Expression of pituitary adenylate cyclase-activating peptide, vasoactive intestinal peptide and their receptors in diabetic rat retinas

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Pituitary adenylate cyclase-activating peptide (PACAP), vasoactive intestinal peptide (VIP) and their related receptors have already been described in the retina of different mammalian species during development. Based on relative affinity studies, at least three different binding sites have been identified for PACAP and VIP: the PACAP-preferring PAC1 receptors and, VPAC1 and VPAC2 receptors which bind with equal affinity both PACAP and VIP. However, peptides and receptors expression in early diabetic retinopathy remains unknown. In the present study we investigated PACAP, VIP and relative receptors expression and distribution in the retina of streptozotocin (STZ)-induced diabetic rats.

Diabetes was induced in Wistar rats by a single STZ intraperitoneal injection. The expression of PACAP, VIP and receptors in healthy and early diabetic retinas were assayed both after 1 and 3 weeks by quantitative real-time PCR, Western blot and immunohistochemical analyses. VPAC1 and VPAC2 receptors, as well as PACAP and VIP peptides mRNA levels were transiently induced by STZ-treatment within 1 week. After 3 weeks, PAC1, VPAC receptors and their related peptides expression levels were remarkably reduced in diabetic as compared to healthy rat retinas. These findings were confirmed at the protein level by immunoblot. Interestingly, immunohistochemical analyses revealed some peculiar changes in PACAP, VIP and receptors distribution between the two animal groups. In fact, after 1 week treatment, PACAP was more expressed in the retinal pigmented epithelium (RPE) and in the inner segment of diabetic rats. Similarly, VIP reactivity was remarkably increased in the RPE, but also in the inner plexiform layer of STZ-treated rats. No apparent changes were observed in PAC1 immunoreactivity between healthy and diabetic rats, although the RPE was slightly less reactive in the latter group. VPAC2 receptors seemed to be more expressed in the inner nuclear layer but less evident in the outer nuclear layer of STZ-treated rats. VPAC1 receptor retinal distribution was not affected by STZ-treatment. These differences were no longer observable 3 weeks after the STZ injection, although an overall reduction of both peptides and receptors expression occurred.

In conclusion, this study provides evidence of an involvement of both PACAP and VIP peptides in early diabetic retinopathy. Furthermore, the overall downregulation of both peptides/receptors expression in retinas of STZ-treated rats after 3 weeks suggests that some deregulatory mechanisms might be activated in the later stages of retinal degeneration.

Key words
Streptozotocin, diabetes, retina, PACAP, VIP