

Endothelium Dependent Relaxation of Isolated Rat Aorta to Samorin (Isometamidium Chloride)

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Abstract

Relaxation responses to samorin (Isometamidium chloride) were tested on rat aortic rings pre-contracted with 10^{-7} M noradrenaline (NA) and 50mM potassium chloride (KCl).

Samorin (10^{-8} to 10^{-4} M) relaxed aortic rings pre-contracted with 10^{-7} M noradrenaline concentration – dependently but had no effect on KCl contracted rings. A mean relaxation of $69.7 \pm 8.8\%$ was achieved at a concentration of 4×10^{-5} M samorin with EC_{50} of $10.9 \pm 2.39 \times 10^{-6}$ M.

This relaxation response was significantly attenuated by removal of endothelium, since endothelium denuded ring showed a mean relaxation of $35 \pm 8.5\%$ at the same concentration of 4×10^{-5} M samorin with EC_{50} of $37.4 \pm 13.7 \times 10^{-6}$ M ($P < 0.05$). Moreover, endothelium intact rings which were pretreated with 10^{-6} M methylene blue showed a significant attenuated relaxation to 4×10^{-5} samorin with a mean relaxation of $18.3 \pm 2.8\%$ and an EC_{50} of $80 \pm 14 \times 10^{-6}$ M ($P < 0.05$).

The results therefore suggest that samorin-induced relaxation of rat aortic smooth muscle occurs by endothelium dependent mechanism which is probably mediated through the release of endothelium – derived relaxing factor (EDRF).

Keywords: Samorin, endothelium, relaxation, rat aorta

Introduction

Samorin remains a major trypanocidal drug widely used against trypanosomiasis in the livestock industry (Na Isa 1971; Hill and Akpokodje 1971;

Joshua, 1986). However, this drug has been shown to lower blood pressure via a direct relaxant effect on blood vessel (Ajagbonna *et. al.* 1993, 1995). It is also known that vasorelaxation of vascular

smooth muscle in response to a variety of stimuli depends on a functionally intact endothelium and the release of the endothelium derived relaxing factor (Furchgott and Zawadzki 1980). Moreover, agents that lower blood pressure could act via one of several vasodilator mechanisms (Bolton, 1979, Ebeigbe and Aloamaka, 1985). This study therefore examines further, possible mechanism by which samorin may lower blood pressure.

Materials and Methods

Inbred adult male and female sprague Dawley rats (170 - 190g) obtained from the laboratory animal centre of the College of Medicine of the University of Lagos were killed by cervical dislocation. The thoracic aorta was quickly removed, freed of connective tissue, cut into 2mm ring segments and suspended on fine stainless steel rods in a 20ml jacketed tissue bath. Tissues were connected to a Grass FT 03 transducer for isometric tension measurement on a Grass model 7D Polygraph (Grass medical instrument, Quincy Mass) under a resting tension of 2g. The bath contained physiological salt solution (PSS) of the following composition (mM): Sodium chloride 119; Potassium chloride 4.7; Magnesium sulphate 1.2; Potassium dihydrogen phosphate 1.2; Calcium chloride 1.6; Sodium bicarbonate 24.9; glucose 11.5. The physiological salt solution was bubbled continuously with a 95% oxygen and 5% carbon dioxide (Industrial Gases Ltd. Apapa, Nigeria). The pH of the medium was 7.35 - 7.40. The bath temperature was kept at 37°C by continuous circulation of water around the bath from a thermostatically controlled water bath (Grass Instrument Ltd) with the aid of a

roller pump (Watson-Marlow Ltd.). The tissue was allowed to equilibrate for a period of 90 minutes during which it was stimulated on three separate occasions by 10^{-7} M noradrenaline (Sigma Chemical Co., St Louis Missouri U.S.A.). Each tissue was now precontracted with 10^{-7} M noradrenaline or 50mM potassium chloride (Sigma Chemical) and concentration response to cumulative concentrations of samorin (Rhone Merieux-lyon France) were tested on tissues with or without an intact endothelium. The endothelium was removed by gently rubbing the inner lining of the rings with a fine platinum wire and its removal was confirmed by the absence of a relaxation response to noradrenaline precontracted rings to 10^{-5} M acetylcholine (Sigma Chemical). Also in another experiment, some tissue were incubated in physiological salt solution containing 10^{-6} M methylene blue (Sigma Chemical), a blocker of endothelium derived relaxing factor, for 15 minutes. At the end of 15 minute incubation, the aortic rings were stimulated with 10^{-7} M noradrenaline before carrying out observation of effects to samorin as previously done. In other experiments, effect of samorin was also tested on baseline tension.

