BASIC AMINO ACIDS STIMULATE THE ACTIVATION OF THE GLUCOCORTICOID–RECEPTOR COMPLEX

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1. Introduction

Rat liver glucocorticoid receptor has been shown to require 'activation' after complexing with the steroid in order to bind to nuclei [1] or to DNA-cellulose [2]. Activation of the complex can be accomplished not only by heating but also by exposure at 0°C to various agents such as high ionic strength [3], calcium ions [2] and theophylline [4]. Dilution initiates slow activation [5,6] and evidence for the participation of a macromolecule [7] or a small molecule [6] as an inhibitor of the process has been presented. Recent evidence also indicates that the use of Tris buffer increases the level of activation much more than phosphate buffer during dilution or ion exchange chromatography [8]. In this report we show that activation of glucocorticoid receptor can be stimulated by basic amino acids and other primary amines. The amount of stimulation is dependent on the pH of the cytosol.

2. Materials and methods

Cytosol from adrenalectomized Sprague-Dawley male rats were prepared as previously described [4] from 50% homogenates with either TSM buffer (50 mM Tris, 0.25 M sucrose, 3 mM $MgCl_2$; pH 7.5, at 20°C) or with PSM buffer (20 mM potassium phosphate, 0.25 M sucrose, 3 mM $MgCl_2$; pH 6.8) and incubated for 2 h at 0–4°C with 30 mM [^{3}H]dexamethasone (Amersham/Searle, 28.6 Ci/mmol). Steroid binding to glucocorticoid receptor was determined by the dextran-coated charcoal procedure [4]. Radioactivity was measured by liquid scintillation spectrometry as previously described [9]. Receptor was activated by heating the cytosol for 30 min at 25°C, by a 5-fold dilution with buffer at 0°C or by the addition of reagents as indicated in the table legends. Receptor activation was assayed by measuring its ability to bind to DNA-cellulose [6]. L-Amino acids and other reagents were obtained from Sigma.

Abbreviation: Tris, Trishydroxymethylaminomethane

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3. Results

3.1. *Activation by Tris buffer*

As previously reported [6,8], dilution of liver cytosol with Tris buffer results in a dramatic increase in the degree and rate of activation of the glucocorticoid–receptor complex. That the level of activation is, at least in part, due to the Tris molecule is shown in table 1. Dilution of Tris-buffered cytosol with Tris or phosphate buffer at comparable pH-values resulted in consistently higher levels of activation when the diluent was Tris. The degree of activation was enhanced at the more basic pH for each buffer. Neither dilution with water alone nor 50 mM KCl caused an increase in activation (data not shown). These findings suggest that the Tris molecule itself may have a stimulatory effect on the activation process. When cytosols were prepared with PSM instead of TSM, the amount of activation produced by dilution was further reduced (data not shown).

The conclusion that phosphate is not inhibitory to activation is supported by the results shown in

Table 1
pH Effect on activation

Sample	pH	% Receptor complex bound to DNA–cellulose
Undiluted control	6.9	2.4
Diluted 1:5 (a) TSM	6.9	16
(b) TSM	7.5	30
(c) PSM	6.9	5
(d) PSM	7.5	13

A 50% cytosol of liver in TSM was labeled with [^{3}H]dexamethasone diluted as shown. The pH of the resulting samples was as indicated. Samples were assayed for steroid binding and DNA–cellulose binding 24 h after dilution

table 2. Activation by heat treatment can be achieved with undiluted cytosol prepared in phosphate buffer, the level of activation again being dependent on pH. Table 2 also shows that activation by heat treatment is stimulated by the presence of Tris at the lower pH.

3.2. *Activation at 0°C by amino acids*

Amines other that Tris were screened for effects on receptor activation. In particular, basic amino acids

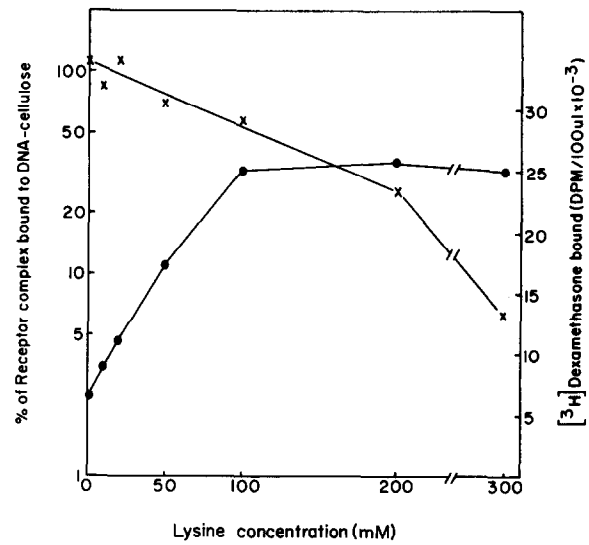

Fig.1. Effect of lysine concentration on steroid binding and receptor activation. Measured quantities of lysine–HCl were added to labeled, PSM-buffered cytosol to yield final lysine concentrations of 10, 20, 50, 100, 200 and 300 mM. After disolving the lysine, pH-values were readjusted at 0°C to 7.3 and incubated 24 h in an ice bath. Samples were then assayed as in table 1. (X) [^{3}H]Dexamethasone bound; (•) % Receptor complex bound to DNA–cellulose.

