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Short Communication

EFFECT OF GENISTEIN ON BOTH BASAL AND GLUCAGON-INDUCED LEVELS OF cAMP IN RAT HEPATOCYTES

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Abstract—Using rat liver hepatocytes, we studied the effect of the tyrosine kinase inhibitor genistein on the Ca^{2+}/IP_3 (inositol 1,4,5-trisphosphate) and the cAMP (adenosine 3:5-cyclic monophosphate) transduction mechanisms. Genistein specifically blocked the activation of glycogen phosphorylase after EGF (epidermal growth factor). Genistein on its own partially activated phosphorylase and inactivated glycogen synthase. Genistein did not influence levels of IP_3, but increased those of cAMP. This was especially clear when genistein was given together with glucagon. The data suggest an effect of a tyrosine kinase on the synthesis/degradation of cAMP.

Key words: genistein; cAMP; IP_3; hepatocytes; EGF, glucagon

Rat hepatocytes respond to a great number of hormones and/or agonists exerting many different effects. These range from the control of fast, mostly transient metabolic processes to more lasting effects on cell cycle or protein synthesis. The agonists can be classified into different groups according to the transduction mechanism they use to produce a biological effect. One group is linked to adenylate cyclase and increases cAMP.* Other agonists increase IP_3 and diacylglycerol as second messengers. IP_3, diacylglycerol, and (especially) cAMP are now considered the "classical" second messengers. An alternative and recently discovered transduction mechanism is used by yet another group of hormones (e.g. growth factors), and consists of a direct stimulation by the agonist of a tyrosine kinase, eventually leading to the biological effect associated with these agonists (see [1] for references).

During our studies on the biological effects of EGF in liver, we used genistein as an inhibitor of tyrosine kinase (see [2] for references). We report here that, apart from preventing the activation of phosphorylase by EGF, genistein also increases both basal cAMP levels and those observed after glucagon.

Materials and Methods

Compounds. EGF and genistein were from Sigma Chemical Co., St. Louis, MO (U.S.A.). The sources of other chemicals were given previously [3].

Isolation of hepatocytes. We used male Wistar-strain albino rats (200–250 g body weight) that were fed *ad libitum*. Liver cells were isolated and incubated in a Krebs-Henseleit bicarbonate buffer equilibrated with O_2/CO_2 (19:1, v/v) as previously described [4], but without bacitracin.

Enzyme and metabolite assay. Glycogen phosphorylase activity was determined as described [4]. Adenosine 3′:5′-cyclic monophosphate (cyclic AMP) and IP_3 were measured with a competitive protein-binding technique by using assay kits from the Radiochemical Centre (Amersham, Bucks, U.K.). Glycogen synthase activity was determined as described [5].

Results and Discussion

We first tested the specificity of genistein by treating hepatocytes with different glycogenolytic agonists and studying their effect in the presence and absence of genistein. Table 1 summarises these data. From this table it is clear that (a) genistein specifically inhibits EGF and (b) genistein on its own slightly activates glycogen phosphorylase.

The partial activation of phosphorylase is rapid in onset (maximum after approx. 2 min) and declines slowly (not illustrated). Table 2 shows the activation of phosphorylase 2 min after genistein. This table further reveals that genistein is also able to inactivate glycogen synthase (in the end, genistein leads to a quasi complete inactivation of synthase). Similarly, genistein slows down the glucose-induced inactivation of glycogen phosphorylase (active in freshly isolated hepatocytes), and prevents the activation of synthase under this condition (Fig. 1). It should be mentioned that the effect of genistein on synthase may be a secondary phenomenon, possibly linked to the relative presence of active phosphorylase. It has indeed been shown that active phosphorylase is an inhibitor of glycogen synthase phosphatase, catalysing the activation of synthase [6].

We next investigated whether the effect of genistein was mediated through increased levels of either IP_3 or cAMP. Since there was no interference with the effects of ATP or vasopressin, genistein does not seem to impair the IP_3/Ca^{2+} system.

Table 1. Effect of genistein on the activation of phosphorylase by EGF, ATP, vasopressin, and glucagon in rat hepatocytes

	Phosphorylase (% of control)	
	(–)	(+) Genistein
Control	100	126 ± 7 (13)
EGF	173 ± 10 (10)	129 ± 9 (9)
ATP	168 ± 28 (5)	166 ± 25 (5)
Vasopressin	176 ± 25 (5)	171 ± 25 (5)
Glucagon	181 ± 20 (5)	196 ± 24 (5)

Cells were preincubated for approx. 30 min with 10 mM glucose at 37°C and then challenged without or with 800 μM genistein. Ten min thereafter, these cells were treated for 1 min with EGF (0.5 μg/ml), glucagon (20 nM), vasopressin (20 nM), or for 20 sec with ATP (10 μM). Phosphorylase values are expressed as % of the respective controls in the different (n) experiments.

