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ORIGINAL PAPER

Relaxin 2 fails to lower intraocular pressure and to dilate retinal vessels in rats

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Abstract
Purpose Recently, the vasodilator relaxin 2 has been introduced as a treatment for acute heart failure. However, its role on vessels of the eye and intraocular pressure (IOP) remains unclear though it has been hypothesized to induce a decrease IOP after intramuscular injection in humans. We aimed to test whether the hormone relaxin 2 lowers IOP and dilates retinal vessels in animals.
Methods The IOP of female Sprague-Dawley rats before and after application of relaxin 2 was measured using an Icare Tonolab device calibrated for rats. Recombinant human relaxin 2 in phosphate-buffered saline with 0.1% bovine serum albumin was either applied as eye drops (1000, 2000 or 3000 ng/ml), injected intravitreally (500 ng/ml) or intravenously (13.3 µg/kg body weight). Retinal vessel thickness was monitored using infrared fundus images compiled with optical coherence tomography (Heidelberg Engineering) before and several time points after application of relaxin 2.
Results Neither topical nor intravitreous or intravenous application of relaxin 2 lowered the IOP or changed the arterial or venous vessel diameter after 1 or 3 h after application.
Discussion Now that relaxin 2 is more easily available, the hormone came again into focus as a potential glaucoma therapeutic. However, our study in rats could not support the hypothesis that relaxin 2 lowers IOP or dilates retinal vessels.

Keywords IOP · Relaxin · Glaucoma · Vessel dilation

Introduction

The peptide hormone relaxin was first known as a pregnancy hormone, but recently came into focus as a therapeutic for acute heart failure [1]. Numerous studies show that relaxin inhibits endothelin and angiotensin II, induces nitric oxide as well as the production of VEGF and matrix metalloproteinases leading to vasodilation [2]. Relaxin was tested for the treatment of scleroderma, a progressive fibrosis of the skin and internal organs. However, it was well tolerated relaxin failed in a phase III study for the treatment of scleroderma after long-term injection [1]. Vasodilatory and antifibrotic properties make relaxin a

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potential candidate for treatment of glaucoma. Primary open-angle glaucoma (POAG) is a neurodegenerative disease leading to irreversible retinal ganglion cell loss. The standard treatment to prevent retinal ganglion cell loss aims to lower the intraocular pressure (IOP) as the main risk factor [3]. However, the demand for additional IOP lowering agents is high due to limited spectrum of it. Nevertheless, despite lowering of IOP RGC loss may occur, lighting the demand for additional therapies. Within recent years the role of the vasculature has been suggested to play a key role in this context with people with vasospasms or reduction of ocular blood flow being at higher risk to develop glaucoma or progress [4–6]. In a former study from 1963 intramuscularly injected relaxin decreased the IOP probably due to increased outflow measured by tonography [7]. However, the study included seven individuals only, therefore the evidence is limited. To further test whether relaxin decreases IOP and dilates retinal vessels, rats received relaxin 2 either topically, intravitreally or intravenously.

Methods

Experimental animals

All animals were treated in accordance with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Ophthalmic. The experiments had received governmental approval (Landesuntersuchungsamt Rheinland-Pfalz permission number: 14-1-085). Only female Sprague-Dawley rats from Janvier Labs were used due to easier handling. Rats were housed under standardized conditions with a 12-h light/dark cycle and with free access to food and tap water. For experiments, female male rats at the age of 4–5 months and the weight of 279 ± 23 g were used. Experiments started between 10 and 12 a.m. to avoid influences of the circadian rhythms.

Topical application of relaxin 2

Human recombinant relaxin 2 (Bachem) was dissolved in phosphate-buffered saline with 0.1% bovine serum albumin. Relaxin was applied as an eye drop on the left eye (1000, 2000 or 3000 ng/ml). The right eye received the solvent as internal control. IOP was measured in awake rats with the help of the Icare Tonolab device calibrated for rats (10 measurements per eye) before, 1, 3 and 6 h after application of relaxin. The measurement required no drops such as local anesthesia or the use of a speculum. Readings that were identified as outliers by the instrument were excluded from analysis.

