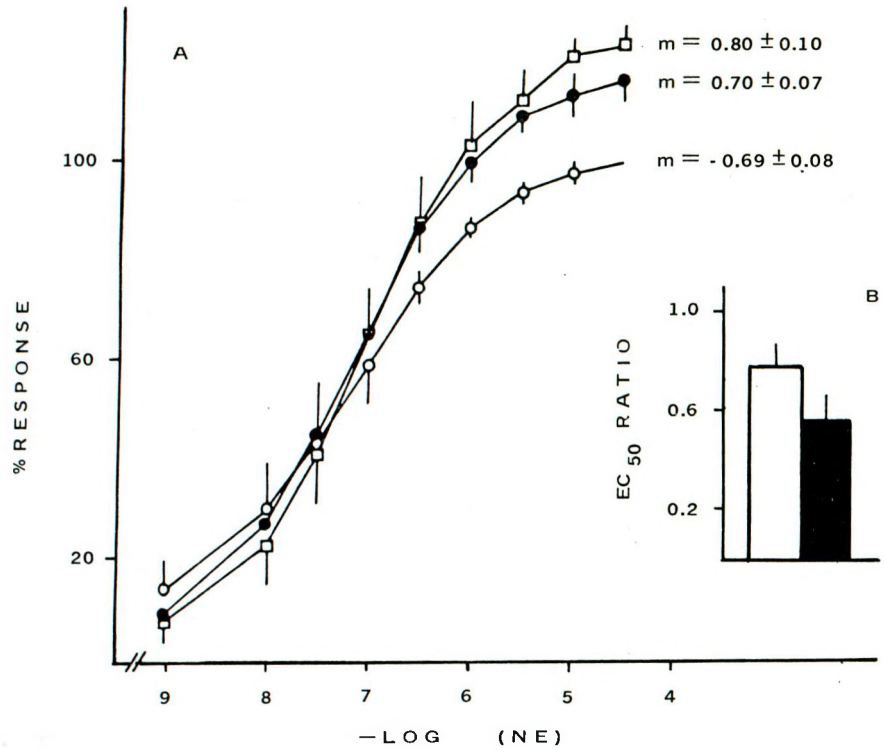

Fig 1. Repetitive concentration-response curves to norepinephrine (NE) of the isolated rabbit thoracic aorta with intact endothelium (A). Responses to the first (o---o), second (●---●) and third (□---□) application of NE with one-hour intervals. The slopes (m) of the curves are written on the figure. The ratios of the EC_{50} values (B) were calculated from the first/second (open columns) and first/third (dark columns) concentration-response curves.

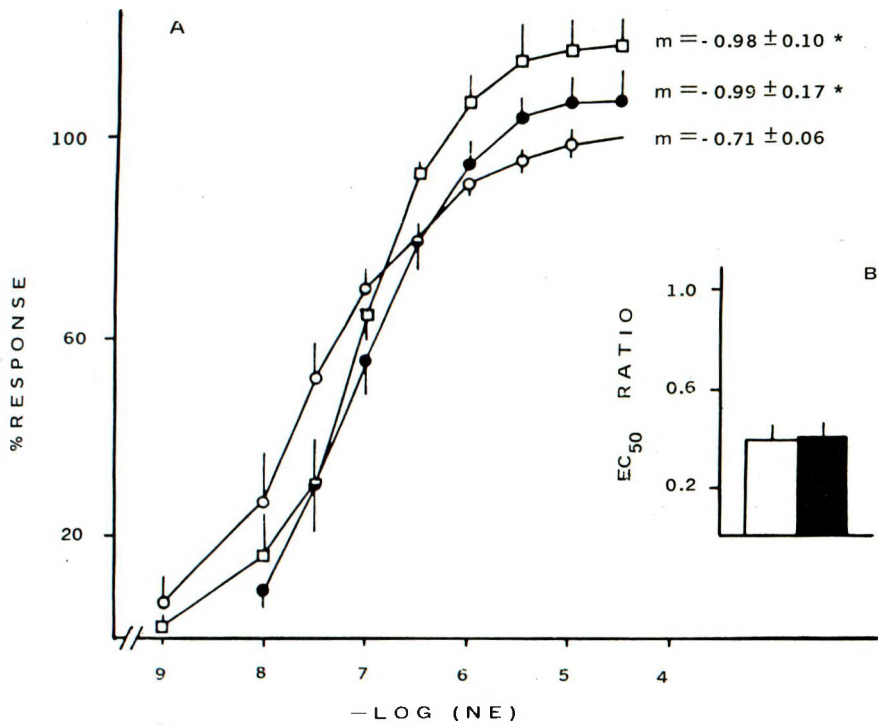

Fig 2. Repetitive concentration-response curves to norepinephrine (NE) of the isolated rubbed rabbit thoracic aorta (A). Responses to the first (o---o), second (●---●) and third (□---□) application of NE with one-hour intervals. The slopes (m) of the curves are written on the figure. (x) significantly different from that of the first curve ($P < 0.05$).
The ratios of the EC_{50} values (B) were calculated from the first/second (open columns) and first/third (dark columns) concentration-response curves.

lative applications of NE was greater than that of the first by 16.1 ± 2.8 and 23.7 ± 2.6 % respectively (Fig 1A). The EC_{50} values of NE increased with time because of desensitization, expressed as EC_{50} ratios which are less than one (Fig 1B).

Isolated rubbed aorta: The slope of the first concentration - response curve to NE in rubbed aortic strips was similar to that of unrubbed preparations (Fig 2A). The slopes of the second and third concentration-response curves to NE in these strips, however, were found to be significantly higher than that of the first, and than the corresponding values of unrubbed strips (Figs 1A and 2A). The EC_{50} ratio obtained from the first/second cumulative applications of NE in rubbed strips was significantly lower than that of the unrubbed strips (Figs 1B and 2B), suggesting facilitated desensitization.

DISCUSSION

Isolated rabbit thoracic aorta is a good pharmacological model to investigate the effects of various vasoactive agonists and their relationship with various antagonists. The slope of the concentration-response curves to an agonist and the EC_{50} values are important in understanding the type of the antagonism, whether it is competitive or not.

On the other hand, there is growing evidence about the importance of the endothelium: It is known today that the endothelium affects the contractile responses of the vascular smooth muscle[4,5] as well as the relaxation in response to various agonists[1, 2, 3]. Unfortunately, endothelial cells may be easily removed or damaged, for example, mechanically while preparing the strip, or by hypoxia during the experiment.

As shown in this study, the removal of the endothelium changes the characteristics of the concentration-response curves to NE in rabbit thoracic aorta. Interestingly, the concentration-response curve obtained by the first cumulative application of NE in rubbed aortic strips was similar to that of unrubbed preparations. The differences between rubbed and unrubbed aortas became apparent for the second cumulative application of NE; the degree of desensitization expressed by EC_{50} ratios was higher and the slope of the curve increased after the first cumulative application of NE for rubbed strips.

Under the light of these observations, if the investigator damages the endothelium accidentially, the first concentration-response will be similar to those of the preparations with intact endothelium. If an antagonist will be added to the bath, then the increase in the slope and the marked decrease in the EC_{50} ratio observed, can wrongly be interpreted as the effect of the antagonist.

In conclusion, it is obvious that the characteristics of the concentration-response curves to NE in rabbit thoracic aorta are affected by the removal of the en dothelium, though the matter needs further investigation. The investigators should keep this in mind while preparing isolated vascular strips.

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