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DEPARTAMENTO DE ODONTOLOGIA

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Trabalho de Conclusão de Curso apresentado ao Departamento de Odontologia do Centro de Ciências Biológicas e da Saúde da Universidade Federal de Sergipe, como requisito parcial para a conclusão do curso de Odontologia, sob orientação da Profª Liane Maciel de Almeida Souza.

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LEVOBUPIVACAINE INDUCES RELAXATION, BUT NOT CONSTRICTION, IN RAT ARTERY

Liciane dos Santos Menezes[*], Liane Maciel de Almeida Souza[†], Márcio Roberto Viana dos Santos[‡].

*Undergraduate Student, School of Dentistry, The Federal University of Sergipe, Aracaju-SE; [†]Associate Professor, School of Dentistry, The Federal University of Sergipe, Aracaju-SE; [‡]Associate Professor, Department of Physiology, The Federal University of Sergipe, São Cristóvão-SE, Brazil.

Address correspondence to Liciane dos Santos Menezes, School of Dentistry, Rua Cláudio Batista s/n. Sanatório, Aracaju-SE, Brazil, 49060-100; licianesmenezes@gmail.com; +55 79 21051821.

Abstract:

The aim of this study was to evaluate the effects of levobupivacaine (LEVO) using the vascular reactivity technique in isolated rat superior mesenteric artery and also compare this effect to lidocaine (LIDO). Rings were obtained by the mesenteric artery of male Wistar rats and were kept in organ baths. For the recording of isometric contractions, each ring was suspended by cotton lines to a force transducer connected to a data acquisition system. Both the LIDO and LEVO showed no vasoconstrictor effect on the basal tone of rings with functional endothelium. However, when the rings were pre-contracted with phenylephrine, both drugs were able to induce concentration-dependent vasorelaxation. The vasorelaxant effect caused by LEVO has not changed after removal of the endothelium, or after tetraethylammonium (1mM), a non-selective blocker of K^+-channels. In rings without functional endothelium and pre-contracted with depolarizing Tyrode solution (80 mM KCl), the LEVO-induced vasorelaxation was no significantly different than observed in rings pre-contracted with phenylephrine. This study demonstrated that LEVO produces vasorelaxant effect in rat superior mesenteric artery, which is endothelium-independent. This effect seems to involve Ca^{2+}-channel blocker in smooth vascular muscle cell.

KEY-WORDS: levobupivacaine vasoactivity; mesenteric artery; vasorelaxant.

Introduction

The advances in operative techniques and the concern in pain control have served to support and sustain several pharmacological and clinical studies of local anesthetics[1]. It is the most used method in dentistry to minimize the painful phenomena, in addition to providing conditions for a safe and effective treatment, becoming an ally in demystifying the association between dentistry and pain[2].

The local anesthetics are classified according to their chemical structure - ester or amide, the latter being of greatest clinical application, comprised by prilocaine, lidocaine, mepivacaine, articaine, ropivacaine and bupivacaine[3]. Bupivacaine (BUPI) is a long-lasting anesthetic, which enables the achievement of longer surgery without the need of anesthetic complementation. However, the same presents certain cardiotoxicity, determined by its dextrorotatory component (S50-R50), encouraging researchers to develop an anesthetic with a lower toxic potential[4-7].

Thus, it was introduced levobupivacaine (LEVO), a local anesthetic with properties similar to those of bupivacaine (BUPI), however with less toxicity to the central nervous and cardiovascular system, due to its levorotatory excess in 50% (S75-R25)[6,8-12].

LEVO presents itself as a new alternative of analgesia associated with low toxicity in dental area, in addition to expressing particular vasoconstriction[13-17], being a satisfactory option for subjects who present contraindications to the use of vasoconstrictors[18-20].

Considering that, the present study aimed to evaluate a possible vasoconstrictor effect of LEVO using the technique of vascular reactivity in isolated superior mesenteric artery of rat and also compared this effect induced by lidocaine (LIDO).

Methods

Animals

Twelve male Wistar normotensive rats (250 – 300g) were obtained from colonies maintained in the Department of Physiology of the Federal University of Sergipe, Sergipe, Brazil. They were maintained in a large cage under controlled conditions of temperature and lighting (lights on: 06:00-18:00 h), fed with rodent diet and tap water *ad libtum*. All procedures were approved by the Animal Research Ethics Committee of the Federal University of Sergipe (Protocol number 27/2012) and were in compliance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996).

