ACTIVATION OF RAT ADIPOCYTE PLASMA MEMBRANE ADENYLATE CYCLASE BY SODIUM AZIDE*

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Summary: The adenylate cyclase of rat adipocyte plasma membrane is stimulated by sodium azide with a half maximal activation of 100-150% occuring at 50 mM NaN_3. Studies of the effects of azide and fluoride indicate different mechanisms of stimulation of the enzyme by these ions. Comparable stimulation of the activity is obtained by 100 mM NaN_3 or 10 mM NaF but unlike azide, higher concentrations of fluoride cause inhibition of the enzyme. Fluoride activated adenylate cyclase is further stimulated by azide. Epinephrine stimulation of the enzyme is absent in the presence of fluoride but the hormone enhances the activity in the presence of azide. Reversal of the inhibitory action of GTP on adenylate cyclase by epinephrine is demonstrated even in the presence of azide but not in the presence of fluoride.

INTRODUCTION

The mechanism of action of fluoride on adenylate cyclase is not established. It is generally believed that fluoride stimulates adenylate cyclase by direct action on the catalytic site (1, 2). However, recent studies have indicated a noncatalytic site of action (3) and induction of uncoupling of the hormone receptor and the catalytic site (4).

This study reports on the discovery of azide stimulation of rat adipocyte plasma membrane adenylate cyclase. In light of the multiple effects of fluoride on this enzyme, a comparative study of the effects of azide and fluoride was carried out. It appears that the modifications of basal, epinephrine stimulated and GTP inhibited adenylate cyclase activities by azide and by fluoride are mechanistically different.

METHODS

Isolated fat cells and fat cell plasma membranes were prepared from male

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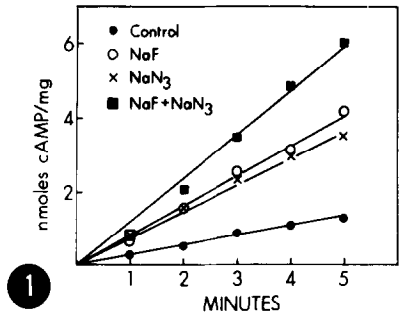
Wister rats as described previously (5). The adenylate cyclase assay was a modification of the method of Krishna, et al (6). The standard assay mixture contained 50 mM tris-HCl, pH 7.4, 0.25 M sucrose, 5 mM MgCl, 1 mM EDTA, 0.1% bovine serum albumin and 1 mM cAMP in a final volume of 0.1 ml. ATP and App(NH)p* concentrations were 0.4 mM with α-^{32}P specific activity of 50-100 cpm/pmole. App(NH)p and [α-^{32}P] App(NH)p were obtained from International Chemical and Nuclear Corp. One μmole of phosphoenolpyruvate and 1 unit of pyruvate kinase were added to maintain constant ATP concentration during the reaction. The reaction was carried out at 30°C with 10-25 μg of enzyme protein and terminated with 0.1 ml of 2% sodium dodecylsulfate containing 5 mM ATP and 1 mM cAMP. [^{32}P]cAMP was isolated and quantitated by Dowex-50 and aluminum oxide chromatography as described by Salomon, et al (7). All incubations were carried out in duplicate with less than 5% difference between duplicates.

RESULTS

The time course of activation of plasma membrane adenylate cyclase by NaN_3 and NaF is shown in Figure 1. Basal and stimulated adenylate cyclase activities are linear under these conditions for at least 5 minutes. Marked stimulation of the activity is obtained by 100 mM NaN_3 or 10 mM NaF. Note that an additive response to NaF (10mM) and NaN_3 (100 mM) is demonstrated indicating different modes of action of these ions. Stimulation of adenylate cyclase by various concentrations of NaF and NaN_3 is shown in Figure 2. Sodium fluoride at concentrations greater than 10 mM results in typical inhibition of the enzymatic activity. In contrast near maximal stimulation occurs with 100 mM NaN_3 and higher concentrations are not inhibitory. Such contrasting effect of NaN_3 and NaF further suggests different mechanisms responsible for stimulation of the enzymatic activity. Although the degree of maximal stimulation by NaF and NaN_3 may vary 2.5 - 3.5 fold in different plasma membrane preparations, equivalent stim-

* Abbreviation. App(NH)p, 5′-adenylylimidodiphosphate.



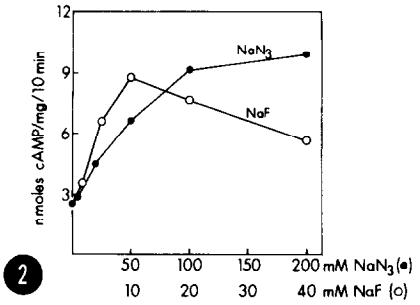


Figure 1. Additivity of Fluoride and Azide Stimulation of Plasma Membrane Adenylate Cyclase. Enzyme activity was determined in the absence (solid circles) or presence of 10 mM NaF (open circles), 100 mM NaN_3 (crosses) or 10 mM NaF + 100 mM NaN_3 (closed squares).

Figure 2. Effects of Fluoride and Azide on Plasma Membrane Adenylate Cyclase. Enzyme activity was determined in the presence of various concentrations of NaN_3 (closed circles) or NaF (open circles).

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ulation is obtained by 10 mM NaF and 100 mM NaN_3 and the stimulatory effects of the two ions are additive. Stimulation by sodium azide is specific for the azide ion since sodium chloride does not appreciably affect the enzyme.

