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INHIBITION OF PROTEIN KINASE C ACTIVITY BY THE ANTIRHEUMATIC DRUG AURANOFIN

MARIO FROSCIO,*§ ANDREW W. MURRAY† and NIGEL P. HURST‡

* Dept. of Medical Biochemistry, Flinders Medical Centre, and † School of Biological Sciences, ‡ Rheumatology Unit, The Queen Elizabeth Hospital, Woodville, S. Australia 5011

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Abstract—The Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C; PKC) has a central role in the transmission of extracellular signals. The orally active anti-rheumatic drug, auranofin, has been shown to modulate PKC-mediated cell responses. In this study, we report that auranofin directly inhibits PKC in a dose-dependent manner; inhibition can be overcome by mercapto-ethanol. Proteolytically-activated PKC is also inhibited by auranofin excluding an effect of the drug on the regulatory domain of the enzyme. These data clearly show that auranofin inhibits the catalytic activity of PKC, probably by interacting with thiol groups.

The Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C; PKC) has a central role in the transmission of extracellular signals. A number of functional substrates for PKC have been identified including proteins of 40–45 kD in human platelets [1]. Activation of platelets by thrombin or 12-O-tetradecanoyl-phorbol-13-acetate (TPA) results in rapid and specific phosphorylation of these proteins by PKC [1].

Auranofin (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-*S*-[triethylphosphine]gold) is an orally active gold complex widely used in the treatment of rheumatoid arthritis. Although the mechanism of action of this and other antirheumatic gold (1) complexes remains uncertain it probably depends on sequential thiol exchange reactions which result in the covalent modification of cellular proteins [2]. Auranofin has been reported to modulate PKC-mediated cell responses. For example, auranofin is a potent inhibitor of platelet aggregation [3] a function which is associated with PKC-mediated phosphorylation of a 40 kD protein [4]. We have reported that auranofin unexpectedly stimulates phosphorylation of both 40 kD and 20 kD proteins in intact platelets, apparently by activating PKC [5]. Similarly auranofin stimulates PKC-dependent phosphorylation of the EGF receptor [6]. The purpose of these present experiments was to investigate whether auranofin has a direct effect on PKC.

MATERIALS AND METHODS

Washed human platelets were prepared as described [5]; platelets were suspended at 1–2×10^9 ml in buffer containing glucose (5.5 mM), Tris (15 mM), NaCl (0.14 M) and BSA (0.35%) at pH 7.4.

PKC was partially purified from washed human platelets. After sonication in Tris–Cl (25 mM), EDTA (2 mM), EGTA (5 mM), 2-mercaptoethanol (10 mM) and leupeptin (100 μg/ml) at pH 7.4, platelet extracts were centrifuged to obtain a 100,000 *g* supernatant which was applied to a DEAE-cellulose column. Protein was eluted with a linear salt gradient (0–0.4 M) and fractions assayed for PKC for 10 min at 30° as previously described [7]. Peak fractions were pooled and used for experiments. When different substrates were tested, lysine-rich histone (histone IIIS) was replaced with an equivalent amount of whole histone (histone IIA) or kemptide. Assays using kemptide were adsorbed to Whatman P81 paper and washed in 75 mM phosphoric acid [8]. Auranofin was added from a stock solution in DMSO giving a final solvent concentration of 0.1%.

Proteolytic cleavage of PKC was achieved by limited tryptic digestion. Pooled PKC fractions (0.42 mg/ml protein) was incubated with TPCK-trypsin (8 μg/ml) for 5 min at 37°. The reaction was stopped by addition of 70 μg/ml soybean trypsin inhibitor and aliquots removed for PKC assays.

RESULTS AND DISCUSSION

As reported previously, auranofin (up to ≈ 40 μM) increased phosphorylation of 40 kD protein in intact human platelets [5]. In contrast, auranofin inhibited the Ca^{2+}/phospholipid-dependent activity of partially purified PKC in a dose-dependent fashion but did not affect basal activity measured in the absence of Ca^{2+} and phospholipid (Fig. 1A). Since both the regulatory and catalytic domains of PKC contain thiol groups with which auranofin might interact [9], PKC was subjected to limited tryptic proteolysis to separate these domains. Proteolysis results in a polypeptide fragment with kinase activity which is independent of Ca^{2+} and phospholipid (PKM) [10]. As indicated, auranofin also inhibited the activity of this proteolytically-activated form of PKC (Fig. 1B).

§ To whom correspondence should be addressed.

