Inhibition of L-type and cyclic nucleotide-gated calcium channels demonstrates synergistic mechanisms for prolonging vascular contractions induced by a mimetic of thromboxane A_2

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Abstract

Previous experiments have demonstrated that the rates of relaxation of blood vessels treated with the thromboxane-A_2 mimetic, U-46619, are significantly lower when compared to vessels treated with other vasoactive agents (e.g. α-adrenergic agonists). As a means of investigating the molecular mechanisms responsible for this prolonged contraction, we examined the roles of two types of calcium channels. L-type Ca^{2+} channels have long been associated with the U-46619 contraction, while cyclic nucleotide-gated (CNG) Ca^{2+} channels have only recently been shown to be involved. We tested the hypothesis that functioning of both channels is necessary to prolong the U-46619 contraction. An isolated organ bath preparation was used to measure the rates of relaxation (g/min) in aortic vessel segments obtained from euthanized rabbits. Isolated vessels contracted with U-46619 were treated with either L-type channel inhibitor (nifedipine, 200 μM) or CNG channel inhibitor (L-cis-dilitiazem, 140 μM), or both inhibitors simultaneously. Mean rates of relaxation were obtained for the four treatment groups: nifedipine only ($7.95 \times 10^{-2} \pm 0.562 \times 10^{-2}$ g/min, n = 10), L-cis-diltiazem only ($6.36 \times 10^{-2} \pm 0.603 \times 10^{-2}$ g/min, n = 10), both inhibitors simultaneously ($6.93 \times 10^{-2} \pm 0.875 \times 10^{-2}$ g/min, n = 12), and a control with vehicle only ($3.94 \times 10^{-2} \pm 0.494 \times 10^{-2}$ g/min, n = 15). Statistical analysis of the data indicated that the mean relaxation rate for the vehicle-treated group differed significantly from the relaxation rates of the experimental groups ($P < 0.001$), which were deemed statistically synonymous ($P = 0.324$). These data indicate that inhibition of either calcium channel alone or both channels simultaneously leads to equivalent increases in the rate of relaxation. This demonstrates that optimal functioning of both channels is necessary for the prolonged contraction, characteristic of U-46619-treated vessels. These results may have implications for reversing the contractions in vessels during myocardial infarction or stroke.

Background

- Thromboxane A_2 (TxA_2) is a metabolite of arachidonic acid released by activated platelets in response to tissue trauma.
- Thromboxane (TxA_2) binds to the TxA_2 receptor (TP) which is a G-protein coupled receptor known to be coupled to a $G_{\alpha q}$ subunit. When activated, the $G_{\alpha q}$ subunit activates phospholipase C leading to cleavage of PIP_2 into DAG and IP_3. The latter diffuses to the sarcoplasmic reticulum (SR) in smooth muscle cells and opens ligand gated Ca^{2+} channels. Increases in intracellular Ca^{2+} activates cellular processes leading to vasoconstriction.
- Vasoconstriction elicited U-46619 is significantly prolonged in comparison to that seen in response to other vasoconstrictors, such as KCl, phenylepinephrine, and others. This characteristic is clinically important when considered in the context of myocardial infarction
- Experiments inhibiting L-type Ca^{2+} channels effectively prevented vascular contractions induced by other agents, but only partially attenuated those in response to U-46619. Subsequently, experiments utilizing Ca^{2+} chelating EGTA buffer prevented vasoconstriction by all agents investigated. This suggested the involvement of more than one type of membrane Ca^{2+} channel.
- Nifedipine is an L-type Ca^{2+} channel blocker, enabling investigation of vascular contraction in the absence of L-type Ca^{2+} channel function.
- L-cis-diltiazem is a cyclic nucleotide-gated (CNG) Ca^{2+} channel inhibitor.

Aim of Experiment

- To inhibit L-type and CNG Ca^{2+} channels—both separately and simultaneously—as a means of investigating their roles in prolonging vascular contractions induced by thromboxane-A_2 mimetic, U-46619.

Methods

To test the effects of L-type and CNG Ca^{2+} channel inhibitors (nifedipine and L-cis-diltiazem, respectively) on U-46619-induced vascular contractions, segments of aorta were removed from euthanized New Zealand white rabbits (IACUC: AUS 42-02). Vessels were placed in a modified Krebs solution and kept overnight at 40°C. Experiments were carried out the following day using a conventional isolated organ bath preparation. 95% O_2/5% CO_2 was bubbled through a physiological buffer (pH ~ 7.4) which was maintained at 37°C. The vessels were held in place by hooks, which applied tension in opposite directions. At the beginning of each experiment, a baseline tension of 2 grams of force was established to mimic the basal muscle tone that occurs *in vivo*. To begin each experiment all vessels were subjected to a series of treatments with 60 mM KCl to induce maximal contraction. Following return to baseline tension, each was treated with 5μM U-46619 to induce the contraction being investigated. Each bath was washed with Krebs buffer solution subsequent to each drug treatment mentioned above.

Each vessel was treated with four different combinations of drugs and respective vehicles. Drugs used included nifedipine, an L-type Ca^{2+} channel inhibitor at 200 μM (dissolved in 0.4% ethanol/99.6% water), and L-cis-diltiazem, a CNG Ca^{2+} channel inhibitor, at 140 μM (dissolved in 100% water). Nifedipine and L-cis-diltiazem were purchased from Enzo Life Sciences, Farmingdale, NY. U-46619 was purchased from Cayman Chemical Co., Ann Arbor, MI.

