Angiotensin II inhibits hepatic cAMP accumulation induced by glucagon and epinephrine and their metabolic effects

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Incubation of isolated hepatocytes containing normal Ca^{2+} levels with angiotensin II, vasopressin or A23187 caused significant inhibition of the cAMP response to glucagon. Angiotensin II also inhibited cAMP accumulation induced by either glucagon or epinephrine in Ca^{2+}-depleted hepatocytes. When submaximal doses of hormone were employed such that cell cAMP was elevated only 3–4-fold (~2 pmol cAMP/mg wet wt cells) inhibition by angiotensin II was correlated with a decrease in phosphorylase activation. The data demonstrate that inhibition of hepatic cAMP accumulation results in reduced metabolic responses to glucagon and epinephrine and do not support the contention that the hepatic actions of glucagon are independent of cAMP.

Hepatocyte *Glucagon* *Angiotensin II* *cAMP* *Phosphorylase* *Gluconeogenesis*

1. INTRODUCTION

Epinephrine, angiotensin II and vasopressin can inhibit glucagon induced cAMP accumulation in liver [1–4]. However, controversy has arisen concerning the ability of these hormones to inhibit the physiological responses induced by glucagon. Using rat hepatocytes containing normal Ca^{2+} levels, we reported that epinephrine, angiotensin II and vasopressin were able to inhibit glucagon-stimulated conversion of [^{14}C]lactate to [^{14}C]glucose [2]. However, in Ca^{2+}-depleted hepatocytes physiological responses induced by glucagon were unaffected by angiotensin II despite a marked reduction in cAMP levels [4]. This led to the proposition that, in hepatocytes, 'the effect of glucagon on cAMP levels can be dissociated from its metabolic effects' [4]. Here, we investigate the apparent discrepancy between these conclusions. We demonstrate that in Ca^{2+}-depleted hepatocytes angiotensin II is able to inhibit both glucagon- and epinephrine-stimulated cAMP accumulation and phosphorylase activation if submaximal doses of agonists are used and cAMP levels are < 2 pmol/mg wet wt cells.

2. EXPERIMENTAL

Parenchymal cells from the livers of fed male Sprague–Dawley rats (body wt 200–220 g) were isolated and incubated as in [5]. Calcium-depleted hepatocytes were prepared by washing and incubating the cells in Krebs–Henseleit bicarbonate buffer containing 1 mM EGTA without added Ca^{2+} [6]. Methods for the measurement of phosphorylase *a* and cAMP levels have been detailed in [5–7].

(−)Epinephrine bitartrate, angiotensin II and arginine–vasopressin were from Sigma. Glucagon and A23187 were gifts from Lilly (IN). α-D-[U-^{14}C]Glucose-1-P was from New England Nuclear (MA).

3. RESULTS

The data in fig.1 show the effect of 10^{-8} M angiotensin II on the dose response for elevation of cAMP and activation of phosphorylase by glucagon, in Ca^{2+}-depleted hepatocytes. Under these conditions angiotensin II itself does not activate phosphorylase (fig.1A). Glucagon at 5 ×

