Adenylyl cyclase activity and glucose release from the liver of the European eel, Anguilla anguilla

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Fabbri, Elena, Laura Barbin, Antonio Capuzzo, and Carla Biondi. Adenylyl cyclase activity and glucose release from the liver of the European eel, Anguilla anguilla. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1563–R1570, 1998.—The properties of adenylyl cyclase (AC) in liver membranes of the European eel (Anguilla anguilla) and the involvement of cAMP in glucose release from isolated hepatocytes in response to catecholamines were studied. Basal enzyme activity seemed essentially unaffected by GTP, while a biphasic response to increasing nucleotide concentrations was obtained in the presence of epinephrine. Eel liver AC was dose-dependently stimulated by guanosine 3′-5′-O-(2-thiodiphosphosphate), AC activity, intracellular cAMP levels, and glucose release from isolated hepatocytes were significantly enhanced by NaF, forskolin, epinephrine, and phenylephrine. The rise in cAMP production stimulated by catecholamines was counteracted by propranolol, but not by phentolamine. Catecholamine-induced glucose output was instead partially antagonized by both phentolamine and propranolol. Complete inhibition was obtained only by the simultaneous presence of the two adrenergic antagonists. Glucose release from the cells was induced by dibutyryl cAMP and by the calcium ionophore ionomycin. In summary, these data provide the first characterization of eel liver AC system and suggest a direct role for cAMP in the catecholamine-dependent glucose output. Furthermore, the involvement of calcium ions in this cellular response is hypothesized.

Since the pioneering studies of Sutherland (41), the liver has been widely used as a model for studying the interactions between catecholamines (CA) and plasma membrane receptors, and most of the steps involved in the signaling transduction pathway relative to these hormones have been elucidated in mammals (9). In the last few years, our understanding of hepatic metabolism in some fish species has greatly increased (14, 16, 28), and the interaction of CA with both α- and β-adrenergic receptor subtypes has been demonstrated in eel and bullhead hepatocytes, where epinephrine (Epi) and norepinephrine (NE) increase cytosolic levels of cAMP (13) and Ca2+ (29).

Glycogenolysis has been shown as a major effect exerted by CA in vivo as well as in vitro in many fishes, including the northern pike, Esox lucius (42); rainbow trout, Oncorhynchus mykiss (32); catfish, Ictalurus melas (36); and carp, Cyprinus carpio (23). Such a response seems to be chiefly due to the interaction of the hormones with β-adrenergic receptors, as it is blocked by the simultaneous infusion of Epi and the β-adreceptor antagonist propranolol (Pro) (44). In support of this hypothesis, Foster and Moon (16) have demonstrated that dibutyryl cAMP, a permeant analog of cAMP, significantly stimulates glycogen phosphorylase activity and enhances glycogen breakdown in eel hepatocytes.

Several data are available on the functionality and hormonal control of glucose metabolism in the liver of the eel, where cAMP has been shown to play a pivotal role in the adrenergic transduction mechanisms (16, 28). However, neither adenylyl cyclase (AC) activity nor its involvement in the glycogenolytic function ascribed to CA have been studied in the liver of this teleost. Therefore, the aim of the present investigation was to characterize the properties of the hepatic AC system in the European eel (Anguilla anguilla) and to evaluate its response to some exogenous compounds known to affect the activity of the enzyme in mammalian tissues. The same compounds have also been used to test the possible coupling between AC activation and glucose release.

MATERIALS AND METHODS

Animals. European eels (A. anguilla), weighing 200–300 g, were obtained from a commercial distributor. Fish were kept in indoor tanks containing 400 liters of well-aerated, dechlorinated, and continuously depurated tap water at environmental temperature (18–20°C) and under natural photoperiod. Animals for experiments were randomly selected from tanks after at least 2 wk of laboratory acclimation before death. Eels were not fed throughout the period of this study.

Chemicals. [3H]cAMP was a product of Amersham International. Adrenergic agonists and antagonists, HEPES, aminophylline, nucleotides, amyloglucosidase, and collagenase (type IV) were from Sigma (Milan, Italy). The glucose test kit was purchased from Boehringer Mannheim (Milan, Italy). The liquid scintillation solution Ready Protein was a product of Beckman (Nervine, Galway, Ireland). All other reagents were of the highest available purity.

