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Particularities of vascular reactivity of the conjunctiva and iris in rats

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Abstract

Considering that the conjunctiva and iris are developed from different embryological origins, an analysis of any possible differences in the density and type of adrenoceptors between these two areas was tried. An experimental study on male adult Wistar rats was performed. Six groups (six rats per group) were used by recording the iris and conjunctival vessels after eye instillations with solutions of adrenaline 0.1%, isoprenaline chlorhydrate 0.00002% and distilled water. The image analyze were carried out using VirtualDub 1.5.1 and Adobe PhotoShop 6.0., measuring the variations of the vessels diameters before and after the solutions instillations at fixed time intervals. The means of each eye values were compared with the control value using a statistical significance T test. The analysis of the two groups treated with adrenaline 0.1% showed a different reactivity of the vessels from the conjunctiva and iris, respectively. The iris vessels performed a gradual vasoconstriction. On the contrary, the conjunctival vessels showed initially mild, but significant vasodilatation, while vasoconstriction started later and was not as intense as for the iris vessels. The iris vessels treated with isoprenaline 0.00002% showed no significant changes in the vascular diameters. On the contrary, the conjunctival vessels showed a significant vasodilatation. The differences in the vascular reactivity of the two ocular areas (conjunctiva and iris) to vasoactive amines (epinephrine, isoprenaline) support the idea that beta 2 adrenergic receptors are present only in conjunctiva vessels but not in iris vessels, while alpha adrenoceptors are present in both vascular territories.

***Keywords*:** adrenaline, epinephrine, isoprenaline, iris, conjunctiva, rat, vascular reactivity.

Introduction

The role of catecholamines in the tissular control of the body is a well-known subject. During the phylogenetic evolution, epinephrine developed as a stress-hormone, offering a better adaptation of animal beings to stress.

Practically, epinephrine causes vasoconstriction in some vascular beds and vasodilatation in others, resulting in a reorientation of the blood-flow from tissues not involved in defense to other regions whose function is very important in such acute conditions. Therefore, the blood flow is directed from the skin, spleen and splanchnic vessels to the muscular tissue, brain and heart [1–2]. This is possible because the density of various adrenergic receptors is different between various body regions. In tissues in which epinephrine causes vasoconstriction there is a higher density of α-adrenoceptors, while in regions where hormones cause vasodilatation there are more beta 2 adrenoceptors. Considering that the conjunctiva and iris are developed from different embryological origins, an analysis of any possible differences in the density and type of adrenoceptors between these two areas was tried.

Material and methods

Male adult Wistar rats, weighting 250 g to 300 g (average 271 g), brought in the laboratory facilities with a minimum of three days before the beginning of the experiments and kept on a standard diet, were used. All the experiments were performed during the daytime (12:00 to 18:00 hrs). All rats were anaesthetized with chloralhydrate (produced by Redox) 20% (0.1 ml/100 g body weight) i.p. injected, and after five minutes pancuronii bromidum (Pavulon, produced by Organon Holland) 0.02%, 0.1 ml/100 g body weight i.p. injected, was used to induce myorelaxation. Data recording was started after 10 minutes.

The anaesthetized rats were placed in a contention plate in lateral decubitus and two clamps were placed on each side of the eyelid in order to liberate the ocular globe which was visualized at the maximum magnitude with an optical system (NAVITAR) connected to a video camera (TOSHIBA–IK642E) and a computer. After selecting a zone of interest, manual adjustments of the magnitude, clarity and brightness of the image were made and the experiment began. The image recording lasted 6 minutes; after the first 5 seconds the test drugs solutions were instilled in the eye without touching the conjunctiva.

Six groups (six rats per group) were used as follows:

- Group 1 – epinephrine (produced by Terapia SA), 0.1%, 2 drops/eye – recording of the iris vessels;
- Group 2 – epinephrine 0.1%, 2 drops/eye, recording of the conjunctival vessels;
- Group 3 – isoprenaline chlorhydrate (produced by Redox) 0.00002%, 2 drops/eye, recording of the iris vessels;

▪ Group 4 – isoprenaline chlorhydrate 0.00002%, 2 drops/eye, recording of the conjunctival vessels;

▪ Group 5 – distilled water, 2 drops/eye, recording of the iris vessels;

▪ Group 6 – distilled water, 2 drops/eye, recording of the conjunctival vessels.

