Extracellular cAMP inhibits proximal reabsorption: are plasma membrane cAMP receptors involved?

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Bankir, Lise, Mina Ahloulay, Peter N. Devreotes, and Carole A. Parent. Extracellular cAMP inhibits proximal reabsorption: are plasma membrane cAMP receptors involved? Am J Physiol Renal Physiol 282: F376–F392, 2002; 10.1152/ajprenal.00202.2001.—Glucagon binding to hepatocytes has been known for a long time not only to stimulate intracellular cAMP accumulation but also, intriguingly, induce a significant release of liver-borne cAMP in the blood. Recent experiments have shown that the well-documented but ill-understood natriuretic and phosphaturic actions of glucagon are actually mediated by this extracellular cAMP, which inhibits the reabsorption of sodium and phosphate in the renal proximal tubule. The existence of this “pancreato-hepatorenal cascade” indicates that proximal tubular reabsorption is permanently influenced by extracellular cAMP, the concentration of which is most probably largely dependent on the insulin-to-glucagon ratio. The possibility that renal cAMP receptors may be involved in this process is supported by the fact that cAMP has been shown to bind to brush-border membrane vesicles. In other cell types (i.e., adipocytes, erythrocytes, glial cells, cardiomyocytes), cAMP egress and/or cAMP binding have also been shown to occur, suggesting additional paracrine effects of this nucleotide. Although not yet identified in mammals, cAMP receptors (cARs) are already well characterized in lower eukaryotes. The amoeba Dictyostelium discoideum expresses four different cARs during its development into a multicellular organism. cARs belong to the superfamily of seven transmembrane domain G protein-coupled receptors and exhibit a modest homology with the secretin receptor family (which includes PTH receptors). However, the existence of specific cAMP receptors in mammals remains to be demonstrated. Disturbances in the pancreato-hepatorenal cascade provide an adequate pathophysiological understanding of several unexplained observations, including the association of hyperinsulinemia and hypertension, the hepatorenal syndrome, and the hyperfiltration of diabetes mellitus. The observations reviewed in this paper show that cAMP should no longer be regarded only as an intracellular second messenger but also as a first messenger responsible for coordinated hepatorenal functions, and possibly for paracrine regulations in several other tissues.

Dictyostelium discoideum; liver; adipose tissue; glucagon; epinephrine; parathyroid hormone; insulin; hypertension; hepatorenal syndrome; diabetes mellitus; sodium; phosphate

Address for reprint requests and other correspondence: L. Bankir, INSERM Unité 367, 17 Rue du Fer à Moulin, 75005 Paris, France (E-mail: bankir@ifm.inserm.fr).
First, we established that the natriuretic, phosphaturic, and renal hemodynamic actions of glucagon required increases in plasma glucagon that were only three- to fivefold above physiological circulating levels but were physiological for the liver. Indeed, the liver is exposed to higher concentrations of pancreatic hormones than are peripheral tissues because these hormones are released directly in the portal vein and are subsequently partially degraded or internalized in hepatocytes and diluted in peripheral blood (5, 7). The marked increase in phosphate excretion induced by glucagon could originate only from an inhibition of solute reabsorption in the proximal tubule, the main site of phosphate reabsorption in the kidney, as confirmed by micropuncture experiments (147). However, no specific binding of radiolabeled glucagon has been found in this nephron segment (38). Because glucagon was known to induce a rise in the concentration of cAMP in the blood, due to the release of cAMP by the liver (30, 77, 159, 162), we hypothesized that cAMP could have a direct influence on renal function. In support of this hypothesis, we observed in our experiments a highly significant correlation between the glucagon-induced changes in plasma cAMP concentration \( [\text{P}_{\text{cAMP}}] \) and in fractional phosphate excretion (Fig. 1A) (6). Next, we showed that iv infusion of exogenous cAMP induced marked increases in sodium and phosphate excretion (Fig. 1B), very similar to those observed after glucagon infusion (6). An increase in GFR occurred only after the combined infusion of cAMP (mimicking glucagon-induced hepatic release) and of a low dose of glucagon, inducing a physiological increase in peripheral glucagon concentration susceptible to act directly on distal segments of the nephron where specific receptors, glucagon-responsive adenylyl cyclase, and transport responses have been identified (6, 7, 38, 117).

These studies revealed that glucagon participates in the regulation of several aspects of renal function by a novel pathway, the “pancreato-hepatorenal cascade” (16). This cascade provides adequate explanations for a number of as yet-ill-understood pathophysiological ob-

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Fig. 1. A: relationship between changes in plasma cAMP and changes in fractional excretion of phosphates \( (\text{FE}_{\text{PO}_4}^\% ) \) induced by infusion of isotonic saline (control, ○) or glucagon at 1.25 or 12.5 ng·min·100 g body wt\(^{-1} \) in 7 anesthetized rats. Basal plasma cAMP and \( \text{FE}_{\text{PO}_4}^\% \) were 49 nmol/l and 6.0%, respectively. The regression line (dashed line) and correlation coefficient \( (r) \) are shown. B: changes in the excretion of water \( (V = \text{urine flow rate} \) ), urea, phosphates, sodium, and potassium induced by intravenous (iv) infusion of either isotonic saline \( (n = 4) \) or cAMP \( (5 \text{ nmol/min, } n = 4) \) in anesthetized rats \( (\text{SE}) \). Because glomerular filtration rate was not altered by the infusions, the increases in excretion seen after cAMP infusion originate only from a reduction in the tubular reabsorption of the different solutes. Adapted from Ref. 6. \*P < 0.05, \**P < 0.01: paired t-test between control and experimental periods in the same rats \( (\text{control} = 40 \text{–} 80 \text{ min before, and experimental} = 40 \text{ to } 80 \text{ min after the beginning of experimental infusions}) \).
servations. The cellular mechanism by which cAMP influences the function of the renal proximal tubule is not yet elucidated, but we postulate that it might involve the binding of the nucleotide to specific membrane receptors, alike those characterized in the amoeba Dictyostelium discoideum (described below). This paper will review a number of observations that support this novel regulatory pathway and provide evidence that extracellular cAMP plays an important role as an interorgan and/or a paracrine extracellular mediator in the kidney and other mammalian tissues.

