European Journal of Pharmacology, 219 (1992) 473–476

EJP 21105

Short communication

Inhibitory and contractile effects of okadaic acid on rat uterine muscle

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Received 17 June 1992, accepted 30 June 1992

The effects of okadaic acid and its interactions with various agents known to increase, by different mechanisms, the intracellular levels of cyclic AMP and/or cyclic GMP were investigated in isolated strips of rat myometrium. Okadaic acid showed inhibitory effects at concentrations between 10^{-7} M and 3×10^{-6} M. At higher concentrations, a biphasic, contractile and then relaxant response was observed. The results obtained suggest that, in rat uterine smooth muscle, the inhibitory effects of okadaic acid are not entirely mediated by the activation of cyclic AMP- and/or cyclic GMP-dependent pathways. The data also point to the existence of a clear interaction between okadaic acid and methylxanthines, although further studies are needed to clarify the mechanisms involved in this interaction.

Okadaic acid; cAMP; cGMP; Xanthine derivatives; Uterine smooth muscle

1. Introduction

Okadaic acid, a polyether derivative of a 38 carbon monocarboxylic fatty acid, has been obtained in our laboratory from a culture of the marine dinoflagellate *Prorocentrum lima* (Norte et al., 1991). Okadaic acid is responsible for diarrhetic shellfish poisoning (DSP) and has been found to be a potent tumour-promoting agent. In smooth muscle, okadaic acid has been reported to have both excitatory and inhibitory actions. Thus, in vascular and intestinal smooth muscles, okadaic acid induces tonic, long-lasting contractions which are highly resistant to the absence of extracellular calcium and are accompanied by only a small increase in $[Ca^{2+}]_i$ (Shibata et al., 1982; Takai et al., 1987; Hirano et al., 1989). At concentrations lower than those needed to evoke a contractile response, okadaic acid inhibits contractions induced by KCl and receptor-specific agonists in different smooth muscle preparations (Ashizawa et al., 1989; Karaki et al., 1989). All these effects are most likely related to its activity as a potent and highly selective inhibitor of protein phosphatases, with okadaic acid being the first substance in which this mode of action has been identified (Takai et al., 1987).

The aim of the present study was to characterize the effects of okadaic acid in rat uterine smooth muscle, and to investigate the possible effects of several agents known to increase the intracellular levels of cAMP and/or cGMP by different mechanisms, on the contractile responses to okadaic acid.

2. Materials and methods

2.1. Tissue preparation and protocols

Longitudinal strips of uterine smooth muscle were obtained from the uterine horns of estrogen-dominated virgin female Sprague-Dawley rats (200–250 g). The strips were mounted in tissue baths containing 4 ml of Sund's physiological salt solution with the following composition (mM): NaCl 154; KCl 5.6; $CaCl_2$ 0.54; $MgCl_2$ 0.95; $NaHCO_3$ 5.95 and glucose 2.78. The preparations were suspended under an initial tension of 0.5 g, maintained at 32°C and continuously gassed with 95% O_2 and 5% CO_2. Changes in isometric tension were recorded by means of a force-displacement transducer (Grass FT-03) connected to a LETICA amplifier and an ABB GOERZ SE-130 multichannel recorder.

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At the start of the experiment and after a 45-min equilibration period, the strips were repeatedly exposed for a short time (3 min) to oxytocin (10^{-6} M) until responses were reproducible. The last response to oxytocin served as an internal standard for contraction in all experiments. The tissue was then allowed to equilibrate for a further 45-min period before addition of okadaic acid. Only one dose of okadaic acid was applied to each strip because okadaic acid could not be removed by washing. In some experiments, the okadaic acid-induced response was obtained in uterine strips preincubated with saline or drug vehicle (time-matched paired control tissues) or drugs for 30 min. The contractile responses to okadaic acid are expressed as a percentage of the maximum tension evoked by oxytocin (10^{-6} M) or as a percentage of the maximum tension induced by okadaic acid.

2.2. Drugs and solutions. Statistical analysis of results

The drugs used were okadaic acid, oxytocin, papaverine hydrochloride, forskolin, (−)isoproterenol hydrochloride, N-6,2′-O-dibutyryl adenosine 3′,5′-cyclic monophosphate sodium salt (db-cAMP), 8-bromoguanosine 3′,5′-cyclic monophosphate sodium salt (8-Br-cGMP), sodium nitroprusside, caffeine, all from Sigma (St. Louis, USA), theophylline sodium anisate (Bruneau, France) and enprofylline (Astra, France). A stock solution of isoprenaline was prepared daily in deionized water containing 1 mg · ml^{-1} ascorbic acid. Caffeine was dissolved in physiological solution. Okadaic acid and forskolin were dissolved in ethanol, and enprofylline was dissolved in dimethylsulphoxide. The final dimethylsulphoxide or ethanol concentration in the bath medium (lower than 0.4%) had no effect on mechanical responses.

The data are expressed as means ± S.E.M. The significance of differences between means was assessed with Student's t-test for unpaired data, with significance achieved at $P < 0.05$.

3. Results

The mechanical effect of okadaic acid on rat uterine smooth muscle varied greatly with the concentration used. Hence, 10^{-7} M okadaic acid did not induce a significant effect whereas at a concentration of 10^{-6} M okadaic acid did not modify the resting tone of the preparation but reduced the subsequent contraction induced by various agonists. This inhibitory action developed slowly. At least 20 min were needed for 10^{-6} okadaic acid to inhibit partially the contraction elicited by 10^{-6} M oxytocin, 10^{-4} M acetylcholine or 60 mM K^+, and the inhibition was greater when the time of preincubation with okadaic acid was increased (data not shown).

At concentrations of 5×10^{-6} M and higher, the response to okadaic acid consisted of a slow contraction which, after reaching a plateau, was followed by a

TABLE 1

Responses of rat myometrium to okadaic acid (5×10^{-6} M to 5×10^{-5} M). The effects of cAMP- and/or cGMP-increasing agents on the 2×10^{-5} M okadaic acid-induced response.

Okadaic acid (M)	Pretreatment	n	E_{max} (% of oxytocin 1 μM)	Time for 50% contraction (min)	Time to peak (min)	Duration of plateau (min)	Time for 50% relaxation (min)
5×10^{-6} (control)	–	7	20.95 ± 3.06	22.57 ± 1.97	50.50 ± 3.89	11.14 ± 0.94	60.29 ± 1.97
10^{-5} (control)	–	10	46.87 ± 4.65	19.50 + 1.29	42.50 ± 2.27	9.80 ± 0.47	49.60 ± 2.35
2×10^{-5} (control)	–	15	102.60 + 3.22	18.00 + 0.75	40.43 ± 1.53	6.13 + 0.33	39.73 ± 1.33
	Papaverine 10^{-4} M	5	105.96 + 4.72	18.00 ± 1.67	41.60 + 3.39	7.80 + 0.66	42.00 + 4.27
	Isoprenaline 10^{-5} M	4	91.97 ± 3.36	17.00 + 0.82	39.75 ± 2.97	6.70 ± 0.48	45.00 ± 2.52
	Forskolin 3×10^{-5} M	5	98.26 + 2.36	16.10 ± 0.81	34.70 + 2.52	6.00 + 0.45	35.80 ± 3.63
	db-cAMP 10^{-3} M	3	103.90 + 7.23	19.50 ± 1.50	42.67 + 4.34	4.83 + 0.60	37.00 + 2.04
	Sodium nitrop. 10^{-4} M	5	92.19 + 4.27	15.20 ± 0.80	34.20 + 2.29	5.20 ± 0.86	38.00 ± 2.60
	8-Br-cGMP 10^{-3} M	3	104.90 ± 3.50	20.33 + 2.32	39.50 ± 2.47	4.67 ± 0.66	35.67 + 5.61
	db-cAMP 10^{-3} M + 8-Br-cGMP 10^{-3} M	5	106.97 ± 5.04	19.30 + 0.89	42.30 ± 2.44	5.90 ± 0.46	38.00 ± 1.92
	Okadaic acid 10^{-6} M	5	75.44 ± 6.04 [c]	11.00 ± 1.06 [c]	24.00 + 2.19 [c]	3.80 ± 0.37 [c]	25.90 ± 2.14 [c]
	Theophylline 3×10^{-3} M	8	73.25 ± 5.04 [c]	10.63 ± 1.07 [c]	22.13 + 1.72 [c]	3.56 ± 0.35 [c]	21.31 ± 1.76 [c]
	Caffeine 5×10^{-3} M	6	97.87 ± 4.20	10.83 + 1.07 [c]	20.67 ± 2.01 [c]	3.00 + 0.37 [c]	31.67 ± 1.05 [b]
	Caffeine 10^{-2} M	6	95.73 ± 6.04	11.58 − 0.72 [c]	22.67 − 1.14 [c]	2.75 ± 0.31 [c]	21.92 ± 1.10 [c]
	Caffeine 2×10^{-2} M	5	69.38 ± 7.22 [c]	8.80 + 0.84 [c]	17.90 + 1.97 [c]	2.60 + 0.51 [c]	19.10 ± 1.12 [c]
	Enprofylline 10^{-3} M	5	66.11 ± 12.78 [c]	12.90 ± 1.71 [b]	30.10 ± 2.77 [b]	4.70 ± 0.92	26.10 ± 2.17 [c]
5×10^{-5} (control)		7	142.27 ± 6.09	9.57 ± 0.72	26.14 ± 1.89	4.43 ± 0.65	30.86 + 2.84

Values are means ± S.E.M. of n experiments. Differences from control tissues: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$. Time for 50% contraction and relaxation (% of maximal tension induced by okadaic acid) was measured from the onset of tension development and tension decrease respectively.

slow relaxation. The maximal relaxation reached approximately 80% of maximal tension induced by okadaic acid. As shown in table 1, the characteristics of this response (maximal contractile activity, time for 50% contraction, time to peak of maximum tension, duration of plateau of contraction and time for 50% relaxation) were all dependent on the dose of okadaic acid. Table 1 also shows that 2×10^{-5} M okadaic acid elicited a maximal contractile effect of a similar magnitude to that induced by oxytocin 10^{-6} M.

After pretreatment of the uterine strip for 30 min with the direct activator of adenylate cyclase forskolin (3×10^{-5} M), the non-specific phosphodiesterase inhibitor papaverine (10^{-4} M), the β-adrenoceptor agonist isoprenaline (10^{-5} M), or the membrane-permeable derivative db-cAMP (10^{-3} M), the response to 2×10^{-5} M okadaic acid was not significantly different from that observed in time-matched paired control tissues (table 1). All these agents produced dose-dependent relaxation in KCl (60 mM)-depolarized uterus and, with the exception of isoprenaline, for which the concentration needed was significantly lower (10^{-7} M), the concentrations of relaxants used in the experiments with okadaic acid were found to be approximately the IC_{100}, i.e. the concentrations which fully relaxed the 60 mM K^+-induced contraction. The same concentrations of these agents almost or completely abolished the phasic component of the oxytocin-induced response and reduced by 100% (10^{-4} M papaverine, n = 5), 71.06 ± 3.96 (10^{-5} M isoprenaline, n = 10), 62.08 ± 6.74 (3×10^{-5} M forskolin, n = 3) and 56.00 ± 3.83 (10^{-3} M db-cAMP, n = 5) the amplitude of the contraction evoked by 10^{-6} M oxytocin, when added to the preparation 30 min before oxytocin. Similarly, the uterine response to 2×10^{-5} M okadaic acid was not significantly modified in the presence of the direct activator of guanylate cyclase sodium nitroprusside (10^{-4} M) or the membrane-permeable derivative 8-Br-cGMP (10^{-3} M), alone or in combination with db-cAMP (10^{-3} M) (table 1). Sodium nitroprusside (10^{-4} M) and 8-Br-cGMP (10^{-3} M) relaxed the high K^+-induced sustained contraction by 38.69 ± 7.81% (n = 7) and 95.67 ± 4.40% (n = 4) and only slightly decreased the amplitude of the contraction evoked by 10^{-6} M oxytocin in rat myometrium.

The biphasic uterine response to 2×10^{-5} M okadaic acid was significantly modified after preincubation of the tissue for 30 min with 10^{-6} M okadaic acid, as shown by the significant reduction in the maximal contractile effect and the increase in the velocity of contraction and relaxation (table 1). Practically identical modifications were observed after pretreatment of the uterine strip for 30 min with theophylline (3 mM), enprofylline (1 mM) or caffeine (20 mM). Caffeine (5 mM and 10 mM) did not modify the E_{max} (% vs oxytocin 1 μM) of the response induced by 2×10^{-5} M okadaic acid, but significantly increased the rate of contraction and relaxation (table 1) One micromolar okadaic acid, 10 mM caffeine, 1 mM enprofylline and 3 mM theophylline were found to be the concentrations required to produce 100% inhibition of the tonic spasm induced by 60 mM K^+ and reduced the amplitude of the contraction evoked by 1 μM oxytocin by 47.67 ± 8.29% (n = 6), 86.34 ± 2.27% (n = 10), 58.41 ± 5.78% (n = 4) and 75.30 ± 2.91% (n = 8), respectively, when added to the uterine strip 30 min before oxytocin. The time course of the 2×10^{-5} M okadaic acid-induced response in the absence (control) and presence of okadaic acid (1 μM), theophylline (3 mM) and caffeine (10 and 20 mM) is shown in fig. 1.

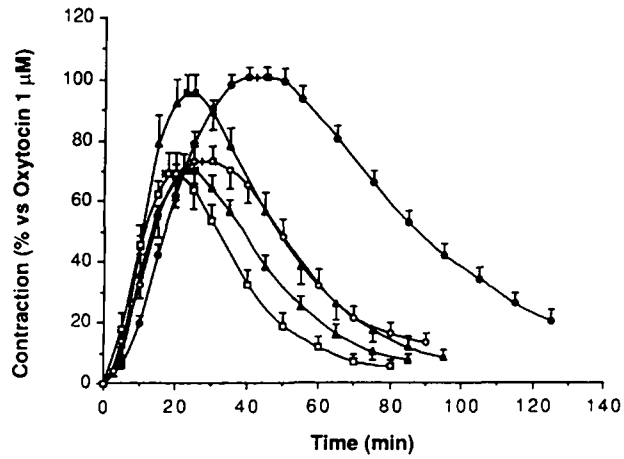

Fig. 1. Time course of 2×10^{-5} M okadaic acid-induced tension changes in rat uterine smooth muscle before (●, control) and after preincubation of the preparation for 30 min with 1 μM okadaic acid (○), 3 mM theophylline (▲) and caffeine 10 mM (△) and 20 mM (□). Abscissa: time (min) from the onset of tension development. Ordinate: per cent of maximum tension induced by 1 μM oxytocin. Points and bars represent the means ± S.E.M. of five to ten experiments.

4. Discussion

We observed that 10^{-6} M okadaic acid did not significantly modify uterine resting tone but reduced, in a time-dependent manner, the subsequent contraction evoked by KCl (60 mM), oxytocin (10^{-6} M) or acetylcholine (10^{-4} M) (unpublished data). A similar inhibitory effect of okadaic acid has previously been found in other smooth muscles and has been attributed to an indirect activation of cAMP- and/or cGMP-dependent protein phosphorylation (Ashizawa et al., 1989; Karaki et al., 1989).

The results obtained in the present experiments show that, at high concentrations, okadaic acid induced a concentration-dependent contraction in rat uterine smooth muscle. The contractile response evoked by okadaic acid at 5×10^{-6} M and higher concentrations

was slow and poorly maintained. The plateau of contraction, the duration of which was inversely related to the concentration of okadaic acid used, was followed by a slow and almost complete relaxation. These results contrast with those previously reported by Shibata et al. (1982) and Hirano et al. (1989) in vascular and intestinal smooth muscles, where okadaic acid induced a tonic contraction which was maintained over several hours, even after washout of the drug. These data confirm that sensitivity to okadaic acid is related to the type of smooth muscle.

The contractile response evoked by 2×10^{-5} M okadaic acid in uterine smooth muscle was significantly modified by a 30-min pretreatment with okadaic acid (1 μM) but not by papaverine, forskolin, db-cAMP or isoprenaline, agents known to increase, by different mechanisms, the intracellular levels of cAMP, leading to smooth muscle relaxation (D'Ocon et al., 1991; see Diamond, 1990). In our experiments, sodium nitroprusside, an activator of guanylate cyclase, and 8-Br-cGMP also failed to modify the contraction evoked by okadaic acid. In further support of our results, the 2×10^{-5} M okadaic acid-induced response was not affected by db-cAMP or 8-Br-cGMP. These data do not exclude the possibility that inhibition of proteinphosphatases by okadaic acid could lead to activation of cAMP- and/or cGMP-dependent protein phosphorilation in rat myometrium, but clearly show that the inhibitory effect of 10^{-6} M okadaic acid on the 2×10^{-5} M okadaic acid-induced biphasic response is not mediated by cAMP- and/or cGMP-dependent pathways.

The present results also show that the xanthine derivatives caffeine, theophylline and enprofylline were able to modify the uterine response to 2×10^{-5} okadaic acid in a similar way as 10^{-6} okadaic acid. Xanthines are known to have a dual spasmogenic and/or spasmolytic effect on the mechanical activity of different smooth muscle cells. It is widely accepted that the spasmogenic action of xanthines is caused by the release of calcium from intracellular stores (Noguera and D'Ocon, 1992). Such an action could explain the facilitatory effects (decrease in time for 50% contraction and time to peak, see table 1 and fig. 1) of xanthines and, particularly, of caffeine on the contractile component of the biphasic response evoked by okadaic acid in rat myometrium. In the existing reports and in our experiments, caffeine was unable to cause spasms either in skinned or unskinned rat uterine smooth muscle (Savineau and Mironneau, 1990). However, the possibility that caffeine and other xanthine derivatives could release calcium from intracellular stores, leading to a functional response masked at the contractile level by activation of opposing mechanisms, must be considered.

The inhibitory effect (reduction of E_{max} and time for 50% relaxation) of xanthine derivatives on the okadaic acid-induced response cannot be explained, on the basis of our results, by their well-documented inhibitory effect on phosphodiesterases, which increase the intracellular levels of cAMP and/or cGMP, or by adenosine antagonism, since enprofylline was able to modify the response to okadaic acid. Our data are not sufficient to establish whether other mechanisms proposed to explain the effects of xanthines, such as alterations in tramsmembrane calcium movements or a decrease in the calcium sensitivity of the contractile proteins, may explain the effects observed in the present report. Further studies are needed to clarify the mechanism involved in this interaction.

Acknowledgements

This work was supported by the Plan Nacional de Investigaciones Farmacéuticas (M:E:C., Spain) through Grants FAR 90-0045-CO2 J.D. Martín) and FAR 91-827 (M. Norte).

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