Cyclic guanosine monophosphate (cGMP) is synthesized by nitric oxide or natriuretic peptide-stimulated guanylyl cyclases and exhibits pleiotropic regulatory functions in the kidney. Hence, integration of cGMP signaling by cGMP-dependent protein kinases (cGKs) might play a critical role in renal physiology; however, detailed renal localization of cGKs is still lacking. Here, we performed an immunohistochemical analysis of cGKI? and cGKI? isozymes in the mouse kidney and found both in arterioles, the mesangium, and within the cortical interstitium. In contrast to cGKI?, the ?-isoform was not detected in the juxtaglomerular apparatus or medullary fibroblasts. Since interstitial fibroblasts play a prominent role in interstitial fibrosis, we focused our study on cGKI function in the interstitium, emphasizing a functional differentiation of both isoforms, and determined whether cGKIs influence renal fibrosis induced by unilateral ureter obstruction. Treatment with the guanylyl cyclase activators YC1 or isosorbide dinitrate showed stronger antifibrotic effects in wild-type than in cGKI-knockout or in smooth muscle-cGKI?-rescue mice, which are cGKI deficient in the kidney except in the renal vasculature. Moreover, fibrosis influenced the mRNA and protein expression levels of cGKI? more strongly than cGKI?. Thus, our results indicate that cGMP, acting primarily through cGKI?, is an important suppressor of kidney fibrosis.