**cAMP IS AN EXTRACELLULAR MEDIATOR**

Several decades ago, the role of cAMP as an intracellular second messenger in the action of peptidic hormones was described (166). Most cells have a relatively low permeability to this nucleotide. The intracellular concentration of cAMP thus usually depends on the balance between the rate of its production through adenyl cyclase and the rate of its degradation into AMP through specific phosphodiesterases. However, some reports mentioned that cAMP was extruded in large amounts from some cell types. For example, addition of glucagon to an isolated, perfused rat liver led to a large increase in cAMP concentration not only in the tissue but also in the perfusate (59, 133), and acute administration of glucagon in humans results in a dose-dependent increase in circulating \([P_{cAMP}]\), as shown in Fig. 2 (1, 30, 77, 159). Some investigators proposed that this egress was used by cells to regulate their intracellular cAMP concentration (72, 177). However, others stated that this was unlikely because of the energetic cost of this pathway, requiring the permanent de novo synthesis of adenine nucleotides. Rather, they assumed that cAMP released by the liver might be involved in an unknown action on a distant organ (18, 94, 99). This daring conclusion received direct support 30 years later (6, 16).

The likelihood that cAMP is an extracellular mediator is supported by the following facts. 1) This nucleotide is actively exported into the blood (or at least into local interstitial fluids) by several cell types, while being impermeant in most others. 2) Extracellular cAMP has been shown to bind specifically to the plasma membrane of several cell types. 3) Extracellular cAMP has been shown to influence several specific functions in distinct cell types.¹

**Egress of cAMP From Various Cell Types**

Demonstration of cAMP egress in vitro and in vivo. Soon after the discovery of the role of cAMP as a second messenger, several studies reported the presence of the cyclic nucleotide in plasma and urine (29, 166). This suggested that cAMP could be exported out of the cells (18, 33). Table 1 lists the tissues and/or cell types from which an egress of cAMP has been demonstrated in vitro and the hormonal factors that stimulate this egress. These cell types include hepatocytes, epithelial cells of the glomerulus, and cells of the renal proximal tubule, erythrocytes (largely studied in birds; only a few studies in mammals), adipocytes, myocardium, fibroblasts, and some cells of the central nervous system. The transport of cAMP out of the cells occurs against a concentration gradient and requires energy (secondary active transport). It is sensitive to temperature and to pH and is inhibited by probenecid. The transporter involved in cAMP exit (or entry in some cases) is the organic acid cotransporter (25, 48, 49, 94, 133, 136, 137, 164, 171, 176, 180). The mechanism of cAMP efflux from cells has recently been reviewed (126).

CAMP release from different organs has also been characterized in vivo. Acute administration of glucagon by iv route in rats, dogs, and humans results in a prompt, dose-dependent, and reversible rise in plasma and urinary cAMP (with a larger rise in plasma than in urine) (30, 77, 148, 159, 162) (Fig. 2). In this case, the liver is responsible for the release of cAMP in the blood, and some of it is further filtered and excreted by the kidney (30, 48, 49, 77). Epinephrine infusion in humans also increases \([P_{cAMP}]\) (15, 21, 30), but to a much lesser extent than glucagon (30), and induces only a modest increase in the urinary excretion of cAMP (15). Note that these glucagon and catecholamine effects are strongly attenuated by prior ethanol ingestion (64). Finally, PTH increases both plasma and urinary cAMP, due to the release of cAMP by its target cells in the renal proximal tubule (43, 92, 102, 168). With this

¹It is conceivable that cGMP, the second messenger of ANP and nitric oxide, may also behave as an extracellular mediator because, like cAMP, it is released in the blood in significant amounts by several cell types, and a few studies have demonstrated functional effects of this mediator when infused intravenously or in the nephron lumen. However, this topic is beyond the scope of the present review.
hormone, the rise in urinary cAMP is larger than that in plasma (167), suggesting that proximal cells secrete intracellularly produced cAMP ("nephrogenous" cAMP) through both their apical and basolateral membranes, but more so through the former than the latter (37, 102). This preferential luminal release was confirmed in vitro in LLC-PK₁ cells and in opossum kidney cells (68, 69, 164).

By measuring arteriovenous concentration differences in dogs, the whole head (probably the brain) has been shown to release cAMP in response to infusion of the β-adrenergic agonist isoproterenol (9). With the use of microdialysis probes implanted in the brain of rats, an ischemia-induced cAMP release was documented in the extracellular space of the striatum (139) and a ischemia-induced cAMP release was documented in the extracellular space of the striatum (139) and a