Liver membrane preparation. After decapitation, the liver was removed, weighed, and homogenized by hand with a Potter-Elvehjem homogenizer in 10 volumes of buffer (5 mM Tris·HCl, 1 mM MgCl2, 0.25 M sucrose, pH 7.4). The homogenate was filtered through a single-layer gauze and centrifuged at 480 g for 10 min. The supernatant was centrifuged at 30,000 g for 10 min, and the precipitate resuspended with 50 mM Tris·HCl, pH 7.4, and washed twice (11). The final pellet was suspended in the same medium to a protein concentration of 3 mg/ml, as determined by the Lowry method (26) using bovine serum albumin as standard.

AC assay. One-hundred fifty micrograms of membrane proteins were incubated in a final volume of 400 μl containing (in mM) 50 Tris·HCl, 1 ATP, 3.75 MgSO4, 0.1 EGTA, 0.5 GTP, 3 aminophylline, pH 7.4, plus test substances or vehicles. Stock solution of forskolin (FSK) was prepared in DMSO. The
The sensitivity of eel liver membrane AC to some exogenous compounds known to affect this transduction system in mammalian preparations was then examined. The enzyme activity was potently stimulated by guanosine 5′-O-(3-thiotriphosphate) (GTPγS), a nonhydrolyzable analog of GTP, reaching a maximum activation of ~400% at 10⁻⁴ M (half-maximal activation at 1.35 × 10⁻⁷ M), whereas it was significantly reduced (~75% at 10⁻⁴ M) by guanosine 5′-O-(2-thiodiphosphate) (GDPβS), a competitive inhibitor of GTP (half-maximal inhibition at 2.1 × 10⁻⁷ M) (Fig. 3). The effects of NaF and FSK on AC activity and cAMP intracellular levels are illustrated in Fig. 4. NaF, which resembles the γ-phosphate of GTP in the interaction with the G protein of the cyclase system (2), induced a maximal stimulation of AC activity of ~400% at a concentration as high as 10⁻² M. The diterpene FSK, widely used to directly stimulate the catalytic subunit of the AC system, dramatically enhanced enzyme activity up to ~3,500% with respect to the basal level. Both compounds were also able to increase cAMP levels in intact hepatocytes, inducing a maximal stimulation of 377% (NaF) and 2,200% (FSK) at 5 min of incubation.

AC activity was significantly stimulated by the physiological adrenergic agonist Epi, reaching a maximum of 240% at 10⁻⁵ M, and also by phenylephrine (PE), although to a lesser extent (142% at 10⁻⁴ M) (Fig. 5A). In Fig. 5B, the remarkable cAMP accumulation in-
duced by CA in intact hepatocytes is illustrated. The stimulation by the adrenergic compounds of AC activity and cAMP production was counteracted by the addition of the \(\beta\)-adrenoceptor blocker Pro, but not by the \(\alpha\)-adrenoceptor antagonist phentolamine (PNT) (Table 1).

To establish a correlation between the activated transduction system and glucose release, the latter parameter was assessed in the presence of FSK and NaF. These substances, previously shown as potent activators of liver membrane AC activity and cAMP accumulation in intact hepatocytes (Fig. 4), also stimulated glucose release from cells (Fig. 6).

As expected from the glycogenolytic role exerted by CA in the liver of mammals and of some fish, Epi was indeed able to induce glucose release from isolated eel hepatocytes. Its stimulatory effect was maximum at 15 min (314% with respect to the relative control value), and decreased thereafter (262% at 30 min incubation) (Fig. 7). Routine evaluations were undertaken at 15 min. As described in Fig. 8, glucose output was increased by Epi and PE in a dose-dependent fashion, with a maximum at \(10^{-5}\) M for both adrenergic agonists.

