NICOTINAMIDE INHIBITION OF 3',5'- CYCLIC AMP PHOSPHODIESTERASE IN VITRO

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SUMMARY

Nicotinamide was found to be potent inhibitor of 3',5'- cyclic AMP phosphodiesterase, but nicotinamide adenine dinucleotide was found impotent. Therefore, it may be presumed that the induction of certain enzymes following the intraperitoneal injection of nicotinamide into rats would due to the elevation of the steady state level of 3',5'- cyclic AMP through the inhibition of phosphodiesterase by nicotinamide.

INTRODUCTION

Nicotinamide induces certain enzymes such as serine dehydratase [EC 4.2.1.14] (1), tyrosine aminotransferase [EC 2.6.1.5] (2) and tryptophan pyrrolase [EC 1.13.1.12] (3). The induction of the first two enzymes by this agent can be seen in both the conventional and the adrenalectomized rat, but that of the third enzyme is not to be found in the adrenalectomized rat, and it has been proposed that nicotinamide may induce the secretion of the adrenocortical hormones (3). On the other hand, 3',5'- cyclic AMP (cAMP) promotes the induction of the first two enzymes but not of the third (4-6), and this cyclic nucleotide participates in stimulating the production of adrenocortical hormones (7) being able to induce tryptophan pyrrolase (8) and tyrosine aminotransferase (9).

These reported data led us to investigate the effect of nicotinamide on cAMP phosphodiesterase in vitro. In this communication, it will be seen that nicotinamide, 3-acetylpyridine and N,N-diethylnicotinamide inhibit rat liver cAMP phosphodiesterase.

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MATERIALS AND METHODS

White male rats of the Wistar strain, weighing from 180 to 200 g, were used. They were maintained on an Oriental Yeast solid diet fed ad libitum, and were allowed free access to drinking water. The ^{3}H-cAMP was obtained from New England Nuclear. The snake venom (Naja naja) was obtained from the Sigma Chemical Company. All the other reagents were purchased from Nakarai Chemicals, Kyoto.

Enzyme assay

The assay for phosphodiesterase activity was carried out by the two stage isotopic procedure described by Thompson and Appleman (10). The first stage reaction mixture of 0.5 ml contained 50 μmoles Tris-Cl buffer, pH 7.5, 0.25 μmoles $MnCl_2$, 2.5 mg of bovine serum albumin, 50 nmoles ^{3}H-cAMP (12,000 cpm), and an appropriate concentration of enzyme. The reaction was initiated by the addition of ^{3}H-cAMP. After incubation for 10 min at 37° C, the tubes containing the reaction mixture were transferred to a boiling water bath for two min to terminate the reaction. The reaction mixture was then further incubated with sufficient snake venom for 30 min at 37° C. The reaction was stopped by the addition of 2.0 ml of a 1:1 slurry Bio-Rad resin AG 1x2, 200-400 mesh. The amount of radioactivity of the ^{3}H-adenosine left in the supernatant after centrifugation in a clinical centrifuge was measured by means of a liquid scintillation spectrometer. In some cases, the assay method described by Krishna et al. (11) was also carried out for the determination of cAMP phosphodiesterase activity. In this procedure, 5'-AMP was involved at a concentration of 2×10^{-3} M in the reaction mixture described above. After incubation for 10 min, the hydrolysis of cAMP was stopped by the addition of 0.2 ml $ZnSO_4$(0.17 M) and the immersion of the tubes in ice water. Subsequent to the addition of $ZnSO_4$, 0.2 ml of $Ba(OH)_2$ (0.15 M) was added resulting in a quantitative precipitate. After centrifugation by clinical centrifuge, the amount of radioactivity of the unhydrolyzed cAMP in the supernatant fraction was measured by same method described above. All assays were carried out at 20 % or less total reaction to

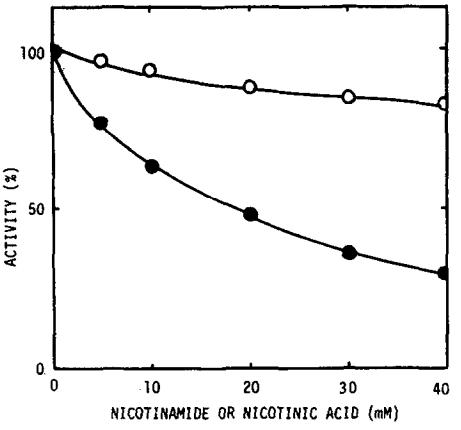

Fig. 1.

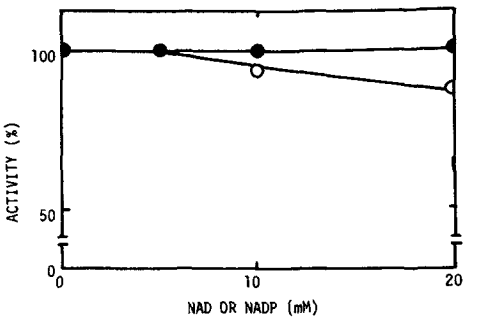

Fig. 2.

Fig. 1. Effect of nicotinamide and nicotinic acid concentrations on phosphodiesterase activity. The two stage isotopic procedure was employed using 25 units of enzyme. Activity in the absence of nicotinamide or nicotinic acid was settled as 100 %. ●——● nicotinamide, o——o nicotinic acid.

Fig. 2. Effect of NAD and NADP concentrations on phosphodiesterase activity. The two stage isotopic procedure was employed using 25 units of enzyme. Activity in the absence of NAD or NADP was settled as 100 %. ●——● NAD, o——o NADP.

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be in the linear portion of the enzyme assay. One unit of enzyme activity was defined as the cleavage nmole of cAMP per hour.