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Cell Type</th>
<th>Species</th>
<th>Stimulating Factor</th>
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<tbody>
<tr>
<td>Liver</td>
<td>Hepatocytes or isolated, perfused</td>
<td>Rat, PTH, epinephrine</td>
<td>Glucagon, epinephrine, 46, 59, 133, 176</td>
</tr>
<tr>
<td>Kidney</td>
<td>Isolated proximal tubule</td>
<td>Pig, Human, Rat</td>
<td>Vasopressin⁺, PGE₂, 164</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Fresh adipocytes or cultured</td>
<td>Rat/pig/human</td>
<td>Glucagon, epinephrine, PGE₁, forskolin, isoproterenol, 35, 36, 40, 62, 94, 181</td>
</tr>
<tr>
<td>Brain</td>
<td>Astrocytes, glial cells</td>
<td>Rat/dog</td>
<td>Norepinephrine, isoproterenol, 139, 145, 146, 161</td>
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<tr>
<td>Blood</td>
<td>Erythrocytes (nucleated in birds)</td>
<td>Pigeon/chicken/turkey</td>
<td>Epinephrine, norepinephrine, 14, 18, 34, 51, 72, 99</td>
</tr>
<tr>
<td>Heart</td>
<td>Isolated, perfused heart</td>
<td>Rat, Isoproterenol</td>
<td>Isoproterenol, 80, 177</td>
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<tr>
<td>Vascularulare</td>
<td>Aortic smooth muscle cells</td>
<td>Rat</td>
<td>Adenosine, 60</td>
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<tr>
<td>Lung</td>
<td>Diploid fibroblasts</td>
<td>Human, PGE₁</td>
<td>94</td>
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*The LLC-PK₁ cell line originates from nonselected pig kidney cells. When cultured on permeant supports, these cells exhibit a mixed proximal/distal phenotype. They respond to vasopressin, whereas native proximal tubule cells do not. PTH, parathyroid hormone.

The existence of extra-renal V₂-like AVP receptors, possibly located in the endothelium, has been proposed (92a). They could mediate the vasodilatory effects observed in the peripheral vascular bed and the kidney after dDAVP infusion (78, 120, 175, 178). dDAVP, which has long been assumed to be a selective agonist of V₂ receptors, has recently been shown to bind with an equally high affinity to vasopressin V₁b receptors (149), which are abundant in pancreatic islets. Moreover, several studies have shown that AVP and dDAVP are able to stimulate insulin and glucagon release from the isolated, perfused rat pancreas (54, 179). This raises the possibility that the dDAVP-induced rise in P_{cAMP} could be indirectly mediated by glucagon and its effects on the liver. Further studies are required to identify the tissue responsible for the dDAVP-induced rise in P_{cAMP}.

**Distinct influence of glucagon, epinephrine, and insulin on cAMP egress from hepatocytes and adipocytes.** In the liver, both glucagon and epinephrine share cAMP as a second messenger and regulate metabolic functions. However, in the 1970s several investigators observed that, for doses of the two hormones inducing the same maximum metabolic effects, glucagon induced a far greater release of cAMP in the perfusate (in vitro), or increase in blood (in vivo), than did epinephrine (22, 30, 59, 74, 128, 133). This suggests that glucagon, but not epinephrine, could initiate liver-dependent actions on a peripheral organ. In contrast to the liver, cAMP release from adipose tissue seems to be greater under the influence of epinephrine than under that of glucagon, despite effects of similar amplitude on lipolysis (181). However, the release of cAMP by adipocytes in vitro was detectable only in the presence of theophylline, a phosphodiesterase inhibitor, suggesting rapid local degradation of the nucleotide (181). Thus, cAMP release by adipose tissue possibly plays only a paracrine role. A preliminary study in four healthy volunteers after an overnight fast revealed a modest positive venoarterial difference in cAMP con-
concentration (+10%) through adipose tissue (115). To our knowledge, the influence of epinephrine administration on cAMP release by adipose tissue in vivo has not been studied so far. Nor has any study looked for a possible relationship between \([P_{cAMP}]\) and obesity, but it is interesting to note that adipocytes isolated from a woman with a high body mass index released twice as much cAMP in vitro than adipocytes from two other women with lower body mass indexes (40). In summary, glucagon and epinephrine, two hormones that both act simultaneously on liver and adipose tissue and induce similar metabolic effects with the same second messenger, might additionally exert different endocrine and/or paracrine actions because in each of these two tissues only one of them also stimulates the release of cAMP from the cells sufficiently to alter \([P_{cAMP}]\).

It is well documented that the magnitude of the glucagon-induced release of cAMP (as well as intracellular cAMP concentration) is reduced dose dependently by insulin because insulin activates a membrane phosphodiesterase that degrades the cAMP formed in hepatocytes in response to glucagon (110, 133). Accordingly, the intensity of the metabolic actions of glucagon, the hepatic release of cAMP (or release by adipose tissue), and the rise in plasma (or medium) cAMP depends on the ratio between glucagon and insulin concentrations rather than on glucagon concentration alone (39, 57, 58, 109, 129, 133, 157, 181). This fact may explain why the intensity of some effects suspected to depend on glucagon (e.g., the rise in GFR) did not correlate with the rise in plasma glucagon concentration (20). When insulin secretion was also altered by the experimental protocol, the observed response was most likely dependent on the glucagon-to-insulin ratio, not just on glucagon (44).

**Binding of cAMP to Various Cell Types**

Several studies have shown that cAMP can bind to the plasma membrane of different cells. In 1975, Insel et al. (87) demonstrated that cAMP binds to brush-border membranes isolated from rabbit renal cortex in a temperature-sensitive and reversible manner (Fig. 3A), and Forte et al. (63) reported similar findings with a plasma membrane-enriched fraction issued from porcine renal cortex. The range of cAMP concentrations used in these studies corresponds to physiological concentrations encountered in mammalian plasma (10–50 nM). Several other cyclic purine nucleotides can displace cAMP binding, however, with a much lower affinity (87) (Fig. 3B).

More recently, a significant and specific binding of cAMP in murine bone marrow cells was reported (131). This binding is saturable, reversible, inhibited by cAMP analogs, and prevented by trypsin. The apparent dissociation constant \(K_d\) at a temperature of 0°C was \(2.7 \times 10^{-6}\) M and the calculated number of receptors \(1.8 \times 10^6\) molecules/cell. Experiments with various analogs and antagonists led the authors to conclude that extracellular cAMP interacts with unconventional purinoreceptors, different from previously known P1 and P2 receptors (131).