* Abbreviations: EGF, epidermal growth factor; IP_3, inositol 1,4,5-trisphosphate; cAMP, adenosine3:5-cyclic monophosphate.

Table 2. Activation of glycogen phosphorylase and inactivation of glycogen synthase by genistein

	Phosphorylase (% of control)	Synthase (% of control)
Control	100	100
Genistein	131* ± 4	51* ± 13

Hepatocytes were preincubated for ±25 min with 30 mM glucose. They were then challenged with or without 800 μM genistein. Two min thereafter, samples were taken for the assay of phosphorylase and synthase.

Phosphorylase: data are from 9 independent experiments. Basal ± SEM activity of phosphorylase was 60 ± 14 mU/mg of protein amounting to 89* ± 16 mU/mg of protein 2 min after genistein.

Synthase: Data are from 4 different experiments. Since the degree of activation of synthase varied considerably from one preparation to another (factor 4), proportional values are given (the starting value is taken as 100).

* Indicates a significant difference from the control values (paired student t-test; $P < 0.05$).

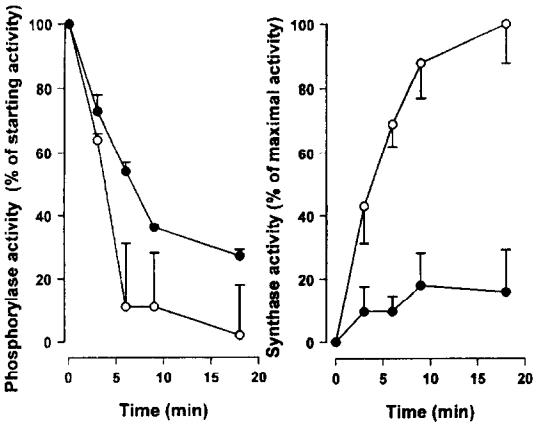

Fig. 1. Effect of genistein on the inactivation of phosphorylase and the activation of synthase in rat hepatocytes. Hepatocytes were taken immediately after isolation. These cells contain active phosphorylase and inactive synthase. The cells were incubated at 37°C in the presence of 30 mM glucose and with (closed symbols) or without 800 μM genistein. Phosphorylase and synthase activity were measured at the indicated times. Data are expressed as means (+ or − SEM) of the proportional inactivation of phosphorylase (starting value = 100, complete inactivation = 0) or proportional activation of synthase (starting value = 0, maximal activity = 100) of three independent experiments.

Indeed, treatment of hepatocytes with genistein did not change the IP_3 levels, either after 10 min (Table 3)—a time at which genistein exerted a maximal inhibition of the EGF effect—or after shorter treatment times (not illustrated). Table 2 further shows that genistein increased cAMP levels in hepatocytes. Basal levels were slightly but significantly increased, which possibly accounts for the effects on phosphorylase and synthase. This effect of genistein was even clearer in the presence of glucagon (Table 3).

Table 3. Effect of genistein on levels of IP_3 and cAMP

	IP_3 (pmoles/mg protein)	cAMP (pmoles/mg of protein)	
		(−)	+ Glucagon
Control	15.6 ± 3.6	1.95 ± 0.23	22.20* ± 2.53
Genistein	14.1 ± 2.4	2.46* ± 0.30	40.10† ± 5.44

IP_3: Cells were incubated in the presence or absence of 800 μM genistein for 10 min. Samples (in triplicate) for the assay of IP_3 were then taken. The values are means ± SEM from 11 independent experiments.

cAMP: After a preincubation of approx. 20 min, hepatocytes were treated with or without 50 nM glucagon for 1 min. They were then challenged or not with 800 μM genistein. One min later, samples (in dupli- or triplicate) were taken for the assay of cAMP. The results are the means ± SEM from 12 independent experiments.

* Significant difference from control values (paired student t-test; $P < 0.01$).

† This value is not only different from control (1.95), but also significantly different ($P < 0.01$) from the cAMP value observed after glucagon under control conditions (22.20).

These data clearly show that (a) genistein specifically inhibited the EGF activation of glycogen phosphorylase, and (b) increased the levels of cAMP, possibly explaining its effect on glycogen phosphorylase and synthase.

Whether or not cAMP is increased by inhibiting a tyrosine kinase or is the consequence of an aspecific effect of genistein is not clear from the data. Some aspecific effects of genistein have indeed been reported by Young *et al.* [2], but since genistein specifically counteracted the EGF effect (and not that after vasopressin, ATP, or glucagon), the data suggest an as yet unknown and certainly indirect tyrosine kinase effect on the synthesis/degradation of cAMP.

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