Synergistic Effects of Cyclic AMP-Related Vasodilators and the Phosphatase Inhibitor Okadaic Acid

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ABSTRACT—The phosphatase inhibitor okadaic acid at 100 nM slowly but completely inhibited high K⁺-induced contraction in the rat aorta (t₁/₂ = 118.9 min). High K⁺-induced contraction was partially inhibited (to 37 ± 65%) by 1 μM forskolin, 100 μM dibutyryl cyclic AMP, 100 nM atrial natriuretic peptide, 1 μM nitroglycerin, 10 nM sodium nitroprusside, 300 nM nicardipine or 100 nM verapamil. The rate of relaxation due to okadaic acid became faster when the contraction was partially inhibited by these compounds. Augmentation of the relaxation was greater with forskolin and dibutyryl cyclic AMP than with the other inhibitors. These results support the suggestion that okadaic acid inhibits phosphatase to augment the phosphorylation due to cyclic AMP-dependent kinase, resulting in smooth muscle relaxation.

Keywords: Okadaic acid, Cyclic AMP, Smooth muscle (vascular)
(300 nM) more rapidly inhibited the contraction with a shorter t1/2 (58.9 ± 1.9 min, n=4). From these results, we decided to use t1/2 rather than contractile force as an indicator of the inhibitory effect of okadaic acid.

Previously, we have shown that the relaxant effects of forskolin and db-cAMP are inversely proportional to the magnitude of contractile tension; that is, the relaxant effect is greater when the contraction before the addition of the relaxant was smaller (9). To examine if the inhibitory effect of okadaic acid is also modified by the magnitude of contraction, we partially inhibited the high K+-induced contraction by adding Ca2+ channel blockers. As shown in Fig. 1B, 100 nM verapamil was added during the high K+-induced contraction which inhibited the contraction to a new steady level of 36.5 ± 2.7% (n = 4). Then 100 nM okadaic acid was sequentially added. In the presence of verapamil, the rate of okadaic acid-induced relaxation became slightly faster. T1/2 was decreased by 19.2 min or to 85.0% of the t1/2 in the absence of verapamil. Nicardipine (100 pM) showed a similar effect as verapamil, inhibiting the high K+-induced contraction to 52.7% and decreasing t1/2 to 82.2 ± 6.1% (n = 4). The relationship between the magnitude of contraction (T in Fig. 1) and potency of okadaic acid (t1/2 in Fig. 1) in Fig. 2 shows that the relaxant effect of okadaic acid is greater when the magnitude of contraction before the addition of okadaic acid was smaller. The similarity between the effects of okadaic acid and cyclic AMP-related vasodilators supports the suggestion that okadaic acid inhibits phosphatase to augment the phosphorylation due to cyclic AMP-dependent kinase.

In the next experiments, we determined if the increase in cyclic AMP level further augments the effect of okadaic acid. To increase the cyclic AMP level, we added forskolin or db-cAMP during the high K+-induced contraction. As shown in Fig. 1C, addition of 1 μM forskolin inhibited the high K+-induced contraction to 49.2 ± 6.1% (n = 4). In the presence of forskolin, the rate of okadaic acid-induced relaxation became faster and shortened t1/2 to 37.5 ± 1.4 min (n = 4). The effect of db-cAMP was similar to that of forskolin. The relationship between the magnitude of contraction (T in Fig. 1) and potency of okadaic acid (t1/2 in Fig. 1) is shown in Fig. 2. The slope of the correlation was steeper in the presence of cyclic AMP-related
relaxants than in the presence of Ca\(^{2+}\) channel blockers, suggesting that the inhibitory potency of okadaic acid is greatly augmented when the cyclic AMP level is increased.

To determine if the inhibitory effect of okadaic acid is selectively augmented by cyclic AMP, we examined the effects of cyclic GMP. To increase the cyclic GMP level, we used 10 nM sodium nitroprusside, 100 nM atrial natriuretic peptide and 1 μM nitroglycerin. As shown in Fig. 2, these compounds inhibited the high K\(^{+}\)-induced contraction to 45.4–64.7% and shortened τ\(_{1/2}\) to 65.1–82.0% of the respective control level. The slope of the correlation for cyclic GMP-related relaxants is only slightly steeper than that for Ca\(^{2+}\) channel blockers, suggesting that cyclic GMP has only a small, if any, potentiating effect on okadaic acid-induced relaxation. It is generally believed that cyclic GMP activates protein kinase to phosphorylate functional proteins and relaxes smooth muscle. However, the site of action of cyclic GMP seems to be different from that of cyclic AMP because cyclic GMP relaxes smooth muscle with little effect on myosin phosphorylation (11), and cyclic AMP but not cyclic GMP augmented the effect of okadaic acid (present result). It is possible that okadaic acid inhibits the phosphatase antagonizing the cyclic AMP-dependent kinase with little effect on the phosphatase antagonizing the cyclic GMP-dependent kinase.

In the rat aorta, the major mechanism of the effect of cyclic AMP on high K\(^{+}\)-induced contraction is to decrease the Ca\(^{2+}\) sensitivity of contractile elements without changing the cytosolic Ca\(^{2+}\) level (9). Cyclic AMP has been shown to inhibit myosin phosphorylation to relax smooth muscle (10). This effect may be antagonized by the increase in cytosolic Ca\(^{2+}\) (9), and this may be the reason why the relaxant effect of cyclic AMP is stronger on a smaller contraction (or in the presence of lower cytosolic Ca\(^{2+}\) level). In the present experiments, we found that the inhibitory effect of okadaic acid is also stronger when the contraction was partially inhibited by Ca\(^{2+}\) channel blockers or nitro-vasodilators. Furthermore, the inhibitory effect of okadaic acid was more strongly augmented when the contraction was partially inhibited by cyclic AMP-related relaxants. These results support the suggestion that okadaic acid inhibits phosphatases and thus augments cyclic AMP-dependent phosphorylation to induce vasodilation. This effect may be antagonized by the increase in cytosolic Ca\(^{2+}\) level and augmented by the increase in cyclic AMP level.

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**REFERENCES**