Intravitreal application of relaxin 2

IOP was measured in awake rats with the help of the Icare Tonolab device before anesthesia. Then, rats were anesthetized by an intraperitoneal injection of ketaminhydrochlorid (75 mg/kg body weight, Ketamin 50 mg/ml; Inresa Arzneimittel) and medetomidin (0.2 mg/kg body weight, Dorbene Vet 1 mg/ml; Pfizer). Oxybuprocainhydrochlorid eye drops (Novesine 0.4%, Omnivision) as a topical anesthetic for pain relief before the use of a contact lens and mydriaticum (Pharma Stulln) eye drops were applied to the cornea. Retinal blood vessel thickness was monitored using infrared fundus images compiled with spectral-domain optical coherence tomography (Heidelberg Engineering) in follow-up mode to track the same retinal area and for constant distance. Arterial and venous diameters were measured in the position of the circle scan (see Fig. 2a, b). Fundus images and IOP measurements were taken before intravitreal injection as well as 1 and 3 h after injection. Relaxin was injected intravitreally into the left eye (500 ng/ml). The right eye received the solvent as internal control.

Intravenous application of relaxin 2

IOP was measured in awake rats with the help of the Icare Tonolab device before anesthesia followed by anesthesia as described above. Fundus images and IOP measurements were taken before intravenous injection as well as 1 and 3 h after injection. Relaxin was given intravenously (13.3 µg/kg body weight). Controls received the solvent intravenously.

Statistics

Statistical analysis was performed using Statistica Version 12 (Dell Inc. Round Rock, TX, USA). A one-way analysis of variance, followed by Tukey's HSD post hoc (equal or unequal N) test, was applied to

detect differences across multiple groups. $P < 0.05$ was considered to be statistically significant, $P < 0.01$ as statistically highly significant. Columns are presented as mean ± standard deviation (SD).

Results

The topical application of relaxin 2 failed to change the IOP after 1, 3 or 6 h of application (Fig. 1a–c). Neither intravitreal nor intravenous application of relaxin lowered the IOP (Fig. 1d, e) nor changed the arterial and venous vessel diameter (Fig. 2) after 1 or 3 h after application. The IOP decreased significantly after anesthesia in control and relaxin 2 treatment groups.

Discussion

Decreasing IOP during pregnancy has been demonstrated in healthy women and women with glaucoma (for review see [8]). More recent studies indicate lowest IOP during the third trimester of pregnancy [9, 10]. It was speculated whether hormones such as relaxin influence IOP during pregnancy. Interestingly,