**Effects of Extracellular cAMP on Effector Tissues**

In many experiments, permeant analogs of cAMP, such as dibutyryl cAMP or 8-bromo-cAMP have been used to mimic the effects of hormonal stimulation on target tissues because cAMP was known not to penetrate cells to a significant extent. However, in a few cases, cAMP itself was used, leading to the disclosure of a well-characterized influence of extracellular cAMP on several aspects of renal, metabolic, and cardiac functions (6, 37, 83, 101, 103).
Effects on renal function. Early studies clearly established that iv cAMP infused in parathyroidectomized animals induced a marked increase in phosphate excretion, mimicking the effects of PTH or glucagon (19, 37, 83, 101). In rats with intact parathyroid glands, recent experiments also revealed that cAMP infusion increased fluid, sodium, and phosphate excretion (Fig. 1B) and, in some conditions, also contributed to increase GFR (6). The molecular mechanism by which cAMP exerted these effects could not be deduced from those studies. Ahloulay et al. (6) proposed that cAMP could be transported into proximal tubule cells by the organic acid cotransporter and that its accumulation in these cells could mimic that induced by adenylyl cyclase activation by PTH. However, this explanation seems unlikely for the following reasons. First, the affinity of the organic acid cotransporter for cAMP is not high enough to enable the uptake of a sufficiently large amount of cAMP into the cells (136, 171). Second, the maximum rise in fractional phosphate excretion induced by glucagon infusion occurs with only a doubling of [PcAMP] (Fig. 1A). This twofold increase, even if followed by a proportional rise in cAMP uptake by proximal tubule cells, could not provide an increase in intracellular cAMP concentration sufficient to mimic that resulting from adenylyl cyclase activation and to account for the large reduction observed in phosphate reabsorption. Finally, concentrations of cAMP as low as 10^{-10} M (i.e., lower than usual plasma levels and much lower than intracellular levels reached after hormonal stimulation), when applied luminally, were shown to inhibit fluid reabsorption in rat proximal tubule. This suggests the existence of receptor sites on the surface of the luminal cell membrane with a high affinity for cAMP (19).

Thus we now favor the hypothesis that cAMP could act on the proximal tubule by binding to specific membrane receptors, probably located in the brush border (87) (see below). Accordingly, filtered cAMP rather than peritubular cAMP could be involved in the regulation of proximal reabsorption. The fact that both sodium and phosphate excretions are altered in parallel and to a similar extent suggests that extracellular cAMP inhibits the Na-Pi cotransporter, as does PTH (4, 69). This is also suggested by recent studies in opossum kidney cells (68). It has been proposed that adenosine, resulting from cAMP degradation, could mediate this effect (69). In vitro studies have shown some degradation of extracellular cAMP to adenosine (112, 176). On the other hand, in vivo studies with radiolabeled cAMP have shown that extracellular cAMP does not undergo extensive degradation in biological fluids because these fluids contain only little phosphodiesterase activity (37, 47). Moreover, although adenosine infusion tends to lower renal hemodynamics, cAMP contributes to its increase (6), and degradation of endogenous adenosine by adenosine deaminase actually potentiates glucagon-induced hyperfiltration (11). Most probably, both nucleotides have their own independent influence on renal function.

Once filtered in the glomerulus, cAMP is not significantly reabsorbed or degraded. Thus it should be progressively concentrated (3- to 4-fold) in the lumen of the proximal tubule as a result of fluid reabsorption. Accordingly, cAMP concentration should increase progressively toward the pars recta, the segment in which the strongest change in phosphate transport is observed in response to either glucagon or PTH infusion and in which the final regulation of phosphate excretion is achieved (101, 147). Possibly, in addition to filtered cAMP, nephrogenous cAMP, extruded into the tubular lumen after PTH action on the proximal tubule, may also act downstream in the proximal straight tube (69). In the aggregate, this information suggests that the cAMP-induced reduction in proximal reabsorption could be mediated by luminal cAMP rather than (or in addition to) plasma cAMP, as already discussed (19, 37, 65, 69). It is conceivable that the putative cAMP receptors are coupled to adenylyl cyclase and result in further cAMP formation and secretion (as in D. discoideum; see below) because glucagon infusion in parathyroidectomized rats as well as in patients with hypoparathyroidism has been shown to induce a marked, but delayed, increase in nephrogenous cAMP excretion (urinary excretion largely exceeding the filtered amount) (148). Of note is the observation that cAMP addition by the proximal tubule in the urine was also observed in chronically parathyroidectomized rats, suggesting the existence of another source of nephrogenous cAMP besides PTH (102).

In summary, glucagon appears to exert simultaneous and coordinated actions on the liver and the kidney through a pancreato-hepatorenal cascade. The effects on the liver are mediated by the usual binding of the hormone to its specific receptors whereas the effects on the kidney depend, at least in part, on the release and subsequent distant action of liver-derived CAMP on the proximal tubule, possibly by binding to CAMP receptors. Several findings suggest that luminal (filtered) rather than peritubular CAMP could be involved, binding to specific membrane receptors in the brush-border membrane. This interorgan link creates a synergy between the metabolic functions of the liver and the excretory functions of the kidney, which are both required to be simultaneously stimulated after the ingestion of a protein meal, or during a prolonged fast, when endogenous tissues rather than nutrients are catabolized (5, 7, 17).

Effects on other tissues. Besides its influence on the renal proximal tubule, extracellular cAMP has been shown, or is suspected, to affect various aspects of cell functions in other mammalian cell types, including hepatocytes, adipocytes, glial cells in certain areas of the brain, cardiomyocytes, smooth muscle cells of the coronary artery, endothelial cells of the pulmonary artery, bone marrow cells, and erythroblasts (see details below). Although the effects of cAMP on renal function are well demonstrated in vivo and occur for plasma concentrations within the physiological range, the effects of cAMP on other cell types have been documented only in vitro and are often enhanced by
the addition of phosphodiesterase inhibitors. It is thus conceivable that, in these cells, cAMP could exert paracrine actions requiring much higher local concentrations of the nucleotide to be active, and controlled by local cAMP degradation. If these different cells were sensitive to changes in cAMP concentrations within the range of circulating levels, their function would always be influenced by liver-borne cAMP in conjunction with that of the kidney. The evidence for such paracrine action in nonrenal tissues is detailed below.