Drugs

The drugs used were levobupivacaíne in enantiomeric excess 50% (Cristália), lidocaine (Cristália), L-phenylephrine chloride (Phe), acetylcholine chloride (Ach) and tetraethylamonium (TEA) (all from Sigma-Aldrich).

Tissue preparation

Rats were killed by exsanguination under anesthesia and the superior mesenteric artery was removed, cleaned from connective and fat tissues and sectioned in rings (1-2 mm). These rings were suspended in organ baths containing 10 mL of Tyrode's solution, gassed with carbogen and maintained at 37 ºC under a resting tension of 0.75g for 60 min (stabilization period). The isometric tension was recorded by a force transducer (Letica, Model TRI210, Barcelona, Spain) coupled to an amplifier-recorder (AECAD 0804, AVS, São Paulo – SP, Brasil). When necessary, endothelium was removed with a fine steel wire and its functionality was assessed by the effect of Ach (1 µM) of reducing more than 75% of the tonus pre-induced by Phe (1 µM). The absence of the relaxation by Ach was considered as evidence that the rings were functionally denuded of endothelium.

Characterization of the effect of the LIDO and LEVO on basal tone of arterial rings

After verification of the presence of functional endothelium and complete recovery to basal tone, concentrations of LIDO ($3 \times 10^{-7} – 3 \times 10^{-5}$ M, n = 4) or LEVO ($3 \times 10^{-7} – 3 \times 10^{-4}$ M; n=9) were cumulatively and separately added to the bath to obtain a control concentration-response curve.

Characterization of the effect of the LIDO and LEVO on pre-contracted arterial rings

After verification of the presence of functional endothelium, intact rings were pre-contracted again with Phe (1 µM) and in the tonic phase of contraction, concentrations of LIDO ($3 \times 10^{-7} – 3 \times 10^{-5}$ M, n = 6) or LEVO ($3 \times 10^{-7} – 3 \times 10^{-4}$ M; n = 6) were cumulatively added to the bath to obtain a concentration-response curve.

Assessment of the role of vascular endothelium in the responses induced by LEVO

After verification of the absence of functional endothelium, the rings were pre-contracted with Phe (1 µM) and on tonic phase of contraction, increasing concentrations of LEVO ($3 \times 10^{-7} – 3 \times 10^{-4}$ M; n = 4) were cumulatively added to bath. The concentration-response curve for this experimental condition was compared with the concentration-response curve with functional endothelium rings.

Assessment of the role of Ca^{2+} in the responses induced by LEVO

A possible effect of LEVO on Ca^{2+}-channels was investigated obtaining concentration-response curves for LEVO ($3 \times 10^{-7} – 3 \times 10^{-4}$ M; n = 4) in rings without endothelium in the presence of high concentrations of potassium. In this protocol, the normal Tyrode's solution

was replaced by K^+-depolarizing Tyrode's solution with 80 mM of KCl and the preparations remained in this solution until the end of the experiment.

Assessment of the contribution of K^+-channels in the responses induced by LEVO

A possible effect of LEVO on K^+-channels was investigated obtaining concentration-response curves for LEVO (3×10^{-7} – 3×10^{-4} M; n = 7) in rings without endothelium incubated with 1 mM of TEA for 30 min, which, at this concentration, is a non-selective K^+-channel blocker. The concentration-response curve for this experimental condition was compared with those in absence of this blocker.

Statistical analysis

Values were expressed as mean ± S.E.M. The results were analyzed with one or two-way ANOVA followed by Bonferroni post-test. All analyses were performed by using GraphPad Prism™ 5.0.

Results

As you can see in Figure 1A and 2, both LIDO and LEVO were unable to induce a vasoconstriction in the vessel on basal tone. However in rings with functional endothelium pre-contracted with phenylephrine, both LIDO and LEVO were capable of inducing concentration-dependent relaxations (E_{max} = 35.3 ± 4.4 %; n = 6 and 120.5 ± 15.9 %; n = 6, respectively) (Fig 1B and 3).

