It has been demonstrated that red blood cells (RBC) express an active and functional nitric oxide synthase (RBC-NOS). RBC-NOS derived NO is thought to play a major role in the regulation of RBC deformability in healthy individuals. Sickle cell anemia (SCA) is a genetic disease characterized by a reduction of RBC deformability, which is at the origin of chronic anemia and acute vaso-occlusive crises. We aimed to 1) characterize RBC-NOS activity in SCA patients and 2) test the effects of RBC-NOS activity modulation on sickle RBC deformability. We showed that while RBC-NOS expression is not different between SCA and healthy RBC, RBC-NOS activation is increased in the former population leading to higher production of NO in RBC. However, the increased oxidative stress in SCA induced the rapid formation of RBC peroxynitrite, which probably further participates to RBC alterations. The incubation of SCA RBC with sodium nitroprusside failed to improve RBC deformability and lead to a 10-fold increase of nitrotyrosine levels. In contrast, although incubating SCA RBC with insulin increased nitrotyrosine levels, it also increased RBC deformability. This positive effect of insulin is not fully understood but enhanced RBC-NOS dependent NO production could lead to increased S-nitrosylation of the a- and b-spectrins, and consequently improvement of RBC deformability. Stimulating RBC-NOS activity and limiting oxidative stress could be an interesting strategy to improve SCA RBC rheology.