Possible Role of Endogenous Prostaglandins in Glucagon Secretion by Isolated Guinea-Pig Islets

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Summary. Previous studies have demonstrated that prostaglandins stimulate glucagon secretion *in vitro* and *in vivo*. The present work was aimed at investigating the influence of two inhibitors of prostaglandin synthesis, isopropyl-2 nicotinoyl-3 indole (L8027) and indomethacin, on basal and arginine- or noradrenaline-stimulated glucagon release from isolated guinea-pig islets incubated in the absence of glucose. L8027 (10^{-4} and 10^{-5} mol/l) did not alter basal glucagon release, blocked almost completely the glucagon response to arginine (10^{-2} mol/l), had no effect on the glucagon release induced by noradrenaline (10^{-4} mol/l), but reduced the stimulatory effect of a lower concentration of noradrenaline ($5 \cdot 10^{-7}$ mol/l). The kinetic study of this inhibitory effect demonstrated that (1) it necessitates preincubation of the islets with L8027 for 30 minutes before the addition of arginine, (2) after a short preincubation period (30 minutes) in the presence of L8027, removal of the inhibitor at the time of arginine stimulation resulted in enhanced glucagon response, (3) on the contrary, after a prolonged incubation period (75 min) with arginine and L8027, the inhibitory effect remained transiently detectable after removal of L8027. Indomethacin similarly blocked arginine- and noradrenaline-induced glucagon secretion. These results suggest that an intra-insular synthesis of prostaglandins is involved in the A cell response to arginine and noradrenaline.

Key words: Isolated islets, guinea-pig, prostaglandin synthesis, glucagon secretion, arginine, noradrenaline, indomethacin.

augment the glucagon response to other secretagogues such as L-arginine [1]. On the other hand, Sacca and Perez [2] reported that PGE₁ intravenous infusion significantly increased glucagon plasma levels in the rat while PGA₁ had no effect. Although these results are in keeping with the hypothesis that prostaglandins (PGs) may be involved in the regulation of glucagon secretion, they do not clarify the possible role of intra-islet synthesis of PGs in the stimulus-secretion coupling of A cells.

The present work was aimed at investigating the influence of inhibitors of PG synthesis on glucagon release by isolated guinea-pig islets incubated *in vitro*. The effects of two such inhibitors, L8027 (isopropyl-2 nicotinoyl-3 indole) [3, 4] and indomethacin [5] were tested under basal conditions as well as under conditions of stimulation by arginine and noradrenaline.

Material and Methods

Separation of Guinea-Pig Islets

Male guinea-pigs weighing 300–400 g were used. The animals, who had free access to food until the time of the experiment, were killed by a blow on the head immediately prior to removal of the pancreas. Islets were isolated by the collagenase technique [6, 7, 8]. Small pieces originating from three pancreases were incubated in Hanks' solution which included 26 mg collagenase (CLS IV Worthington Biochemical Corporation, New Jersey, USA.) in a total volume of 9 ml at 37°C and shaken vigorously for 8–10 minutes. Yields of 400–500 islets were obtained from 3 pancreases. Digestion was stopped by diluting with chilled Hanks' buffer and washing 5 times with fresh buffer. The last precipitate, consisting of intact islets and small fragments of acinar tissue, was resuspended and examined in a Petri dish against a black background.

Incubation of Islets

Islets were identified under the stereo-microscope and drawn up with the aid of finely drawn Pasteur pipettes [7]. Two or three

Previous studies have demonstrated that the prostaglandins PGE₁, E₂ and F_{2α} stimulate the release of glucagon by the isolated perfused rat pancreas and

Fig. 1. Effect of arginine and noradrenaline on the glucagon release during the two successive periods of incubation. Results are expressed as mean $\pm$ SEM. The number of incubation vials for each condition is indicated at the bottom of the columns

