Moderate Hypothermia Increases Donepezil-Induced Relaxation in Calf Cardiac Vein: The Role of Nitric Oxide

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Abstract – At present very little is known about the vascular effects of donepezil. The aim of the present study was to evaluate the effects of donepezil on calf cardiac vein. The effects of hypothermia (to 28 ˚C) on the vasodilatation induced by donepezil (10-9 - $3x10^{-4}$ M) on carbachol (10^{-6} M) - pre-contracted calf cardiac vein and the role of nitric oxide in these effects were also analyzed. Donepezil induced concentration dependent relaxations on carbachol (10^{-6} M) - pre-contracted vein preparations. During hypothermia, the sensitivity to donepezil was significantly higher than at 37 ˚C. L-NAME-incubation decreased the sensitivity at both temperatures. These results suggest that hypothermia increased the sensitivity to donepezil furthermore, nitric oxide plays an essential role in the donepezil-induced relaxation in calf cardiac vein. Furthermore, the results of this study demonstrate for the first time that hpothermia-induced changes of donepezil in calf cardiac vein is independent of nitric oxide.

Keywords – Carbachol, Cardiac vein, Donepezil, Hypothermia, Nitric oxide

1. Introduction

Temperature has been shown to induce significant changes in cutaneous and non-cutaneous vascular smooth muscle responses to various drugs [1, 2]. The effects of hypothermia have mostly been investigated in cutaneous vessels [3,4]. Furthermore, most of the previous studies examining the effect of hypothermia on smooth muscle responses have focused on the effects of contractile agents and information about vasodilators is rather limited. It's reported that in guinea-pig myocardium and aorta the vasorelaxant effects of NIP-121, cromakalim, and pinacidil were greatly reduced during cooling [5]. Thus, the investigators demonstrated that the effects of these K+ channel openers on the myocardium and vascular smooth muscle are temperature sensitive. Previously we observed that hypothermia decreased the sensitivity, but not maximal relaxation to diazoxide in calf cardiac vein and coronary artery [6].

Also, it becomes appearent that nitric oxide is a relaxant factor that produces a basal vasodilator tone and is involved in the response of different vascular beds to vasoactive stimuli. Nitric oxide is an important cellular signaling molecule and synthesized in the endothelium from L-arginine and this synthesis can be stimulated by several types of stimuli [7]. Moreover, it was recently reported that cooling facilitates the stimulated release of nitric oxide from the endothelium in cutaneous, but not in noncutaneous vessels [8-11].

Donepezil is an acetylcholinesterase inhibitor and currently used to treat patients with Alzheimer disease [12]. However, its direct effect on vascular blood vessels during cooling has not been evaluated.

In contrast to the effect of hypothermia on cutaneous smooth muscle reactivity to vasoactive agents, the influence on non-cutaneous vessels is unclear because of limited information and disparate results. Therefore, the purpose of this study was to determine the effects of hypothermia (28°C) on vascular smooth muscle responses to donepezil in calf cardiac vein, analyzing the role of nitric oxide in these effects. This vessel was selected because this is an easily accessible vessel and is appropriate for studying the direct effect of agents on the vascular smooth muscle. Therefore, carbachol was used as contractile agent.

2. Materials and Methods

2.1. Materials

Carbachol chloride, NG nitro-L-arginine methlyl esther, acetylcholine chloride (all dissolved in distilled water), donepezil dissolved in dimethyl sulphoxide; DMSO. Donepezil was kindly provided by Ilko Drug Industry (Turkey). All other drugs were obtained from Sigma (St Louis, MO, USA).

2.2. Tissue preparation

Calf hearts were obtained from a slaughterhouse and were immediately placed in Krebs-Henseleit solution (KHS). Segments of the great cardiac vein was removed and cut into rings of 2.5 mm in length. Care was taken not to damage the endothelium. Each ring was mounted in 25 ml organ baths containing KHS, aerated with 95% O_2 and 5% CO_2. KHS was composed of (mM): NaCl 119, KCl 4.70, $MgSO_4$ 1.50, $KH2PO_4$ 1.20, CaCl2 2.50, $NaHCO_3$ 25, and Glucose 11. The responses were recorded isometrically by a force-displacement transducer (Grass FT04; Grass Instruments Co W., Warwick, RI, USA) connected through amplifiers to a polygraph (Grass 7D; Grass Instrument Co). The tissues were allowed to equilibrate for 60 mins under a resting tension of 1g with repeated washing every 15 mins.

The endothelial cell integrity was determined in each ring before all experiments. Relaxation responses to acetylcholine (10^{-6} M) in rings pre-constricted with 5-hydroxytryptamine (5-HT, 10^{-6} M) were used to test endothelial cell integrity. Preparations which were relaxed by > 70% of the

5-HT-induced tone after addition of acetylcholine were considered to have undamaged endothelium.

