GLUCAGON STRUCTURE AND FUNCTION. II.

INCREASED ACTIVITY OF IODOGLUCAGON

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SUMMARY: Iodination of glucagon with ICl markedly enhances biological activity as measured by activation of rat liver adenylate cyclase and by hyperglycemia in rabbits. Adenylate cyclase activity is increased about 5-fold after monoiodination of glucagon and about 10-fold after more extensive iodination. Our finding that iodoglucagon exhibits enhanced biological activity may affect the interpretations of recent receptor studies involving the binding of [^{125}I]glucagon to plasma membranes.

We previously reported (1) that administration of I_4-glucagon[1] produced a greater response than glucagon itself in increasing activity of hepatic l-phenylalanine:pyruvate aminotransferase. This increase in hepatic enzyme activity is probably mediated by cAMP[1] formed from the activation of hepatic adenylate cyclase by iodoglucagon. Several investigators (2-8) are studying the activation of adenylate cyclase by glucagon and are attempting to correlate this activation with the binding of [^{125}I]glucagon to plasma membranes; these correlations are based on a finding (5) that monoiodination of glucagon does not alter hormonal activity. It is apparent that the interpretation of such studies could be altered if iodoglucagon were more active than glucagon itself. The purpose of this report is to describe our findings that iodination enhances the ability of glucagon to activate hepatic

[1]The abbreviations used are: I-, I_2-, I_3-, I_4-, I_5-glucagon= mono-, di-, tri-, tetra-, and pentaiodoglucagon, respectively; cAMP=cyclic adenosine 3',5'-monophosphate.

adenylate cyclase and also increases the hyperglycemic response in rabbits.

METHODS

Hyperglycemic assays were performed as described in the first paper of this series (9). Adenylate cyclase activation was measured using the method of Krishna *et al.* (10) as modified by Pohl *et al.* (6). Partially purified rat liver plasma membranes were prepared by the method of Neville (11), through step 12. Our membrane preparations without hormonal stimulation converted [α-^{32}P]ATP to [^{32}P]cAMP at a rate of 0.6 to 1.2 nmoles cAMP per mg membrane protein per 10 minutes. Addition of one μM glucagon during the reaction gave a 2 to 5-fold stimulation of the enzyme. Since we wished to compare the activity of a number of glucagon derivatives and since each membrane preparation gave slightly different basal and maximal responses, we expressed the result as percent of maximal activation provided by μM glucagon after subtracting the basal value. Each assay contained 4 to 6 replicates each of the basal and glucagon responses.

Iodination of glucagon was carried out in an ice bath at pH 8-8.5 using a nearly stoichiometric amount of ICl prepared by the method of McFarlane (12). About 0.04 μCi Na^{125}I was added per nmole of ICl as an aid to determining the extent of iodination. Iodoglucagon was purified by gel filtration through columns of Sephadex G-10 in 1M acetic acid. We prepared iodinated glucagon mixtures containing an average of about one through five g-atoms iodine per mole. Details of the preparation and characterization of these products will be described in a later report.

RESULTS AND DISCUSSION

Fig. 1 shows the activation of rat liver adenylate cyclase by

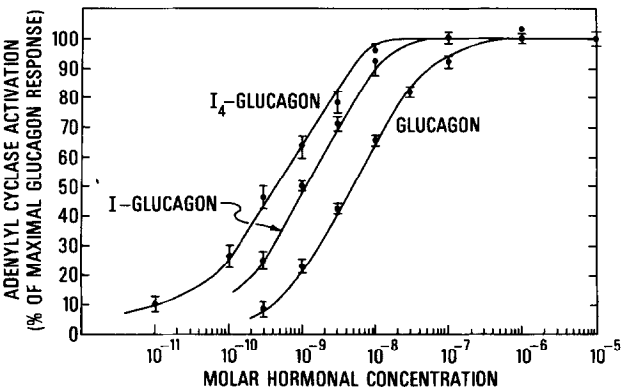

Fig. 1. Activation of adenylate cyclase by glucagon and derivatives. Dose-response curves for glucagon, I-, and I_4-glucagon. Bars represent standard errors. Each point represents the mean of at least six replicates.

