Glaucoma is second leading cause of blindness worldwide which is characterized by progressive degeneration of retinal ganglion cells (RGCs). Although an elevated intraocular pressure (IOP) is major risk factor, the mechanism for IOP regulation has not fully understood. Here we report that the P2Y$_1$ receptor (P2Y$_1$R) is essential for IOP reduction and its dysfunction causes ocular hypertensive glaucoma-like phenotypes. P2Y$_1$R activation by MRS2365, a selective agonist for P2Y$_1$R, significantly reduced IOP in wild-type (WT) mice but not in P2Y$_1$KO mice. P2Y$_1$R was dominantly expressed in the ciliary body and the angle tissue including trabecular meshwork and Schlemm’s canal, essential for aqueous humor (AH) production and draining, respectively. Supporting this observation, P2Y$_1$R activation suppressed production and enhanced draining of AH, respectively. We also found that aquaporin 4 (AQP4) is downstream target of P2Y$_1$R. AQP4 was expressed in the ciliary body, which was well co-localized with P2Y$_1$R-positive signals. The findings that an AQP4 blocker significantly suppressed AH production, which was not reduced further by MRS2365 suggest that P2Y$_1$R and AQP4 should share the same pathway for the AH reduction. P2Y$_1$KO mice showed chronic ocular hypertension. P2Y$_1$KO mice at 12 months old showed significantly lower RGC number, thinner retina, optic nerve atrophy and impaired visual function. Taken together, our results demonstrated that P2Y$_1$ receptor activation reduces IOP and P2Y$_1$KO mice show hypertensive glaucoma-like phenotypes.