Rat adipocyte plasma membrane contains a high specific activity Mg^{+2}-ATPase which is strongly inhibited by NaN_3 (8). Inhibition of ATPase by NaN_3 may result in higher concentration of ATP available for adenylate cyclase activity producing an apparent stimulatory effect on adenylate cyclase. However, in the presence of an ATP regenerating system the concentration of ATP was shown to remain constant during the course of adenylate cyclase reaction.

The use of App(NH)p as a substrate for adenylate cyclase further distinguishes between 1) direct stimulation of adenylate cyclase and 2) competition between ATPase and adenylate cyclase for the available substrate. The imido group between the terminal phosphate groups of App(NH)p prevents hydrolysis of this compound by ATPase and therefore provides a specific substrate for adenylate cyclase (9). The effect of NaN_3 on adenylate cyclase using ATP or App(NH)p as substrate is shown in Table I. Stimulation of the enzyme activity with ATP as substrate is higher than with App(NH)p but the marked stimulation in the pre-

TABLE I

Effect of Azide on Adenylate Cyclase with ATP or App(NH)p as Substrate

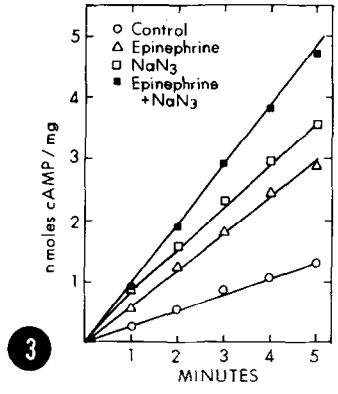
Addition	Percent of Control Activity	
	ATP	App(NH)p
None	100 (2.33)*	100 (1.17)
NaN_3, 20 mM	162	134
40 mM	205	180
60 mM	232	194
100 mM	272	201

* Values in parentheses represent control specific activities in nmoles cAMP/mg protein/5 min.

sence of the latter substrate indicates that azide affects adenylate cyclase directly and not by inhibition of ATPase activity.

One curious effect of fluoride on adenylate cyclase is the abolishment of the response of the enzyme to hormones. This phenomenon has been attributed to maximal stimulation of the catalytic activity (1, 2) or to uncoupling of the hormone receptor and the catalytic site by fluoride (4). In contrast to fluoride, however, hormone stimulation of the enzyme occurs in the presence of NaN_3 (Figure 3). The stimulatory effects of NaN_3 (100 mM) and epinephrine (10 ug/ml) are at least partially additive (Figure 3). In the presence of 10 mM NaF no response of the enzyme to epinephrine (0.1 to 10 ug/ml) is observed whereas in the presence of 100 mM NaN_3, epinephrine produces a significant and concentration dependent stimulation of the activity (data not shown).

The effects of epinephrine and GTP on the adenylate cyclase activity in the absence and presence of fluoride or azide are shown in Figure 4. In each



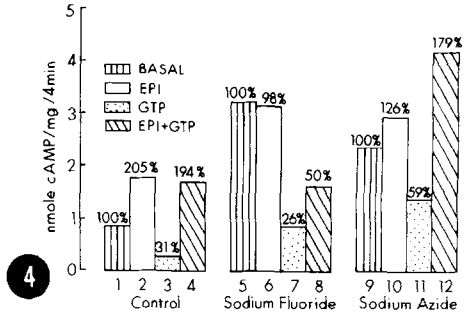


Figure 3. Additivity of Epinephrine and Azide Stimulation of Plasma Membrane Adenlyate Cyclase. Enzyme activity was determined in the absence (open circles) or presence of 10 ug/ml epinephrine (triangles), 100 mM NaN_3 (open squares) or 10 ug/ml epinephrine + 100 mM NaN_3 (closed squares).

Figures 4. Effects of Fluoride and Azide on Epinephrine Stimulation and GTP Inhibition of Plasma Membrane Adenylate Cyclase. Concentrations of reagents in the final assay mixture were: 10 ug/ml epinephrine, 10 uM GTP, 10 mM NaF and 100 mM NaN_3. In each set of columns activities are shown as percent of basal activity on the top of each column.

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group of columns relative activites are shown on top of the columns in percent of activity in the absence of epinephrine or GTP (basal). In the control experiment typical stimulation by epinephrine (column 2) and inhibition by GTP (column 3) are observed. Column 4 shows that in the presence of epinephrine the inhibitory effect of GTP is abolished. The lack of response to epinephrine in the presence of fluoride is seen by comparison of columns 5 and 6. This is in contrast to the persistant stimulation by epinephrine in the presence of azide (columns 9 and 10). Marked disparity of fluoride and azide is apparent in the effects of these ions on modulation of the activity by epinephrine and GTP. GTP inhibition of the basal activity persists in the presence of fluoride and azide (columns 3, 7 and 11). However, epinephrine reversal of GTP inhibition is prevented by fluoride (column 8) but not by azide (column 12). In fact the highest activity is observed in the presence of GTP, epinephrine and azide.

DISCUSSION

In light of the synergistic stimulation of adenylate cyclase by azide and

fluoride demonstrated in Figure 1 the contention of maximal stimulation of the enzyme by fluoride is apparently invalid. Consistant with previous suggestions (4) it appears that fluoride causes complete uncoupling of the hormone receptor and the catalytic site of the enzyme. Azide, however, does not appear to interfere with this coupling process although the possibility of partial uncoupling by azide can not be discounted at this time. Together with the comparative effects of fluoride and azide on the response of the enzyme to epinephrine and GTP, these results tend to suggest a more direct effect of azide than fluoride on adenylate cyclase.

The recent suggestion of the presence of phosphorylated and nonphosphorylated forms of adenylate cyclase (10, 11) offers an attractive working model for the control of this enzyme system. Further studies on the effects of azide on adenylate cyclase may prove beneficial in understanding of this complex control mechanism.

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