Inhibition by Fluoride Ion of Hormonal Activation of Fat Cell Adenylate Cyclase

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SUMMARY

The adenylate cyclase system in a plasma membrane-rich fraction termed “ghosts” from rat adipocytes is activated by epinephrine, adrenocorticotropic hormone, glucagon, and secretin, and is also stimulated in the presence of fluoride ion. Studies of the temperature dependency of activation by these agents showed that whereas hormonal activation occurs down to 10°, activation by fluoride ion is minimal below 25°. When the enzyme system is incubated at temperatures below 25° with combinations of fluoride and each of the hormones, no hormonal activation is observed. At higher temperatures, enhanced activity observed in the presence of combinations of fluoride and hormones is probably due to fluoride alone. Thus fluoride abolishes hormonal activation of the adenylate cyclase system. The inhibitory effect of fluoride ion is independent of the concentration of the hormone. The inhibitory effect is also independent of the concentration of either ATP or magnesium ion in the assay system. The concentration dependency of the inhibitory effect is similar to that required for fluoride activation of the system. These observations provide the first evidence that fluoride exerts some effect on a process related to hormone action on adenylate cyclase. It is suggested that fluoride acts at some point on the pathway by which hormonal interaction with the receptor leads to an increase in the catalytic activity of the enzyme. This process may involve the “coupling” between the receptor and catalytic components of this complex enzyme system.

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EXPERIMENTAL PROCEDURES

The sources of radiochemicals and other reagents were the same as those reported in previous publications from this laboratory (5, 8). Fat cells “ghosts” were prepared from fat cells isolated from epididymal fat pads of 175- to 200-g rats. The procedures used were essentially those described previously (5, 9) except that the medium used for lysing the fat cells consisted of 2.5 mM MgCl2, 0.1 mM CaCl2, 1 mM KHCO3, 1 mM mercaptoethanol, and 2 mM Tris-HCl, pH 7.4. Ghosts were incubated in medium containing 1 mM mercaptoethanol and 1 mM KHCO3 and were assayed for adenylate cyclase activity within an hour of preparation.

Adenylate cyclase activity was assayed by a modification (8) of the method of Krishna et al. (10). Ghosts were incubated in 50 μl of medium containing 5 mM MgCl2, 2.5 mM cyclic AMP, 0.1 or 1.0 mM [α-32P]ATP (specific activity not less than 50 cpm per pmole), 25 mM Tris-HCl, pH 7.4, and a regenerating system consisting of 20 μl creatine phosphate and 1 mg per ml of creatine kinase. Incubation time was 10 min; temperature was varied as indicated in the figures and table. Adenylate cyclase activity is expressed as picomoles of cyclic AMP formed per mg of protein in 10 min.

RESULTS AND DISCUSSION

The ability of fluoride ion to stimulate the activity of adenylate cyclase is one of the most interesting and still puzzling features of this membrane-bound enzyme system (1, 2). When tested on hormonally responsive adenylate cyclase systems, fluoride ion frequently induces activity that is similar to that given by hormones which exert the greatest effect on the system (3-5). Several studies have also shown that combinations of fluoride ion with hormones do not yield additive activities (3, 6). It has been assumed that maximal activity of the enzyme was induced by either fluoride or hormone and that further addition of either agent could not give added activity. An alternative explanation of the lack of additivity is that fluoride inhibits the activating effects of hormones at the same time as it exerts an independent stimulatory effect on the enzyme. Since fluoride stimulates enzyme activity under normal assay conditions at 30 or 37°, it was not possible to investigate the latter hypothesis. Now however conditions have been found under which stimulatory and inhibitory effects of fluoride ion can be differentiated. We have found that the multisreceptor adenylate cyclase system in rat fat cells (3, 7) does not respond to fluoride ion at low temperatures whereas the hormone response is maintained. When fluoride and hormones are incubated together at low temperatures with adenylate cyclase, fluoride completely inhibits the stimulatory effects of all the hormones. This finding will be discussed in terms of the possible inter-relationship between the mechanism of adenylate cyclase activation by hormones and fluoride.
when the incubation temperature is decreased from 37 to 30 °C whereas the stimulatory effect of ACTH on the system remains essentially unchanged. These findings have been extended in the present study by examining the effects of lower temperatures of incubation on both fluoride activation and activation of the fat cell system by glucagon, epinephrine, and ACTH. Fig. 1 shows that each of the hormones stimulates the enzyme system at temperatures as low as 10 °C whereas fluoride ion exerts virtually no stimulatory effect at this temperature and only begins to activate the system at temperatures above 25 °C. These findings provided a means of examining whether the stimulatory effect of fluoride on the system. The stimulatory effect of ACTH on the system remains essentially unchanged. These findings have been extended in the present study by examining the effects of lower temperatures of incubation on both fluoride activation and activation of the fat cell system by glucagon, epinephrine, and ACTH. Fig. 1 shows that each of the hormones stimulates the enzyme system at temperatures as low as 10 °C whereas fluoride ion exerts virtually no stimulatory effect at this temperature and only begins to activate the system at temperatures above 25 °C. These findings provided a means of examining whether the stimulatory effect of fluoride on the system. The stimulatory effect of ACTH on the system remains essentially unchanged. These findings have been extended in the present study by examining the effects of lower temperatures of incubation on both fluoride activation and activation of the fat cell system by glucagon, epinephrine, and ACTH. Fig. 1 shows that each of the hormones stimulates the enzyme system at temperatures as low as 10 °C whereas fluoride ion exerts virtually no stimulatory effect at this temperature and only begins to activate the system at temperatures above 25 °C. These findings provided a means of examining whether the stimulatory effect of fluoride on the system.