The effects of Epi and PE were then tested in the presence of PNT and Pro (Fig. 9) to assess the relative contribution of the two receptor subtypes to the adrenergic stimulation of glucose release from isolated hepatocytes. Neither of the two antagonists, when added alone, was able to completely prevent glucose release; this effect was achieved only by the simultaneous presence of \(\alpha\)- and \(\beta\)-adrenergic receptor antagonists. These data prompted us to hypothesize that Epi and PE might regulate glucose metabolism in fish hepatocytes through both \(\alpha\)- and \(\beta\)-adrenoceptor-coupled transduction mechanisms, involving \(Ca^{2+}\) and cAMP as second messengers, respectively. Hence, the possible direct involvement of the two messengers was tested by using the calcium ionophore ionomycin and the permeant analog of cAMP, dibutyryl cAMP. The results shown in Fig. 10 indicate that \(Ca^{2+}\) and cAMP were both independently able to provoke glucose release from isolated eel hepatocytes.
AC properties have been widely studied in the liver of mammals, but little attention has been devoted to nonmammalian vertebrates. As to fish, a characterization of this enzyme has only been performed on catfish hepatocyte membranes (10, 34). Enhancement of cAMP intracellular levels by Epi has been previously reported in isolated hepatocytes from the American eel, *A. rostrata* (13). In similar preparations, PE was able to significantly increase cAMP concentrations by 20-fold, and glycogenolytic rate by 3-fold (31). PE, generally regarded as an \( \alpha \)-adrenergic agonist in mammals, has also a well-defined \( \beta \)-action in fish liver. In fact, it displaced both prazosin and dihydroalprenolol, \( \alpha \)- and \( \beta \)-adrenergic receptor antagonists, respectively, from their binding sites in catfish liver membranes (11, 15). Other glucoregulatory hormones have been shown to enhance cAMP production in fish liver cells. Incubation of eel hepatocytes with glucagon, for example, led to a large increase (almost 20-fold) in intracellular cAMP concentration, whereas exposure to glucagon-like peptide was accompanied by a less than twofold increase in cAMP levels, although gluconeogenetic and glycogenolytic effects of the two treatments were similar (28). These results, however, did not indicate any simple relationship between changes in cAMP levels and metabolic actions of the hormones, raising the question of whether the cyclic nucleotide is the only second messenger responsible for glucose release, or instead other messengers are involved.

This paper represents a first attempt to study the properties of AC in the liver of the European eel (*A. anguilla*), and to correlate adrenergic activation of the enzyme with glucose release from the hepatocytes.

Table 1. Effects of \( \alpha \)- and \( \beta \)-adrenergic antagonists on AC activity and cAMP levels in the presence of Epi and PE in the liver of the European eel

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>AC Activity, pmol cAMP·mg protein(^{-1})·10 min(^{-1})</th>
<th>cAMP Levels, nmol/g cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21.2 ± 0.9 (5)</td>
<td>1.01 ± 0.06 (4)</td>
</tr>
<tr>
<td>10 ( \mu )M Epi</td>
<td>48.0 ± 1.9* (5)</td>
<td>39.2 ± 2.80* (4)</td>
</tr>
<tr>
<td>10 ( \mu )M Epi + 100 ( \mu )M PNT</td>
<td>51.1 ± 2.5* (4)</td>
<td>40.3 ± 3.12* (4)</td>
</tr>
<tr>
<td>10 ( \mu )M Epi + 100 ( \mu )M Pro</td>
<td>22.3 ± 0.5 (4)</td>
<td>1.12 ± 0.08 (4)</td>
</tr>
<tr>
<td>10 ( \mu )M PE</td>
<td>31.4 ± 1.8* (5)</td>
<td>3.32 ± 0.18* (4)</td>
</tr>
<tr>
<td>10 ( \mu )M PE + 100 ( \mu )M PNT</td>
<td>30.3 ± 2.0* (4)</td>
<td>3.41 ± 0.24* (4)</td>
</tr>
<tr>
<td>10 ( \mu )M PE + 100 ( \mu )M Pro</td>
<td>20.6 ± 0.4 (4)</td>
<td>1.15 ± 0.09 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of experiments run in duplicate indicated in parentheses. Adenylyl cyclase (AC) activity was assessed on liver membrane preparations and cAMP levels in intact hepatocytes; for incubation conditions see MATERIALS AND METHODS. Epi, epinephrine; PE, phenylephrine; PNT, phentolamine; Pro, propranolol. *Statistically different from control values (\( P < 0.01 \)).

Fig. 5. Dose-response effect of epinephrine and phenylephrine on adenylyl cyclase activity in eel liver membranes (A) and on cAMP levels in intact hepatocytes (B). Mean values ± SE of 5 separate experiments run in duplicate are shown. Levels of significance: \( \ast P < 0.05 \) and \( \ast\ast P < 0.01 \) compared with basal values (AC: 21.2 ± 0.9 pmol cAMP·mg protein\(^{-1}\)·10 min\(^{-1}\); cAMP intracellular levels: 1.01 ± 0.06 nmol/g cell).

**DISCUSSION**

AC properties have been widely studied in the liver of mammals, but little attention has been devoted to nonmammalian vertebrates. As to fish, a characterization of this enzyme has only been performed on catfish hepatocyte membranes (10, 34).