In artery rings without functional endothelium and pre-contracted with FEN (1 µM) (Fig. 4) concentration-dependent relaxations induced by LEVO were no different from that obtained in rings with functional endothelium (E_{max} = 120.5 ± 15.9 %; n = 6 and 132.2 ± 15.5 %; n = 4, respectively).

A possible involvement of Ca^{2+}-channels was investigated with curves in which the rings were pre-contracted with depolarizing Tyrode solution (KCl 80 mM), and as showed in Figure 5, LEVO was capable of inducing vasorelaxation similar to that achieved in rings without functional endothelium and pre-contracted with FEN (1 µM) (E_{max} = 132.2 ± 15.5 %; n = 4 and 117.9 ± 6.2 %; n = 4, respectively).

To assess a possible participation of K^+-channels in vasorelaxant effect induced by LEVO, rings without functional endothelium were pre-incubated with 1 mM of TEA. The TEA, at the concentration used in this study is able to inhibit, non-selectively, the K^+ channels. In this protocol, the vasorelaxant effect induced by LEVO was significantly higher when compared to that presented by the drug in the absence of inhibitor (E_{max} = 132.2 ± 15.5 %; n = 4 *vs* E_{max} = 157.7 ± 6.7 %; n = 7) (Fig. 6).

Discussion

Studies show that levobupivacaine in 50% enantiomeric excess seems to present certain intrinsic vasconstriction *in vitro*[13-15,17,21] and *in vivo*[22-26], favoring those subjects who cannot make use of vasoconstrictor substances. Therefore, this study aimed to assess the vasoactivity of LEVO through experiments *in vitro* using preparations rings isolated of rat superior mesenteric artery that as a resistant artery, can reflect the possible effects on vascular reactivity in smaller beds such as those present in the area covered by clinical anesthetic in dentistry. Furthermore, this effect was compared to lidocaine, a standard local anesthetic used in dentistry.

In this study, it was shown that both LEVO and LIDO showed no contractile effect on basal tone in rat mesenteric artery. These studies corroborate the findings of Chang et al.[27], which demonstrated that LEVO on isolated rat tracheal smooth muscle did not present contractile effect. Conversely, Iida et al[28] in studies with dog cerebral arterioles and

Mukozawa et al[14,17] in experiments on isolated rat thoracic aorta, has shown the LEVO was able to induce vasoconstriction. It is possible to suggest that the discrepancy presented in these results can be justified by differences in the structures of the chemical agents, in the vascular bed, conditions of the experiments or species used.

In addition, these contrary effects presented by LEVO can be also justified by the fact that some anesthetics, such as LEVO and BUPI, present biphasic activity depending on the concentration in which they are used. It was observed that both *in vivo* and *in vitro*, these anesthetics cause vasodilation in high concentrations, and vasoconstriction in smaller concentrations[21-24,26]. The biphasic vasoactivity has also been reported in other amide-type local anesthetics after intradermal injection, such as lidocaine, that at concentrations lower than 1% seems to cause cutaneous vasoconstriction on visual inspection of skin colour[25].

As vasoconstrictor activity was not presented on the basal tonus, experiments were conducted to evaluate a possible vasorelaxant effect of LEVO and compare it to LIDO. In this way, in pre-contracted rings preparations with 1 μM of phenylephrine, an agonist of the Alpha 1-adrenergic receptor, both LIDO and LEVO induced concentration-dependent relaxations in rings isolated of rat superior mesenteric artery with functional endothelium.

Considering the effect presented by LEVO for the largest equivalent concentration of lidocaine used in this study, the vasorelaxation presented by both drugs was similar. Therefore, the vasodilation presented by the both drugs possibly are similar, however, greater LIDO concentrations needed to confirm this hypothesis.

As LEVO presented a vasorelaxant effect, we sought to assess the mechanism of action involved in this effect. Since the endothelium is an important regulator of vascular tone by releasing endothelium-derived relaxing factors, mainly NO and products derivate of the activation of cyclo-oxygenase (COX), such as prostacyclins, experiments were carried out to assess the participation of the endothelium in this response[29,30].

For this, curves were obtained to LEVO in artery rings without functional endothelium. In these conditions, the vasorelaxant response was similar to that obtained in rings with functional endothelium. Thus, suggesting that the endothelium does not seem to be relevant to the expression of this effect.