Table 2
Effect of buffer type and pH on heat activation

Sample	Final pH	Treatment	% Steroid–receptor complex bound to DNA–cellulose
PSM cytosol	6.8	0°C	0.4
		25°C	10.2
	7.8	0°C	3.1
		25°C	53
TSM cytosol	6.8	0°C	2.2
		25°C	53
	7.8	0°C	5.9
		25°C	54

Labeled cytosols prepared with either TSM or PSM were adjusted to the indicated pH at 0°C with concentrated HCl or 1 M KOH and aliquots then heat activated. Samples were then assayed as in table 1

Table 3
Amino acid activation in PSM buffer

Sample	pH	% Steroid–receptor complex bound to DNA–cellulose
Control	6.5	0.8
Control (after pH change)	7.3	2.4
+ Additions		
Alanine	7.3	2.4
Alanine (100 mM)	7.3	2.3
Monosodium glutamate	7.3	3.9
Lysine–HCl	7.3	11
Arginine–HCl	7.3	9.6
KCl	7.3	3.7
KCl (200 mM)	7.3	20

Labeled, PSM-buffered cytosol (at pH 6.5) was adjusted to pH 7.3 with KOH. The additions were made as 1/10 vol. dilutions of stock reagents giving a final concentration of 50 mM, except where indicated. Samples were assayed after 24 h as in table 1

were found to have stimulatory affects on receptor activation (table 3). At all pH levels (only pH 7.3 data are shown) there was an increase of receptor activation at 0°C in the presence of 50 mM lysine or arginine but not alanine or glutamate. However, the degree of activation increased markedly with increasing pH.

Since 50 mM KCl did not enhance activation, it seems unlikely that the stimulatory effect of the basic amino acids is due solely to an increase in ionic strength. The concentration dependence for receptor activation by lysine is shown in fig.1. An approximately logarithmic increase in activation levels occurs at lower concentra-

Table 4
Amine stimulation of the heat activation process

Sample	Temperature	% Steroid–receptor complex bound to DNA–cellulose
Series 1		
Control	0°C	0.28
Control	25°C	4.8
+ Tris	25°C	14
+ Lysine · HCl	25°C	22
+ Ornithine · HCl	25°C	22
+ Urea	25°C	5.6
+ Cytidine	25°C	5.4
Series 2		
Control	0°C	0.50
Control	25°C	11
+ Tris	25°C	30
+ Lysine · HCl	25°C	37
+ Ethanolamine · HCl	25°C	42

Labeled, PSM-buffered cytosol (pH 6.8) was diluted with 0.5 M solutions of the indicated reagents which had been dissolved in PSM and adjusted to pH 6.8. The final concentration of all reagents was 50 mM. Samples were heat activated at 25°C for 30 min and assayed as in table 1

tions of lysine with an apparent plateau reached above 100 mM lysine.

3.3. *Influence of amines on heat activation*

The effectiveness of heat activation, at physiological pH levels, was greatly increased not only by Tris but also by other charged amines, including lysine, ornithine and ethanolamine (table 4). Neither cytidine, which has an uncharged amine at physiological pH, nor the amides urea of glutamine (not shown) were able to stimulate the heat activation of receptor.

4. Discussion

One possible mechanism of receptor activation by amines is to cause the exposure of positive residues on the receptor protein by removing an inhibitor bound to these sites. A low molecular weight inhibitor has been proposed to explain activation by dilution and gel filtration [6]. The basic amines could form a complex with such an inhibitor, thus favoring its dissociation from the unactivated receptor. These basic amines may act by competing with the receptor for binding by reversible formation of a Schiff's base with a carbonyl compound such as pyridoxal phosphate, which would occupy the DNA binding site of the receptor protein in the unactivated state. Indeed pyridoxal phosphate can reversibly prevent activated receptor from binding to DNA cellulose [10]. In this context, since Tris is a reactive primary amine and forms a Schiff's base with pyridoxal phosphate [11], the stimulatory effect of Tris on receptor activation becomes understandable.

The data presented in this report are consistent with a model in which a low molecular weight inhibitor of activation is a negatively charged compound which interacts with the receptor protein via a Schiff's base. Even if the basic amines activate in some other manner, the use of Tris buffer should be avoided in order to maintain the receptor in the unactivated state.

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