The first treatment group consisted of vessels treated with both inhibitors, namely 200 μM nifedipine, followed by 140 μM L-cis-diltiazem. All vessels were allowed to relax to baseline tension and the relaxation time was recorded. The second group consisted of vessels treated with 200 μM nifedipine, followed by L-cis-diltiazem vehicle (100% water). The third group consisted of vessels treated with nifedipine vehicle (0.4% ethanol/99.6% water), followed by 140 μM L-cis-diltiazem. The fourth group consisted of vessels treated with nifedipine vehicle, followed by L-cis-diltiazem vehicle. This was the control set for this experiment.

As stated previously, vessel tension was monitored electronically throughout the duration of the experiment (see figure 1). At the conclusion of each experiment, the graphs obtained were analyzed to yield quantitative data in the form of rates of relaxation for each vessel. This method of data analysis was chosen to normalize vessels that varied in the magnitude of the contraction.

Results

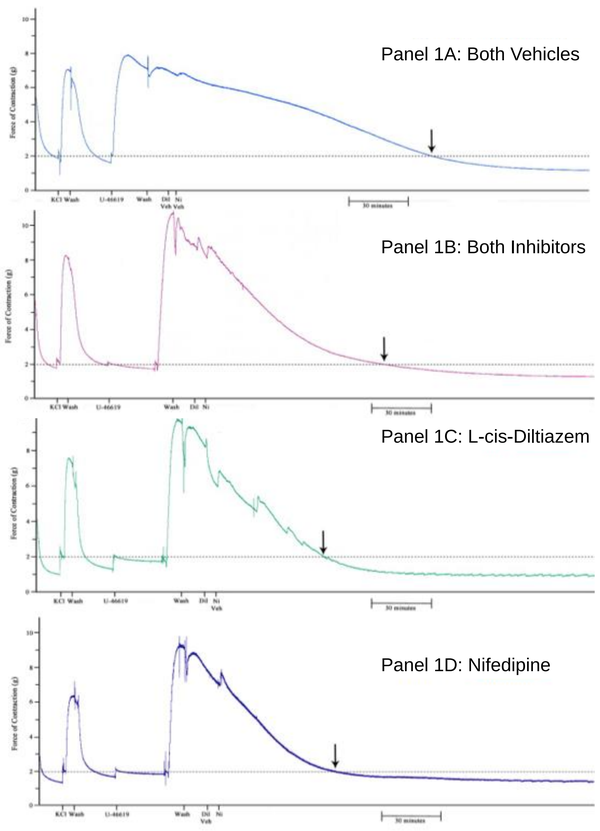

Figure 1: Data representing the contractions and relaxations of four vessels are shown. The panels are labeled according to which drugs they received prior to the relaxation phase. Notice how the vessels treated with various combinations of drugs reached baseline tension much more quickly than the vessel treated with vehicles only. On the x-axis, the treatment given at each point in time is indicated. Ni and Dil represent nifedipine and L-cis-diltiazem, respectively. Also, note that the arrows indicate the time at which each vessel returned to baseline tension after treatment with U-46619. Baseline tension (2 g) is indicated by the dotted horizontal line

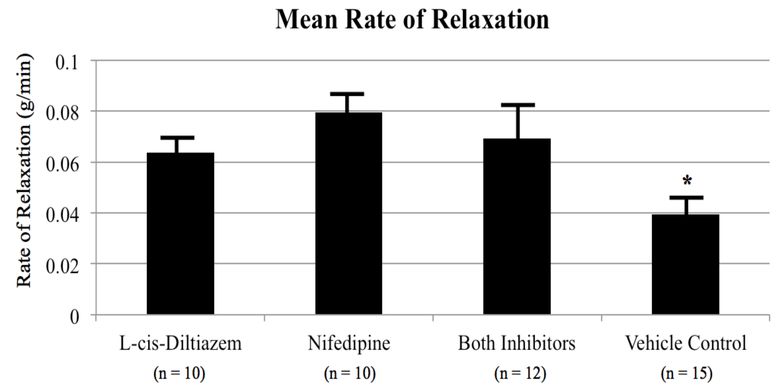

Figure 2: The figure above is a graphical representation of the mean rates of relaxation (g/min) for each treatment group of interest, with bars representing the standard error of the mean. Statistical analysis of these data using an unstacked ANOVA indicated that the inhibitor-treated groups (L-cis-diltiazem, nifedipine, and both inhibitors) were statistically synonymous (F-statistic = 1.17; 31 degrees of freedom; P = 0.324). The same test, with the addition of the vehicle control treatment, indicated a strong statistical discrepancy between the four means (F-statistic = 7.61; 46 degrees of freedom; $P < 0.001$).

Conclusion

- Based upon the results obtained in this experiment, it can be concluded that both L-type and CNG Ca^{2+} channels are necessary, but not sufficient for prolonging TxA_2/U-46619-induced vascular contractions.
- Following this logic, it can be speculated that these two types of Ca^{2+} channels are linked in an interdependent, cooperative mechanism whose optimal functioning is necessary for prolonging the vascular contractions traditionally observed in TxA_2/U-46619-treated vessels.

Significance

- Clinical significance with respect to disorders in which tissue is damaged as a result of inadequate oxygen and nutrient supply due to decreased blood flow.
- Acute cases of vessel constriction resulting in myocardial infarction or stroke. This trauma prompts the release of TxA_2, which elicits powerful, prolonged vasoconstrictive effects that further decrease blood supply. By elucidating the mechanisms responsible for significantly prolonging TxA_2-induced vascular contractions, it is possible that effective treatments for the inhibition of this phenomenon could be developed.
- Outlining unique components of these mechanisms could prove especially important for providing treatments capable of specifically targeting TxA_2-induced vascular tension; thereby avoiding negative side effects resulting from interfering with basal systemic vascular tension.

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