The EC_{50} (concentration of samorin required to produce 50% relaxation) of 10^{-7} M noradrenaline induced tone was graphically determined using chart recorder data. Values are expressed as means \pm SEM. Statistical significance was assessed using student's t-test and probability values less than 0.05 were considered significant.

Results

Cumulative addition of samorin caused a concentration dependent relaxation of aortic rings precontracted

smooth muscle in response to a variety of stimuli depends on a functionally intact endothelium and the release of the endothelium derived relaxing factor (Furchgott and Zawadzki 1980). Moreover, agents that lower blood pressure could act via one of several vasodilator mechanisms (Bolton, 1979, Ebeigbe and Aloamaka, 1985). This study therefore examines further, possible mechanism by which samorin may lower blood pressure.

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The EC_{50} (concentration of samorin required to produce 50% relaxation) of 10^{-7} M noradrenaline induced tone was graphically determined using chart well graph data. Values are expressed as means \pm SEM. Statistical significance was assessed using student's t-test and probability values less than 0.05 were considered significant.

Results

Cumulative addition of samorin caused a concentration dependent relaxation of aortic rings precontracted

with 10^{-7} M noradrenaline (Fig.1). Samorin achieved $69.7 \pm 8.18\%$ relaxation of maximal contraction at a concentration of 4×10^{-5} M.

At this concentration, endothelium denuded tissues showed $35 \pm 8.56\%$ relaxation and methylene blue treated rings showed $18.3 \pm 2.18\%$ relaxation ($P < 0.05$). The result in Table 1 shows the EC_{50} of control rings, endothelium denuded and methylene blue treated rings. Those results demonstrated the effectiveness of samorin in causing relaxation of different rings, however, the EC_{50} of methylene blue and endothelium denuded rings differed significantly ($P < 0.05$) from that of the control rings (Fig.2).

Discussion

Results from this study demonstrate that samorin has a vasorelaxant effect on the rat aortic rings and this was highest in aorta with intact endothelium. This may suggest an endothelium dependent mechanism in its relaxation.

Since the discovery that muscarinic agent acetylcholine induces relaxation of vascular smooth muscle by stimulation of endothelium to release endothelium derived relaxing factor (Furchgott and Zawadzki, 1980; Furchgott, 1983), a number of other vasodilators have been shown to lower vascular resistance through the release of endothelium derived relaxing factor e.g histamine and hydralazine (Van de Vorde and Leusen, 1983; Ebeigbe and Aloamaka 1985). It is also known that acetylcholine will relax a variety of vascular smooth muscle precontracted by specific receptor agonists e.g noradrenaline, serotonin or Angiotensin II but potassium chloride induced contraction are relatively resistant to relaxation by acetylcholine (Furchgott, 1983; Ebeigbe

and Aloamaka 1987). The result from our present study (Fig. 1) shows that samorin relaxed aortic ring precontracted with noradrenaline but not potassium chloride precontracted ring. This may suggest an acetylcholine like activity.

The attenuation of the relaxation responses to samorin in aortic rings denuded of endothelium (Fig. 1 & 2) demonstrate that samorin induced relaxation is largely dependent on endothelium dependent mechanism. Furthermore, the attenuation of samorin induced relaxation by methylene blue provides additional evidence to support the assertion that samorin relaxation is partly mediated by the release of endothelium derived relaxing factor. Agents that elicit endothelium dependent relaxation cause elevation of cyclic guanosine monophosphate level within the smooth muscle and methylene blue prevent formation of cyclic guanosine monophosphate by inhibiting guanylate cyclase (Rapoport and Murrad, 1983) but in endothelium denuded aortic ring acetylcholine produced neither a relaxation nor a change in cyclic guanosine monophosphate level (Furchgott, 1988). The presence of methylene blue in this experiment significantly attenuate samorin induced relaxation (Fig.2) suggesting therefore that the endothelium dependent relaxation observed here was linked to intracellular elevation of cyclic guanosine monophosphate in smooth muscle cells. However, result from this study where samorin caused greater relaxation in endothelium denuded rings than in methylene blue treated rings (Fig.1) suggest that there may be other component to the endothelium dependent relaxatory response to samorin, the explanation for this may be seen in our earlier study where samorin

also relaxes vascular smooth muscle via a mechanism that involves inhibition of Ca^{2+} influx through receptor operated channel (Ajagbonna *et. al.* 1995).