In the liver itself, the organ that, because of its large mass, probably contributes to most of the plasma cAMP, the release of the nucleotide into the extracellular space could also play a paracrine role, enabling cell-to-cell interactions and communication (46, 154). cAMP infusion in isolated perfused liver reproduced the metabolic actions of glucagon (46), and cAMP infusion in rats resulted in hyperglycemia, especially if coinfused with theophylline (which retards cAMP degradation) (123). An increase in blood glucose was also observed in 20 human subjects 10 min after acute cAMP administration (8–12 mg/kg) (103). In the same study, the authors reported that cAMP increased heart rate in seconds and cardiac output a few minutes later. We found no other study describing an effect of cAMP on cardiac function in vivo. However, extracellular cAMP was shown to modulate the activity of the sodium channel in rat, guinea pig, and frog cardiomyocytes in a rapid (<50 ms), reversible, and dose-dependent manner. This effect seems to be mediated by the interaction of cAMP with cell membranes, resulting in the activation of a pertussis toxin-sensitive G protein (158). In the isolated, perfused canine coronary artery, extracellular cAMP was shown to induce vasodilation, but in this case, the effect seemed to depend on prior cAMP degradation into adenosine (121).

Extracellular cAMP was recently shown to influence endothelial cells from human pulmonary microvessels in which it exerts a negative regulation on the inducible expression of prostaglandin H synthetase (55). In the brain, the fact that cAMP is released by glial cells in intercellular spaces suggests that this nucleotide could play a role in neuromodulation of some other signaling molecules in certain brain areas (139, 146). The well-documented egress of cAMP from adipocytes, with a selective hormone dependence, also suggests that extracellular cAMP could exert a paracrine action in the adipose tissue. Whether adipocytes contribute to significantly influence plasma cAMP level and could thus influence renal function remains to be determined.

In murine bone marrow cells, a paracrine effect of the nucleotide could also take place because extracellular cAMP was shown to induce the expression of the lipopolysaccharide receptor CD14 in vitro (131). Finally, extracellular cAMP is suspected to play a role in the regulation of erythropoiesis. cAMP and several analogs have been shown to stimulate erythropoiesis in plethoric mice (132, 155) and hemoglobin synthesis in bone marrow cells from humans, dogs, sheep, and rabbits, but not in cells isolated from rodents (32). However, a rise in hemoglobin, hematocrit, and red cell mass was also observed in mice after chronic dibutyryl-cAMP treatment (144). The functional consequences of the cAMP release by erythrocytes are not yet known, but it is unlikely that it is purposeless, especially in some birds (e.g., chickens, pigeons) in which adenyl cyclase activity is very high and cAMP is released in large amounts (18, 51, 72). Aurbach et al. (14) observed that cAMP enhanced sodium and potassium transport in avian erythrocytes in the same fashion as did catecholamines. King and Mayer (99) assumed that cAMP extruded from erythrocytes could carry a message from red cells to some other cell type.

Taken together, the effects induced by cAMP in the kidney and other tissues suggest that this nucleotide binds to specific receptors to influence various cell functions. It seems unlikely that any transporter could make enough extracellular cAMP enter the cells uphill so as to achieve an intracellular concentration high enough to mimic that induced by activation of adenylate cyclase. Actually, specific cAMP receptors have been identified in lower eukaryotes, as described below.

cAMP RECEPTORS IN THE SOCIAL AMOEBA D. DISCOIDEUM

The amoeba D. discoideum lives two independent life cycles (24, 95). Vegetative or growth-stage cells function independently and use phagocytosis or pinocytosis to ingest bacteria or liquid media. On starvation, the cells enter a developmental program that leads to the formation of a multicellular organism composed of a spore head atop a stalk of vacuolated cells, the so-called "fruiting body." cAMP plays a central role in the transition from single cells to multicellularity. As in most mammalian cells, after its synthesis, some of the nucleotide remains inside the cells where it binds and activates protein kinase A. However, in D. discoideum, a significant fraction of cAMP is also secreted outside the cells, where it acts as a chemoattractant by binding to specific surface receptors called cARs (for cAMP receptors). cAMP binding to the receptors activates a multitude of signaling pathways, giving rise to chemotaxis, the synthesis and secretion of additional cAMP (signal relay), and changes in gene expression.

Over the years, D. discoideum has proven to be an invaluable model system to elucidate the molecular mechanisms of complex cellular responses, including cytokinesis, motility, phagocytosis, chemotaxis, and signal transduction, as well as aspects of development such as cell-type determination and pattern formation (93). With its highly accessible biochemistry and genetics, this simple organism has brought unique advantages for the study of these fundamental cellular processes, and important generalizations for eukaryotic cells have been derived from studies using D. discoideum. Indeed, cloning and deletion of cARs showed that these receptors are essential for chemotaxis. It is now clear that a family of 20 G protein-linked “chemokine” receptors mediate chemotaxis in leukocytes (see
Discovery and Diversity

Cell-surface binding sites for cAMP have been measured throughout D. discoideum’s developmental program, although they are most abundant during the aggregation events (89). At this early stage of development, cAMP binds with high affinity ($K_d \sim 300$ nM) to both whole cells (~50,000 sites/cell) and membrane preparations (90). Biochemical evidence has suggested that the cAR is coupled to a GTP-dependent regulatory protein. First, the affinity for cAMP has been shown to be reduced in the presence of GTP and, second, cAMP stimulates the binding of GTP to membranes (88, 174). Moreover, the cDNAs for two G protein-α-subunits and one β-subunit were identified in D. discoideum before the first cAR was cloned.

