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EFFECT OF EPINEPHRINE AND NOREPINEPHRINE ON IMMUNO-REACTIVE INSULIN SECRETION FROM ISOLATED ISLETS OF LANGERHANS

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Abstract—Earlier studies have demonstrated the inhibitory nature of epinephrine and norepinephrine on pancreatic insulin release. The present study reports their effect on beta-cell IRI release in an isolated islet system. Results show that epinephrine and norepinephrine inhibit islet-IRI release at the 100–10 μM level. The alpha adrenergic blocker phentolamine (100 μM) but not the beta adrenergic blocker propranolol (100 μM) can reverse this catecholamine induced inhibition of islet-IRI release. This clearly suggests that epinephrine and norepinephrine inhibit insulin release via alpha-adrenergic pathway.

Since the direct inhibitory role of epinephrine on pancreatic immuno-reactive insulin (IRI) release was traced out [1–3] attempts have been made to establish a causal relationship between stress and the diabetic syndrome. The level of blood catecholamine has been found to be higher in diabetics both during rest and exercise [4]. Robertson *et al.* [5] also found that in diabetics the catecholamine level remained significantly elevated in comparison to healthy controls both before and after glucose administration. Intravenous phentolamine administration increased the circulating insulin level both in diabetics and non-diabetics but the rate of increase of insulin level in diabetics was significantly higher than that in non-diabetics [5]. These findings indicate that excessive catecholamine secretion may lead to an abnormal alpha-adrenergic activity that contributes to a defective insulin secretion. These *in vivo* studies are not conclusive, as they cannot rule out the effect of several internal variables. The aim of the present study was to investigate the effect of epinephrines and norepinephrines used singly and in presence of the blockers on the β-cell IRI release in a system which is free from the influence of the neuro-endocrinal circuit.

MATERIALS AND METHODS

Chemicals. The fine chemicals used were obtained from the following sources: collagenase-type-V, (−) arterenol hydrochloride, DL-propranolol HCl, epinephrine bitartrate (all from Sigma Chemical Co., U.S.A.), phentolamine (CIBA–Geigy, Bombay, India), insulin radioimunoassay kit (BARC, Bombay, India).

Other reagents used were of analytical grade.

Animals. Adult male albino rats (200–250 g) of Charles Foster strain were used.

Estimation of islet-IRI release. Islets were collected by collagenase digestion method [6]. Batches of 10 islets were incubated in 0.5 ml of Kreb's Ringer bicarbonate buffer (pH 7.4, previously gassed with $O_2:CO_2::19:1$), containing 1 mg/ml bovine serum albumin (BSA) for 1 hr at 37° with gentle shaking (90 cycles/min). The media were supplemented with 8.33 mM of glucose and varying concentrations of different substances as indicated in the results. Control media were supplemented with 8.33 mM glucose only.

After incubation, media was collected to assess the islet IRI release by radioimmunoassay [7]. Results were expressed in terms of micro units of IRI release/islet.

Data analysis. Mean, S.D. and S.E. of each set of data were calculated and statistical evaluations were made by Student's *t*-test.

RESULTS

The basal value of IRI release from glucose (8.33 mM) challenged islets was 3.898 ± 0.43 μU/islet. In the presence of 1, 10 and 100 μM of epinephrine the values of IRI were 4.058 ± 0.485, 1.281 ± 0.085 and 1.037 ± 0.084 μU/islet respectively (Table 1). This indicates that epinephrine at 10 and 100 μM inhibited glucose-induced IRI release but had no effect at 1 μM.

In the presence of 1, 10 and 100 μM of norepinephrine, IRI released were 2.617 ± 0.469, 1.425 ± 0.12 and 1.125 ± 0.116 μU/islet respectively (Table 2). This also shows that norepinephrine inhibited glucose-induced IRI release at 10 and 100 μM level but not at 1 μM level.

When 10 μM of epinephrine and 100 μM propranolol were added to the medium the release value of islet-IRI is 1.782 ± 0.206 μU/islet whereas when 10 μM epinephrine and 100 μM of phentolamine were added the release values of IRI was 4.078 ± 0.477 μU/islet (Table 3). This indicates that phentolamine but not propranolol could reverse the epinephrine effect on islet-IRI release.