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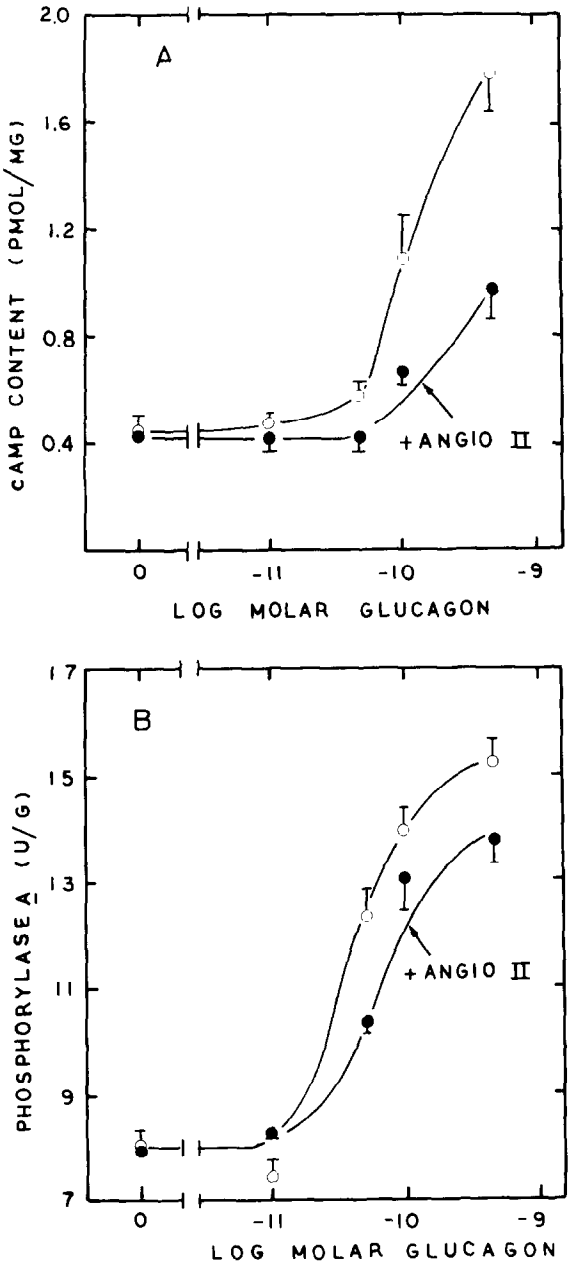

Fig.1. Effect of angiotensin II on cAMP accumulation (A) and phosphorylase activation (B) induced by glucagon in Ca^{2+}-depleted hepatocytes. Isolated hepatocytes were incubated with increasing doses of glucagon in the presence or absence of angiotensin II (Angio II, 10^{-8} M). After 2 min, samples were removed for determination of cAMP and phosphorylase *a*. Values are means ± SEM of triplicate incubations each assayed in duplicate from an experiment representative of 2.

10^{-10} M raised cAMP to ~1.8 pmol/mg wet wt of cells (fig.1A) and produced maximum activation of phosphorylase (fig.1B). Angiotensin II was able to inhibit cAMP accumulation and phosphorylase activation at all concentrations of glucagon used.

A similar inhibition of cAMP accumulation and phosphorylase activation was observed when (−)epinephrine was used as the stimulus in Ca^{2+}-depleted hepatocytes (fig.2A,B). In the Ca^{2+}-depleted situation, epinephrine acts via both β_2- and α_1-adrenergic receptors to elevate cAMP [6,8].

In hepatocytes containing normal levels of Ca^{2+}, angiotensin II, vasopressin and A23187 inhibited the ability of both glucagon and exogenously added cAMP to stimulate synthesis of [^{14}C]glucose from [^{14}C]lactate [2]. Hepatocyte cAMP levels were measured in some, but not all, of those experiments. The data in table 1 show that angiotensin II, vasopressin and A23187 were all able to inhibit hepatic cAMP accumulation elicited by glucagon. However, cAMP levels under these conditions were > 2 pmol/mg wet wt of cells, which is enough to stimulate gluconeogenesis maximally [5]. Thus, other factors besides cAMP must have been responsible for the inhibition of gluconeogenesis observed in these studies [2].

