THE EFFECT OF DIMETHYL 3,3'-DITHIOBISPROPIONIMIDATE ON THE ADENYLATE CYCLASE ACTIVITY OF BOVINE CORPUS LUTEUM

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Received 6 June 1979

1. Introduction

In a previous report from this laboratory [1] evidence was presented for the requisite association of two or more components of the luteal adenylate cyclase complex in order for a high activity state of the enzyme to be maintained. Ross and Gilman [2] have suggested that the lability of solubilized adenylate cyclase might be explained by the dissociation of two protein components and Pfeuffer [3] showed that the activity of soluble adenylate cyclase is dependent on the association of two protein fractions, one of which contained the guanyl nucleotide-binding protein. This paper describes the effect of the cleavable, bifunctional cross-linking agent, dimethyl 3,3'-dithiobispropionimidate (DTP) on the activity of luteal adenylate cyclase and on the stability of enzyme activity to dispersion in Lubrol-12A9.

2. Materials and methods

ATP (disodium salt) was obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey. Guanosine 5-[β,γ-imido]triphosphate (p[NH]ppG, tetralithium salt) was purchased from Boehringer Corp. (London) Ltd., London, W.5. Lubrol-12A9 was a gift from ICI, Alderley Park, Macclesfield, Cheshire. Methyl acetimidate hydrochloride and dimethyl 3,3'-dithiobispropionimidate dihydrochloride (DTP) were obtained from Pierce and Warriner (UK) Ltd., Chester, Cheshire.

The washed 600 × *g* sediment of bovine corpus luteum homogenate was prepared as described previously [4]. The adenylate cyclase of the 600 × *g* sediment was activated by incubation at 37°C for 20 min with 0.1 mM p[NH]ppg in 40 mM Tris–HCl (pH 7.5) 6 mM $MgSO_4$, followed by washing (twice) with p[NH]ppG-free buffer at 4°C. Portions of the washed p[NH]ppG-activated 600 × *g* sediment were resuspended in 40 mM Tricine–NaOH buffer (pH 7.5) containing various concentrations of DTP (see fig.1). Cross-linking was carried out at 4°C for 18 h, after which time the sediment was washed twice with buffer (40 mM Tricine–NaOH (pH 7.5), followed by 40 mM Tris–HCl, pH 7.5). Four tissue fractions were prepared from each cross-linked sediment: (1) sediment resuspended in 40 mM Tris–HCl, pH 7.5; (2) sediment resuspended in 40 mM Tris–

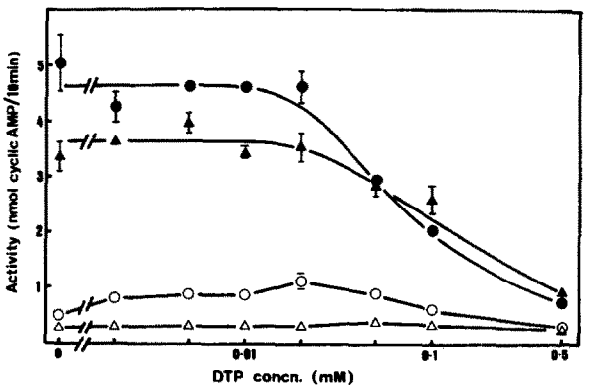

Fig.1. The effect of dimethyl 3,3'-dithiobispropionimidate (DTP) on the activity of adenylate cyclase. nmol cyclic AMP produced/10 min v. concentration of DTP (mM). Symbols: cross-linked sediment was resuspended in ●, 40 mM Tris–HCl (pH 7.5); ○, 40 mM Tris–HCl (pH 7.5) containing Lubrol-12A9 (10 g/l); ▲, 40 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol; △, 40 mM Tris–HCl (pH 7.5) containing Lubrol-12A9 (10 g/l) and 1 mM dithiothreitol.