Fig. 2. Influence of L8027 on basal (upper panel) and arginine-stimulated (lower panel) glucagon release during the two successive periods of incubation. * $p < 0.001$ versus islets incubated in the absence of L8027

groups of 150–200 islets were then preincubated for 30 min in Packard vials containing 15 ml of Gey and Gey buffer [9]. The flasks were stoppered with a rubber cap and the medium was thoroughly gassed with O_2 – CO_2 (95 : 5 v/v) during the preincubation. At the end of the preincubation period, the groups of islets were transferred to Petri dishes and from there were picked out individually and distributed, in groups of 15 into Packard vials containing 2 ml of the incubation medium (Incubation I). The flasks were stoppered, gassed for 5 min and incubated for 45 min in a water bath at 37.5°C with gentle shaking at 80 cycles/min. The incubation medium was carefully removed at the end of the first incubation period and a second 45 min incubation was carried out under conditions identical to those of the first, with 2 ml of fresh medium (Incubation II). At the end of the experiment, all the islets incubated in the same medium were pooled in 3 ml of buffer and submitted to ultrasonic disruption for 30 sec. The resulting mixture was assayed for glucagon at a dilution 1/100 or 1/200 with the assay buffer.

Incubation Medium

The bicarbonate-buffer salt solution according to Gey and Gey [9] was used, containing in mmol/l Na: 149; K: 5.2; Cl: 131; Mg: 1.3; Ca: 2 and HCO_3^- : 27. Bovine crystallised albumin (Armour Pharmaceutical Company, England) was added at a concentration of 2 g/l and the pH was adjusted to 7.4. The medium was gassed by bubbling for at least 30 min with O_2 – CO_2 (95 : 5 v/v) immediately before use. According to the basic experimental protocols, the secretagogues (either L-arginine monohydrochloride or L-noradrenaline, BDH Biochemicals Ltd, England) were present in the incubation medium during incubation periods I and II, while the inhibitors of prostaglandin synthesis (indomethacin and L8027) were added to the preincubation medium as well. Indomethacin (Merck, Sharp and Dohme, Haarlem, Nederland) and L8027 (Labaz, Bruxelles, Belgium) were dissolved in methanol 35.8 and 88.0 mg/ml respectively and then diluted in the incubation medium to reach the appropriate concentrations. Noradrenaline was first dissolved in buffer containing 6 g/l ascorbic acid to reach a concentration of 1 mmol/l and this solution was diluted 1/10 in the incubation medium immediately before use. In this case, methanol or ascorbic acid were always added to the incubation medium used for control islets. All incubations were carried out in the absence of glucose in order to favour glucagon secretion. Indeed, it has been reported that a glucose concentration as low as 2.5 mmol/l suppresses amino acid stimulated glucagon release by 50% [10].

Assays

Glucagon was measured by radioimmunoassay [11] using ^{125}I -glucagon (NEN, Boston, USA) as tracer and 30K antiserum (Dr R. H. Unger, Dallas). The standard curve was prepared with MC pork glucagon (Batch 6770, kindly given by Dr Schlichtkrull, Novo Copenhagen) dissolved in an aliquot of the incubation medium. The results must therefore be understood to be expressed as "pork-glucagon equivalents" since marked species differences exist between pork and guinea-pig glucagons [12].

Calculations

Glucagon release was calculated as pg/islet/45 min. All data were expressed as mean $\pm$ SEM and were analysed using the Student *t* test for paired and non-paired data [13].

