Abstract: $\mathcal{O}$-operators are important in broad areas in mathematics and physics, such as integrable systems, the classical Yang-Baxter equation, pre-Lie algebras and splitting of operads. In this paper, we establish a deformation theory of $\mathcal{O}$-operators which is consistent with the general principles of deformation theories. On the one hand, we show that $\mathcal{O}$-operators are characterized as the Maurer-Cartan elements in a suitable graded Lie algebra. We also construct a differential graded Lie algebra from an $\mathcal{O}$-operator and show that deformations of the $\mathcal{O}$-operator are characterized as Maurer-Cartan elements in this differential graded Lie algebra. On the other hand, we identify a Lie algebra with a representation induced from an $\mathcal{O}$-operator T such that the corresponding Chevalley-Eilenberg cohomology controls deformations of T, thus can be regarded as an analogue of the Andrè-Quillen cohomology for the $\mathcal{O}$-operator. Thereafter, we study infinitesimal and formal deformations of $\mathcal{O}$-operators. In particular, we introduce the notion of Nijenhuis elements to characterize trivial infinitesimal deformations. Formal deformations and extendibility of order n deformations of an $\mathcal{O}$-operator are also characterized in terms of the new cohomology theory. Applications are given to deformations of Rota-Baxter operators and skew-symmetric r-matrices for the classical Yang-Baxter equation. For skew-symmetric r-matrices, there is an independent Maurer-Cartan characterization of the deformations as well as an analogue of the Andrè-Quillen cohomology controlling deformations, which turn out to be equivalent to the ones obtained as $\mathcal{O}$-operators associated to the coadjoint representation. Finally, we study infinitesimal deformations of skew-symmetric r-matrices and the corresponding triangular Lie bialgebras.