Activating effect of destomycin A on adenylate cyclase from several animal tissues

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1. INTRODUCTION

Adenosine 3′,5′-monophosphate (cAMP), which is formed from adenosine 5′-triphosphate (ATP) in a reaction catalyzed by adenylate cyclase [1,2], plays various important roles in cell function [3–5]. Peptide or neurohormone application always causes an increase in the cAMP content of the target cell, because the hormone activates adenylate cyclase of the cell [6]. Since hormones only activate adenylate cyclase in the intact cell, the cAMP-mediated reaction has been investigated by artificial blockers of cAMP breakdown.

Here, we observed a stimulatory effect of destomycin A, which is a water-soluble aminoglycoside antibiotic, isolated from the cultured filtrate of *Streptomyces rimofaciens* [7,8], on the adenylate cyclase activity of several animal tissues.

2. MATERIALS AND METHODS

2.1. *Materials*

The brain and liver of 7–8-week old male Wistar rats and the skin of bull frog tadpole *Rana catesbeiana* were used as materials.

2.2. *Incubation of tissue and cAMP extraction*

The brain and the liver were sliced in 1 mm thickness. The skin of the frog was cut in pieces of 5 mm × 5 mm.

The tissue was incubated in Kreb's ringer bicarbonate buffer containing various concentration of destomycin A in a glass petri dish for 10 min at 30°C. The tissue was then quickly frozen with liquid nitrogen, and cAMP was extracted from the tissue with 6% $HClO_4$ as in [9].

2.3. *Enzyme preparation*

Tissue was homogenized with 0.25 M sucrose/5 mM $MgCl_2$/5 mM β-mercapto ethanol for preparation of adenylate cyclase or with 20 mM Tris–HCl (pH 7.8) containing 5 mM $MgCl_2$ for preparation of phosphodiesterase, respectively. The enzyme preparation was obtained from the homogenate by the same procedures as in [9].

2.4. *Enzyme assay and cAMP determination*

Appropriate concentrations of the adenylate cyclase preparation (3–5 mg protein) were mixed with the solution containing 5 mM $MgCl_2$/2 mM KCl/10 mM caffeine/2 mM phosphoenolpyruvate/5 μg pyruvate kinase in 1 ml solution/20 mM Tris–HCl (pH 7.8) and various concentration of destomycin A, and the final volume was adjusted to 900 μl. The enzyme reaction was initiated by addition of 100 μl ATP at 10 mM into the mixture and stopped by addition of 1 ml 6% $HClO_4$ after 20 min incubation at 30°C. The mixture was then prepared for cAMP extraction as above. The enzyme activity was calculated by determination of the amount of cAMP formed during the incubation.

Cyclic AMP was determined spectrophotometrically by the same method in which the minimum measurable range was 0.5 nM as in [9].

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Table 1

Content of cAMP in the tissue after incubation with destomycin A

	cAMP content (nmol/mg tissue protein)			
Destomycin A (mM):	0	0.1	1.0	10.0
Brain	16.1 ± 8.1	17.3 ± 9.1	65.5 ± 22.6	105.9 ± 35.2
Liver	8.9 ± 8.3	19.4 ± 10.4	45.3 ± 11.2	72.2 ± 19.4
Skin	6.7 ± 4.1	18.1 ± 8.6	52.2 ± 19.6	70.4 ± 20.1

All values are means of 3 expt ± SEM

2.5. *Enzymes and chemicals*

All enzymes, ATP, phosphoenolpyruvate and cAMP were purchased from Boehringer Mannheim Co. Destomycin A was a kind gift of Meiji Seika Kaisha Ltd. Other chemicals were obtained from Wako Pure Chemicals Co.

3. RESULTS

cAMP in the liver and the skin was elevated ~2-fold when treated with 0.1 mM destomycin A (table 1). Destomycin A at this concentration did not increase the content of cAMP in the brain. The content of cAMP of all tissues was evidently increased when incubated with the above antibiotic at 1 mM. About 4–7-fold elevation in cAMP content was observed at 1 mM, and 10 mM destomycin A increased cAMP level by 7–10-fold. Destomycin A at high concentrations (examined up to 50 mM) showed no more elevation (not shown).

Activities of the adenylate cyclase of the brain and the liver were enhanced ~2-fold by destomycin A at 0.1 mM. Destomycin A at 1 mM maximally activated the enzyme (~4-fold). If antibiotic became 1 mM (up to 10 mM), an apparent inhibitory effect was observed (not shown). The adenylate cyclase of frog skin was more sensitive to the antibiotic than that of the rat tissues (table 2). At 0.1 mM, destomycin A fully activated the adenylate cyclase (~5-fold).

On the contrary, phosphodiesterase activity was never influenced at the concentration of destomycin A which activated the adenylate cyclase (table 2).

Since no effect of destomycin A on ATP regen-

Table 2

Percent activation of the adenylate cyclase and the phosphodiesterase

	Adenylate cyclase			Phosphodiesterase		
Destomycin A (mM):	0.01	0.1	1.0	0.01	0.1	1.0
Brain	0.3 ± 8.1	102 ± 76	276 ± 75	− 0.5 ± 8.2	0.8 ± 7.7	− 1.0 ± 7.3
Liver	0.9 ± 6.2	90 ± 36	321 ± 84	0.7 ± 8.0	− 7.1 ± 10.1	− 6.0 ± 6.4
Skin	18.0 ± 9.6	402 ± 104	398 ± 97	4.3 ± 9.2	0.4 ± 7.0	0.8 ± 5.3

All values are means of 3 expt ± SEM

erating system was noticed (not shown), destomycin A might be a specific activator of the adenylate cyclase.

4. DISCUSSION

The stimulatory effect of many peptide and neurohormones on the functions of the target tissues has been believed to be via an increase in cAMP content [6]. Here, destomycin A activated the adenylate cyclase, which resulted in the increase of cAMP content as if the tissue were treated by a specific hormone. The increase in cAMP content with destomycin A may be caused by the activation of the adenylate cyclase located on the plasma membrane, because the adenylate cyclase is activated significantly and phosphodiesterase is not affected by destomycin A. The effective concentration for the increase in cAMP content of frog skin was evidently higher than the concentration of destomycin A which stimulated the adenylate cyclase. This may be due to a permeability barrier inhibiting the uptake of destomycin A across frog skin.

One possible explanation for the activating effect of this antibiotic on the adenylate cyclase may be due to the structure of itself. Since destomycin A is an aminoglycoside, the receptor site of the adenylate cyclase may mistake the antibiotic for a specific hormone. This hypothesis is partly supported by: extract of sea urchin eggs containing an aminoglycoside compound stimulated the cleavage cycle [10]; the cleavage cycle of the sea urchin egg was accelerated by cAMP or dibutyryl cAMP [11].

Destomycin A is expected to become a powerful agents and specific hormone to investigate the structure of the receptor site of the adenylate cyclase.

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