Table 1. Influence of the duration of the exposure of the islets to L8027 (10^{-5} mol/l) upon the subsequent glucagon release induced by arginine (10 mmol/l). Results are expressed as pg/islet/45 min

Experimental Conditions	Presence of L8027			Number of vials	First incubation	Second incubation
	PRE (30 min)	I (45 min)	II (45 min)			
A	—	—	—	42	23.2 ± 1.4	32.9 ± 1.5
B	+	+	+	8	11.9 ± 1.3^b	17.5 ± 1.9^c
C	+	—	—	8	31.5 ± 5.5^a	47.8 ± 5.1^c
D	—	+	+	9	23.9 ± 4.0	25.9 ± 5.0
E	+	+	—	9	15.6 ± 1.8^b	13.7 ± 1.5^c

In all experimental conditions, arginine 10 mmol/l is present during incubations I and II; ^a $p < 0.05$, ^b $p < 0.02$, ^c $p < 0.001$ versus the corresponding incubation period in Condition A

Results

1. Influence of Arginine and Noradrenaline

Figure 1 illustrates the influence of arginine and noradrenaline on glucagon release. Under control conditions, glucagon release averaged 15 pg/islet/45 min and was almost identical for incubations I and II. Arginine 10 mmol/l elicited a significant stimulation of glucagon release during incubation I ($p < 0.001$ versus controls) and the stimulation persisted and was significantly greater during incubation II ($p < 0.005$ versus incubation I). For noradrenaline 10^{-4} mol/l, the stimulation did not reach the level of statistical significance during incubation I but became highly significant during incubation II ($p < 0.001$ versus controls). Expressed as % of the glucagon content of the islets, the glucagon release during the second incubation averaged 0.67% in 45 min under control conditions and 1.1% or 1.2% when the islets were stimulated by arginine ($p < 0.001$) and noradrenaline ($p < 0.005$) respectively.

2. Influence of L8027 on Basal and Arginine-Stimulated Glucagon Release

In this series of experiments (Fig. 2), L8027 was added at concentrations of 10^{-4} or 10^{-5} mol/l to the medium used for preincubation as well as for incubations I and II. The two concentrations of L8027 did not significantly alter basal glucagon release. When arginine was added during incubations I and II, the glucagon release was moderately reduced during incubation I; paradoxically, this reduction was not statistically significant for L8027 10^{-4} mol/l but was unequivocal for L8027 10^{-5} mol/l ($p < 0.001$). Both concentrations of the inhibitor abolished arginine-induced glucagon release during incubation II ($p < 0.001$ versus islets preincubated and incubated in the absence of L8027).

3. Influence of the Duration of Contact of the Islets with L8027 on the Arginine-Induced Glucagon Release

The purpose of these experiments was to evaluate the time needed for L8027 inhibition of arginine-induced glucagon release to develop as well as to test the reversibility of this inhibition. Three conditions were used: L8027 added during preincubation alone (C), L8027 absent during preincubation but present during incubations I and II (D), and L8027 added during preincubation and incubation I, but not during incubation II (E). For this last experiment, islets were washed carefully between incubations I and II. Table 1 compares the results obtained in conditions C, D and E with the reference values of glucagon release obtained either in the absence (A) or in the presence (B) of L8027 during the whole experiment. When L8027 was present only during the preincubation period (C), there was no inhibition of the arginine-induced glucagon release; on the contrary, a significantly greater response to arginine was recorded ($p < 0.05$ during incubation I and $p < 0.001$ during incubation II versus absence of L8027). When L8027 was not present during the preincubation period and was only added together with arginine for incubations I and II, the arginine-induced glucagon release remained unaltered (condition D). Finally, careful washing of islets which had been incubated for 75 min in the presence of L8027 (30 min preincubation + 45 min incubation I) did not restore their capacity to respond to arginine during incubation II (condition E). Another series of experiments was designed in order to determine whether the inhibitory effect of L8027 [1] was completely or transiently irreversible and [2] was relatively specific for the arginine stimulus, leaving unaltered the response to noradrenaline. For this purpose, islets were preincubated for 75 min (instead of 30 min) in the presence of a higher concentration of L8027 (10^{-4} mol/l),

Table 2. Influence of a prolonged preincubation (75 min) of the islets in the *presence* of L8027 upon the subsequent glucagon release induced by arginine alone and by arginine plus noradrenaline. Incubations themselves are carried out in the *absence* of L8027. Results are expressed as pg/islet/45 min