The image analyze were carried out using VirtualDub 1.5.1 and Adobe PhotoShop 6.0., by measuring the variations of the vessels' diameters before and after the solutions' instillations, at fixed time intervals, every one minute, so seven different measurements were made for each eye. The first value of the vessel diameter (V_i) was considered a control value for each eye registration. The following six values, considered actual values (V_a), were analyzed in relation with the initial value (V_i) by the following formula:

$$\frac{(V_i - V_a)}{V_i} \times 100$$

The six means of each eye values were compared with the control value (before the solutions' instillations) using a statistical significance t-test.

Results

The analysis of the two groups treated with adrenaline 0.1% showed a different reactivity of the vessels from the conjunctiva and iris, respectively. The iris vessels reacted by a gradual vasoconstriction (reduction of the vascular diameter), significant (P=0.0068), starting from the first minute after the solutions' instillation.

On the contrary, the conjunctival vessels reacted by mild, but significant vasodilatation (increase of the vascular diameter), (P=.003) 30 seconds after the solutions' instillation, while significant vasoconstriction started later, at 3 minutes (P=.008), and was not as intense as for the iris vessels (Table 1, Figure 1).

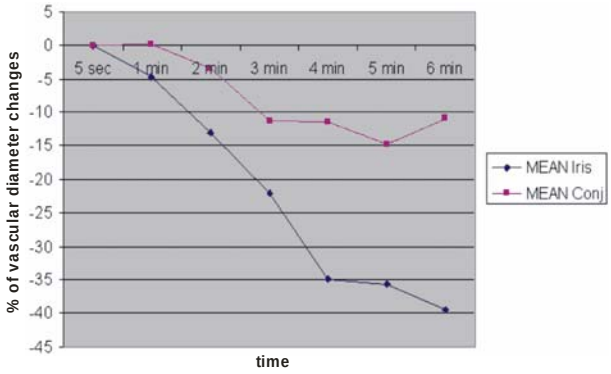

Figure 1 – ***Evolution of iris and conjunctiva vascular diameters after adrenaline instillation. The iris vessels reacted by a gradual vasoconstriction (reduction of the vascular diameter), significant (P=.006), starting from the first minute after the solutions' instillation. The conjunctival vessels reacted by mild, but significant vasodilatation (increase of the vascular diameter), (P=.003) 30 seconds after the solutions' instillation while significant vasoconstriction started later, at 3 minutes (P=.008), and was not as intense as for the iris vessels***

Table 1 – ***Variations in iris and conjunctiva vascular diameters after adrenaline, isoprenaline and distilled water instillation (the test drugs solutions were instilled in the eye after the first 5 seconds of records). Variations of vascular diameter are expressed as percents***

Time	Adrenaline iris	T-test/5 sec.	Adrenaline conjunctiva	T-test/5 sec.	Isoprenaline iris	T-test/5 sec.	Isoprenaline conjunctiva	T-test/5 sec.	Control iris	T-test/5 sec.	Control conjunctiva	T-test/5 sec.
5 sec.	0	–	0	–	0	–	0	–	–	–	–	–
30 sec.	–	–	0.704375	0.003409	–	–	–	–	–	–	–	–
1 min.	-4.709101367	0.006843	1.132462	0.14735	-0.95009	0.195808	3.757	0.1082	0.025686	0.485421	-0.01909	0.488484
2 min.	-13.12122292	0.00764	-3.420935	0.178619	-3.39108	0.003125	8.714383	0.028207	-0.40156	0.140203	-0.94804	0.050509
3 min.	-22.1151586	0.001157	-11.38936	0.008827	-1.50272	0.149275	10.51229	0.028866	-0.11701	0.449082	-0.71266	0.264587
4 min.	-34.98620953	0.001308	-11.54084	0.009606	-0.77404	0.261019	3.036319	0.234249	0.996444	0.156106	-0.00083	0.499683
5 min.	-35.7018817	0.0002	-14.78724	0.002336	-1.2906	0.168256	-4.95261	0.102032	0.072959	0.452886	-0.66324	0.244039
6 min.	-39.37668094	0.000125	-10.95908	0.016565	-1.05704	0.199827	-9.73731	0.024145	-0.40951	0.327319	-0.40218	0.132981

As for the isoprenaline treated groups, the concentration of 0.00002% was chosen because higher concentrations induced vasoconstriction in both vascular areas, probably due to the loss of its beta-receptor selectivity, acting on the alpha-receptors (data not shown).

