Okadaic acid (OA; C₃₈H₆₆O₁₃) is a polyether derivative of a C₃₈ fatty acid first isolated from marine sponges of the genus *Halichondria* (Tachibana et al. 1981). It is thought to be synthesized by marine diflagellates and to accumulate in the other marine organisms such as sponges and shellfishes which feed on them (Yasumoto et al. 1985). Physiologically, OA has been known to have a marked contractile effect on smooth muscles (Shibata et al. 1982) and heart muscle (Kodama et al. 1986). Recent results strongly suggest that these contractile effects are due to the inhibitory action of OA on the intracellular protein phosphatase activity (Takai et al. 1987; Bialojan et al. 1988; Hescheler et al. 1988; Bialojan & Takai, 1988).

In the intact smooth muscle fibres of human umbilical artery, rabbit aorta and guinea-pig taenia coli, Shibata et al. (1982) first demonstrated that OA (0.1–100 μM) produced a long lasting and reversible contraction even under severe Ca²⁺-deficient conditions. They also determined that OA had no effect on the ATPase activities of the Na,K-pump or native myosin. Some years later, Ozaki et al. (1987) showed that OA produced a marked leftward shift of the Ca²⁺-tension relation of the saponin-skinned taenia coli. These initial works suggested that OA acts on the intracellular regulatory site(s) rather than on the plasma membrane or contractile proteins of the smooth muscle tissues. This possibility was investigated by Takai et al. (1987), who examined the effect of OA on calmodulin, myosin light chain kinase (MLCK) and phosphatase as well as on the isometric tension in the guinea-pig taenia coli skinned with Triton X-100. They showed that micromolar concentrations of OA potently inhibited the myosin light chain phosphatase activity of the crude extract of the skinned taenia and of a purified aortic enzyme whereas it did not affect either calmodulin or MLCK. Since maximal tension development and a nearly complete inhibition of the phosphatase activity were both observed with 5 μM OA, they speculated that the two phenomena were causally related.

Figure 1 is a schematic representation of the current theory of smooth muscle activation. According to that, contractile force depends on the state of myosin light chain phosphorylation which is determined by the activity balance of myosin light chain kinase and phosphatase(s) (for review see, e.g., Hartshorne & Mrwa, 1982). The postulated site of action of OA is indicated in Fig. 1. To examine the hypothesis, Bialojan et al. (1988) analysed the change of myosin phosphorylation level during the OA-induced contraction in the Triton X-skinned taenia by means of two-dimensional gel electrophoresis. They showed that the contractile effect of OA was well correlated with an increase of phosphorylated myosin light chains and a concomitant decrease of unphosphorylated light chains. The effect on myosin phosphorylation as well as that on tension was completely reversed when OA was washed out. They also found that OA strikingly slowed down relaxation and myosin dephosphorylation induced by Ca²⁺ removal following prior activation with 30 μM Ca²⁺. OA enhanced the isometric tension and increased the myosin phosphorylation even under low Ca²⁺ concentration conditions. However, Ca²⁺ was necessary for OA to produce the effects; i.e. OA had very little effect on tension and phosphorylation in Ca²⁺-free solutions. These findings strongly support the postulated mechanism for the contractile action of OA (Fig. 1). Since the contractile response and the susceptibility of myosin light chain phosphatase activity to OA are very similar between the Triton X-skinned fibres of guinea-pig taenia coli and bovine aorta (A. Takai and M. Troschka, unpublished observations), the same explanation may apply to the contractile effects of OA on various types of smooth muscle. In the Triton X-skinned fibres, however, the plasma membrane and intracellular Ca²⁺ store sites have been destroyed. Therefore, it is not impossible
that OA may have some effects on plasma membrane and/or sarcoplasmic reticulum which are directly or indirectly produced through phosphatase inhibition. The tension enhancement by OA in intact muscles (Shibata et al. 1982) or in saponin skinned fibres (Ozaki et al. 1987; Shibata, 1987) may be partly due to such possible effects of OA.

In heart muscle, OA increases the duration of action potential and enhances the contraction (Kodama et al. 1986). Hescheler et al. (1988), in their whole-cell clamp experiments on isolated guinea-pig cardiac myocytes, have demonstrated that OA (5-100 μM) strongly and reversibly increases the L-type calcium current which is thought to be regulated by the cAMP-dependent phosphorylation system (Fig. 2; see Reuter et al. 1983; Bruin et al. 1984). They have also shown that the time required to wash out the β-adrenergic effect of isoprenaline on the calcium current is markedly prolonged by micromolar concentrations of OA. In the ferret papillary muscle, the inotropic action of OA (>5 μM) is concomitant with a reversible increase of the Ca^{2+} transient (D. G. Allen & J. Lee, personal communication). These observations strongly support the idea that OA, a protein phosphatase inhibitor, produces its inotropic effect through suppression of dephosphorylation mechanism resulting in elevated phosphorylation of the Ca^{2+} channel related protein(s), which in turn increases the opening probability of calcium channels (Fig. 2). The effect of OA on calcium current can be produced by extracellular application, suggesting a comparatively high membrane permeability of OA in cardiac muscle (Hescheler et al. 1988). In heart muscle, too, OA increases myosin light chain phosphorylation (I. Morano, personal communication). It has been reported that light chain phosphorylation may increase the calcium sensitivity of the chemically skinned ventricular fibres (Morano et al. 1985). Therefore, it is possible that the increase of myosin phosphorylation may be partly involved in the inotropic action of OA. OA has no effect on cAMP-dependent protein kinases from bovine heart and rabbit skeletal muscle (A. Takai & M. Troschka, unpublished observations). The effect of OA on adenylate cyclase complex remains to be examined.

The specificity and kinetics of the inhibitory action of OA on various types of phosphatase were recently studied using purified enzymes (Hescheler et al., 1988; Bialojan & Takai, 1988). The results show that OA has a relatively high affinity to type 2A- and type 1-protein phosphatases (for classification of protein phosphatases, see Ingebritsen & Cohen, 1983). These phosphatases were potentily inhibited by nanomolar concentrations of OA. Of the phosphatases of physiological interest, the following were not affected by up to 10 μM OA: phosphotyrosyl phosphatase, inositol 1,4,5-trisphosphate phosphatase, and acid and alkaline phosphatases. As for kinases, OA affects neither myosin light chain kinase nor cAMP-dependent kinase (see above). OA has no effect on protein kinase C, either (Suganuma et al. 1988). Kinetic studies have shown that OA acts as a non-competitive or mixed inhibitor on the OA-sensitive enzymes (Bialojan & Takai, 1988). This suggests that the binding site for OA is different from that for the substrate.

Apart from the contractile effects on muscles, OA is attracting the attention of cell pathologists as a potent tumour-promoter (Suganuma et al. 1988). Although the detail is beyond the scope of this article, recent reports suggest that this effect might be also related to phosphatase inhibitory action (see Issinger et al. 1988). To my knowledge, OA is the first exogenous substance that potently inhibits the protein phosphatases. The inhibitory effect is fairly specific for some restricted types of protein phosphatases and can be reversed by washing out (see above). These are advantageous features of OA as a tool for analysing the mechanisms of muscle contraction and cell motility.

Fig. 2. Symbols: R, β-adrenergic receptor; G, GTP-binding protein and E, effector (= catalytic subunit of adenylate cyclase complex).

Akira Takai is at The II. Physiologisches Institut, Universität Heidelberg, Im Neuenheimer Feld 326, 6900 Heidelberg, FRG.