2.3. Experimental procedure

First, the cardiac vein rings were contracted with 10−6 M carbachol. After the contraction had reached steady state, donepezil was added to the organ bath cumulatively (10−9-3 × 10^{-4} M) at 37 °C. The maximal carbachol contraction was used as a standart by which subsequent responses of the tissue could be expressed (as a percentage of this contraction).

After the first concentration-response curve was completed, preparations were washed and allowed to reestablish resting tension. The same procedure was repeated in the presence of the nitric oxide synthase inhibitor NG nitro-L-arginine methlyl esther (L-NAME, 10^{-4} M) [14] at 37 °C.

In another group, after the contractile responses to carbachol, the temperature was changed from 37 - 28°C, 28 C was considered to be "moderate cooling" temperature according to the previous studies [6,13]. Cooling was rapidly achieved, and preparations were allowed to equilibrate at this temperature for 30 mins before a second concentration-response curve was determined for donepezil. Donepezil was added to the organ bath cumulatively, n=6 in each group.

Also, the influence of nitric oxide on relaxations to donepezil at 28°C was investigated. Again, after the contractile responses to carbachol, the temperature was changed from 37 to 28 °C. The tissues were allowed to equilibrate at 28°C for 30 mins. L-NAME was added to the organ bath 20 mins before concentration-response curves were obtained. Endothelium was not denuded because only the role of endothelial nitric oxide was examined in this study.

2.4. Statistical analysis

Relaxation responses to donepezil are expressed as percentages of the carbachol (10^{-6} M) induced contraction. Concentrations of donepezil causing 50% of the maximal response (IC_{50}) were calculated from each individual concentration-response curve. pIC_{50} (−log IC_{50}) values for curves obtained before and during cooling were compared using Student's t-test. Statistical significance was set at $P<0.05$

3. Results and Discussion

In the present study, donepezil applied cumulatively to the calf cardiac vein and produced no response. Then the tissues were washed and rested. After the stabilization period, cardiac vein preparations were contracted with carbachol (10^{-6} M). Carbachol produced reproducible contractions and time-dependent changes were not observed in response to this agonist. Cumulative addition of donepezil (10−9–3×10^{-4} M) produced concentration-dependent relaxation of cardiac vein preparations pre-contracted with carbachol at both 37 and 28 ºC (Figure 1). During hypothermia the pIC_{50} value of the calf cardiac vein was significantly higher than at 37ºC (pIC_{50} = 5.66±0.11 at 37 ºC and 7.30 ± 0.02 at 28ºC, $p<0.05$).

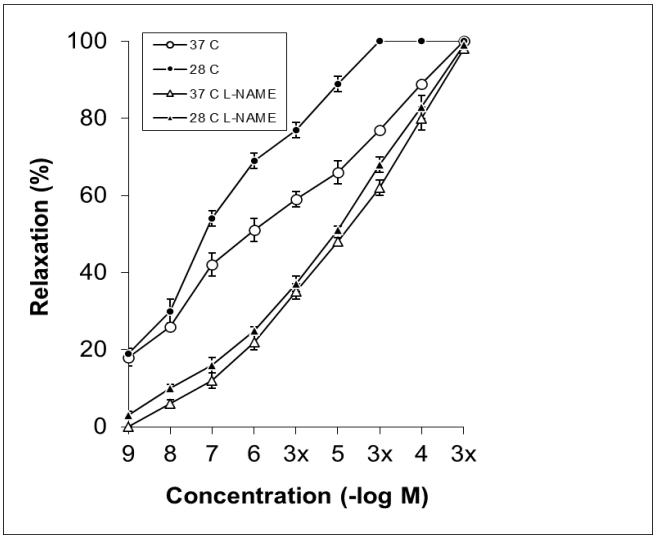

Fig. 1. Concentration-response curves for donepezil at 37 and 28 °C and in the presence of N^G nitro-L-arginine methyl esther (L-NAME, 10^{-6} M), at 37 and 28 °C, in calf cardiac vein. The preparations were pre-constricted with carbachol (10^{-6} M). Each point is the mean ± SEM of six experiments.