4. DISCUSSION

Controversy has arisen concerning the ability of the hormone angiotensin II to inhibit physiological responses induced by glucagon in hepatocytes. Cardenas-Tanus et al. have claimed that angiotensin II is able to inhibit glucagon induced cAMP accumulation in hepatocytes without modulating physiological responses to this hormone [4]. However, these workers used high concentrations of glucagon which are known to induce cAMP levels in liver far in excess of those needed to maximally activate physiological processes [9]. As we demonstrate here, when low doses of glucagon are employed such that cAMP levels are ≤2.0 pmol/mg cells, the inhibitory effect of angiotensin II on cAMP shows a close correlation with the extent of phosphorylase activation. Inspection of the data in [4] reveals that, in their experiments, hepatic cAMP was still elevated by 6.4-fold (2.3 ± 0.23 pmol/mg cells) in the presence of angiotensin II. As demonstrated here (fig.1) and

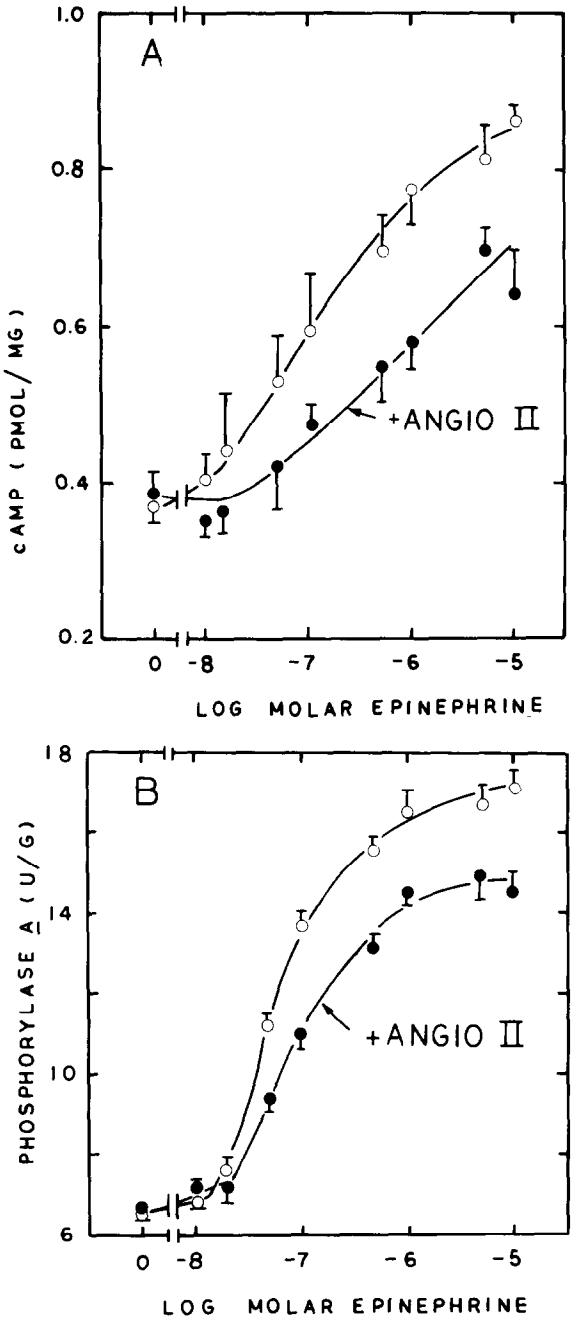

Fig.2. Effect of angiotensin II on cAMP accumulation (A) and phosphorylase activation (B) induced by epinephrine in Ca^{2+}-depleted hepatocytes. Isolated hepatocytes were incubated with increasing doses of epinephrine in the presence or absence of angiotensin II (Angio II, 10^{-8} M). After 2 min, samples were removed for determination of cAMP and phosphorylase *a*. Values are means ± SEM of triplicate incubations each assayed in duplicate from an experiment representative of 2.