In conclusion, this study demonstrate further that samorin casued vaso-relaxation of the rat aorta via an endothelium dependent release of endothelium derived relaxing factor which may be linked to intracellular elevation of cyclic guanosine monophosphate.

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TABLE 1: Values (mean \pm SEM) for EC_{50} (M) and absolute response to 4×10^{-6} M isoproterenol and absolute response to 4×10^{-6} M isoproterenol in the presence of endothelial dysfunction.

| EC ₅₀ (M) | Maximum response (Mg) |
|---------------------------------|--------------------------------|
| Endothelium intact (+E) control | $10.9 \pm 2.30 \times 10^{-6}$ |
| Endothelium denuded (-E) | $37.4 \pm 13.7 \times 10^{-6}$ |
| Methylene blue treated (+MB) | $80 \pm 14 \times 10^{-6}$ |

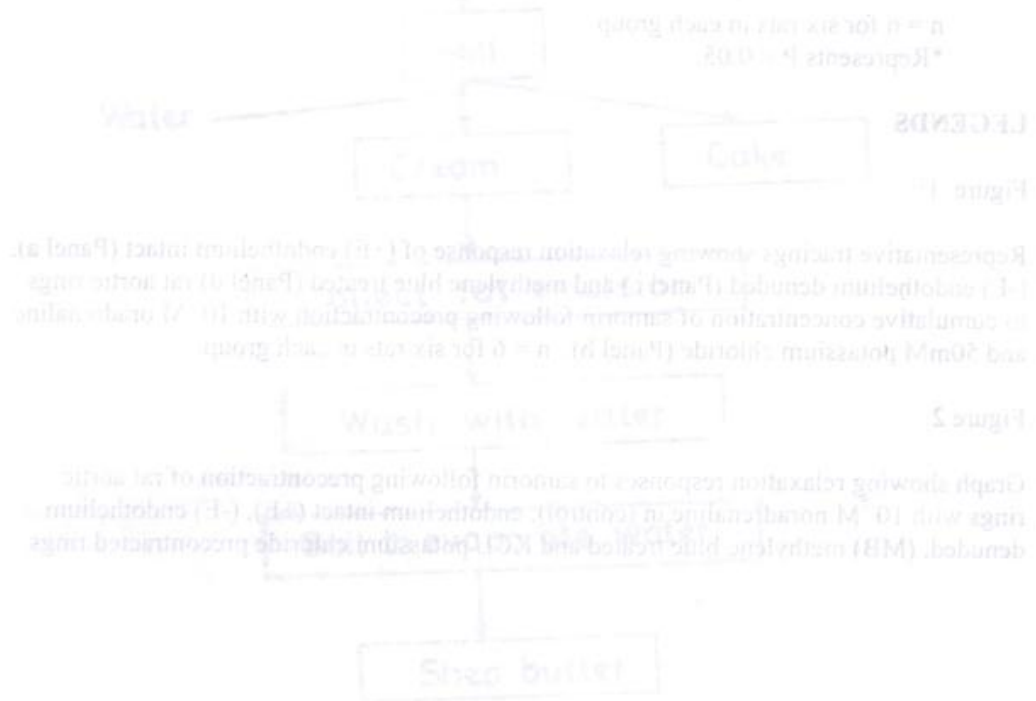


Fig 1 The relaxation process of shear stress in blood.

TABLE 1: Values (mean \pm SEM) for EC₅₀ (M), and maximal relaxation (%) in response to 4×10^{-5} M samorin and absolute tension developed during 10^{-5} M noradrenaline precontractions.

| | EC ₅₀ (M) | %Maximum relaxation | Absolute Tension Developed (Mg) |
|---------------------------------|--------------------------------|---------------------|---------------------------------|
| Endothelium intact (+E) control | $10.9 \pm 2.39 \times 10^{-6}$ | 69.7 ± 8.18 | 1330 ± 25 |
| Endothelium Denuded (-E) | $37.4 \pm 13.7 \times 10^{-6}$ | $35 \pm 8.56^*$ | 1345 ± 45 |
| Methylene Blue Treated (+MB) | $80 \pm 14 \times 10^{-6}$ | $18.3 \pm 2.18^*$ | |

n = 6 for six rats in each group

*Represents P < 0.05.