Similarly to the present study, LEVO presented endothelium independent effect on rat thoracic aorta[17] as well as in the experiment *in vitro* performed on isolated human umbilical artery and vein[16]. However, in the studies with isolated rat aorta it was demonstrated that the effect of LEVO was dependent on nitric oxide released by endothelium[13]. These findings reinforce even more that the vascular effects of LEVO may differ depending on some variables, such as the vascular bed used.

We also evaluated whether the effects induced by LEVO involves another endothelium-independent signaling pathway, such as Ca^{2+}-channel blocker. It is known that increased external K^+ concentration induces smooth muscle contraction through the activation of voltage-operated calcium channels (Cavs) and subsequent release of calcium from the sarcoplasmic reticulum. The contractions induced by high concentrations of K^+ are inhibited by Ca^{2+} -channel blockers or removal of Ca^{2+} of external environment, and are thus totally dependent on the influx of Ca^{2+} [31].

Thus, curves to LEVO were obtained in pre-contracted rings with depolarizing solution of Tyrode (KCL 80 mM). In these preparations, LEVO induced vasorelaxation that was not much different from that obtained in rings without pre-contracted functional endothelium with phenylephrine, suggesting that the vasorelaxation induced by LEVO possibly involves the Cavs.

Studies performed by with Choi et al[13,], Baik et al[15] and Mukozawa et al[17] demonstrated that vasoconstriction induced by LEVO in aorta preparations is mediated mainly by intracellular calcium levels and by the influx of these ions through the Cavs[13, 15,17] .

However, our results suggest that LEVO acts inhibiting the Ca^{2+}-influx through these channels. These discrepancies might be related to the different used beds and experimental conditions. Nevertheless, other studies are necessary for a better understanding of this mechanism of action.

Literature reports that the K^+-channels also play an important role in the regulation of vascular tone [32]. The opening of these channels in smooth muscle cell membrane causes efflux of ions K^+, generating a hyperpolarization that closes the Cavs, resulting in decreased influx of ions Ca^{2+} and causing relaxation of smooth muscles[33].

Departing of this assumption, the participation of K^+-channels in the vasorelaxant effect induced by LEVO was investigated preincubating rings without functional endothelium with TEA at concentration of 1 mM that was able to inhibit, non-selectively, potassium channels[34]. In this condition, the LEVO was able to induce vasodilation greater than that obtained in rings without endothelium functional, suggesting the non-involvement of these channels in the effect induced by this drug.

The results obtained in this study demonstrate that LEVO was unable to contract rings of rat superior mesenteric artery, but it was able to produce vasorelaxant effect. The vasorelaxation was endothelium and K^+-channel independent and seems to be caused by the blockage of the Cavs in smooth vascular muscle cells. It is possible to suggest that LEVO presents several vascular effects and these effects mainly depend on the vascular bed used. Even not showing the expected vasoconstriction in our studies, LEVO stills beneficial and could be spread in dentistry as it is less cardiotoxic and neurotoxic than the BUPI, and it also provides long-lasting anesthesia.

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Figures

Figure 1: Original registration showing the effect of LIDO in isolated rings of rat superior mesenteric artery on basal tone **(A)** and pre-contracted with 1µM of Phe **(B)**.

Figure 2: Concentration-response curves for LIDO (3×10^{-7} - 3×10^{-5} M; n = 9) and LEVO (3×10^{-7} - 10^{-4} M; n = 4) on basal tone of isolated rings of rat superior mesenteric artery with functional endothelium. Values are expressed as mean ± S.E.M.

Figure 3: Concentration-response curves for LIDO (3×10^{-7} - 3×10^{-5} M; n = 6) and LEVO (3×10^{-7} - 10^{-4} M; n = 6) in isolated rings of rat superior mesenteric artery with functional endothelium, pre-contracted with 1µM of Phe. Values are expressed as mean ± S.E.M. The data were analyzed with two-way ANOVA followed by the Bonferroni post-test.

Figure 4: Concentration-response curves for LEVO (3×10^{-7} - 3×10^{-5} M; n = 6) in isolated rings of rat superior mesenteric artery with (n = 6) and without (n = 4) functional endothelium, pre-contracted with 1 µM of Phe. Values are expressed as mean ± S.E.M. The data were analyzed with two-way ANOVA followed by the Bonferroni post-test.