Enhancement of cAMP intracellular levels by Epi has been previously reported in isolated hepatocytes from the American eel, *A. rostrata* (13). In similar preparations, PE was able to significantly increase cAMP concentrations by \( \sim \)20-fold, and glycogenolytic rate by \( \sim \)3-fold (31). PE, generally regarded as an \( \alpha \)-adrenergic agonist in mammals, has also a well-defined \( \beta \)-action in fish liver. In fact, it displaced both prazosin and dihydroalprenolol, \( \alpha \)- and \( \beta \)-adrenergic receptor antagonists, respectively, from their binding sites in catfish liver membranes (11, 15). Other glucoregulatory hormones have been shown to enhance cAMP production in fish liver cells. Incubation of eel hepatocytes with glucagon, for example, led to a large increase (almost 20-fold) in intracellular cAMP concentration, whereas exposure to glucagon-like peptide was accompanied by a less than twofold increase in cAMP levels, although gluconeogenetic and glycogenolytic effects of the two treatments were similar (28). These results, however, did not indicate any simple relationship between changes in cAMP levels and metabolic actions of the hormones, raising the question of whether the cyclic nucleotide is the only second messenger responsible for glucose release, or instead other messengers are involved.

This paper represents a first attempt to study the properties of AC in the liver of the European eel (*A. anguilla*), and to correlate adrenergic activation of the enzyme with glucose release from the hepatocytes.

Fig. 6. Dose-response effect of NaF and FSK on glucose release from isolated eel hepatocytes. Each column represents mean ± SE of 4 separate experiments run in duplicate. Levels of significance: \( \ast P < 0.05 \) and \( \ast\ast P < 0.01 \) compared with basal values (5.0 ± 0.3 \( \mu \)mol/g cell).
This study indicates that eel hepatocyte membranes contain an active AC whose characteristics are not substantially different from those of the mammalian and catfish enzyme, at least with regard to ATP and Mg²⁺ requirements (34). As for GTP modulation, it is generally accepted that the nucleotide is required for the full expression of agonist effect on AC (39). The sensitivity of basal enzyme activity to GTP appears to differ according to the animal species and the tissue examined, as reported for salmon granulosa cells (27), catfish and rat liver (34), fish gill (18), and frog liver (19). In eel liver, the basal AC activity was unaffected by increasing concentrations of GTP added to the incubation medium, whereas the enzyme activity was finely regulated by the nucleotide when the receptor was activated. Such a modulation was well described...
by a diphasic dose-response curve: maximum stimulation by the catecholamine was achieved at 10^{-5} M GTP, generally regarded as the physiological concentration of the nucleotide (4); at higher concentrations, capable of inducing full activation of the inhibitory G protein (1), the enzyme stimulation was progressively reduced.

To further characterize AC activity, GTPγS and GDPβS were used, because these stable analogs of GTP and GDP, respectively, can mimic the natural compounds in their binding to the a-subunits of the G proteins. As expected on the basis of the model of Rodbell et al. (39), GTPγS dose-dependently activated the enzyme, whereas GDPβS led to a significant inhibition of cAMP production.

NaF also increased the enzyme activity, with a maximum effect at a concentration as high as 5 \times 10^{-2} M. It must be emphasized, however, that the active species mimicking the γ-phosphate of GTP is AlF₄⁻, whose effective concentration cannot be calculated but is expected to be in the micromolar range (2). A potent stimulation of AC activity was observed at all FSK concentrations tested. Such a strong effect could be ascribed to the ability of the diterpene to interact with all the AC catalytic subunits of the tissue independently of their coupling or uncoupling to specific receptors. Taken together, these data provide evidence for different potency. Impermeant guanine nucleotide analogs GTPγS and GDPβS could not be tested on cells.

The AC activity was responsive to both Epi and PE, the latter compound being less effective than the natural CA. Moreover, CA potently increased intracellular cAMP production also in eel hepatocytes, as already reported for other fish (5, 31). The action of CA on the AC system could be ascribed to β-adrenergic receptor occupation, because Pro, but not PNT, proved able to counteract the effects of Epi and PE.

As for the glucoregulatory effects of CA, it is well documented that hormone infusion elicits hyperglycemia in teleosts (8, 43), and glycogenolysis has been reported as the main process accounting for CA-stimulated hepatic glucose release (21, 24, 32, 44).