Table 1

Effects of angiotensin II, vasopressin and A23187 on glucagon induced cAMP formation in Ca^{2+}-replete hepatocytes

Additions to incubation medium	Conc. (M)	cAMP level (pmol/mg wet wt cells)
Saline		0.53 ± 0.09
Angiotensin II	10^{-8}	0.50 ± 0.03
Vasopressin	10^{-8}	0.56 ± 0.01
A23187	10^{-5}	0.61 ± 0.07
Glucagon	10^{-9}	3.65 ± 0.39
Glucagon + vasopressin	10^{-9} 10^{-8}	2.77 ± 0.24[a]
Glucagon + angiotensin II	10^{-9} 10^{-8}	2.54 ± 0.14[b]
Glucagon + A23187	10^{-9} 10^{-5}	1.97 ± 0.17[c]

[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.005$ *vs* glucagon alone

Isolated hepatocytes were incubated as shown for 1 min when samples were removed for cAMP determination. Values are means ± SEM of triplicate incubations each assayed in duplicate, from an experiment representative of 2

in [5], this level of cAMP is sufficient to cause maximal activation of phosphorylase and gluconeogenesis. We contend, therefore, that valid support for the suggestion that the hepatic actions of glucagon are independent of cAMP is not provided in [4].

The ability of angiotensin II to lower cAMP in Ca^{2+}-depleted hepatocytes is probably due to an inhibition of adenylate cyclase [1,3]. This is supported by the observation that cAMP increases induced by either glucagon or epinephrine are inhibited by angiotensin II (fig.1,2). Since the former two hormones have independent receptors mediating adenylate cyclase activation in liver cells [10–12] it seems unlikely that angiotensin II would exert its influence directly at the receptor level. Indeed in juvenile hepatocytes, where epinephrine-induced cAMP elevation is mediated entirely by β_2-adrenergic receptors [8] (rather than by both β_2- and α_1-receptors as in the Ca^{2+}-depleted situation [6,8]) angiotensin II also inhibits the response (not

shown). In addition, angiotensin II was unable to inhibit phosphorylase activation elicited by exogenously added cAMP (not shown), suggesting that its action must be at the level of cAMP generation.

In Ca^{2+}-replete hepatocytes, angiotensin II, vasopressin, A23187 and epinephrine can all inhibit gluconeogenesis from [U-^{14}C]lactate [2]. However, cAMP levels were not measured under all conditions in that study. Under similar conditions the glucagon-induced elevation of hepatic cAMP was markedly inhibited by each of these agents (table 1), although cAMP levels still exceeded 2 pmol/mg wet wt cells, a value which would be sufficient to cause maximal stimulation of gluconeogenesis [5]. Furthermore, these agents were also able to inhibit the gluconeogenic effects of exogenous cAMP, a response which would not be expected if they were acting only to inhibit adenylate cyclase. It was proposed that changes in Ca^{2+} redistribution might account for the inhibition, since it is known that Ca^{2+} can regulate the gluconeogenic pathway [13]. When a cAMP stimulus (e.g., glucagon) is combined with a Ca^{2+} mobilising hormone (e.g., epinephrine, angiotensin II and vasopressin) net cellular Ca^{2+} influx is the result [14]. This can be accounted for by a net increase in mitochondrial Ca^{2+} content [14] and we propose that this phenomenon may then be responsible for the observed inhibition of gluconeogenesis [2]. Since [4] was done with Ca^{2+}-depleted hepatocytes, this would account for the failure to observe inhibition of gluconeogenesis by angiotensin II when cAMP levels were still elevated.

Our data support the following conclusions:

(1) In Ca^{2+}-depleted hepatocytes angiotensin II inhibits glucagon and epinephrine stimulated cAMP accumulation by inhibiting adenylate cyclase;

(2) If submaximal doses of hormone are used, inhibition of phosphorylase by angiotensin II is observed, suggesting that elevation of cAMP can entirely account for the glycogenolytic action of glucagon and epinephrine in Ca^{2+}-depleted hepatocytes;

(3) In Ca^{2+}-replete hepatocytes epinephrine, vasopressin, angiotensin II and A23187 all lower glucagon mediated increases in cAMP and stimulation of gluconeogenesis [2];

(4) Since cAMP levels are still more than sufficient to maximally stimulate gluconeogenesis under these conditions it is proposed that changes in Ca^{2+} flux are, at least partially, responsible for the inhibition observed [2].

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