	Number of vials	First incubation	Second incubation	
Islets preincubated (75 min) in the <i>absence</i> of L8027 and then incubated in the presence of				
A. Arginine 10 mmol/l	8	50.0 ± 4.0	45.7 ± 2.6	p < 0.025
Islets preincubated (75 min) in the presence of L8027 10 ⁻⁴ mol/l and then incubated in the <i>presence</i> of:				
B. Arginine 10 mmol/l	7	32.3 ± 5.8	45.6 ± 7.1	p < 0.005
C. Arginine 10 mmol/l + Noradrenaline 10 ⁻⁴ mol/l	7	67.2 ± 7.2	107.3 ± 28.3	

Table 3. Influence of noradrenaline and L8027 upon the glucagon release by isolated guinea-pig islets. Results are expressed as pg/islet/45 min

		First Incubation	Second Incubation
Control medium	n = 13	11.1 ± 1.9	13.7 ± 2.9
Control medium + Noradrenaline 5.10 ⁻⁷ mol/l	n = 15	21.7 ± 1.9 ^a	24.4 ± 2.4 ^a
Control medium + Noradrenaline 5.10 ⁻⁷ mol/l L8027 10 ⁻⁴ mol/l	n = 16	19.4 ± 1.8 ^a	18.4 ± 2.2

^a p < 0.01 *versus* control medium**Table 4.** Influence of noradrenaline and indomethacin upon the glucagon release by isolated guinea-pig islets. Results are expressed as pg/islet/45 min

	Number vials	First Incubation	Second Incubation
Control medium	8	31.4 ± 3.8	31.7 ± 2.8
Control medium + noradrenaline 10 ⁻⁴ mol/l	8	45.8 ± 11.3	49.4 ± 7.5 ^a
Control medium + noradrenaline 10 ⁻⁴ mol/l indomethacin 10 ⁻⁴ mol/l	10	34.0 ± 5.7	33.9 ± 2.5 ^b

^a p < 0.05 *versus* control medium; ^b p < 0.05 *versus* noradrenaline alone. The high glucagon release observed in this experiment is entirely due to the large size of the islets. For some unknown reason, their mean glucagon content was 5950 pg/islet while the mean value for all other experiments was around 3000 pg/islet. Expressed in % of islet glucagon content, the release was similar to that reported in Figure 1 (0.54 and 0.67 % respectively)

washed carefully, and then incubated for two successive periods, either in the presence of arginine alone (10 mmol/l) or in the presence of arginine plus noradrenaline (10⁻⁴ mol/l). As depicted in Table 2, islets preincubated with L8027 release significantly less glucagon in response to arginine than control islets

Fig. 3. Influence of indomethacin on arginine-stimulated glucagon release during the two successive periods of incubation

(p < 0.025) during the first incubation period, but completely recovered their sensitivity to arginine during the second incubation period (Condition B). Moreover, noradrenaline (10⁻⁴ mol/l) was able to overcome the persistent inhibitory action of L8027 during the first incubation and to exert an additional stimulation above that of arginine during the second incubation (Condition C).

4. Influence of L8027 on Noradrenaline-Induced Glucagon Release

As already mentioned (see Fig. 1) noradrenaline 10⁻⁴ mol/l increased glucagon release during incubation II

from a control value of 16.2 ± 1.3 pg/islet/45 min to 39.5 ± 7.0 , $n = 9$, $p < 0.001$). When L8027 was present during preincubation and incubations I and II, the noradrenaline-induced glucagon release was not significantly altered (33.1 ± 2.7 pg/islet/45 min, $n = 8$). A lower concentration of noradrenaline ($5 \cdot 10^{-7}$ mol/l) was still capable of stimulating significantly glucagon release during the two incubation periods (Table 3). This effect was no more significant during the second incubation when the whole experiment was carried out in the presence of L8027 (10^{-4} mol/l).