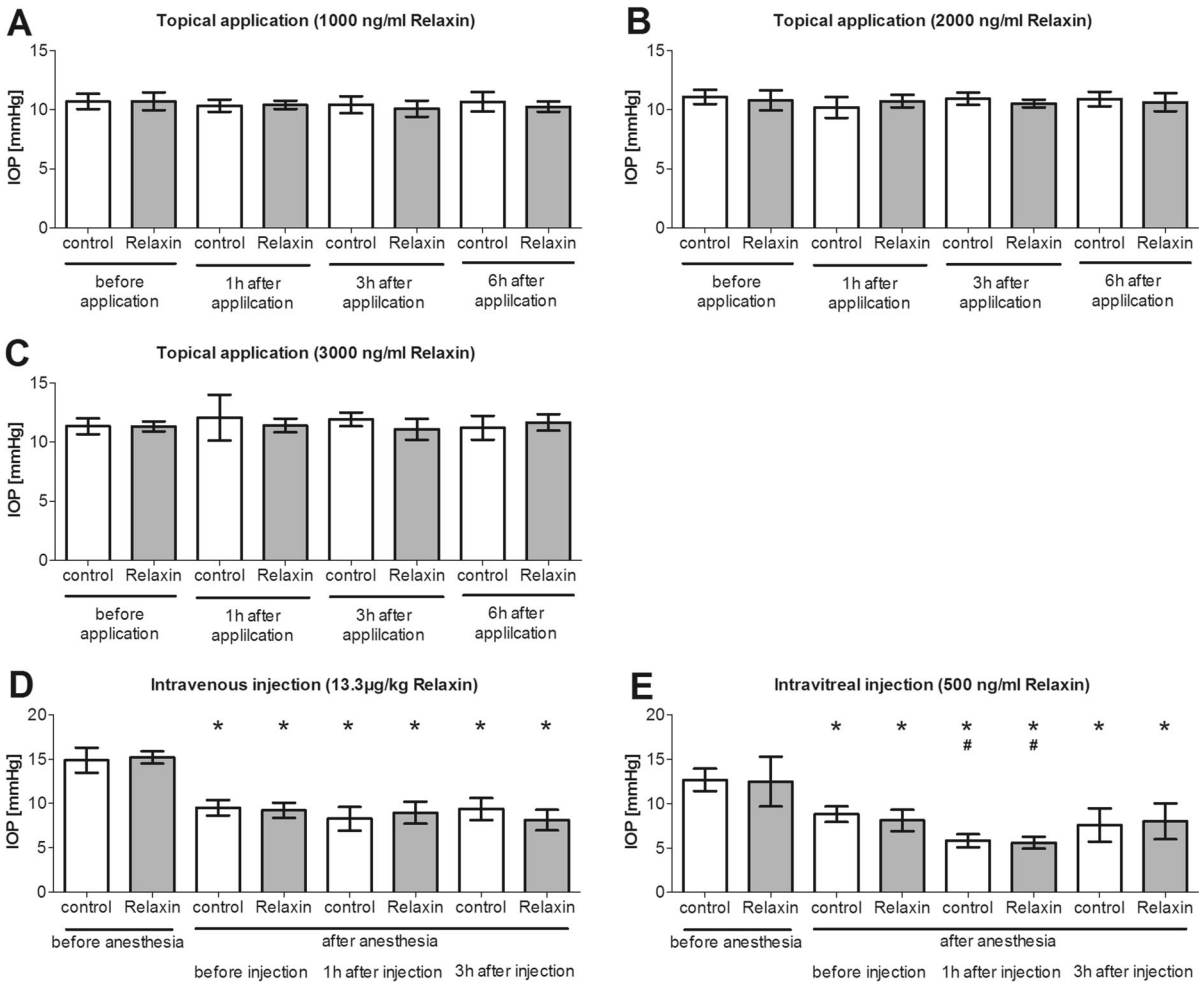

Fig. 1 IOP remains stable after relaxin treatment in rats. Recombinant human relaxin 2 was either applied as an eye drop (**a**–**c**; each group 5 eyes), injected intravenously (**d**; each group 10 eyes) or intravitreally (**e**; control group 6 eyes, relaxin group 4 eyes). Results are representative of 2 separate experiments (gray bars—Relaxin 2, white bars—control; mean ± SD, One-way ANOVA, post hoc test $*P \leq 0.05$ compared with before anesthesia, $\#P \leq 0.05$ compared with after anesthesia and before injection)