Fig. 2 shows the effect of fluoride on the dose response to epinephrine at 20, 25, and 30 °C. No stimulatory effect of fluoride was observed at 20 °C (Panel B) whereas increasing doses of epinephrine caused increased activation of the enzyme. When fluoride was added to the medium with epinephrine, the response to epinephrine was inhibited at each concentration of hormone tested; the activity was that observed in the presence of fluoride alone. At 25 and 30 °C (Panels B and C), the stimulatory effect of fluoride alone became apparent, but again, the activity seen in the presence of combinations of fluoride and epinephrine was always that seen with fluoride alone. At temperatures above 30 °C (not shown), activity observed in the presence of fluoride alone was the same as that observed with combination of fluoride and epinephrine (20 μg per ml), as has been shown previously for the fat cell system (3). As shown in Table 1, this property of fluoride to inhibit epinephrine activation was observed for each of the hormones acting on the system. Fluoride inhibited the action of epinephrine, ACTH, and secretin at maximally stimulating concentrations of the hormones under conditions (25 °C) in which fluoride exerted slight stimulatory effects on the enzyme. Since activation by glucose and fluoride were similar, it was not possible to observe inhibition of the glucagon response. However, at 20 °C, fluoride did abolish the glucagon action (not shown).

The following conclusions can be drawn from the above findings: (a) fluoride stimulates the enzyme system in a temperature-dependent manner; (b) fluoride inhibits the actions of hormones at low temperatures and, by extrapolation, at higher temperatures as well; and (c) the inhibitory effect of fluoride is not competitive with respect to the concentration of hormone and it is not related to the specific type of receptor through which the hormones independently stimulate the adenylate cyclase system (3, 7).

The observation that fluoride does not inhibit the binding of glucagon to its receptor in hepatic plasma membranes (11) may be taken as further evidence for the last conclusion above. It appears, therefore, that the inhibitory effect of fluoride on hormone action is not exerted at the step of hormone-receptor interaction.
Inhibition by fluoride of enzymes requiring divalent cations for activity has been attributed to formation of fluoride complexes with the metal ions or, if the enzyme catalyzes reactions involving inorganic or organic phosphates, to the formation of metallophosphate complexes with fluoride (12). Since adenylate cyclase requires both ATP and magnesium for activity, the enzyme falls into the latter category in any consideration of the inhibitory effects described above. The enzyme in the fat cell system is particularly sensitive to changes in magnesium ion, the most effective cation for hormone action on this system (5). It is therefore probable that the inhibition of fluoride activation by high doses of fluoride (see Fig. 3) can be accounted for by the removal of magnesium by fluoride ion when the latter is present in large molar excess.

To examine the possibility that inhibition of hormonal response by fluoride is also related to removal of magnesium or to the formation of fluoride-magnesium-phosphate complexes, we tested the effects of varying the ratio of magnesium to ATP on the basal activity and the response of the enzyme to fluoride and hormones added individually or combined. Table I shows that at constant magnesium concentration (5 mM), inhibition of hormone action (epinephrine, ACTH, and secretin) by fluoride was effective in the presence of either 1.0 or 0.1 mM ATP. For each concentration of ATP, the activity observed in the presence of fluoride remained unchanged in the absence or presence of hormones. Fig. 4 illustrates the effect of varying the magnesium concentration over a 4-fold range at a fixed concentration of ATP. At any given concentration of magnesium the activation produced by each of the hormones alone was inhibited in the presence of fluoride ion. Increasing the concentration of magnesium resulted in enhanced adenylate cyclase activity in the presence or absence of the hormones or fluoride, as reported previously (5). Thus, fluoride inhibits the actions of the hormones under all conditions of incubation. In the absence of hormones but under otherwise identical incubation conditions fluoride exerted a small stimulatory effect which indicates that the inhibitory effects of fluoride on hormone action are not due to impaired utilization of ATP or magnesium at the catalytic site.