HCl, pH 7.5, containing Lubrol-12A9 (10 g/l); (3) sediment resuspended in 40 mM Tris–HCl, pH 7.5, containing 1 mM dithiothreitol; (4) sediment resuspended in 40 mM Tris–HCl, pH 7.5, containing Lubrol-12A9 (10 g/l) and 1 mM dithiothreitol. Amidination by methyl-acetimidate was carried out in the same manner as treatment with DTP. All tissue extract concentrations were equivalent to 75 mg wet weight of corpus luteum/ml.

Adenylate cyclase activity was assayed as described previously [1,4–6]. The total assay incubation volume of 1 ml contained 40 mM Tris–HCl (pH 7.5), 6 mM $MgSO_4$, 1 mM ATP, 6.7 mM caffeine and 500 μl of enzyme preparation equivalent to 37.5 mg wet weight of tissue. Incubation was carried out at 37°C for 10 min. The cyclic AMP produced was measured by a competitive protein-binding assay [4]. All assays were performed in duplicate and the mean and range of duplicate values are shown.

3. Results and discussion

It can be seen from fig.1 that DTP inhibited adenylate cyclase activity of the 600 × *g* sediment in a dose-dependent manner at concentrations above 0.02 mM. Amidination with the monofunctional imidate, methyl-acetimidate, at a concentration of 10 mM, was without effect on enzyme activity. The effects of DTP, therefore, are probably due to cross-linking of membrane constituents and are not a direct result of amidination.

Treatment with DTP stabilized the enzyme activity to dispersion in detergent at all concentrations of DTP used. For example, using 0.01 mM DTP the percentage yield* of activity following dispersion in detergent was 19.1 in contrast to 9.5% obtained with the control preparation (non cross-linked). In six separate experiments treatment with 0.01 mM DTP increased the total yields** of detergent-dispersed activity by 36.8%, 38.6%, 63.2%, 80.6%, 83.3% and 137% respectively. A paired '*t*'-test of the data obtained in these six experiments showed that treatment with 0.01 mM DTP had a significant effect ($p = < 0.05$) on the stability of enzyme activity to dispersion in detergent. For both cross-linked and control preparations greater than 80% of the detergent-dispersed activity was recovered in the supernatant following centrifugation at 105 000 × *g*.

DTP concentrations greater than 0.02 mM also stabilized enzyme activity to detergent-dispersion, despite the fact that the particulate enzyme was inhibited. For three experiments the percentage yields of activity following exposure to detergent were 9.5, 15.2 and 25.6 for the control preparations and 31.4, 21.8 and 42.1 respectively for the 0.05 mM DTP treated sediment. The corresponding increases in the total yields of activity due to treatment with 0.05 mM DTP were 92.7%, 23.8% and 51.3% respectively.

In a previous report it was shown that preactivation of the particulate enzyme with p[NH]ppG increased the total yield of activity solubilized by detergent but not the percentage yield [5]. It is possible that p[NH]ppG activates adenylate cyclase by promoting the association of the guanyl nucleotide-binding protein with the catalytic unit of the enzyme and that DTP stabilizes this complex to detergent-dispersion by covalent cross-linking of the components.

Two effects of dithiothreitol were evident from the results of the experiment shown in fig.1: (1) in the absence of DTP treatment, dithiothreitol inhibited adenylate cyclase, as was also demonstrated previously [6]; (2) dithiothreitol reversed the effects of DTP, presumably by reductive cleavage of the disulphide bond; the inhibitory effects of DTP on the particulate enzyme were partially reversed and the effect of cross-linking on the stability of the enzyme activity to dispersion in detergent was almost completely reversed. These results also indicate that it is cross-linking and not amidination per se that is responsible for the observed effects of DTP.

On the assumption that the effective cross-links are intermolecular, the results shown herein are consistent with the hypothesis that the high activity state of the adenylate cyclase requires the association of two or more components. However, an effect of intramolecular cross-linking on the activity and stability of adenylate cyclase has not been excluded. The precise nature of the cross-links and the components involved remain to be elucidated.

* Percentage yield = activity in the presence of detergent ÷ activity in absence of detergent × 100

** Percentage increase in total yield = activity of cross-linked preparation in presence of detergent (A) minus activity of control preparation of detergent (C) ÷ C × 100

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