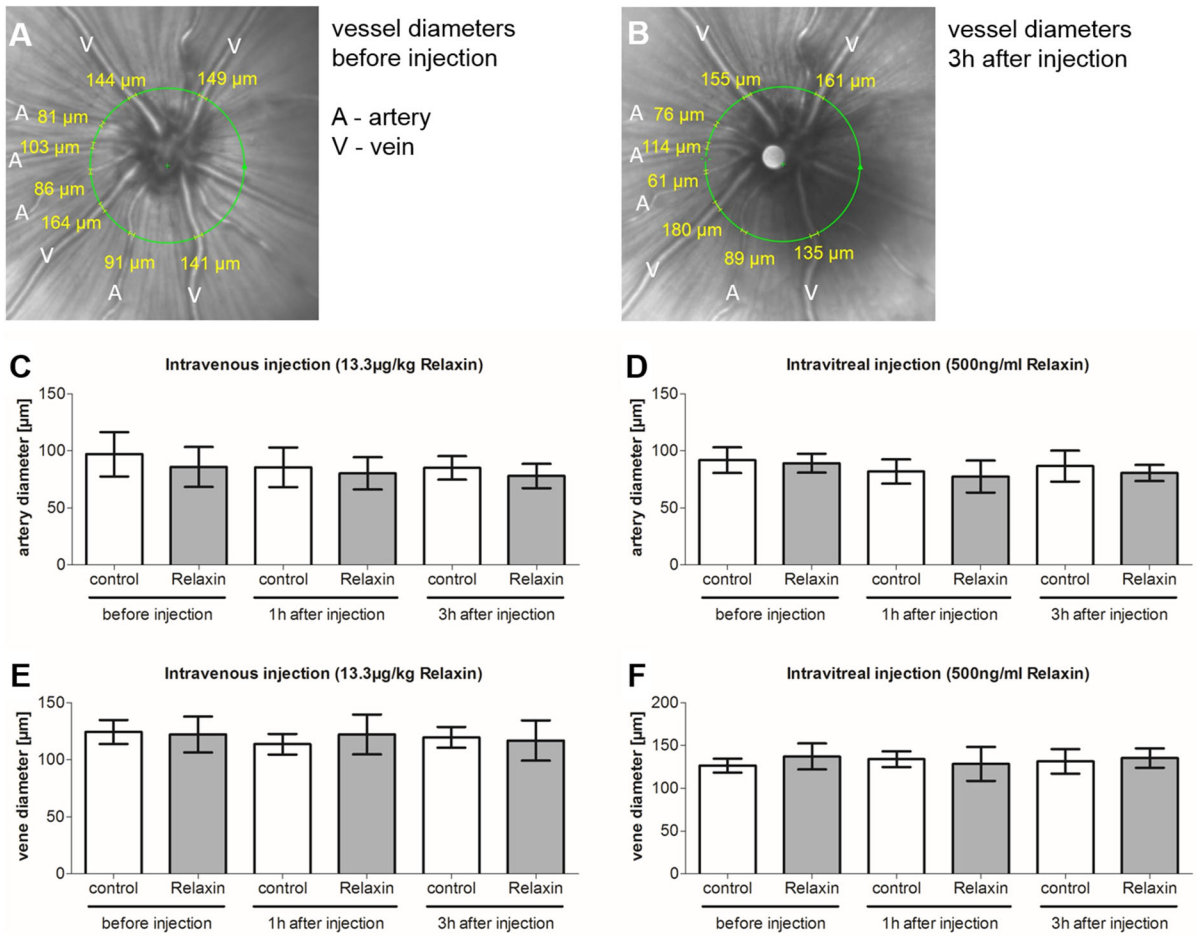

Fig. 2 Retinal artery (A) and vein (V) diameter measured using OCT. Infrared images from confocal scanning laser ophthalmoscope of a rat eye before (**a**) and 3 h after intravitreal injection of relaxin 2 (**b**). Green circles in **a** and **b** indicated peripapillary circular scans. **c**–**f** Unchanged retinal arterial (**a**, **b**) and venous (**c**, **d**) diameters after relaxin treatment in rats. Recombinant human relaxin 2 was either injected intravenously (**c**, **e**; each group 10 eyes) or intravitreally (**d**, **f**; control group 6 eyes, relaxin group 4 eyes). Results are representative of 2 separate experiments (gray bars—Relaxin 2, white bars—control; mean ± SD, One-way ANOVA, post hoc test $P > 0.05$)

highest levels of relaxin 2 in humans were measured during the first semester of pregnancy [1]. Therefore, in humans, relaxin blood levels seem not to correlate with lower IOP during pregnancy.

In rats, the IOP decreasing effect of relaxin that has been demonstrated in humans could not be supported by our study [7]. A possible explanation could be a different receptor expression of RXFP1 in rat and human ocular tissue. Unpublished data show RXFP1 mRNA expression in retina and uvea with sclera of rats; however, RXFP1 mRNA expression in human ocular tissues other than cornea and conjunctiva has not been determined [11]. It is questionable if topically applied relaxin penetrates the eye to influence the ciliary body or the trabecular meshwork or the episcleral veins. However, intravitreal and intravenous injections not just failed to lower IOP but also to change the blood vessel diameters of arteries and veins. Dosages of Relaxin were chosen according to previous publications. In a study by Leo, male rats were injected with 13.3 µg/kg serelaxin into the tail vein and showed a vasorelaxation shortly after injection [12]. Furthermore, plasma serelaxin concentrations after continuous i.v. infusion of 13.3 µg/kg h^{-1} were approximately 60 ng/ml [13]. For intravitreal injection, we chose 500 ng/ml of recombinant relaxin 2, a tenfold higher concentration as was measured in serum under continuous serelaxin injection.

Accordingly, we tested even higher concentrations of 1000, 2000 and 3000 ng/ml relaxin for topical application, which were equivalent to 16-, 33- and 50-fold higher concentrations as used for intravenous injections. Since general anesthesia clearly lowered IOP (see Fig. 1d, e), it seems possible that this effect alone could have masked any effect of relaxin on IOP. Other methods for studying aqueous humor dynamics in rodents might could be use and might detect even smaller changes [14].

Due to the resolution of the fundus images, the measurement of the vessel diameters has a limited sensitivity. Methods like wire myography or cannulated, pressurized vessel measurements are more sensitive and might detect an influence of relaxin on retinal vessels. In addition, animals were dilated with mydriatics that might affect the vascular caliber, or the ability of the vessels to respond to a vasoactive substance. Furthermore, due to the general anesthetic that was necessary for intravitreal und intravenous application and following OCT measurements, the systemic blood pressure might also influence vascular responses since the blood pressure could affect perfusion pressure and retinal blood flow. To avoid these obstacles, experiments with explanted vessels would more sophisticated.

In conclusion, although a previous study showed some effect of relaxin 2 on the IOP, our study in rats could not support the hypothesis that relaxin 2 lowers IOP or dilates retinal vessels.

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Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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