In presence of 100 μM norepinephrine and 100 μM propranolol the release values of islet IRI was

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Table 1. Effect of different concentrations of epinephrine on IRI release at 1 hr incubation by the islets in the presence of glucose

Glucose (mM)	Epinephrine	IRI release (μU/islets)
8.33	—	3.898 ± 0.43 (11)
8.33	1	4.058 ± 0.485 (9)
8.33	10	1.281 ± 0.085 (9)†
8.33	100	1.037 ± 0.084 (6)†

Figure in parenthesis denotes the number of observation. Values are mean ± S.E.M.
† $P < 0.001$ (comparison between the control and treated values).

Table 2. Effect of different concentrations of norepinephrine on IRI release at 1 hr incubation by the normal rat islets in the presence of glucose

Glucose (mM)	Norepinephrine (μM)	IRI release (μU/islet)
8.33	—	3.898 ± 0.43 (11)
8.33	1	2.617 ± 0.469 (10)
8.33	10	1.425 ± 0.12 (10)†
8.33	100	1.125 ± 0.116† (13)

Figure in parenthesis indicates the number of observation. Values are mean ± S.E.M.
† $P < 0.001$ (in comparison to the control value).

Table 3. The effect of adrenergic blockers on epinephrine-induced inhibition of glucose stimulated islet-IRI release at 1 hr incubation

Glucose (mM)	Epinephrine (μM)	Blockers (μM)	IRI release (μU/islet)
8.33	—	—	3.898 ± 0.43 (11)
8.33	10	—	1.281 ± 0.085 (9)†
8.33	10	Propranolol 100	1.782 ± 0.206 (15)†
8.33	10	Phentolamine 100	4.078 ± 0.477 (14)

Figure in parenthesis denotes the number of observation. Values are mean ± S.E.M.
† $P < 0.01$ (comparison between the control and treated values).

1.77 ± 0.121 μU/islet, whereas with 100 μM norepinephrine and 100 μM phentolamine it was 2.667 ± 0.208 μU/islet (Table 4). This demonstrates that phentolamine but not propranolol could disinhibit the norepinephrine-induced inhibition to a significant extent.

Table 4. The effect of adrenergic blockers on norepinephrine induced inhibition of islet-IRI release at 1 hr incubation in presence of glucose

Glucose (mM)	Norepinephrine (μM)	Blockers (μU)	IRI-release (μU/islet)
8.33	—	—	3.898 ± 0.43 (11)
8.33	100	—	1.125 ± 0.116 (13)†
8.33	100	Propranolol 100	1.77 ± 0.121 (10)†
8.33	100	Phentolamine 100	2.667 ± 0.208 (10)

Figure in parenthesis denotes the number of observation. Values are mean ± S.E.M.
† $P < 0.001$ (comparison between the treated and control values).

DISCUSSION

In the present study, the effect of blockers alone on islet-IRI release were not tested because earlier reports [8–11] had shown that propranolol or phentolamine at 100 μM level have no effect on pancreatic-IRI release in intact and *in vitro* systems.

Ashcroft *et al.* [12] had shown that islet-IRI release occurred at a slower rate when challenged by glucose concentration not higher than 5.6 mM. Between 5.6 mM and 16.7 mM glucose the islet-IRI release occurred at a brisk pace. So in the present study glucose concentration was set at 8.33 mM—a level at which any change in secretion due to the administration of test substance could easily be detected.

The inhibitory effect of these two biogenic amines has been shown in intact system [1–3, 13] and also in cut pieces of pancreas [8–10, 14], but not in an isolated islet system. Cut pieces of pancreas do have intact neural connection, which might be a source of endogenous catecholamine secretion through the nerve endings. Thus with the above experimental model it becomes difficult to quantitate the effect of an exogenously added amine as the result may be vitiated by the endogenous amine secretion.

The isolated model of islets of Langerhans used in this experiment were devoid of neural connection as such the effects appeared are entirely due to the influence of exogenous amine administration.

Results obtained from this study clearly demonstrate that both the catecholamines epinephrine and norepinephrine in 10–100 μM in incubation media inhibit islet-IRI release. Phentolamine but not propranolol disinhibits this catecholamine induced inhibition in islet-IRI release. As phentolamine is an alpha-adrenergic blocker it is clear that epinephrine and norepinephrine inhibit insulin release via alpha-adrenergic mechanism.

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