To gain information about the role played by CA in the regulation of eel liver carbohydrate metabolism, glucose release from isolated hepatocytes was evaluated. Time course experiments showed that Epi-stimulated glucose release reached a maximum percent increase at 15 min, decreasing thereafter. Such an effect was probably due not to hormone degradation by piscine hepatocytes (7) but rather to glucose accumulation in the medium, which prevents further glucose release, as previously demonstrated for catfish hepatocytes (35).

The stimulation of AC activity, the rise of intracellular cAMP levels, and the increase of glucose release elicited by FSK suggest a cAMP-dependent effect of the diterpene in our experimental system. cAMP-independent effects of the compound, such as those described in mammalian cells (25), cannot however be excluded. As for NaF, this induced a statistically significant glucose release stimulation only at concentrations as high as 5 \times 10^{-2} M. It has previously been demonstrated that NaF activates G proteins in isolated cells (33), but also that it affects phosphatase activities and ion channels (37). Therefore, it is not yet possible to establish the relative contribution of NaF through direct or indirect effects on cAMP-mediated glucose release.

A lack of proportion between the extent of AC stimulation and the amount of glucose release evoked by the tested compounds can be observed. Such a discrepancy has often been subject to comment (28, 38), and the

### Table 2. Stimulatory effects of Epi and FSK on AC activity, cAMP, IP₃, Ca^{2+} intracellular levels, and glucose release in eel and catfish hepatocytes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Eel</th>
<th>Catfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC activity, pmol·mg protein^{-1}·10 min^{-1}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>21.2 ± 0.9⁹</td>
<td>40.0 ± 3.7⁶–20.2 ± 1.8⁶</td>
</tr>
<tr>
<td>10⁻⁵ M Epi</td>
<td>48.9 ± 0.8⁹</td>
<td>62.4 ± 5.9⁶–32.1 ± 3.0⁶</td>
</tr>
<tr>
<td>10⁻⁵ M FSK</td>
<td>428.5 ± 35.2⁹</td>
<td>213.5 ± 23.0⁴</td>
</tr>
<tr>
<td>cAMP levels, nmol·g cell^{-1}·5 min^{-1}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>1.0 ± 0.06–0.7 ± 0.1⁶</td>
<td>1.1 ± 0.05⁹</td>
</tr>
<tr>
<td>10⁻⁵ M Epi</td>
<td>39.2 ± 2.5–35.8 ± 1.2⁶</td>
<td>14.8 ± 0.5–9.1 ± 1.6⁶</td>
</tr>
<tr>
<td>10⁻⁵ M FSK</td>
<td>16.3 ± 0.9⁹</td>
<td>14.0 ± 3.6⁶</td>
</tr>
<tr>
<td>IP₃ levels, nmol·g cell^{-1}·45 s^{-1}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>2.0 ± 0.05⁹</td>
<td>5.5 ± 0.12⁹</td>
</tr>
<tr>
<td>10⁻⁵ M Epi</td>
<td>4.6 ± 0.24⁹</td>
<td>9.4 ± 0.33⁹</td>
</tr>
<tr>
<td>[Ca^{2+}], mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>79.6 ± 14.6⁷</td>
<td>183.9 ± 23.0⁶</td>
</tr>
<tr>
<td>10⁻⁵ M Epi</td>
<td>184.3 ± 30.0⁷</td>
<td>433.5 ± 135.6⁶</td>
</tr>
<tr>
<td>Glucose release, µmol·g cell^{-1}·15 min^{-1}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No addition</td>
<td>5.0 ± 0.3⁹</td>
<td>5.2 ± 0.6⁹</td>
</tr>
<tr>
<td>10⁻⁵ M Epi</td>
<td>20.3 ± 1.4⁹</td>
<td>30.1 ± 4.4⁹</td>
</tr>
<tr>
<td>10⁻⁵ M FSK</td>
<td>14.1 ± 1.6⁹</td>
<td>25.0 ± 3.3⁹</td>
</tr>
</tbody>
</table>

Values are means ± SE from the following: ⁹ present study; ⁶ Ref. 35; ⁷ Fabbi et al., unpublished observations; ⁵ Ref. 30; ⁴ Ref. 13; ⁸ Ref. 45. The data presented were determined in comparable experimental conditions, except for ⁹ at 30°C and ⁶ at 22°C. All effects are significantly different from respective control values (at least P < 0.05). FSK, forskolin; IP₃, inositol trisphosphate; [Ca^{2+}], intracellular calcium concentration.
answer might lie in any step of the transduction pathway. Several data from our and other laboratories regarding the modulation of the adrenergic transduction pathways by the receptor agonist Epi and the nonreceptor activator FSK in the liver of two different fish species are reported in Table 2. It can be seen that the receptor-regulated AC system seems more sensitive to agonists in eel than in catfish. Assuming that FSK directly interacts with AC (20), the reported comparison suggests that, in eel liver membranes, a greater number of AC moieties are present with respect to catfish. However, the extent of glucose release does not appear proportional to AC activation, and it can be seen that glucose output is more stimulated in the catfish, whereas AC activity is more affected in the eel. As for the $\alpha_1$-adrenergic transduction pathway, evidence that Ca$^{2+}$ and/or inositol trisphosphate (IP$_3$) intracellular changes trigger glucose output from the cells has not yet been found, notwithstanding extensive studies (12, 13, 15, 45).

Intriguing results were obtained when glucose release was evaluated in eel hepatocytes incubated in the presence of adrenergic agonists, together with the antagonists Pro and PNT. Neither of the two adrenoceptor blockers completely prevented the response to Epi and PE, implying that the $\beta$-adrenoceptor and AC are components of the transduction pathway through which CA stimulate glucose release in these cells, but also that further mechanisms might be involved.

The classification and properties of adrenergic receptors present in the liver of nonmammalian vertebrates have been the subject of much debate in recent years. Not so long ago, the glycogenolytic effect exerted by CA on the liver of ectothermal vertebrates was thought to be exclusively mediated via $\beta$-adrenergic receptor occupation and enhancement of cAMP biosynthesis (3, 21–23, 40). The first circumstantial evidence for an $\alpha$-like adrenoceptor system in the liver of ectothermal animals was reported by Moon and Mommsen (31) and by Fabbri et al. (15). However, the presence of the $\alpha_1$-adrenoceptor-IP$_3$-Ca$^{2+}$ transduction system in the hepatocytes of some fish has recently been reported: 1) $\alpha_1$-adrenoceptors have been identified in catfish, eel, and trout liver membranes (12, 13, 15, 17); 2) CA have been reported to increase IP$_3$ levels in eel and catfish hepatocytes (13, 17); and 3) in the hepatocytes of the same fish, CA modulate calcium concentration (29, 45). Despite all these observations, no correlation between $\alpha_1$-adrenergic receptor occupancy and physiological response has been reported in fish. In mammals, on the contrary, experimental evidence has led to a model in which CA activate in parallel $\alpha$- and $\beta$-transduction pathways, each partially accounting for the glycogenolytic response (9). In eel hepatocytes, dibutyryl cAMP has previously been reported to stimulate glycogen phosphorylase activity (16), and ionomycin to act as Ca$^{2+}$ ionophore (45). Our results clearly demonstrate that both compounds are able to dose-dependently increase glucose output in the same preparation. Because $\alpha_1$-adrenergic receptors have been shown in eel liver (13), it can be hypothesized that dibutyryl cAMP and ionomycin induce glucose release through two separate transduction pathways.

**Perspectives**

The present work provides the first characterization of the eel liver AC system and suggests the direct involvement of cAMP in CA-induced glucose release. There is some evidence that also Ca$^{2+}$ may have a role in triggering this cell response to adrenergic agonists. The relationship between intracellular Ca$^{2+}$ changes and physiological effects has not been established in the liver of any fish, and further research is required to quantify the relative contribution of cAMP- and Ca$^{2+}$-dependent pathways to the CA modulation of glucose metabolism in fish hepatocytes. This research has been considerably hampered by the lack of selective $\alpha$-adrenergic agonists specific for fish liver cells, and it is hoped that future work will help to solve this pharmacological problem. The use of adrenergic ligands specific for fish receptors could also definitively clarify the similarities and differences between the CA receptor system of mammals and fish and give information about the evolution of adrenergic receptors within vertebrates. Despite intensive experimentation, a satisfactory description of the adrenergic transduction pathway in ectothermal vertebrates has yet to emerge. The use of in vitro preparations has been of great value to our knowledge of CA control of fish liver metabolism, although the physiological significance might be questioned; in fact, high hormone concentrations are usually used and the influence of integrated endocrine functions are not taken into account. However, a better understanding of hepatic carbohydrate metabolism in fish at a cellular level will surely contribute to revealing some interesting and controversial aspects of their metabolism, such as the ability to survive for a long time without food and with minimal variations of hepatic glycogen.

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