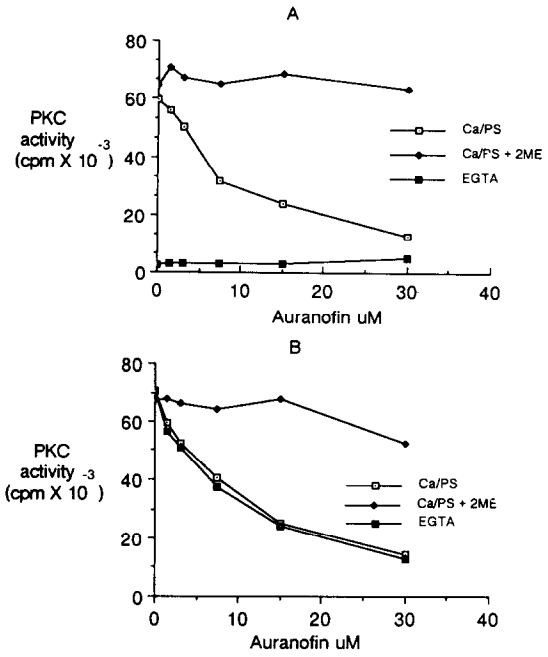

Fig. 1. Effect of auranofin on catalytic activity of (A) PKC or (B) PKM in the presence of Ca^{2+} and phosphatidylserine (Ca/PS) with or without 2-mercaptoethanol (2ME). Ca^{2+} and phosphatidylserine-independent activity was measured in the presence of EGTA. PKM was generated from PKC as described in Materials and Methods. Points are means of duplicate assays.

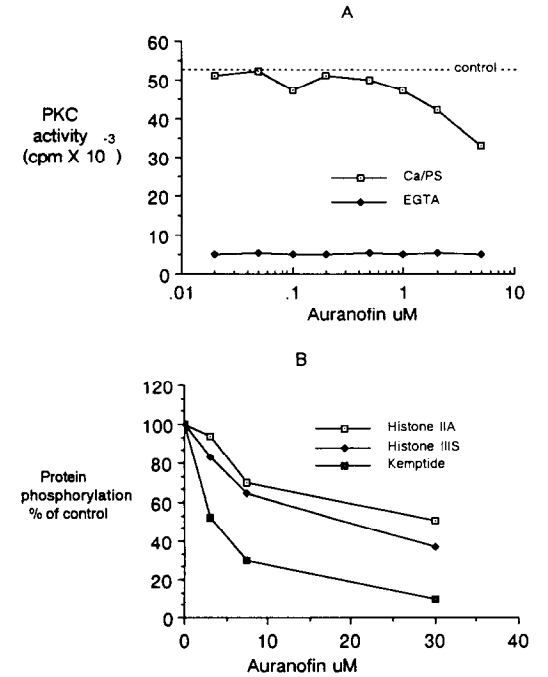

Fig. 2. (A) Effect of low concentrations of auranofin on PKC activity assayed in the presence of Ca^{2+} and phosphatidylserine (Ca/PS). Ca^{2+} and phosphatidylserine-independent activity was measured in the presence of EGTA. (B) Effect of auranofin on PKC activity using different proteins as acceptor substrates. Points are means of duplicate assays.

The inhibitory effect of auranofin on PKC and PKM was abolished by 2-mercaptoethanol (Fig. 1A and B) consistent with an effect of the drug on thiol groups in the protein. To exclude the possibility that low concentrations of auranofin might stimulate PKC activity, an additional dose–response experiment was performed (Fig. 2A). Clearly, no enhancing effect was obtained over the concentration range 0.02–0.5 μM.

Different protein acceptor substrates were also used to test whether auranofin might act on the substrate rather than the kinase (Fig. 2B). Although quantitative variation was observed, auranofin inhibited phosphorylation of whole histone, lysine-rich histone and kemptide. Since kemptide lacks sulphydryl groups substrate interaction with auranofin is unlikely to explain the effect of this drug on ^{32}P-incorporation.

In summary, these data clearly show that auranofin inhibits the catalytic activity of PKC probably by interacting with thiol groups. Furthermore, auranofin appears to interact with thiols present in the catalytic rather than the regulatory domain of PKC. No evidence for an enhancing effect of auranofin on PKC was found even at low concentrations. Consequently, the enhancing effect of auranofin on protein phosphorylation in intact cells [5, 6] is unlikely to be due to a direct effect of auranofin on PKC.

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