The first cAR was identified by photoaffinity labeling and subsequently purified from wild-type cells (100). The cDNA was obtained by expression cloning using a monospecific polyclonal antiserum. Analysis of the nucleotide sequence revealed that the gene encodes a 392-amino acid protein with a predicted topology composed of six highly hydrophobic regions followed by a seventh stretch that is less hydrophobic (Fig. 4). This pattern confirmed that cAR belongs to the superfamily of G protein-coupled receptors such as rhodopsin and the β-adrenergic receptor. Indeed, comparison of the cAR and rhodopsin sequences revealed a 32% amino acid conservation over the first 270 residues, a region encompassing the transmembrane domains and connecting loops. The COOH-terminal tail is more divergent, although for both rhodopsin and cAR it is rich in serine and threonine residues. Expression of the cAR cDNA in vegetative wild-type amoebas recapitulated high-affinity binding sites for cAMP in both whole cells and membranes, thereby proving that the cDNA encoded the cAMP binding protein.

With the use of reduced stringency hybridization, three additional cARs were subsequently cloned (152, 153). The four members were therefore named cAR1, cAR2, cAR3, and cAR4 (Fig. 4). They are 60% identical within their transmembrane domains and connecting loops, but their COOH-terminal tails differ extensively in both length and amino acid sequence. The receptors also differ in their affinities for cAMP: cAR1 and cAR3 possess high-affinity binding sites, and cAR2 and cAR4 bind cAMP, with $K_d$ values in the micromolar range (89). The four receptors are expressed sequentially throughout D. discoideum’s development, and the range of affinities of the different cARs mirrors the cAMP concentrations present during the different developmental stages of the organism. Although the exact binding site for cAMP has yet to be mapped on the cARs, the major determinant of cAMP affinity has been mapped to a domain in the second extracellular loop in which only five residues differ between cAR1 and cAR2 (97).

Functional Effects

Using gene targeting and homologous recombination, the biological functions of the four cAR genes have been thoroughly analyzed. Mutants lacking cAR1 (the first cAR expressed) cannot carry out chemotaxis and remain aggregation deficient (165). However, these cells will respond to higher concentrations of cAMP and, under these conditions, will differentiate into multicellular structures. Cells lacking both cAR1 and cAR3 (the second cAR expressed) are completely insensitive to cAMP and never enter the developmental program (85). These results suggest that these two receptors are functionally redundant. Cells that lack either cAR2 or cAR4 (the third and fourth cAR expressed) are fully capable of aggregating but then arrest at the multicellular stages of development (108, 151). These series of experiments therefore underscored the sequential role of the cARs in the development of D. discoideum and suggested that the receptors are linked to the similar signaling pathways (96).

The addition of cAMP to cells gives rise to the activation of a variety of effectors, each displaying specific kinetics and regulatory patterns (Fig. 5) (13, 127). A few seconds after receptor activation, increases in cGMP and inositol 1,4,5-trisphosphate production, as well as the recruitment of the pleckstrin homology domain-containing proteins protein kinase B and cytosolic regulator of adenylyl cyclase (CRAC) are observed. At the same time, a dramatic rise in the proportion of polymerized actin occurs. This is quickly followed by phosphorylation of myosin I and II as well as a transient influx of calcium. One minute after the addition of cAMP to cells, the production of cAMP through the activation of adenylyl cyclase peaks and a mitogen-activated protein kinase is phosphorylated. All of these responses are transient, i.e., they subside even in the presence of persistent stimulation. The turning off of the response, also called “adaptation,” has been suggested to be dependent on the phosphorylation of the tail of the receptor. Indeed, as shown for other G protein-coupled receptors, cAMP binding to cAR1 leads to phosphorylation of its COOH-terminal tail. With the use of site-directed mutagenesis and deletion analysis, this phosphorylation has been shown...
to be important for the regulation of cAMP binding activity, but, very interestingly, it does not mediate the adaptation response (41, 98). By using cells in which the unique β subunit of the G proteins was deleted by homologous recombination, it was also demonstrated that a subset of the responses elicited by cAMP is independent of G proteins (113). The interplay between all of these diverse signaling events eventually leads to chemotaxis, cell-cell signaling, and gene expression.

The gene encoding the adenylyl cyclase expressed in early aggregation was cloned, and the resulting protein product shares homology and a typical 12-transmembrane topology with the *Drosophila melanogaster* and mammalian G protein-coupled adenylyl cyclases (134). This enzyme is responsible for the synthesis of cAMP that binds to cAR1 and is required for cell-cell signaling. Cells in which the adenylyl cyclase ACA has been deleted by homologous recombination are devoid of cAMP-induced adenylyl cyclase activity and remain aggregation deficient when starved. Because ACA and ATP binding cassette (ABC) transporters share a similar topology, it was proposed that the adenylyl cyclase could also be involved in the secretion of cAMP. However, when *aca*− cells were transformed with ACG, a novel form of adenylyl cyclase expressed in the germinal stage of *D. discoideum* development and predicted to have a single transmembrane domain between large intracellular and extracellular domains, cAMP was normally secreted. To this day, the molecular components involved in the secretion of cAMP remain to be discovered.

**cAMP Receptors in Higher Eukaryotes?**

Two independent groups recently reported the cloning of a putative G protein-coupled receptor from the plant *Arabidopsis thaliana* that displayed 23% amino acid identity (53% similarity) with cAR1 (91, 135). The sequence similarity extended across the entire protein except for the COOH terminus. The gene product, called GCR1, was identified by screening the dbEST database of expressed sequence tags for sequences that contained similarities with known seven-transmembrane-receptor sequences. It is expressed at very low levels, and transformation with an antisense construct gave rise to plants with lower sensitivity to the plant hormones cytokinins. It is not known whether the receptor actually binds cAMP, so for now GCR1 remains a putative cAMP receptor. Searches of both the *D. melanogaster* and *Caenorhabditis elegans* genomes have yielded no homologs of cAR1.