The observations reported above suggest that fluoride ion inhibits the actions of the various hormones acting on the fat cell adenylate cyclase system by a process that is not due to inhibition of substrate utilization at the catalytic site nor to inhibition of hormone interaction with their receptors. It is likely, therefore, that fluoride inhibits at some step intervening between hormone-receptor interaction and the process of hormonal activation of the enzyme; i.e., fluoride “uncouples” the process of hormonal activation subsequent to hormone-receptor interaction. Although inhibition of hormone action by fluoride has not been reported for other adenylate cyclase systems, the observation that fluoride inhibits the effects of four hormones, operating through distinct receptors (3, 7) on the fat cell system, suggests that this phenomenon might occur generally. It should not be concluded that maximal activity of adenylate cyclase has been achieved in those experiments in which combinations of fluoride and hormone fail to give more activity than that given by fluoride alone. The results reported herein suggest that such experiments might be based on an untenable supposition if fluoride ion does indeed inhibit hormonal activation in other adenylate cyclase systems.

The present studies provide the first evidence that fluoride exerts some effect on a process related to hormone action on adenylate cyclase. Until now, comparisons between activation of adenylate cyclase by hormones and fluoride have stressed that, although the kinetic data of substrate utilization at the catalytic site are similar (5, 13), the properties of activation were sufficiently different to suggest that hormones and fluoride were acting through completely independent processes (4, 5). For example, on ghosts prepared from trypsin-treated cells stimulation of adenylate cyclase by polypeptide hormones is decreased, whereas the fluoride stimulation is not affected, suggesting that fluoride acts directly on the catalytic component (7). Treatment of fat cell ghosts or of liver membranes (4) with detergents or phospholipases leads to selective losses in hormone response, suggesting that lipids are required for hormone action but not for stimulation of adenylate cyclase by fluoride. Recently it has been found that the effects of fluoride on adenylate cyclase are reversed by ouabain (14). This may indicate that the effects of fluoride are mediated by a mechanism which is similar to that by which sodium ions and ouabain restore the activity of adenylate cyclase.

**Figure 3** shows the effect of the concentration of fluoride ion on the hormonal stimulation of adenylate cyclase activity of fat cell “ghosts.” “Ghost” protein (35 μg) was incubated in the presence of 1.0 mM ATP with regenerating system essentially as described in the legend to Fig. 1. Incubation was for 10 min at 25°C, and the concentration of epinephrine and secretin was 20 μg per ml.

**Figure 4** illustrates the effect of varying the magnesium concentration on fluoride inhibition of hormonal stimulation of fat cell “ghost” adenylate cyclase activity. “Ghost” protein (21 μg) was incubated in the presence of 1.0 mM ATP with regenerating system essentially as described in the legend to Fig. 1. Incubations were for 10 min at 25°C. The concentration of fluoride ion was 10 mM, and epinephrine, ACTH, secretin, and glucagon were present at a final concentration of 20 μg per ml.

![Figure 3](https://example.com/fig3.png)

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**Figure 4.** Effect of magnesium concentration on fluoride inhibition of hormonal stimulation of fat cell “ghost” adenylate cyclase activity. “Ghost” protein (21 μg) was incubated in the presence of 1.0 mM ATP with regenerating system essentially as described in the legend to Fig. 1. Incubations were for 10 min at 25°C. The concentration of fluoride ion was 10 mM, and epinephrine, ACTH, secretin, and glucagon were present at a final concentration of 20 μg per ml.
been proposed (14) that acidic phospholipids are specifically involved in the interaction of the histidine residue of glucagon to a regulatory site on the glucagon-sensitive adenylate cyclase system; fluoride activation does not require these lipids.

Although fluoride and hormones have differing requirements for activation of adenylate cyclase, the possibility raised from the present findings is that fluoride, in the process of "uncoupling" hormone action, converts the enzyme into a state of activity comparable to that given by the hormones. This conversion is temperature-dependent and occurs by a process not involving lipid participation. This hypothesis is supported by the data in Fig. 2 which provide suggestive evidence that fluoride stimulates adenylate cyclase activity and inhibits hormone response simultaneously. Furthermore, the stimulatory and inhibitory effects of fluoride occur over a comparable dose-response range as shown in Fig. 3. Thus, the sensitivity of both processes to fluoride is comparable. The major difference observed is that the stimulatory effect of fluoride requires increasing temperatures of incubation.

Further experimentation is clearly required for understanding the possible inter-relationship between the "uncoupling" and stimulatory actions of fluoride on adenylate cyclase. Viewed from the perspective that fluoride may act through a process common to hormone action, one can no longer consider that the mechanism by which fluoride activates the system is completely independent of that by which hormones operate. Future studies of fluoride action, hitherto considered to be focused primarily on the catalytic component, may give new insights into the mechanism of hormone action.

REFERENCES

Inhibition by Fluoride Ion of Hormonal Activation of Fat Cell Adenylate Cyclase
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