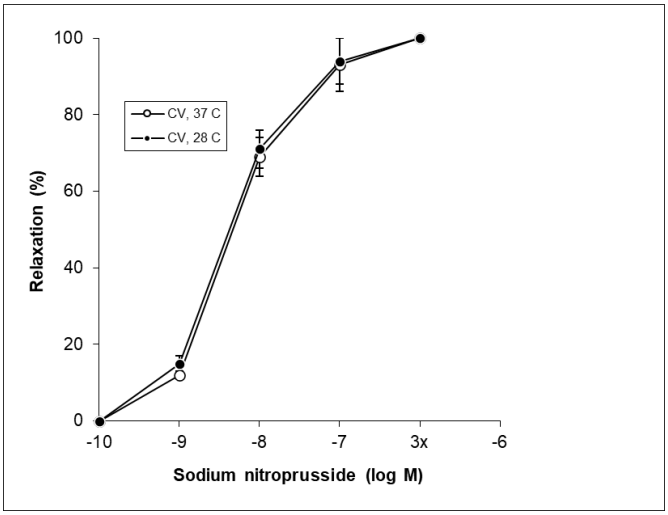

Fig. 2. Responses of calf cardiac vein to sodium nitroprusside at 37 and 28 °C. Each point is the mean ± SEM of six experiments.

Pre-treatment with L-NAME (10^{-4} M) significantly decreased the sensitivity to donepezil at both 37 and 28 ºC ($p<0.05$). The pIC_{50} values at 37 and 28 ºC in the presence of L-NAME were 5.03±0.48 and 5.01±0.86, respectively.

3.1. Effect of sodium nitroprusside

Sodium nitroprusside-induced concentration-dependent relaxation in cardiac vein and the relaxation to this agent was not influenced by hypothermia (Figure 2).

We know responses of vascular smooth muscles caused by agonist and antagonists can be influenced by temperature. The calf cardiac vein is an easily accesible smooth muscle preparation and there is limited information [13,15] about the effect of temperature in this vessel. Donepezil is an acetylcholinesterase inhibitor, and clinically used worldwide for patients with mild to severe Alzheimer disease [12]. Chronic treatment with donepezil is known to preserve regional cerebral blood flow in these patients [16]. There is only one study demonstrating donepezil induced vasodilation and, in that study, cerebral parenchymal arterioles have been used [17]. This result supports our finding.

In our study, compared with the control responses at 37 °C, hypothermia increased the sensitivity, to donepezil in calf cardiac vein. To our knowledge, there are no studies that analyze the effects of hypothermia on the donepezil-induced relaxations. So, this is the first study to show the effects of cooling on donepezil-induced relaxation.

It is known that endothelium plays a crucial role in the regulation of cardiovascular homeostasis through the release of vasoactive factors such as nitric oxide. Nitric oxide directly activates soluble guanylate cyclase in vascular smooth muscle cells and increases intracellular cyclic GMP production, resulting in vasodilation [18]. In this study, pre-treatment with nitric oxide synthase inhibitor L-NAME decresed the sensitivity to donepezil suggesting that nitric oxide is important for donepezil-induced relaxation mechanism. Our results are in aggrement with another report [17] showing that donepezil-induced arteriolar dilation has completely abolished by S-methyl-L-thiocitrulline as well as NG-nitro-L-arginine methyl ester.

Limited data suggest that changing temperature may also alter the ability of the endothelium to generate or release nitric oxide. In this work we also studied the role of nitric oxide on donepezil induced responses during hypothermia. At 28 °C, inhibition of nitric oxide synthesis decreased the sensitivity to donepezil. No previous data on the effects of nitric oxide on the donepezil-induced responses of calf cardiac vein during cooling has been published before. In our study, the effects of hypotermia to sodium nitroprusside, a nitrous compound is known to relax vascular smooth muscle cells in a similar way to nitric oxide [19] were also studied, and it was found that the relaxations to this substance were not influenced by hypothermia and it can therefore be assumed that the ability of the smooth muscle cells to relax to nitrous compounds remains essentially unaltered at lowered temperatures in cardiac vein of calf. With our data we cannot suggest which mechanisms underlie this decreased sensitivity to donepezil during cooling because pre-incubation with L-NAME significantly decreased the sensitivity to donepezil at both temperatures. Limited data suggests that vascular smooth muscle responsiveness to nitric oxide can be influenced by temperature. Furthermore, Monge et al [11] reported that changes in temperature might affect the production of nitric oxide in a different way depending on vascular beds. The investigators reported that the relaxations of ear arteries, but not of femoral arteries, from rabbits to cholinergic stimulation is increased during cooling, probably by an increased production of nitric oxide in the activated endothelium at low temperature. It's also reported that the increased sensitivity of the relaxation in ear arteries, but not in femoral arteries, to histamine found during cooling seems to be independent of the release of nitric oxide [8]. Thus, again in cardiac vein we have reported that the relaxations to rosuvastatin were independent of nitric oxide during cooling [15].

4. Conclusion

In conclusion, the present results demonstrated for the first time that donepezil induced relaxation of calf cardiac vein and hypothermia increased the sensitivity to cilostazol independently on nitric oxide. Further studies must be performed to clarify the mechanism of hypothermia-induced increase in the sensitivity of donepezil

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