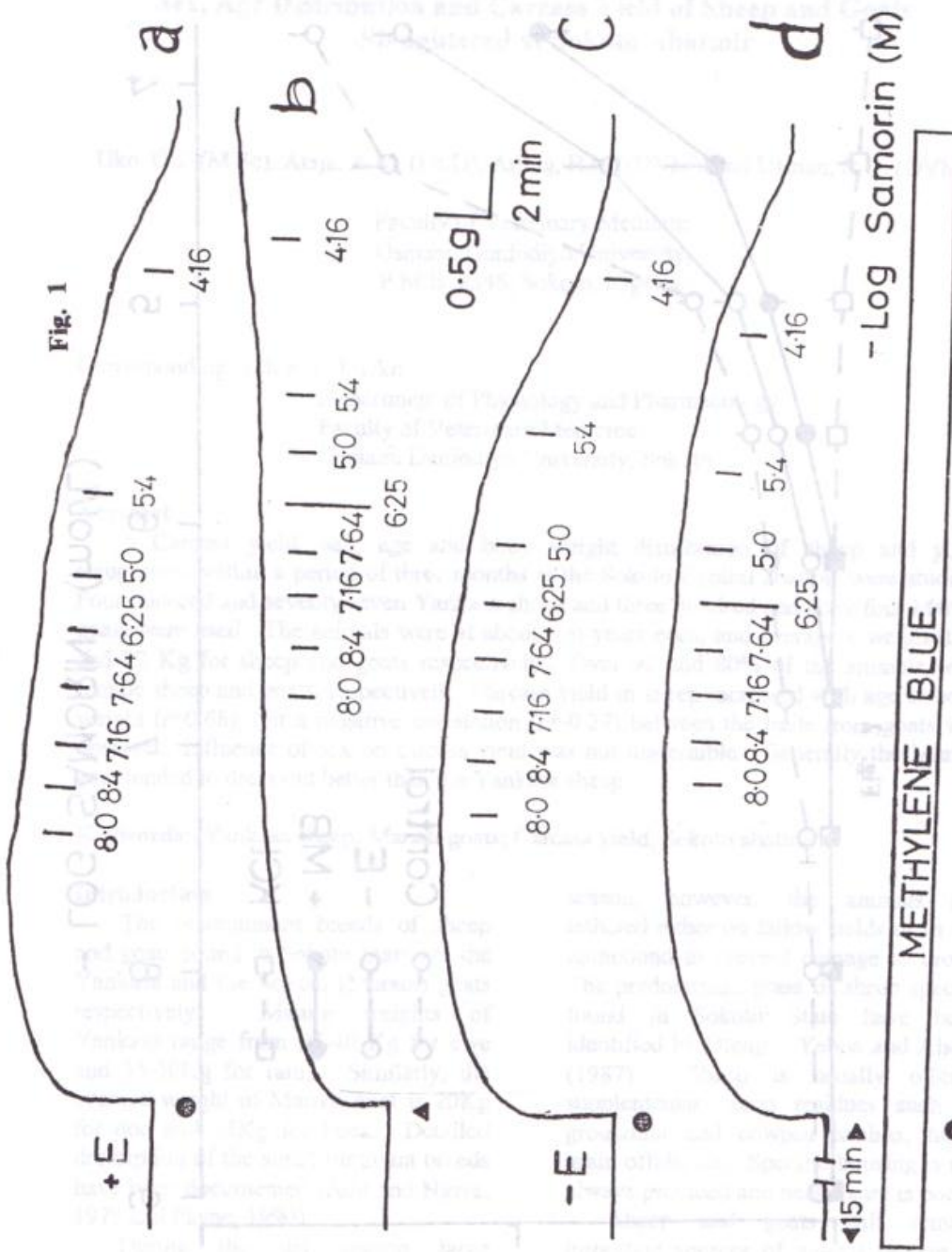
LEGENDS

Figure 1:

Representative tracings showing relaxation response of (+E) endothelium intact (Panel a), (-E) endothelium denuded (Panel c) and methylene blue treated (Panel d) rat aortic rings to cumulative concentration of samorin following precontraction with 10^{-7} M oradrenaline and 50mM potassium chloride (Panel b). n = 6 for six rats in each group.

Figure 2:

Graph showing relaxation responses to samorin following precontraction of rat aortic rings with 10^{-7} M noradrenaline in (control), endothelium intact (+E), (-E) endothelium denuded, (MB) methylene blue treated and KCL potassium chloride precontracted rings.



20mM POTASSIUM CHLORIDE
10mM MORADRENALINE

Fig. 2