different quantities of glucagon, I-glucagon, and I_4-glucagon. Our glucagon dose-response curve is nearly identical to that obtained by Pohl *et al.* (13). They found about 4 nM glucagon to be required for half-maximal stimulation of adenylate cyclase, and our data indicate about 4.8 nM glucagon gives half-maximal activation. However, Rodbell and coworkers (5) obtained identical dose-response curves for glucagon and I-glucagon, which is not in agreement with our data in Fig. 1. We find that the relative potency of I-glucagon (Table I) is nearly five times that of glucagon in the adenylate cyclase assay. We also found the hyperglycemic potency of I-glucagon to be significantly greater than that of glucagon: 183%, with 95% confidence limits of 137-240%. Similar results have been obtained repeatedly with numerous preparations of iodoglucagon. Reasons for the discrepancy between our results and those of Rodbell *et al.* (5) are not readily apparent. However, their studies were made with very small quantities of glucagon and with different methods of iodination. In any case, it will be important to resolve the question to clarify interpretations of [^{125}I]glucagon binding to membranes.

TABLE I. STIMULATION OF ADENYLATE CYCLASE BY IODOGLUCAGONS

Compound	% Activation at 1 μM (±S.E.)	nM Concentration Required for 50% Activation[a]	Relative Potency[b]
glucagon	100	4.8	100
I-glucagon	100 ± 3.7	1.0	480
I_2-glucagon	99 ± 1.9	0.84	570
I_3-glucagon	99 ± 4.4	0.48	1000
I_4-glucagon	103 ± 2.1	0.48	1000
I_5-glucagon	104 ± 2.7	0.48	1000

[a]Determined from dose-response curve.

[b]Calculated from hormone concentration required for 50% activation.

Increasing the iodine content of glucagon further enhances biological activity (Table I and Fig. 2). The adenylate cyclase dose-response curves for I_3- and I_5-glucagon (not reported) are identical within experimental error to that found for I_4-glucagon (Fig. 1); these three iodoglucagons are about 10 times more active than glucagon (Table I). In the bioassay based on hyperglycemia, I_2- through I_5-glucagons produced a steeper dose-response line than U.S.P. glucagon (data not reported), thus preventing us from obtaining a quantitative comparison using this four-point, parallel-line, cross-over bioassay. However, when we administered these iodoglucagon preparations to cortisone-primed rabbits and measured the blood glucose at intervals over four hours (Fig. 2), the enhancing effect of iodination on biological activity was again evident.

No direct evidence is available for the mechanism of the increased biological action of the iodoglucagons. However, among

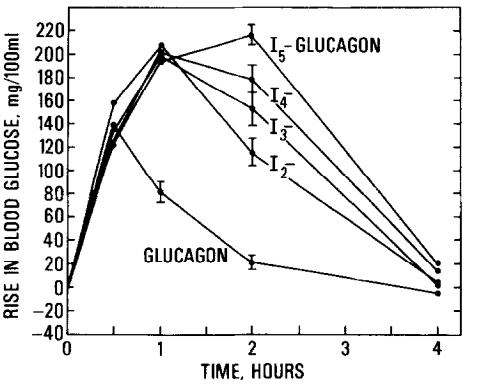

Fig. 2. Rise in blood glucose in cortisone-pretreated rabbits following the subcutaneous injection of 3 μg per kg of glucagon or iodoglucagon. Each point represents the mean results from 24 rabbits except for the glucagon results, wherein 16 rabbits each were used. Bars represent standard errors.

the possible reasons one must consider (A) enhanced binding of iodoglucagon to the glucagon receptor and/or (B) a lower rate of destruction of iodoglucagon. The enhanced activity may have resulted from an increase in the ionized form of the tyrosyl hydroxyl group (depression of pK by iodination) at physiological pH, from increased hydrophobic properties imparted by the iodine, or from combinations of these and other factors.

Whatever the reason for the enhanced activity of iodoglucagon, our finding is clearly important to the interpretation of current membrane receptor studies using [^{125}I]glucagon, particularly since conclusions drawn from these binding studies are based on the idea that [^{125}I]glucagon has behavior identical to glucagon.

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