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REGULATION BY VARIOUS HORMONES AND AGENTS OF ADENOSINE-3',5'-MONOPHOSPHATE LEVELS IN ISLETS OF LANGERHANS OF RATS*

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Abstract—Regulation by various hormones and agents of adenosine-3',5'-monophosphate (cyclic AMP) levels in intact islets of rat pancreas was studied, employing either the prelabeling technique or the protein kinase catalytic method. Many agents were found to increase islet cyclic AMP levels. These include corticotropin, glucagon, isoproterenol, prostaglandins (E_1, E_2, A_1 and $F_{1\alpha}$), secretin, thyroid-stimulating hormone, β-adrenergic agonists, tolbutamide and GTP. Epinephrine caused a slight decrease in islet cyclic AMP level, but in the presence of α-adrenergic blockers, they conversely increased the cyclic AMP levels. Isoproterenol, on the other hand, increased cyclic AMP levels and its effect was abolished by β-adrenergic blocking agents. The evidence presented further substantiates the contention that in rat islet plasma membranes there exist both α- and β-ardenergic receptors and that cyclic AMP levels in islets are closely modulated by α- and β-adrenergic receptor activation.

WE HAVE reported earlier[1] an improved method for the isolation of islets of Langerhans from rat pancreas. The key step in this method, which routinely yielded up to 500 islets per pancreas, involved pretreatment of rats with pilocarpine. This allowed depletion of zymogens from the exocrine pancreatic tissue and thus minimized the destruction of islet membranal structure which could otherwise occur during the collagenase digestion of the tissue. We have shown[1] that many agents, including those shown to stimulate insulin secretion *in vivo* and *in vitro*, activate adenylate cyclase in the homogenates of islets prepared by this method.

In the present study, we have examined the effects of various hormones and agents on the adenosine-3',5'-monophosphate (cyclic AMP) levels in the incubated intact islets, employing either the prelabeling technique[2,3] or the protein kinase catalytic method[4] for the measurement of changes in the cellular cyclic nucleotide levels. The results obtained with the present studies confirm our findings previously made with the broken cell system,[1] clearly suggesting a role for the cyclic AMP system in the action of certain insulinogenic agents.

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MATERIALS AND METHODS

Materials. Adenine-2-[^{3}H], 25 Ci/m-mole, was purchased from Schwarz/Mann and AG50W-X8 (100–200 mesh, hydrogen form) was obtained from BioRad. The following compounds were gifts of the companies mentioned: tolbutamide (Orinase) and prostaglandins (E_1, E_2, A_1 and $F_{1\alpha}$) were from Upjohn; phenoxybenzamine was from Smith, Kline & French; KÖ 592 was from C. H. Boehringer Sohne (Italy); phentolamine was from Ciba; MJ 1999 was from Mead Johnson. Cyclic AMP-dependent protein kinase from bovine heart was prepared according to the procedure described elsewhere.[5] Other materials used were the same as reported earlier.[1]

Isolation and prelabeling of the islets. Pancreatic pieces pooled from three to five rats previously treated with pilocarpine were incubated with collagenase and the free islets were collected as previously described.[1] About 1200–2000 islets were usually collected and they were suspended in 4 ml of Krebs–Ringer bicarbonate buffer containing glucose (2 mg/ml) and 50 mCi of adenine-2-^{3}H. The islets were incubated for 20 min at 37°, with shaking. At the end of the prelabeling period, 40 ml of ice-cold buffer was added, the islet suspension centrifuged briefly, and the supernatant fluid removed by aspiration. The washing procedure was repeated five times, and the islets were finally transferred to an appropriate volume of the same buffer to yield a suspension containing 25–30 islets/ml.

In experiments (see Table 3) in which the absolute levels of intracellular cyclic AMP were measured by the protein kinase catalytic method,[4] the isolated islets were used directly for incubation, as described below, with the omission of the prelabeling step.

Incubation of islets and measurement of cyclic AMP. For experiments involving the studies of the relative levels of cyclic AMP, aliquots (2.5 ml) of the suspension of the prelabeled islets were incubated at 37° for 10 min in the presence of 10 mM theophylline and various agents as indicated. At the end of the incubation period, the reaction mixture was centrifuged and the supernatant removed. One-half ml of ice-cold 5% trichloroacetic acid and 0·01 ml of 1 mM cyclic AMP (as carrier) were then added to the islets. The islets were then quickly sonicated for 0·5 min, the precipitate was removed by centrifugation, and the supernatant fluid was neutralized with 1 M Tris. The radioactive cyclic AMP in the supernatant fluid was purified by the $BaSO_4$ method[6] and the precipitates were removed by centrifugation. The supernatant fluid was lyophilized, the residue was taken up in 0·5 ml water, and the entire volume of the solution was loaded onto columns (0·5 × 2·5 cm) of AG50W-X8; the columns were then eluted with water. Cyclic AMP was collected in the third and fourth ml fraction. One-ml aliquots of the pooled fractions were counted for radioactivity.

In experiments (see Table 3) in which the absolute amounts of cyclic AMP were assayed, aliquots (usually 0·1 to 0·4 ml) of the eluate from the column as described above were lyophilized and the amount of cyclic AMP was assayed by the protein kinase catalytic method.[4] No carrier cyclic AMP was added to the islet samples in these experiments. The islet protein from the trichloroacetic acid precipitate was measured by the method of Lowry *et al.*,[7] using bovine serum albumin as a standard protein.

RESULTS AND DISCUSSION

Corticotropin, glucagon, prostaglandins (E_1, E_2, A_1 and $F_{1\alpha}$), secretin, thyroid-stimulating hormone, GTP and tolbutamide (an oral hypoglycemic drug) were all

TABLE 1. REGULATION BY VARIOUS AGENTS OF THE RELATIVE LEVELS OF CYCLIC AMP IN ISOLATED PANCREATIC ISLETS AS DETERMINED BY THE PRELABELING METHOD*

Additive	Cyclic AMP (cpm × 10^{-2}/mg protein)
None (control)	20·5 ± 1·5
Corticotropin (2 μM)	62·8 ± 3·8†
Glucagon (5 μM)	42·6 ± 3·2†
Prostaglandin E_1 (20 μg/ml)	70·5 ± 4·5†
Prostaglandin E_2 (30 μg/ml)	81·0 ± 6·1†
Prostaglandin A_1 (30 μg/ml)	62·8 ± 6·5†
Prostaglandin $F_{1\alpha}$ (20 μg/ml)	92·5 ± 8·2†
Secretin (20 μg/ml)	45·2 ± 1·6†
Thyroid-stimulating hormone (20 μg/ml)	40·6 ± 3·4†
GTP (7 μM)	85·0 ± 10·2†
Tolbutamide (0·1 mM)	42·6 ± 4·0†
Phenformin (0·1 mM)	21·2 ± 3·5‡
Glucose (10 mM)	20·6 ± 1·8‡
Leucine (10 mM)	21·4 ± 3·9‡

* About 60–70 islets were incubated at 37° for 10 min, with shaking, in the presence and absence of various additives, in 2·5 ml Krebs Ringer bicarbonate buffer containing 10 mM theophylline. The values presented are the means ± standard errors of three to five separate experiments performed on different days.
† Significantly different from the control (P < 0·01).
‡ Not significantly different from the control.

found to increase the levels of radioactive cyclic AMP in intact pancreatic islets incubated in the presence of 10 mM theophylline (Table 1). The formation and accumulation of radioactive cyclic AMP were linear, at least up to 12 min under the incubation conditions. Theophylline (10 mM), when present alone, increased slightly (10

TABLE 2. REGULATION BY ADRENERGIC AGONISTS AND ANTAGONISTS OF THE RELATIVE LEVELS OF CYCLIC AMP IN ISOLATED PANCREATIC ISLETS AS DETERMINED BY THE PRELABELING METHOD*

Additive	Cyclic AMP (cpm × 10^{-2}/mg protein)
None (control)	19·8 ± 1·2
DL-Isoproterenol (0·05 mM)	82·5 ± 6·2†
DL-Propranolol (0·05 mM)	19·1 ± 2·6‡
MJ 1999 (20 μg/ml)	18·8 ± 3·6‡
KÖ 592 (0·1 mM)	20·2 ± 3·1‡
DL-Isoproterenol plus DL-propranolol	31·5 ± 5·2§
DL-Isoproterenol plus MJ 1999	35·2 ± 6·8§
DL-Isoproterenol plus KÖ 592	26·6 ± 3·3§
DL-Epinephrine (0·05 mM)	12·5 ± 3·5‖
Phenoxybenzamine (0·05 mM)	19·6 ± 3·8‡
Phentolamine (0·05 ± mM)	20·8 ± 1·6‡
DL-Epinephrine plus phenoxybenzamine	86·8 ± 6·9†
DL-Epinephrine plus phentolamine	81·9 ± 2·8†

* Incubation conditions were the same as in Table 1 except for the variation in kinds and amounts of additives, as indicated. The values presented are the means ± standard errors of three separate experiments performed on different days.
† Significantly different from the control (P < 0·01).
‡ Not significantly different from the control.
§ Significantly different from isoproterenol alone (P < 0·01).
‖ Significantly different from the control (P < 0·05).

TABLE 3. REGULATION BY VARIOUS AGENTS OF THE ABSOLUTE LEVELS OF CYCLIC AMP IN ISOLATED PANCREATIC ISLETS AS DETERMINED BY THE PROTEIN KINASE CATALYTIC METHOD*

Additive	Cyclic AMP (pmoles/mg protein)
None (control)	52·8 ± 2·8
Glucagon (5 μM)	123·6 ± 5·4†
Prostaglandin E_1 (20 μg/ml)	180·0 ± 12·7†
GTP (7 μM)	198·2 ± 10·2†
Tolbutamide (0·1 mM)	121·6 ± 3·8†
Glucose (10 mM)	51·6 ± 3·2‡
DL-Isoproterenol (0·05 mM)	206·7 ± 15·6†
KÖ 592 (0·1 mM)	50·2 ± 3·8‡
DL-Isoproterenol plus KÖ 592	58·6 ± 6·2§
DL-Epinephrine (0·05 mM)	36·2 ± 3·1
Phenoxybenzamine (0·05 mM)	51·8 ± 6·2‡
DL-Epinephrine plus phenoxybenzamine	165·0 ± 10·2†

* Incubation conditions were the same as in Table 1 except for the variation in kinds and amounts of additives, as indicated. The sample from individual incubation was assayed for cyclic AMP in triplicate. The values presented are the means ± standard errors of four separate experiments performed on different days.

† Significantly different from the control ($P < 0.01$).

‡ Not significantly different from the control.

§ Significantly different from isoproterenol alone ($P < 0.01$).

Significantly different from the control ($P < 0.05$).

15%) the cyclic AMP levels. These agents, with the exception of GTP, have been shown to stimulate insulin secretion *in vitro* or *in vivo*, or both (see Ref. 1 for a survey of the literature). Phenformin, another class of oral anti-hyperglycemic drug believed to exert its effect through a mechanism different from that of tolbutamide, was not effective. Glucose and leucine, both known to stimulate insulin secretion effectively, did not elevate islet cyclic AMP, suggesting that cyclic AMP may not be involved in their actions.

Isoproterenol effectively increased islet cyclic AMP levels and this stimulation was antagonized by all three of the β-adrenergic blockers tested (propranolol, MJ 1999 and KÖ 592) (Table 2). Epinephrine, on the other hand, caused a slight reduction in cyclic AMP levels. In the presence of the α-adrenergic blockers (phenoxybenzamine and phentolamine), however, the inhibition by epinephrine was overcome, and conversely, pronounced increases in cyclic AMP were noted. Neither the α- nor the β-adrenergic blockers have any significant effect when present alone. The present results confirm the findings of Turtle and Kipnis[8] made in a similar study using intact islets and our own findings[1] using islet homogenates. These results also support the contention that rat pancreatic islets contain both α- and β-adrenergic receptors.[1,8,9]

Most of the agents whose effects on the relative levels of cyclic AMP were measured with the prelabeling method (Tables 2 and 3) were re-examined for their effects on the absolute cyclic nucleotide levels in the islets. The results are presented in Table 3. It is clear that the effects of the agents determined by these two methods are closely similar.

Although a rigid relationship between the effects of many hormones and agents on intracellular cyclic AMP levels of islets and their effects on insulin secretion by

the organelle remains to be fully established, several lines of experimental evidence, including our own, appear to support the concept that cyclic AMP may mediate the action of certain insulinogenic agents. It is interesting that the islet cyclic AMP levels are increased by a wide variety of substances, perhaps reflecting a central role of insulin in many homeostatic mechanisms. Increases in islet cyclic AMP levels brought about by those agents are due to their direct stimulation of islet adenylate cyclase activity,[1] since none of them, at the same concentrations tested, had any significant effects on islet phosphodiesterase activity either in the presence or absence of 10 mM theophylline.[1]

REFERENCES

1. W. N. Kuo, D. S. Hodgins and J. F. Kuo, *J. biol. Chem.* **248**, 2705 (1973).
2. J. F. Kuo and I. K. Dill, *Biochem. biophys. Res. Commun.* **32**, 333 (1968).
3. J. F. Kuo and E. C. DeRenzo, *J. biol. Chem.* **244**, 2252 (1969).
4. J. F. Kuo and P. Greengard, *J. biol. Chem.* **245**, 4067 (1970).
5. J. F. Kuo and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **64**, 1349 (1969).
6. G. Krishna, B. Weiss and B. B. Brodie, *J. Pharmac. exp. Ther.* **163**, 379 (1968).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
8. J. R. Turtle and D. M. Kipnis, *Biochem. biophys. Res. Commun.* **24**, 797 (1967).
9. T. Atkins and A. J. Matty, *J. Endocr.* **51**, 67 (1971).