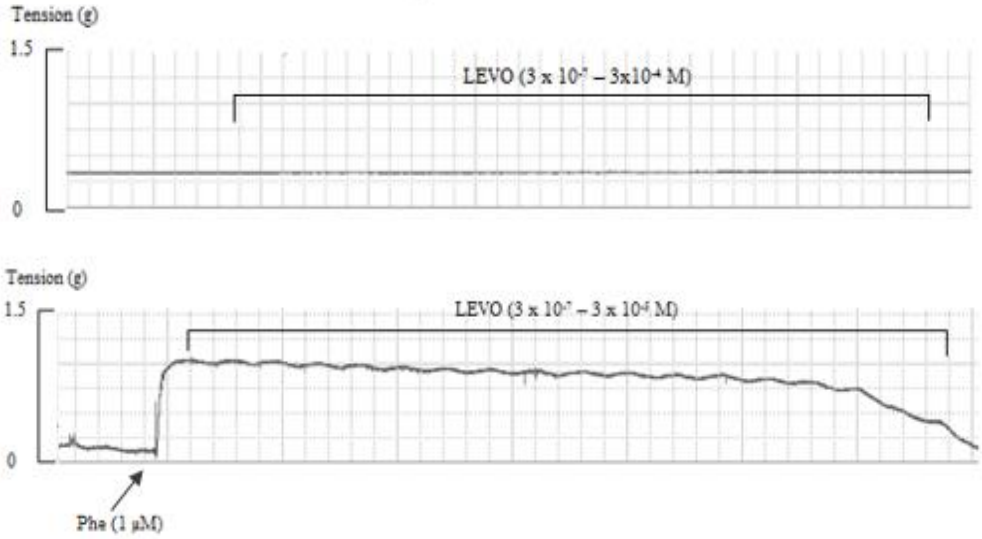
Figure 5: Concentration-response curves for LEVO (3×10^{-7} - 3×10^{-5} M; n = 6) in isolated rings of rat superior mesenteric artery without functional endothelium pre-contracted with Phe (n = 4) or KCL 80mM (n = 4). Values are expressed as mean ± S.E.M. The data were analyzed with two-way ANOVA followed by the Bonferroni post-test.

Figure 6: Concentration-response curves for LEVO (3×10^{-7} - 3×10^{-5} M; n = 6) in isolated rings of rat superior mesenteric artery without functional endothelium pre-contracted with

Phe before (n = 4) and after TEA (1mM) (n = 7). Values are expressed as mean ± S.E.M. Data were analyzed with two-way ANOVA followed by Bonferroni post-test. *p<0.05 and *** P <0.001 *vs* rings without the endothelium pre-contracted with Phe.

Figure 1:

A



B

Figure 2:

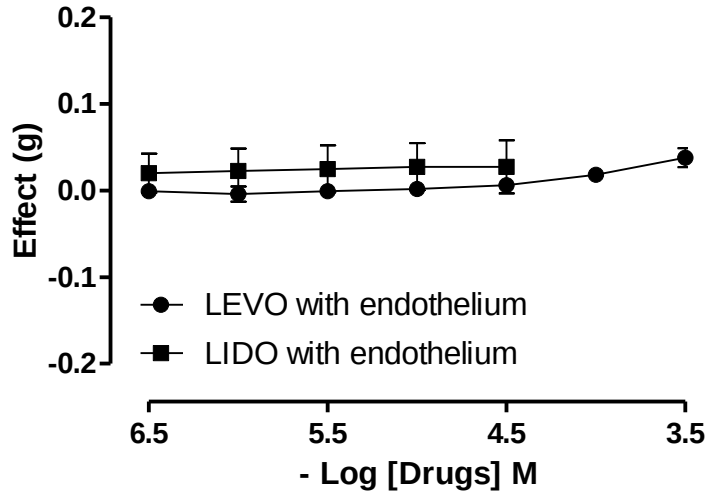


Figure 3:

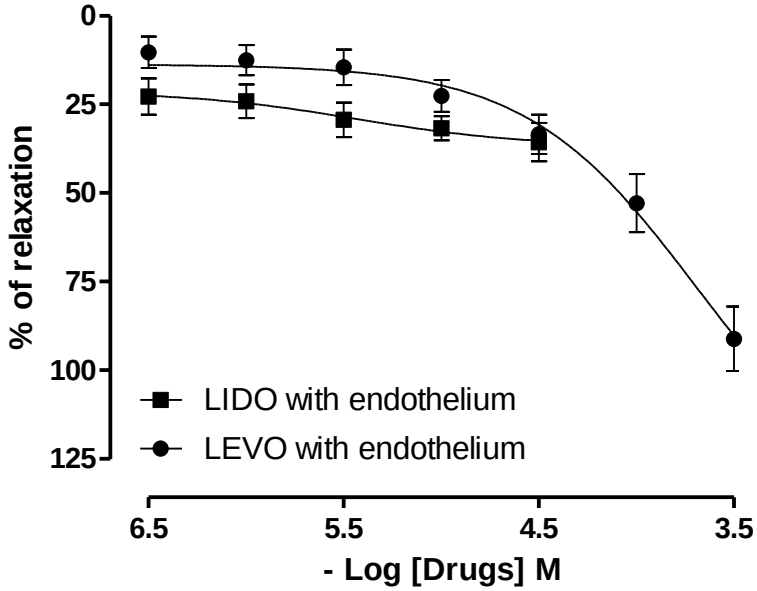


Figure 4:

Vasorelaxant effect of levobupivacaine

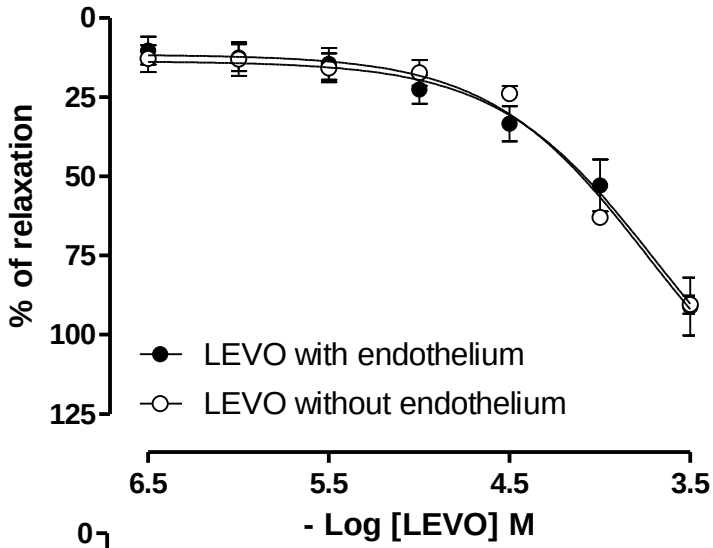


Figure 5:

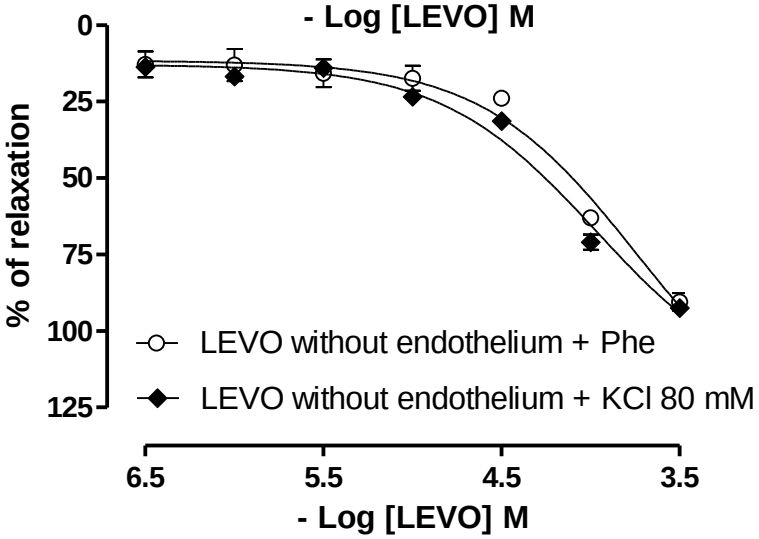


Figure 6: