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Short communication

Chelerythrine, a protein kinase C inhibitor, interacts with cyclic nucleotide phosphodiesterases

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Abstract

Chelerythrine, a potent inhibitor of protein kinase C, was evaluated for its effect on cyclic nucleotide phosphodiesterases (PDE) isolated from bovine aorta. Chelerythrine activated basal PDE2 and inhibited activated PDE2, PDE4 and PDE5. The effect of chelerythrine (10 μM) was also investigated on vasorelaxation induced by a β-adrenoceptor agonist or a PDE3 inhibitor. Chelerythrine attenuated the isoprenaline-mediated relaxation whereas it potentiated the relaxation induced by SK & F 94120 (5-(4)acetamidophenyl)pyrazin-2(1*H*)-one, a PDE3 inhibitor). The present study demonstrates that chelerythrine, at a concentration generally reported in the literature to inhibit protein kinase C, interacts with cyclic nucleotide phosphodiesterases and consequently modulates vasorelaxation. These results cast some doubt on the use of chelerythrine as a specific inhibitor of protein kinase C.

Keywords: Chelerythrine; Protein kinase C; Phosphodiesterase, cyclic nucleotide; Vasorelaxation; SK&F 94120; Isoprenaline

1. Introduction

The Ca^{2+}-phospholipid-dependent protein kinase is a key regulatory enzyme in vasoconstriction. Protein kinase inhibitors are represented by various different classes of compounds such as alkaloids, calmodulin antagonists and isoquinolinesulfonamides. These protein kinase inhibitors interact either with the substrate binding site (ATP, protein) or with the regulatory binding site (cyclic nucleotide, diacylglycerol). Inhibitors of protein kinase C that interact with the catalytic domain of protein kinase often exhibit limited specificity because of the homologous sequence of this domain in various kinases. The closest similarities were found with cyclic AMP-dependent protein kinase (Ohno et al., 1987). Since some structural homologies in the catalytic domain between cyclic AMP-dependent protein kinase and cyclic nucleotide phosphodiesterases have been detected, it could be expected that an interaction between protein kinase C inhibitors and cyclic nucleotide phosphodiesterases may exist (Chen et al., 1986). Cyclic nucleotide phosphodiesterases comprise seven families of isozymes which have been classified from PDE1 to PDE7 according to the cDNA sequence, substrate specificity and differential sensitivity to calmodulin, cyclic GMP and selective inhibitors (Beavo et al., 1994).

The aim of this study was to investigate whether a protein kinase C inhibitor could also behave as an inhibitor of cyclic nucleotide phosphodiesterases. The benzophenanthridine alkaloid, chelerythrine, has been described as a potent ($IC_{50} = 0.7$ μM) and specific inhibitor of protein kinase C that interacts with the catalytic subunit (Herbert et al., 1990). We evaluated its inhibitory effect on the five major families of cyclic nucleotide phosphodiesterases isolated from bovine aorta. To investigate whether this compound could serve as a useful probe for clarifying the physiological role of the protein kinase C pathway in vascular smooth muscle, we tested its ability to modulate vasorelaxation. Since interactions between cyclic AMP and protein kinase C pathways have been described (McKinney and Rubin, 1988), the ability of chelerythrine to antagonize the aortic relaxation induced by a β-adrenoceptor agonist and a PDE3 inhibitor (isoprenaline and SK&F 94120, respectively) was investigated.

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2. Materials and methods

Cyclic nucleotide phosphodiesterase isozymes (PDE1–PDE5) were isolated from bovine aorta and their activities determined as previously described (Lugnier and Schini, 1990; Komas et al., 1991). The concentration of chelerythrine that produced 50% inhibition of 1 μM substrate hydrolysis (IC_{50}) was calculated by nonlinear regression from concentration-response curves for six concentrations of inhibitor. When IC_{50} values could not be determined due to limited solubility, the inhibitory effect was expressed as the percentage of inhibition at 300 μM. The apparent K_i (inhibitor constant toward enzyme) and K_I (inhibitor constant toward enzyme-substrate complex) values of chelerythrine for cyclic nucleotide phosphodiesterase inhibition were determined by using Lineweaver-Burk plots and secondary plots for mixed inhibition according to Palmer (1985).

Aortic rings from male Wistar rats (400–450 g) were mounted under 2 g in organ baths containing Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 1.25, KH_2PO_4 1.14, $MgSO_4$ 1.19, glucose 10, $NaHCO_3$ 25, at 37°C and gassed with 95% O_2 and 5% CO_2, pH 7.4. All experiments were carried out in the presence of 10 μM indomethacin. In some aortic rings the endothelium was intact. The presence of the endothelium was confirmed by an at least 50% relaxation to acetylcholine (1 μM) in rings precontracted with noradrenaline (1 μM).

Tissues were allowed to equilibrate for 75 min before the experiments were started. In treated rings, chelerythrine at 10 μM was added 30 min before addition of noradrenaline. Cumulative concentration-tension dependencies for isoprenaline or SK&F 94120 (5-(4)acetamidophenyl)pyrazin-2(1*H*)-one, a PDE3 inhibitor) were then measured. In control rings, addition of chelerythrine was omitted. Relaxation produced by each concentration of drugs was expressed as the percentage of the contractile force induced by noradrenaline. The selection of the concentration of noradrenaline (0.1 or 1 μM for respectively isoprenaline- or SK&F 94120-mediated relaxation) was guided by the results of earlier relaxant studies (Eckly et al., 1994b). The final concentration of the solvent for cyclic nucleotide phosphodiesterase inhibitor, dimethyl

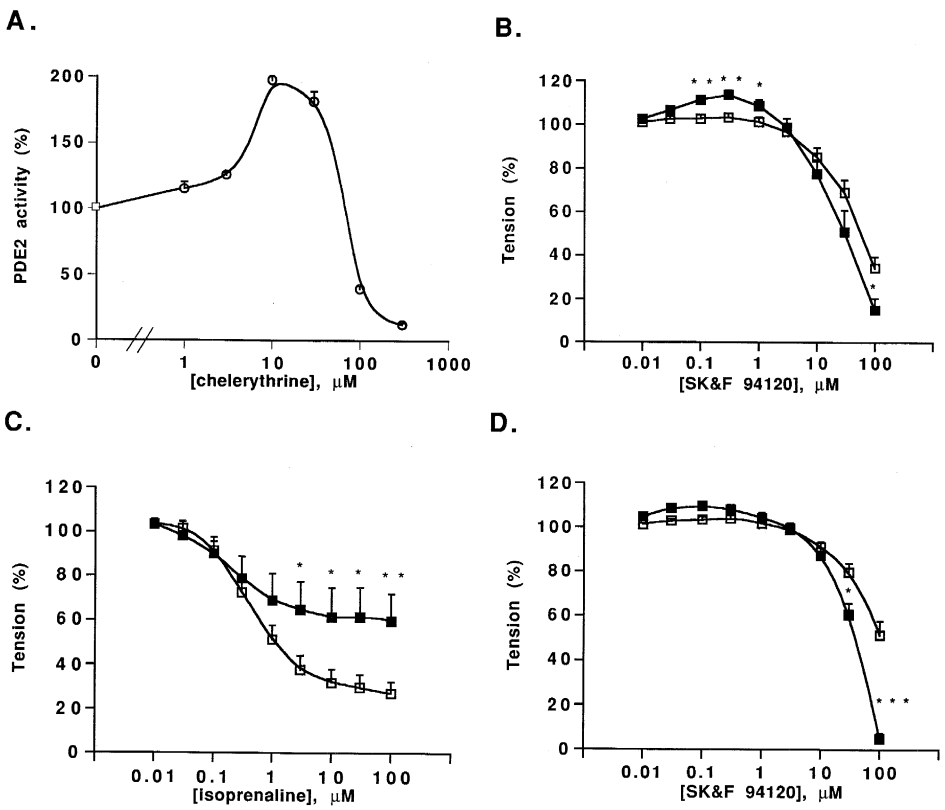

Fig. 1. Effect of chelerythrine on endothelial basal PDE2 activity (A). Hydrolytic activity was determined at 1 μM cyclic AMP and represents the mean ± S.E.M. of three different experiments (100% of activity = 830 pmol/min/mg). Relaxant effect of SK&F 94120 in aortic rings with endothelium (B) and relaxant effects of isoprenaline (C) and SK&F 94120 (D) in aortic rings without endothelium. Concentration-response curves in the absence (□) or in the presence of 10 μM chelerythrine (■). The results are expressed as the mean of 6–10 experiments ± S.E.M. Statistical significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Table 1
Inhibitory effects of chelerythrine on isolated vascular cyclic nucleotide phosphodiesterases

Isozyme	Substrate	Modulator	IC_{50} (μM)
PDE1	cGMP	EGTA	19% [a]
	cGMP	Ca^{2+}-CaM	122 ± 10
PDE2	cAMP	cGMP 5 μM	30 ± 2
PDE3	cAMP		206 ± 10
PDE4	cAMP		18 ± 0.4
PDE5	cGMP		19 ± 1

[a] Percentage of inhibition determined in the presence of 300 μM chelerythrine. Basal PDE1 activity was assayed in the presence of 1 mM EGTA, as Ca^{2+} chelator. Stimulated PDE1 activity was assayed in the presence of calmodulin (CaM, 18 nM) and $CaCl_2$ (10 μM). Results represent the means of three determinations ± S.E.M on different enzyme preparations.

sulfoxide, never exceeded 0.5%. Tension was measured with an isometric force transducer.

Data are presented as means ± S.E.M. Statistical comparisons between control tissue and tissue treated with chelerythrine were performed using Student's test.

3. Results

Chelerythrine significantly inhibited cyclic GMP-activated PDE2, PDE4 and PDE5 with similar IC_{50} values of 30, 18 and 19 μM, respectively (Table 1). Kinetic determinations showed mixed inhibition behaviours related to competitive (K_i)–noncompetitive (K_I) inhibition by chelerythrine of activated PDE2 ($K_i = 17 \pm 2$ μM, $K_I = 43 \pm 5$ μM), PDE4 ($K_i = 15 \pm 2$ μM, $K_I = 58 \pm 5$ μM) and PDE5 ($K_i = 13 \pm 2$ μM, $K_I = 71 \pm 3$ μM). The effect of chelerythrine on basal PDE2 was dependent on the concentration of the protein kinase C inhibitor used (Fig. 1A). From 0.1 to 10 μM, chelerythrine caused a concentration-dependent increase in basal PDE2 activity (maximal response: 197 ± 4.5%). At 300 μM, this compound significantly decreased basal PDE2 activity to 12 ± 1.5%.

The functional effect of chelerythrine on basal PDE2 activity was studied on rat aortic rings with endothelium (Fig. 1B). The protein kinase C inhibitor alone did not significantly affect the precontraction level (data not shown). Chelerythrine significantly decreased the relaxant effect induced by low concentrations of SK&F 94120 (0.01–1 μM) whereas at higher concentrations of SK&F 94120 (100 μM which inhibited isolated PDE1, PDE2, PDE3, PDE4 and PDE5 by 4, 6, 80, 12 and 16%, respectively; from Lugnier and Komas, 1993), chelerythrine potentiated the vasorelaxation (Fig. 1B).

In aortic rings without endothelium, chelerythrine at 10 μM significantly reversed the vasorelaxation induced by 3–100 μM isoprenaline (Fig. 1C). In contrast, chelerythrine significantly potentiated the relaxant effect elicited by 30–100 μM SK&F 94120 (Fig. 1D).

4. Discussion

The ability of chelerythrine to modulate the activities of cyclic nucleotide phosphodiesterases isolated from bovine aorta endothelial cells (PDE2) or bovine aorta media (PDE1, PDE3, PDE4 and PDE5) was investigated. Chelerythrine elicits a biphasic response on basal PDE2 activity and inhibits cyclic GMP-activated PDE2, PDE4 and PDE5 in a competitive-non-competitive manner since apparent K_I values related to enzyme-substrate complexes were greater than apparent K_i values for enzyme for PDE2, PDE4 and PDE5. The question is how chelerythrine could act in an opposite manner on cyclic nucleotide phosphodiesterase activities. It is known that PDE2 contains a non-catalytic binding site for cyclic GMP. The binding of low concentrations of cyclic GMP to this site increases the affinity of the catalytic site for the substrate by allosteric interactions (Yamamoto et al., 1983). As in the case of cyclic GMP, low concentrations of chelerythrine may activate basal PDE2 by interacting directly with the non-catalytic site. At higher concentrations, there may be competition between substrate and excess chelerythrine for the catalytic site, leading to a decrease in PDE2, PDE4 and PDE5 activities. Interestingly, a similar effect on cyclic nucleotide phosphodiesterases has been reported with papaverine, a non-specific inhibitor of cyclic nucleotide phosphodiesterases (Lugnier and Schini, 1990). Chelerythrine and papaverine share a common dimethoxyisoquinoline structure, which may explain the partial competitive effect of chelerythrine on cyclic nucleotide phosphodiesterases.

We next performed a series of experiments aimed to determine whether the modulation of cyclic nucleotide phosphodiesterase activities by chelerythrine could interfere with its functional effect. Chelerythrine was used in these experiments at the concentration of 10 μM, which corresponds to the concentration generally reported in the literature to inhibit protein kinase C. In aorta without endothelium, where PDE1, PDE3, PDE4 and PDE5 have been identified (Komas et al., 1991), chelerythrine significantly decreased the isoprenaline-induced relaxation whereas it potentiated the relaxant effect elicited by SK&F 94120. Experiments were also conducted in aorta rings with an intact endothelium, where PDE2 has been characterized (Lugnier and Schini, 1990). These studies indicated that chelerythrine modulates the relaxation in an opposite manner depending on the concentrations of SK&F 94120 used. How can these different effects of chelerythrine on vasorelaxation be explained? At least two mechanisms of actions of chelerythrine need to be considered. The first concerns the inhibition of protein kinase C and the second the modulation of cyclic nucleotide phosphodiesterase activities, as described in the present study.

If inhibition of protein kinase C represents the only mechanism of action of chelerythrine, then how does inhibition of protein kinase C in rat aorta induce opposite

effects, either inhibitory or facilitatory, on the vasorelaxation elicited by cyclic AMP-elevating agents? The involvement of protein kinase C in cyclic AMP metabolism is supported by previous studies demonstrating that activation of protein kinase C by phorbol ester treatment leads to an increase in adenylyl cyclase and PDE3 activities (Jacobowitz et al., 1993; Michael and Webley, 1991). Even if these observations concerning protein kinase C inhibition seem to be consistent with the effect of chelerythrine on isoprenaline-induced vasorelaxation, they do not support all of the functional effects we observed (for example: opposite effects of chelerythrine according to the concentration of SK&F 94120 used in aorta with endothelium). Therefore, it must be assumed that, at least, another mechanism of action is involved in the effect of chelerythrine on vasorelaxation. The present investigations demonstrate that chelerythrine activated PDE2 at 10 μM. In aorta with endothelium, activation of basal PDE2 should decrease cyclic nucleotide levels and lead to vasoconstriction. This result suggests that, in the presence of endothelium, chelerythrine is able to decrease vasorelaxation through basal PDE2 activation. Moreover, biochemical studies also show that chelerythrine, at 10 μM, is able to inhibit PDE4 and PDE5, which are both present in the vascular media. We have previously reported that inhibition of cyclic nucleotide phosphodiesterases leads to an increase in cyclic nucleotide content and, consequently, to vasorelaxation (Komas et al., 1991; Eckly and Lugnier, 1994a). Thus, it is likely that chelerythrine, by its inhibition of PDE4 and PDE5, could potentiate the effect of SK&F 94120 on relaxation in aorta without endothelium. Taken together, these results support the conclusion that chelerythrine could modulate the relaxant response through two mechanisms of action: (1) protein kinase C inhibition and (2) modulation of cyclic nucleotide phosphodiesterase activities. Interestingly, modulation of cyclic nucleotide phosphodiesterase activities by chelerythrine seems not to be involved in isoprenaline-induced relaxation. The reason is not clear, but this could probably reflect the existence of different cyclic AMP compartments more or less associated with cyclic nucleotide phosphodiesterases (Jurvicius and Fischmeister, 1996).

In conclusion, these results show that chelerythrine at 10 μM, which corresponds to the concentration generally described in the literature to inhibit protein kinase C, significantly modulates the activities of some cyclic nucleotide phosphodiesterase isozymes, thus altering the vasocontrictor potency of this compound. Some other non-specific effects of high concentrations of chelerythrine have been reported in the literature (100 μM inhibits phospholipase C and phospholipase D; Guillemain and Rossignol, 1995). Altogether, these results for cyclic nucleotide phosphodiesterase activities and for relaxation studies cast some doubt on the use of chelerythrine as a specific inhibitor of protein kinase C.

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