Because several types of mammalian cells have been reported to either specifically bind cAMP or somehow respond to extracellular cAMP (see previous section), extensive searches of the mammalian genome databases have been carried out to identify possible homologs to the *D. discoideum* cARs. The most significant result of these searches is the disclosure of a weak homology of cARs with the secretin family of receptors, which includes the PTH and calcitonin receptors. The region of similarity between these receptors and cAR1 falls within the third and fourth transmembrane domains, the second extracellular loop, and the fifth transmembrane domain (Figs. 4 and 6). Interestingly, the region within the second extracellular loop of cAR1 has been shown to be a major determinant for the modulation of affinity for cAMP (97). Because of this homology and of the fact that cAMP exerts PTH-like effects in the mammalian kidney, the possibility that cAMP action in mammals could depend on binding of the nucleotide to a specific region of the PTHR1 receptor (or to another receptor of the same family) deserves to be evaluated.

The essential role of G protein-coupled signaling in *D. discoideum*’s development, together with a mode of functioning that is virtually identical to its mammalian counterpart, underscores the great evolutionary pressure exerted on this signaling cascade. In mammals, the superfamily of G protein-coupled receptors contains >1,000 members and is responsible for a wide range of physiological responses. As presented in this review, a great body of evidence suggests that in mammals cAMP may also act as an extracellular mediator.
INVITED REVIEW

The characteristics of cAMP-mediated signaling in D. discoideum point to the probable existence of cAMP receptors in mammalian systems.

PATHOPHYSIOLOGICAL IMPLICATIONS OF DISTURBANCES IN EXTRACELLULAR cAMP

Whether cAMP exerts its effects on the proximal tubule via specific binding to membrane receptors will probably be clarified in the near future. However, whatever the exact mechanism, it seems now well established that circulating cAMP exerts a permanent PTH-like regulatory action on the intensity of proximal tubule reabsorption and thus on sodium, phosphate, and water excretion. With more cAMP in plasma, proximal reabsorption will be inhibited more strongly, and sodium and phosphate excretion will be facilitated (and vice versa) (Fig. 7A). Simultaneously, it reduces glucagon-dependent reduction in cAMP release by the liver may explain the well-known, but poorly understood, antinatriuretic action of this hormone (52, 73). The natriuresis of fasting (150, 160) (a situation in which glucagon secretion is selectively enhanced) and the subsequent antinatriuresis observed after carbohydrate refeeding (increasing the insulin-to-glucagon ratio) (79) are also easily explained by alterations in the amount of liver-borne cAMP. The edema observed in kwashiorkor (selective protein malnutrition with sufficient caloric intake from carbohydrates) but not in marasmus (protein and calorie malnutrition) (170) could be explained by a high insulin-to-glucagon ratio, most likely occurring in the former but not the latter. The contribution of hyperinsulinemia and insulin resistance to some forms of hypertension (27) could also depend, at least in part, on the pancreato-hepato-renal cascade and the resulting excessive reabsorption in the proximal tubule, because, in this situation, peripheral organs, but not the liver (61, 144a), become resistant to insulin, thus allowing the normal insulin-glucagon interplay in this organ (Fig. 7B).

Recently, a missense mutation in the glucagon receptor gene has been identified (Gly40Ser), present in ~1% of Caucasian subjects (76). When expressed in transfected cells, this mutation results in a reduced cAMP response (76) (effects similar to those induced by an increase in the insulin-to-glucagon ratio) (79) are also easily explained by alterations in the amount of liver-borne cAMP. Edema observed in kwashiorkor (selective protein malnutrition with sufficient caloric intake from carbohydrates) but not in marasmus (protein and calorie malnutrition) (170) could be explained by a high insulin-to-glucagon ratio, most likely occurring in the former but not the latter. The contribution of hyperinsulinemia and insulin resistance to some forms of hypertension (27) could also depend, at least in part, on the pancreato-hepato-renal cascade and the resulting excessive reabsorption in the proximal tubule, because, in this situation, peripheral organs, but not the liver (61, 144a), become resistant to insulin, thus allowing the normal insulin-glucagon interplay in this organ (Fig. 7B).

Receptor carriers of the mutation have a lower increase in plasma glucose concentration in response to glucagon (76) (effects similar to those induced by an increase in the insulin-to-glucagon ratio) (79) are also easily explained by alterations in the amount of liver-borne cAMP. Edema observed in kwashiorkor (selective protein malnutrition with sufficient caloric intake from carbohydrates) but not in marasmus (protein and calorie malnutrition) (170) could be explained by a high insulin-to-glucagon ratio, most likely occurring in the former but not the latter. The contribution of hyperinsulinemia and insulin resistance to some forms of hypertension (27) could also depend, at least in part, on the pancreato-hepato-renal cascade and the resulting excessive reabsorption in the proximal tubule, because, in this situation, peripheral organs, but not the liver (61, 144a), become resistant to insulin, thus allowing the normal insulin-glucagon interplay in this organ (Fig. 7B).

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Recently, a missense mutation in the glucagon receptor gene has been identified (Gly40Ser), present in ~1% of Caucasian subjects (76). When expressed in transfected cells, this mutation results in a lower affinity of the receptor for glucagon and a reduced cAMP response (76) (effects similar to those induced by an increase in the insulin-to-glucagon ratio) (Fig. 7B). Carriers of the mutation have a lower increase in plasma glucose concentration in response to glucagon infusion, thus suggesting that this mutation also results in a reduced cAMP response in the human liver (169). In addition, a significant (although modest) enhancement in proximal tubule reabsorption (measured after an overnight fast) has recently been characterized in a large group of carriers of the mutation, suggesting a reduced cAMP influence also in the kidney (163). In this context, it is interesting to note that this mutation was found to be significantly more frequent in patients with hypertension (26, 42, 118, 119, 163). However, the Gly40Ser mutation alone is not sufficient to induce hypertension as a number of carriers are
normotensive, and no association of this mutation with hypertension was found in other studies (70, 169). Nonetheless, the finding of an association between hypertension and a mutation that has only a modest functional consequence on renal function strongly suggests that the pancreato-hepatorenal cascade and its consequences on sodium reabsorption in the proximal tubule contribute to the regulation of blood pressure in humans. Because the mutation reproduces a liver response similar to that observed when insulin action on this organ is inadequately increased, and because insulin resistance is often observed to occur in peripheral organs but not in the liver (61, 144a), the above observations support the novel pathophysiological mechanism proposed here for explaining the association of hypertension with hyperinsulinemia and insulin resistance.