The iris vessels treated with isoprenaline 0.00002% showed no significant changes in the vascular diameters (Figure 2).

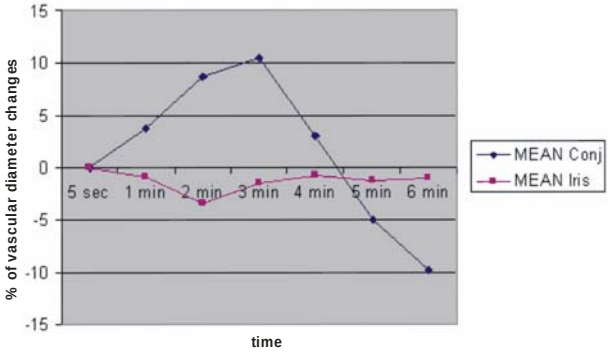

Figure 2 – ***Evolution of iris and conjunctiva vascular diameters after isoprenaline instillation. The iris vessels treated with isoprenaline 0.00002% showed no significant changes in the vascular diameters. The conjunctival vessels showed a vasodilatation (increase of the vascular diameter) significant (P=.02) in minutes 2 and 3 after the solutions' instillation***

On the contrary, the conjunctival vessels showed a vasodilation significant (P=.02) in minutes 2 and 3 after the solutions' instillation.

The control groups showed no changes in the vascular diameter in the 6 recording minutes, proving that the results were obtained only due to the direct effect of the instilled drugs.

Discussions

In the experimental conditions shown, epinephrine caused a small vasodilatation in the conjunctival vessels in the first 2 minutes, but not in the iris vessels. Vasodilatation can be caused by epinephrine by means of beta 2 adrenoceptors. The results prove that such receptors are present only in the conjunctival vessels, but not in the iris vessels [3–5]. For an experimental confirmation, isoprenaline, which is a selective beta receptor agonist, was administered. Isoprenaline did not cause vasodilatation in the iris vessels, but did cause such an effect in the conjunctival vessels.

These results support the idea that the alpha adrenoceptors are present in both vascular territories because epinephrine produced vasoconstriction both on the iris and conjunctiva vessels. On the contrary, beta 2 adrenoceptors are present only in conjunctiva, but not in the iris vessels. Both epinephrine and isoprenaline produced vasodilatation in conjunctiva, but not in iris vessels, suggesting a total absence of beta 2 adrenoceptors in iris vessels.

An interpretation of the biological significance of these differences in vascular reactivity is difficult. It may be a natural defense mechanism against the progression of conjunctival infections, very common, to iridocyclitis, very uncommon. On the other hand, many drugs used in ophthalmology, such as anti-glaucoma drugs, cause conjunctival vasodilatation, as an unwanted effect [6, 7]. It is not clear if vasodilatation is important as a mechanism for the expected therapeutic effect, and so, which vascular territory must be dilated [8–11]. The presented results may prove that vasodilatation in all areas could not be necessary. The results also prove that a possibility of pharmacological manipulation exists, aiming to prevent an unwanted effect due to vasodilatation. The experimental model used may be a good instrument for such research.

The differences in the vascular reactivity of the two ocular areas (conjunctiva and iris) to vasoactive amines (epinephrine, isoprenaline) support the idea that beta 2 adrenergic receptors are present only in conjunctiva vessels but not in iris vessels, while alpha adrenoceptors are present in both vascular territories [12–15]. These aspects open the perspective of a more detailed study with drugs used in different ophthalmologic diseases, concerning their wanted or unwanted pharmacological effects.

Conclusions

In the present experimental conditions, the use of epinephrine 0.1% or isoprenaline 0.00002% showed a different reactivity of the iris and conjunctiva vessels.

Instillation of epinephrine 0.1% induced a gradual vasoconstriction in the iris vessels and an initial vasodilatation, followed by a mild vasoconstriction in the conjunctival vessels.

Instillation of isoprenaline 0.00002% induced changes in vascular diameter only at the level of conjunctival vessels, which showed a significant vasodilatation.

These results support the idea that beta 2 adrenergic receptors (that produce vasodilatation) are present only in conjunctiva vessels but not in iris vessels, while alpha adrenoceptors (that induce vasoconstriction) are present in both vascular territories.

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