5. Influence of Indomethacin on Arginine- or Noradrenaline-Induced Glucagon Release

The significant stimulation of glucagon release induced by arginine 10 mmol/l was completely blocked by indomethacin 10^{-4} mol/l. This inhibition was statistically significant for incubations I and II (Fig. 3). Similarly, indomethacin blocked the glucagon release stimulated by noradrenaline 10^{-4} mol/l (Table 4). The experimental protocol used for indomethacin was similar to that used for L8027 in Fig. 2: the inhibitor of PG synthesis was present during preincubation, and incubations I and II whereas arginine or noradrenaline were added after preincubation and thus present during incubations I and II.

Discussion

L8027 is a compound with anti-inflammatory properties which has been shown to inhibit the synthesis of PGE_2 and $\text{PGF}_{2\alpha}$ [4]. The prostaglandin synthetase from microsomal preparations of bovine seminal vesicles is markedly inhibited by concentrations 5×10^{-6} to 10^{-5} mol/l L8027 [4].

The present work demonstrates that this compound does not alter basal glucagon release, blocks almost completely the glucagon response to arginine, reduces the glucagon release evoked by a low noradrenaline concentration, but does not alter the stimulatory effect of a high noradrenaline concentration in the incubation medium. Indomethacin, a classical inhibitor of PG synthesis (review in 5), similarly abolishes arginine- and noradrenaline-induced glucagon release. In the case of L8027, we observed that the inhibitory action was not immediate and was only displayed when the islets were preincubated with the compound for 30 minutes before arginine was added. Moreover, a significant rebound phenomenon was observed when, after 30 minutes contact, L8027 was removed at the time of arginine stimulation. A more prolonged incubation of the islets with L8027 (75 minutes) resulted in a transient insensitivity to

arginine despite the removal of the inhibitor. That the islets retain their response to noradrenaline and subsequently recover their sensitivity to arginine suggests that these observations do not correspond to a damaged islet system but rather to various states of interaction between L8027 and the prostaglandin synthetase system but our protocol does not bring any information about the molecular mechanism(s) underlying this interaction. Although the possibility exists that indomethacin and L8027 might have membrane effects *per se*, independent of their specific action on the prostaglandin synthetase system, the most likely explanation for our results is that endogenously synthesized prostaglandins are involved in the A cell secretory response. Thus, not only can exogenous PGE_1 , PGE_2 and $\text{PGF}_{2\alpha}$ stimulate the release of glucagon, as demonstrated by Pek *et al.* [1], but we suggest that prostaglandins could be generated within the islets and exert their biological effect locally. Another possibility is that, besides prostaglandins themselves, other derivatives of arachidonic or dihomo- γ -linolenic acids metabolism such as endoperoxides, thromboxanes or prostacyclins are involved. Several studies have suggested that some interaction of catecholamines and prostaglandins may occur. This was extensively studied on the kidney where it was shown that renal nerve stimulation, as well as norepinephrine infusion, resulted in a prostaglandin release [14]. Using the isolated perfused rabbit kidney, Needleman *et al.* [15] demonstrated that the prostaglandin release was blocked with indomethacin and α -adrenergic blockade with phenoxybenzamine. A similar α -adrenergic blockade on splenic nerve stimulation-induced prostaglandin release was reported by Ferreira *et al.* [16]. Confirming previous results [17, 18, 19] we have demonstrated here that noradrenaline stimulates glucagon release. Both inhibitors of prostaglandin synthesis interfere with this stimulatory effect, but indomethacin seems more potent than L8027. One could speculate that prostaglandins are involved in the activation of A cell adenylate cyclase by catecholamines. In fact, this hypothesis would be in agreement with previous results obtained with a rat islet membrane adenylate cyclase and suggests that hormone-receptor units may be influenced by PGE_1 [20].

Finally, although isolated guinea-pig islets have proven to be a valuable model for studying glucagon secretion by several group of investigators [8, 21, 22], the possible species specificity of the phenomena must be taken into consideration.

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