**Cirrhosis and Hepatorenal Syndrome. Congestive Heart Failure**

The hepatorenal syndrome is characterized by severe fluid and sodium retention due, at least in part, to excessive salt and water reabsorption in the proximal tubule, and by an intense selective renal vasoconstriction. It may be assumed that the severely diseased liver is no longer able to produce sufficient amounts of cAMP (Fig. 7B). The role of extracellular cAMP
renal function described above may explain the high avidity of the proximal tubule for sodium and the fall in GFR. Actually, patients with various liver diseases may, in some cases, exhibit a blunted cAMP response to glucagon stimulation (2, 45, 114). An involvement of liver-borne cAMP in the glucagon-induced hyperfiltration is suggested by the observations of Levy et al. (104, 105), who showed that an iv infusion of glucagon raises GFR in normal dogs but not in cirrhotic dogs, in which the hepatic release of cAMP may be blunted. Resistance of the kidney to the action of cAMP may also be suspected in cirrhosis as a consequence of the well-documented respiratory alkalosis (116), because a resistance to the phosphaturic effects of PTH, cAMP, dibutyryl-cAMP, or glucagon has been observed in rats with respiratory alkalosis (82, 83, 138). In support of this hypothesis, Ahloulay et al. (8) observed that cAMP infusion in cirrhotic rats with ascites failed to increase phosphate and sodium excretion as it did in control rats.

Preliminary results in rats with cirrhosis (induced by chronic bile duct ligation) show that $[P_{\text{cAMP}}]$ is actually raised above normal when the disease is moderate but falls significantly when rats exhibit a strongly positive sodium balance and develop ascites (Ahloulay M, Bankir L, Déchaux M, and Lebrec D, unpublished observations; Ref. 8). The initial rise in $[P_{\text{cAMP}}]$ might be related to the marked hyperglucagonemia that characterizes the early phase of cirrhosis. Similarly, in humans, $[P_{\text{cAMP}}]$ appeared to be higher in 50 cirrhotic subjects than in healthy individuals, but, among cirrhotic patients, it was lower in those with severe ascites than in those with mild ascites (Ahloulay M, Bankir L, Déchaux M, and Lebrec D, unpublished observations). Whether this late fall in $[P_{\text{cAMP}}]$, a resistance to the proximal tubular effects of extracellular cAMP, or an increased formation of adenosine (resulting from cAMP catabolism) plays a role in sodium retention and abnormal fluid accumulation remains to be determined.

The similarity of edematous symptoms observed in hepatic cirrhosis with ascites and in congestive heart failure (CHF) suggests that these disorders could result from a common pathophysiological mechanism. The heart has been shown to release cAMP in vitro (124). Defective cAMP production in failing human hearts (23) or resistance of the kidney to cAMP could thus be involved in the edema of CHF. However, this remains speculative at present.

Glomerular Hyperfiltration in Diverse Situations

As explained above, an increase in $[P_{\text{cAMP}}]$ contributes to glomerular hyperfiltration when combined with an elevation of plasma glucagon (6). In several pathophysiological situations, such parallel increases in the two mediators are observed and may explain a rise in GFR. In diabetes mellitus, elevated glucagon secretion is observed in the absence (type 1) or lack of effects of insulin on the liver (type 2) (3, 81, 111, 172, 173), resulting in elevated $[P_{\text{cAMP}}]$ (107). The combination of high glucagon and high $[P_{\text{cAMP}}]$ could contribute, at least in part, to the hyperfiltration known to occur in the early phase of the disease.

In chronic renal insufficiency, hyperfiltration in residual intact nephrons is thought to compensate, at least partially, for the reduced filtration in sclerotic glomeruli. Increases in both plasma cAMP and glucagon have been reported in renal failure (75). Thus a combined and parallel elevation of these two mediators could account, at least partially, for the progressive hyperfiltration seen in remnant nephrons. Finally, the compensatory adaptation of GFR seen after acute uninephrectomy could also be explained by this same mechanism because the reduced excretion of cAMP and the reduced renal clearance of peptidic hormones resulting from the sudden halving of functioning renal mass will result in a rapid rise in their plasma concentrations.

**CONCLUSION**

In conclusion, a wide body of experimental observations suggests that extracellular cAMP could exert endocrine and paracrine actions in several mammalian tissues. These actions could result from the binding of cAMP to specific cell membrane receptors, like those identified in *D. discoideum*, and subsequent transduction of the signal by as yet unidentified pathways. Specific receptors also exist for a number of other adenine (50) and purine nucleotides (53, 86, 156). Calcium, another second messenger, has also been shown to behave as a first messenger, binding to specific membrane receptors (“calcium sensor”) (31). The modest homology between the mammalian PTHR1 receptor and the cloned cAMP receptors in *D. discoideum*, together with the similarity between the effects of extracellular cAMP and those of PTH in mammals, raises the possibility that the PTH receptor (or related member of the same family) could be also a cAMP receptor. Whether this hypothesis receives confirmation, cAMP may no longer be regarded only as a second messenger but should also now be looked at as an interorgan mediator ensuring coordinated actions in liver and kidney, and as a paracrine factor in several other tissues.

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