Phosphodiesterase preparation

The livers were isolated immediately after perfusion with ice-cold sterilized 0.9 % NaCl solution through the portal vein. Livers were homogenized in 5 volumes of 0.05 M Tris-Cl buffer containing 0.5 mM $MnCl_2$, pH 7.5, and centrifuged at 105,000 x g for 120 min. 20 ml of the supernatants were applied to an Agarose A-5m column (2.5x90 cm), which was eluted with the same buffer. The flow rates for the separation did not exceed 10 ml per 30 min. Under these conditions, single fractions presenting phosphodiesterase activity were isolated in the manner described by Thompson and Appleman (12).

RESULTS AND DISCUSSION

As shown in Fig. 1, nicotinamide was found to be potent inhibitor of cAMP phosphodiesterase. Nicotinic acid also inhibited this enzyme activity but

to a much milder degree. Furthermore, 3-acetylpyridine and N,N-diethylnicotinamide produced a marked inhibition as seen with nicotinamide (data not shown). The effect of NAD and NADP on the phosphodiesterase was also tested. NADP was found to be week inhibitor, but NAD was found impotent (Fig. 2).

To make certain that nicotinamide and nicotinic acid inhibition was not due to the influence of these agents on the conversion of 5'-AMP to adenosine by the snake venom added to the 2nd stage reaction mixture, nicotinamide and nicotinic acid were added to the 2nd stage reaction mixture, respectively, however no detectable effect of these compounds was observed. Furthermore, nicotinamide and nicotinic acid inhibition of phosphodiesterase activity was confirmed by the estimation of the amount of unhydrolyzed cAMP in the reaction mixture according to the $ZnSO_4$-$Ba(OH)_2$-method (11) (data not shown).

Lineweaver-Burk plots of 1/v versus 1/[S] in both nicotinamide absence and presence are shown in Fig. 3. The inhibition of phosphodiesterase activity seems to be competitive.

Little is known about the regulation of the activity of cAMP phosphodiesterase by physiologically relevant compounds. A majority of mammalian cAMP phosphodiesterase is stimulated by imidazole and inhibited by methylxanthine derivatives (13). The significance in vivo of phosphodiesterase inhibition by nicotinamide in vitro is unclear. From the data described here, however, one can speculate that nicotinamide would participate in the regulation of the intracellular cAMP levels through inhibitory action on phosphodiesterase, although the concentrations of nicotinamide required for a significant inhibition of phosphodiesterase activity are relatively high as compared to the concentration of nicotinamide normally found in cells (14). Recently, we had observed that adrenocortical cAMP phosphodiesterase from rat was also inhibited by nicotinamide (1). Therefore, it is possible that relatively large injections of nicotinamide would serve to elevate the cAMP levels in the adrenal cortex and that consequently, the induction of certain enzymes might occur through the stimulatory secretion of adrenocortical hormones.

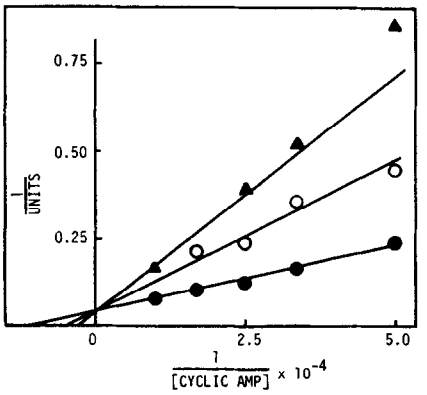

Fig. 3. Double reciplocal plots of the initial velocity of phosphodiesterase versus, cAMP concentration at a constant nicotinamide level. The initial reaction velocity was determined by utilizing the two stage isotopic procedure with 11.8 units of enzyme preparation. The nicotinamide concentrations used were ; ●——● none, o——o 20 mM, and ▲——▲ 40 mM.

Studies of the effect of nicotinamide and its analogue such as N,N-diethylnicotinamide on tissue cAMP levels will be required to the hypothesis that nicotinamide produces significant inhibition of phosphodiesterase activity in vivo; such investigations are now in progress in our laboratory.

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REFERENCES

1. Shimoyama, M., and Ueda, I., unpublished data.
2. Yamaguchi, K., Sakakibara, S., Koga, K., and Ueda, I., Biochim. Biophys. Acta, 237 : 502, 1971.
3. Yamaguchi, K., Shimoyama, M., and Gholson, R. K., Biochim. Biophys. Acta, 146 : 102, 1967.
4. Wicks, W. D., Science, 160 : 977, 1968.
5. Wicks, W. D., J. Biol. Chem., 244 : 3941, 1969.
6. Wicks, W. D., Kenney, F. T., and Lee, K-L., J. Biol. Chem., 244 : 6008, 1969.
7. Haynes, R. C., Koritz, S. B., and Peron, F. G., J. Biol. Chem., 234 : 1421, 1959.
8. Lin, E. C. C., and Knox, W. E., Biochim. Biophys. Acta, 26 : 85, 1957.
9. Thompson, J. F., and Mikuta, E. J., Endocrinology, 55 : 232, 1954.
10. Thompson, W. J., and Appleman, M. M., Biochemistry, 10 : 311, 1971.
11. Krishna, G., Weis, B., Brodie, B. B., J. Pharmacol. Exp. Ther., 163 : 379. 1968.
12. Thompson, W. J., and Appleman, M. M., J. Biol. Chem., 246 : 3145, 1971.
13. Bucher, R. W., and Sutherland, E. W., J. Biol. Chem., 237 : 1244, 1962.
14. Grunicke, H., Liersh, M., Hinz, M., Pushendorf, B., Richter, E., and Holzer, H., Biochim. Biophys. Acta, 121 : 228, 1966.